

Bioaccessibility of Dietary Carotenoid Compounds

Assessed from Durum Wheat

By

Hannah Oduro-Obeng

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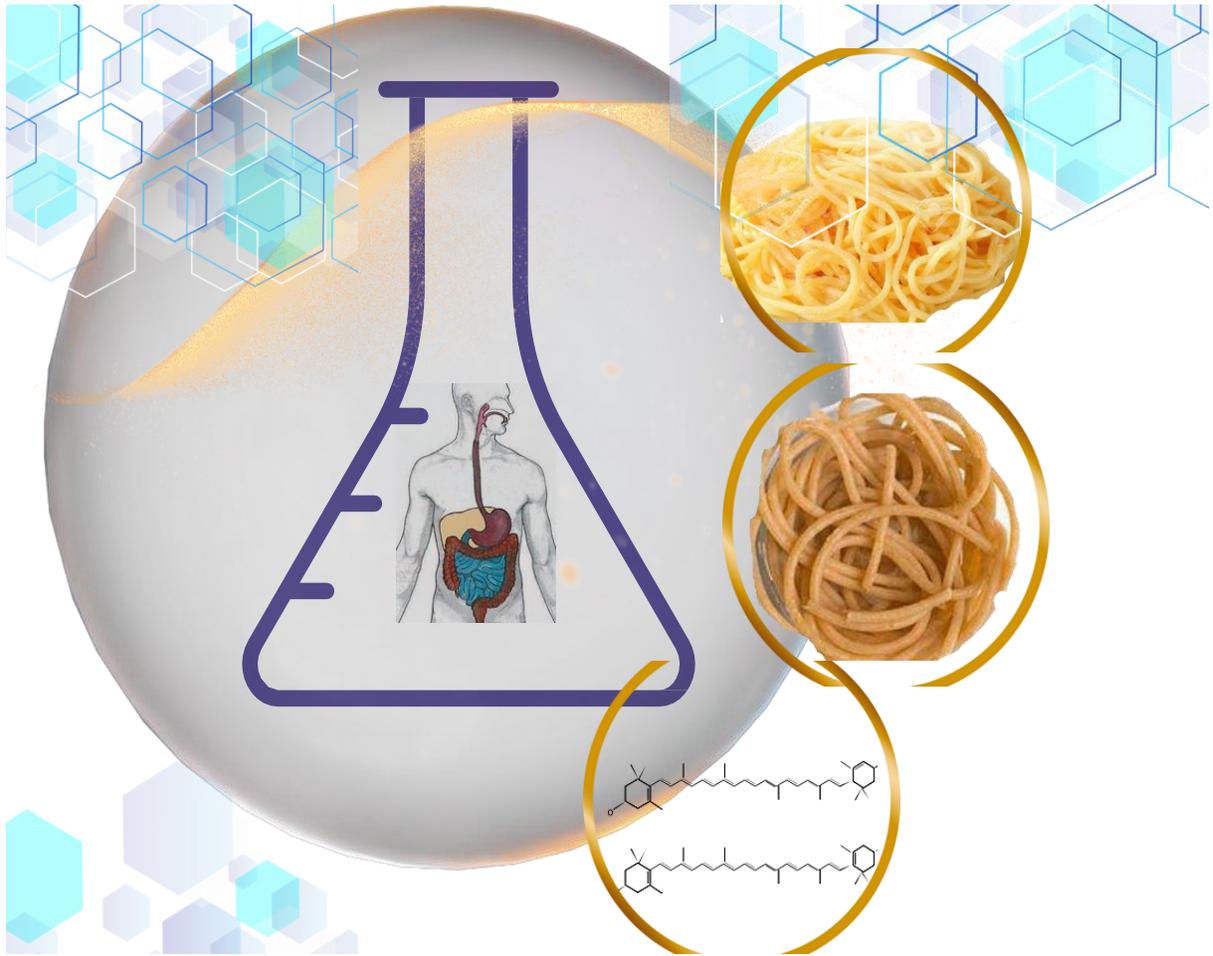
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ABSTRACT

Wheat is an economically important crop worldwide and is the most consumed cereal in Canada. Among the tetraploid wheat species, durum wheat is predominantly cultivated and accounts for about 5-8 % of the wheat produced worldwide. Statistics show that Canada is a significant durum wheat producer, leading the world in exports. Nutritionally, durum wheat is a source of both macronutrients and micronutrients, including starch, proteins, fiber, vitamins and minerals. It is also an important source of grain phytochemicals, including carotenoids. Indeed, its high concentration of carotenoids makes it an ideal choice for producing certain foods such as pasta. Although semolina is the preferred ingredient for pasta production due to its chemical composition, whole wheat pasta consumption is equally encouraged due to the health benefits of whole grains. Some health benefits associated with the intake of carotenoid-rich foods include antioxidative and anti-inflammatory effects. To exert any of these health benefits, however, potentially absorbed dietary carotenoids must first be bioaccessible. Therefore, the objectives of this research were to 1) characterize the wheat varieties in terms of their carotenoid contents in milled flour, dry pasta, and cooked pasta, 2) evaluate the effects of cooking duration and the food matrix on the retention and chemical transformation of carotenoids, 3) evaluate the effect of the food matrix and food processing on carotenoid bioaccessibility, digestive stability and absorption efficiency, examining matrix composition properties which may influence carotenoid bioaccessibility, 4) determine the antioxidant capacity of carotenoids as influenced by the food matrix and pasta cooking duration before and after digestion. Firstly, the identification and quantification of carotenoids were accomplished using HPLC. The wheat varieties differed in their initial carotenoid contents. Carotenoid composition also differed among grain flour types with

lutein being the predominant carotenoid compound. Upon pasta extrusion, carotenoid contents decreased, and pasta cooking induced further decreases from their initial contents in flour. Nonetheless overcooking (OC) pasta promoted a higher extractability of carotenoids than when cooked to al dente (AI) or fully cooked (FCT). Cooking generally increased the cis-carotenoid content from their uncooked samples, which was significantly higher in the OC samples. Carotenoids were greatly retained in the Transcend variety after cooking its pasta despite its low inherent carotenoid profile. A static model was used to simulate pasta gastrointestinal digestion and determine carotenoid bioaccessibility. Furthermore, this was coupled with an in vitro bioavailability assessment using human Caco-2 cells to determine carotenoid uptake. All carotenoid isomers were bioaccessible. However, the magnitude of bioaccessibility depended on the wheat flour type, the carotenoid physical properties, and the wheat variety. For lutein, Transcend and Spitfire had the highest and lowest bioaccessibilities, up to 156.94 % and 124.45 %, respectively, in refined semolina (RS) and up to 139.06 % and 104.03 %, respectively, in whole flour (WWF) pasta. The variation observed between varieties indicates that durum wheat chemical composition influences the effects of pasta cooking duration on the bioaccessibility of its carotenoids. Cooking RS pasta to AI and OC had the highest and lowest bioaccessible lutein content, respectively, suggesting that OC caused extensive degradation (less digestive stability) of carotenoid, leading to a reduction in their transfer to mixed micelles or OC modified the food matrix entrapping released carotenoids during digestion. The effect of cooking was not seen among WWF pasta. Transcend was subsequently selected for the cellular uptake study. Lutein was the only carotenoid taken up by the caco-2 cells. Its uptake efficiency was low, ranging from 3.37-7.95 % for RS pasta and 3.65-5.24 % for WWF samples. The cellular antioxidant activity of the carotenoids revealed that undigested and digested carotenoids were capable of attenuating reactive

oxygen species (ROS)-induced oxidative stress. However, after digestion, the protective capacity of the carotenoids slightly reduced. Unlike the undigested samples, digested RS and WWF were not significantly different in attenuating oxidative stress. At high doses, digested carotenoids in RS but not WWF exhibited prooxidant activity, suggesting that carotenoid composition or structure influenced its antioxidant activity. It can be concluded that durum wheat is a source of bioaccessible carotenoids. However, the magnitude of carotenoid bioaccessibility will depend on the effects of food processing on the wheat food matrix, which will ultimately influence carotenoid stability and its bioaccessibility after digestion. This research is the first to report on the bioaccessibility and bioavailability of carotenoids in different durum wheat varieties, comparing whole and refined semolina flour matrices. The findings provide insights into the factors that influence the bioaccessibility of carotenoids in cereal grains, further laying the foundation for additional research into these factors. Knowledge gained facilitates the selection of appropriate grain varieties or food matrices and food processing techniques for high carotenoid functional food development and their health-promoting effects.

Dedication

*To my ever-loving father, George Oduro (late) and my wonderful family for your love, support,
and prayers*

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Thesis Format

This thesis has been prepared in the manuscript format. The referencing style is that of the Food Chemistry Journal. The thesis has six chapters, of which chapters 1 and 2 provide the introduction and literature review, respectively. Chapters 3, 4, and 5 are experimental chapters that contain published manuscripts. Chapter 6 summarises the main findings of the research, gives a general conclusion, and proposes recommendations for future research. The following are the experimental chapters found in this thesis and information on the contribution of the authors.

Experimental chapters and contributions of authors

Chapter 3: The influence of cooking duration on carotenoids, physical properties and in vitro antioxidant capacity of pasta prepared from three Canadian durum wheat varieties.

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- Dr. Bin Xaio Fu: Sample acquisition, reviewing and editing.
- Dr. Trust Beta: Supervision, funding acquisition, project administration, reviewing, and editing the manuscript.

Chapter 4: The effect of cooking duration on carotenoid content, digestion, and potential absorption efficiencies among refined semolina and whole wheat pasta products.

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- Dr. Kun Wang: Sample preparation, reviewing and editing.
- Dr. Bin Xiaio Fu: Sample acquisition, reviewing and editing.
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- Dr. Daniel Zogona: Methodology, reviewing and editing.
- Dr. Jithendra Paliwal: Methodology, reviewing and editing.
- Dr. Bin Xiaio Fu: Sample acquisition, reviewing and editing.

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CHAPTER 1

General Introduction, Hypothesis and Objective

1. Introduction

Wheat (*Triticum* spp) is one of the top three cultivated cereal grains in the world with key exporting countries constituting Canada, Russia, and Australia (Shewry, 2024). Globally, among the cultivated wheat species, durum wheat (*T. turgidum* L. subsp. *durum*, tetraploid, genome AABB) contributes about 5-8% of the total wheat produce (Shewry, 2009). According to the 2023 new wheat crop report from Cereals Canada, 12% of the global wheat trade is accounted for by Canada with Canada leading the world in durum wheat exports. About 17% of the total wheat produced in Canada was durum wheat (Cereals Canada, 2023).

Unlike common wheat, durum wheat is mainly grown for food. Durum wheat is utilized in the production of couscous, bulgar and some speciality breads with pasta being the main food product produced from durum wheat (Kadkol & Sissons, 2016). Semolina is the choice ingredient for the processing of these food products mainly due to its unique chemical composition and characteristics which are inherited from its raw material (Fu, Chiremba, Pozniak, Wang, & Nam, 2017). Ideally, durum related food products such as pasta require grains with high protein content, gluten strength, and high yellow pigment content.

Nutritionally, durum wheat grains are a major source of carbohydrate (mainly starch). Nonetheless, other macronutrients such as non-starch polysaccharides (fibre), and essential micronutrients such as thiamin, tocopherols iron, calcium, zinc, and potassium can be found in appreciable amounts (Apea-Bah & Beta, 2021; Ferruzzi, Kruger, Mohamedshah, Debelo, & Taylor, 2020; Tian et al., 2022). Due to the numerous health benefits associated with the consumption of whole grains, investigations into their phytochemical composition beyond basic nutrition has been a paramount agenda among many research groups. Durum wheat is a rich source of cereal carotenoids, mainly the xanthophylls (lutein and zeaxanthin) (Fig. 1.1). It has about 2-3

times (an approximation of about 5-6 μ g/g) the content of carotenoids found in common wheat (Abdel-Aal, Young, Rabalski, Hucl, & Fregeau-Reid, 2007). Compared to fruits and vegetables, however, carotenoid content in durum wheat is low. Nonetheless, regular consumption of staple foods produced from durum wheat can significantly enhance the daily intake of these carotenoids.

The distribution of carotenoids, within and between different durum wheat genotypes or varieties is uneven (Ndolo & Beta, 2013). Hence the utilization of the whole grain as an ingredient in food production may be more beneficial. Carotenoids such as lutein and zeaxanthin are considered natural antioxidants, and most of the health benefits associated with carotenoids are ascribed to their antioxidative properties. Carotenoids act as quenchers of singlet oxygen species as well as scavengers of peroxy radicals through their electron transfer and hydrogen donation mechanisms (Cooperstone & Schwartz, 2016; Edge, 1997; Moran, Mohn, Hason, Erdman, & Johnson, 2018). Due to their antioxidative properties, the regular and high dietary intake of carotenoids has been associated with the reduced incidence of several non-communicable diseases such as cancer, cardiovascular diseases (CVD) and other inflammatory conditions (Amorim-Carrilho, Cepeda, Fente, & Regal, 2014; Eroglu, Al'Abri, Kopec, Crook, & Bohn, 2023). Lutein and zeaxanthin, for instance, have been found to accumulate in high amounts in the eye serving as a protectant of the eye against photooxidation (Nwachukwu, Udenigwe, & Aluko, 2016).

To be able to mediate any of these health benefits and confer protection, carotenoids must be released from their food matrices, digested, and absorbed through the epithelial cells of the small intestine. Their fate through digestion up until absorption is of crucial importance in the determination of their activity *in vivo* (Granado-Lorencio, Blanco-Navarro, Pérez-Sacristán, & Hernández-Álvarez, 2017; Rošul et al., 2022). Nonetheless carotenoid solubility in the aqueous environment of the gastrointestinal tract can be low and varied (Eroglu et al., 2023; Moran et al.,

2018). Several dietary and non-dietary factors perpetuate this variability. Hence, the effectiveness of carotenoid release and its functionality largely depends on an understanding of some these diet-related factors.

In most plant foods including cereals, carotenoids are found as complex structures with proteins or in lipid- dissolved crystalline structures (de Oliveira et al., 2020; Schweiggert & Carle, 2017). These structures physically restrict their efficient release and subsequent utilization. According to several studies, the food matrix appears to be one of the most relevant food-related factors limiting the availability of dietary carotenoids at the pre-absorptive stages (Ornelas-Paz, Failla, Yahia, & Gardea-Bejar, 2008; Yonekura & Nagao, 2007) and its linkages at molecular levels might explain the high variability of micellization and bioaccessibility of the same carotenoid in different foods or even in the same food (Cervantes-Paz et al., 2017).

Aside from the food matrix, other notable factors reported to mediate carotenoid availability include carotenoid type (species) and source (food and non-food born), other pre-harvest factors such as variety or genotype variations as defined by cultivation techniques and environmental conditions and the physical disposition of the carotenoid within the food (Amorim-Carrilho et al., 2014; Cilla, Bosch, Barberá, & Alegría, 2018; Granado-Lorencio et al., 2017; Rodrigues, Mariutti, & Mercadante, 2016; Schweiggert & Carle, 2017; Zaripheh & Erdman, 2002).

More importantly, post harvest factors such as food processing techniques including type or intensity and/or time or duration, carotenoid saturation, interactions with other carotenoids or biopolymers for transport, absorption modifiers (e.g. lipids, fibre, phytosterols and metals) and other intrinsic host-related factors such as gender, age, and genetic variations can also impact carotenoid release, uptake, and subsequent bioactivity (Borel, 2003; Cardoso, Afonso, Lourenço,

Costa, & Nunes, 2015; Desmarchelier & Borel, 2017). For example, Colle, Lemmens, Van Buggenhout, Van Loey, and Hendrickx (2010), in a recent study, revealed that the improvement of the bioaccessibility of lycopene from thermally processed tomatoes was only significant after intense thermal processing. Therefore, the duration or intensity of food processing does play a significant role in influencing carotenoid availability for absorption (Palmero, Lemmens, Hendrickx, & Van Loey, 2014).

Food processing has the potential to facilitate an increase or decrease of carotenoid content, release, or even change their profile (Cilla et al., 2018; Tian et al., 2022). Processing may physically modify ultrastructural features in food, affecting enzyme accessibility, which can further influence the stability and release of these lipophilic bioactive compounds going through the gut (Ficco et al., 2019). The process-induced structural transformations to food can also influence the digestive profile (chemistry) of carotenoids (O'Sullivan, Galvin, Aisling Aherne, & O'Brien, 2010; Schieber & Carle, 2005; Updike & Schwartz, 2003). Indeed, the incidence of carotenoid chemical changes (isomerization) has been related to the intensity and time of processing, and their occurrence might also be a function of their molecular structure or cellular localization within the food matrix (Nguyen, Francis, & Schwartz, 2001).

Bioaccessibility by definition is the maximum fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (Cardoso et al., 2015).

Despite the reported low bioaccessibility of dietary carotenoids, de Oliveira et al. (2020) reveals that the effect of food processing on carotenoid micellization and bioaccessibility may be tissue-specific and may appear to favor non-photosynthetic plant tissues such as seeds where carotenoids are found as structural complexes compared to photosynthetic tissues such as leaves. Generally,

the effects of some of the food-related factors including the food matrix and food processing on carotenoid retention, digestive stability, bioaccessibility, and absorption have been investigated separately to a large extent and especially among non-cereal grains or photosynthetic foods (Barba et al., 2017; Lemmens et al., 2014; O'Brien, 2008; O'Connell, Ryan, & O'Brien, 2007; Thakur et al., 2020; Tyssandier et al., 2003). Studies are lacking on the effects of these factors on the bioaccessibility and bioavailability of carotenoids from wheat grain-based foods (Tian et al., 2022).

Pasta is distributed and consumed worldwide and is an important component of our daily diet. It can be made from different cereals, and fortified with different nutrients, bringing reasonable nutritional advantages to different populations. Even though durum wheat pasta is considered a low-cost food as well as a healthy one among diabetics due to its low glycemic index (Fares et al., 2008; O'Hearn et al., 2023), its phytochemical benefits, aside from basic nutrition, have not been explored in depth. Before pasta is consumed, it is cooked. Structural-induced changes caused by pasta processing can impact the digestibility of carotenoids. Moreover, the food matrix of pasta, including its macronutrient composition, can affect carotenoid digestibility. In this study, durum wheat pasta was used as a model food to help understand the influence of the food matrix and processing conditions, such as cooking duration on the mechanism of carotenoid release, bioaccessibility and potential bioavailability during digestion. To do this, pasta processed from different durum wheat flours was explored.

Over the past few years, the use of various *in vitro* digestion models to assess carotenoid bioaccessibility and to predict subsequent bioavailability have been developed and reviewed extensively (Etcheverry, Grusak, & Fleige, 2012). Essentially, these models have been used as a surrogate to evaluate the natural phenomena underlying nutrient bioaccessibility. Their use may

serve as high throughput screening tools, enabling the control of numerous variables and ensuring high reproducibility while cutting down cost and bioethical issues related to the use of human and animal subjects (Dupont et al., 2019; Minekus et al., 2014). Nonetheless, the heterogenous and complex conditions, including physiological factors and fluid-mechanic forces characteristic of in vivo conditions, are not entirely replicated, and variations in the use of these in vitro model parameters such as pH, enzyme and bile concentrations, digestion duration exist among different labs (Wright, Pietrangelo, & MacNaughton, 2008). To resolve these differences and to make results comparable across research labs, the INFOGEST 2.0 standardized in vitro gastrointestinal protocol was developed (Brodkorb et al., 2019; Minekus et al., 2014).

In this study, we sort to determine the multi factorial influence of the food matrix and food processing on pasta carotenoid content, stability, bioaccessibility and cellular uptake after simulated digestion using the standardized INFOGEST protocol coupled with the human intestinal Caco-2 cell line. According to Payne, Zihler, Chassard, and Lacroix (2012) and Etcheverry and his colleagues (2012), this combination of an in vitro digestion model and the human cell line is a much better and complete approach to assessing the relative availability of carotenoids. We envisage that using the above-combined model will facilitate an understanding of the release and absorption dynamics of durum wheat carotenoids after their oral administration. Knowledge from this study will provide insights into food matrix components that influence carotenoid bioaccessibility of cereal grain while facilitating the selection of appropriate varieties or food matrices and food processing techniques for high carotenoid functional food development and their health-promoting effects.

To facilitate the investigation of this study, the following hypotheses and research objectives were garnered.

1.1. Hypothesis

- a) The different cooking durations employed will differentially affect the physical and technological properties of pasta and influence its chemical composition and contents from the different pasta food matrices.
- b) The different cooking durations will also influence carotenoid isomer profile changes, micellization efficiency and bioaccessibility at the end of in vitro digestion through pasta food matrix modification.
- c) Pasta structural changes through food processing will affect pasta digestion behaviour and further influence carotenoid species stability.
- d) Moreover, digested carotenoids will have similar absorption efficiencies irrespective of the food matrix and cooking duration.
- e) Antioxidant carotenoids from digested and undigested pasta samples will attenuate free radical-induced oxidative stress using both cellular and chemical antioxidant assays.

1.2. Objectives

The main aim of this research was to determine the concentration of carotenoids in durum wheat accessible for absorption after digestion and to assess to what extent food processing and the food matrix may mediate carotenoid bioaccessibility using whole wheat and refined semolina pasta produced from different wheat varieties. Our secondary objective was to determine antioxidative capacity of these carotenoid isomers before and after in vitro digestion using different biochemical assays.

Specifically, we evaluated.

- a) The carotenoid profile of pasta before and after cooking to al dente, fully cooked time and overcooked to identify the impact of cooking duration on carotenoid stability, pasta

cooking quality and in vitro antioxidant capacity from three durum wheat varieties (AAC Spitfire, CDC Precision and Transcend) milled to refined semolina and whole wheat flour.

- b) The effects of cooking duration on the digestive stability, bioaccessibility (solubility) and potential absorption efficiencies of carotenoid isomers among refined semolina and whole wheat flour pasta samples.
- c) Pasta matrix components and establish potential correlation with carotenoid bioaccessibility while proposing release mechanisms.
- d) Carotenoid cellular uptake and determined its cellular antioxidative capacity as affected by cooking duration using human Caco-2 intestinal cells.
- e) The relationship between durum wheat food structure, carotenoid bioaccessibility, and uptake.

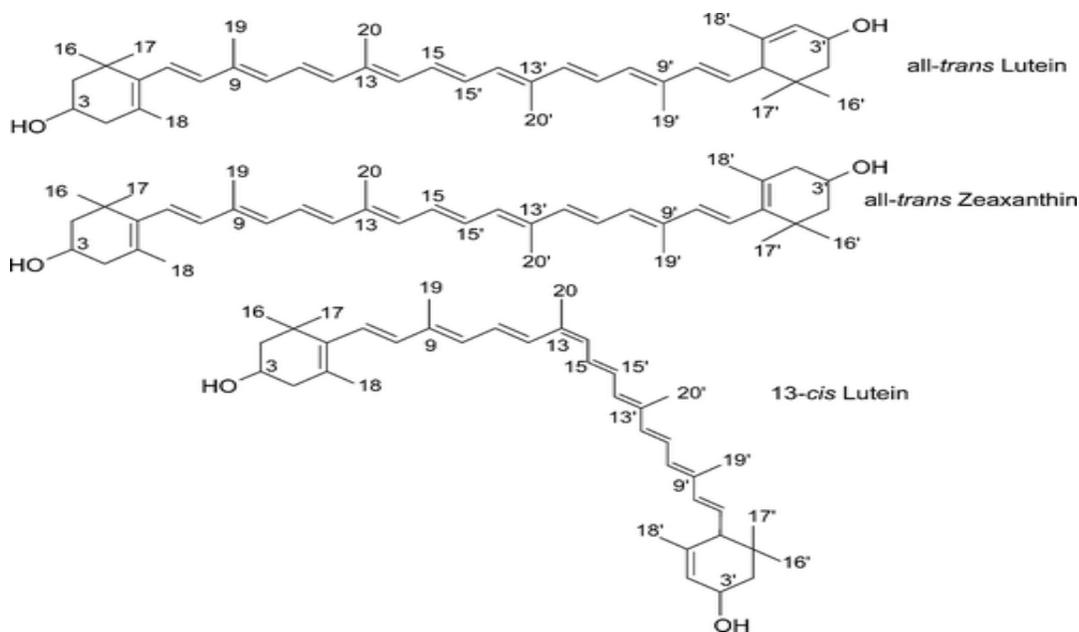


Figure 1.1. Chemical structures of the main carotenoid species (all-trans lutein and zeaxanthin) and a minor component (13-cis lutein) in durum wheat. Adapted from Abdel-Aal el, Young, Akhtar, and Rabalski (2010) and used with permission.

CHAPTER 2

Literature Review

2. Literature Review

2.1. Description of study varieties used in this study

Data from this study was obtained from three durum wheat varieties that were released in 2010 from various breeding programs and research activities and met the grading system of the Canadian Western Amber Durum (CWAD) wheat market. They are AAC Spitfire, CDC Precision, and Transcend. With reference to the Canadian Grain Commission (2023) these wheat varieties ranked among the top 4 CWAD varieties grown in western Canada from 2019 to 2023, due to their relatively high protein and yellow pigment content. Specifically, Transcend was the top variety grown in 2023. Historically, these wheat varieties were developed from several genetic enhancements of the earlier durum wheat cultivars Wascana and Wakooma. Subsequent significant changes occurred with the release of Kyle in 1991. Kyle accounted for about 80% of the total durum wheat seeded area in western Canada. After Kyle was AC Avonlea, which became the most abundantly grown cultivar from 2005 to 2006 because of its higher grain yield and protein concentration. AC Avonlea was quickly replaced by the first low cadmium uptake cultivar Strongfield which combined the traits, high grain yield, high grain protein concentration, good standability, and strong gluten. For the period 2007 to 2015, Strongfield was the most widely grown cultivar, occupying from 40% to 80% of the seeded area. Over the years, the dynamic change in durum wheat cultivars occurred mostly between preceding dominant cultivars and the ones with various phenotypic traits. In 2017, three cultivars accounted for more than 70% of Canadian durum wheat seeded area: Transcend, about 43%; Strongfield at 16%; and Brigade at 14%. Transcend and Brigade both have lower *Fusarium* head blight (FHB) and trichothecene deoxynivalenol (DON). More recently, several varieties have been released with higher grain yield

than Strongfield (Li et al., 2018). The following gives a brief description of the agronomic characteristics of AAC Spitfire, CDC Precision, and Transcend, their respective year of release, and grain yield relative to the check cultivar Strongfield, as shown in Table 2.1.

1. Transcend: This variety was released from an array of genetic enhancement techniques resulting in its double haploid genetic makeup. In 2017, Transcend accounted for two-thirds of all the durum wheat seeded area. It has a high grain yield of 3.1 % and a high grain and dough pigment content. Agronomically, it has a strong straw, is significantly taller, and takes slightly more days to mature. Grain protein concentration is about 14.1 %. Moreover, it is resistant to FHB and has low mycotoxin (DON) and cadmium content. It was registered by the Canadian Food Inspection Agency (CFIA) in 2011 (Singh et al., 2012) .
2. AAC Spitfire had a 9.0 % increase in grain yield but a significantly low protein concentration when averaged over a four-year duration (2010-2013). It also has a high pigment content and contains a low cadmium concentration. On average, it matures within 105 days after planting. It is a short plant with strong straw strength. It was registered by the CFIA in 2014 (Singh et al., 2016).
3. CDC Precision is also high yielding. It recorded a 10.0 % increase in grain yield over the check durum wheat variety, Strongfield. Agronomically, it expresses a conventional height of 97 cm and matures in 103 days. Protein content recorded from 2012-2014 was lower in relation to Strongfield. It is also low in cadmium and was registered by the CFIA in 2015 (Pozniak & Clarke, 2016).

Table 2.1. Study varieties released since 2010 in Canada, their yields and protein deviation relative to the check cultivar Strongfield.

Name	Grain yield (%) increase over Strongfield	Protein deviation from Strongfield	Key traits	Release
Transcend	3.1	-0.3	Improved FHB resistance	2010
CDC Precision	10.0	-0.6	High test weight	2015
AAC Spitfire	9.0	-0.5	High pigment content, strong straw	2014

Source: Adapted from Li et al., *Agronomy* **2018**, 8(9), 193; FHB, *Fusarium* head blight

2.2. Durum wheat structure and composition

2.2.1. Structure

Unlike bread or common wheat, structurally, durum wheat has large kernels, is hard and vitreous (Kadkol & Sissons, 2016). These endosperm characteristics of durum wheat influence milling which is an important criterion for the end-use application of durum wheat (Autio & Salmenkallio-Marttila, 2003; Fu, Schlichting, Pozniak, & Singh, 2013). In durum wheat grains the length of brush hairs is usually short. Also, the shape of the base of the grain is straight or incurved with a long glume beak (Wrigley, Batey, & Miskelly, 2017). Durum wheat has a crease or a re-entrant on the ventral side that runs across the entire cereal length, deepest in the middle and extends the bran layer deep into the kernel (Evers, O'Brien, & Blakeney, 1999; Evers & Millar, 2002). Figure 2.1 (a) shows the structure of a typical wheat grain with all its botanical parts.

Just like most cereals, industrial processing typically produces three botanical components namely, the endosperm, bran, and germ which make up about 80-85%, 13-17% and 2-3% respectively of the whole kernel (Fig. 2.1a). The endosperm mainly contains starch and storage proteins (Evers & Millar, 2002). The peripheral endosperm cells have the most protein content and the least starch content (Autio & Salmenkallio-Marttila, 2003; Evers et al., 1999). The bran fraction is a composite multilayer tissue which mainly consists of the aleurone layer, seed coat, and the pericarp complex (Autio & Salmenkallio-Marttila, 2003). Anatomically these tissues closely adhere to each other with varying thickness. The aleurone layer is the outermost layer of the endosperm (Autio & Salmenkallio-Marttila, 2003). It is a unicellular tissue with cuboid cell sizes of approximately 50 μm (Barron, Surget, & Rouau, 2007; Evers et al., 1999; Evers & Millar, 2002). Various nutrients and bioactive compounds can be found in the aleurone layer such as B-

vitamins, carotenoids (zeaxanthin), protein bodies, phenolic acids, minerals and lipid droplets (Autio & Salmenkallio-Marttila, 2003; Brouns, 2012).

The germ is highly concentrated with nutrients though it makes up a small percentage of the durum wheat kernel. Biological constituents such as protein, lipids, sugar, minerals and vitamin E, B-group vitamins, carotenoids (lutein and zeaxanthin), flavonoids, phytosterols, hydrolytic and oxidative enzymes are found in this tissue. The distinctive nature of these industrial fractions in terms of organization, distribution of chemical composition and gross biochemical and/ or phytochemical composition influences their utilization in food processing and during food consumption as the food matrix of different milling fractions will impact differently on the digestion of their food components.

2.2.2. Composition

The biochemical composition of durum wheat consists primarily of starch and protein, with minor constituents of lipids and non-starch polysaccharides. In terms of durum wheat techno functionality, protein content and quality have received in-depth investigations due to their role in the quality of durum wheat foods like pasta. Until recently, little information on the influence of starch on pasta quality was available even though starch constitutes the majority in grains and flour. Moreover, equally little attention has been given to research on the role of the other minor components (lipids and fiber) and their impact on the quality of durum wheat-related food products. Changes in these components, as mediated by food processing and the digestion process, can influence the release and bioaccessibility of carotenoids in a complex food structure such as pasta.

2.2.2.1. Protein

Durum wheat proteins play a crucial role in the techno-functional properties of wheat-based foods, such as pasta cooking quality. Generally, durum wheat proteins are classified into fractions based on their solubility. Albumins and globulins, typically being salt and water-soluble account for about 20-25% of the total wheat protein content (Pronin, Börner, Weber, & Scherf, 2020), whereas the rest (75-80 %) consist of the storage proteins, gliadins, and glutenins, which are generally alcohol soluble (De Santis et al., 2017). These proteins can be separated by size-exclusion chromatography. The interaction of these endosperm storage proteins to form disulfide bonds upon semolina hydration produces the viscoelastic matter of the dough termed gluten (Sissons et al., 2008). Glutenin is responsible for the elastic properties of dough and gliadin, the dough's extensibility and viscosity (Sissons, Soh, & Turner, 2007).

Gliadins are monomeric proteins and constitute the majority of the two storage proteins. They are separated into α , β , γ , and ω groups. Glutenins, on the other hand, are polymeric proteins stabilized by inter and intrachain disulfide bonds. Upon reduction of these bonds, high molecular weight (HMW) and low molecular weight (LMW) polypeptides are released from glutenin. The low molecular weight glutenin subunits comprise about 60% of the glutenin fraction, while the high molecular weight subunits constitute 30%. The rest comprises gliadin-like structures that are part of glutenin because they have been modified by cysteine residues (Lafiandra et al., 2012).

In durum wheat, high protein content is a preferred quality trait that affects pasta processing and cooking quality (Sissons et al., 2008). High protein durum wheat varieties yield semolina of high protein content and uniform particle size which facilitates homogenous hydration during mixing. A protein content of 11-16% is considered ideal for milled semolina, with a minimum of 12% for grain protein content (Kaplan Evlice, 2022). Protein content can be measured by the

Kjeldahl method or near-infrared (NIR) spectroscopy. However, the Dumas method (combustion nitrogen analysis) is currently considered a standard method by many laboratories due to its precision, less use of hazardous chemicals, and high turnaround time.

Uniformly hydrated semolina forms a continuous gluten matrix around starch granules during pasta extrusion, which maintains the integrity of cooked pasta by preventing starch swelling, thus reducing cooking loss (Fu, Wang, Dupuis, Taylor, & Nam, 2018). During pasta drying, especially at high-temperature drying conditions, these gluten proteins aggregate, further reducing starch swelling, which makes the pasta firmer and less sticky. It is believed that while the total protein content influences the extent of the protein matrix, the protein quality or gluten strength influences the properties or integrity of this matrix (Marchylo & Dexter, 2001). Grain protein content is highly impacted by environmental conditions and agronomic practices (Kaplan Evlice, 2022). On the other hand, gluten quality is observed as a highly heritable character and minimally influenced by the environment (Liu, Shepherd, & Rathjen, 1996).

Generally, there is a positive relationship between protein content and pasta firmness. However, the degree of increase in firmness is affected by both the durum wheat variety and the cooking time of pasta (Wang, Pozniak, Ruan, & Fu, 2023). In this study by Wang et al. (2023), pasta firmness linearly increased with an increase in semolina protein content for wheat varieties with moderate to strong gluten strength. When a weak gluten variety was assessed, the relationship between pasta firmness and semolina protein content was curvilinear. Moreover, with increased cooking time, pasta firmness gradually reduced, and the degree of increase in firmness in relation to protein content was less evident. The authors concluded that gluten strength only appeared to have a positive effect on pasta firmness when pasta was overcooked, overriding the impact of

protein content. The impact of gluten strength on pasta texture appeared to be minimized at high protein levels and high drying temperatures (Wang et al., 2023).

Currently, in Canada, commercially grown Canadian Western Amber Durum (CWAD) wheat varieties are all reported to have moderately strong to very strong gluten properties (Canadian Grain Commission, 2023). Differences in pasta texture will affect its digestion process, causing structural modifications that will influence its breakdown and modulate the release of both macro and micronutrients such as carotenoids.

Protein type and content in food have been shown to positively or negatively influence carotenoid bioaccessibility even though it may depend on the carotenoid species (Iddir et al., 2021). According to Iddir, Vahid, Merten, Larondelle, and Bohn (2022), this influence depends on protein digestibility and digestive conditions. Food protein can influence digestive enzymes' access to lipid droplets when they bind to the surface, negatively affecting carotenoid bioaccessibility. In one of the studies by Iddir and coworkers (Iddir et al., 2020), in which whey protein isolate, soy protein isolate, sodium caseinate, and gelatin were assessed for their influence on carotenoid bioaccessibility when carotenoids were added as pure compounds in oil. They observed that higher carotenoid polarity (being the xanthophylls) was associated with a strong negative influence of proteins, while nonpolar carotenoids such as carotenes appeared to benefit from the presence of proteins in their micellization. Xanthophylls such as lutein are solubilized on the surface of lipid droplets, hence, the presence of proteins at the oil/water interface may result in a stronger negative interaction of proteins and lutein, thus affecting their extent of micellization.

This notwithstanding, peptides produced during digestion may have emulsifying properties that may protect carotenoids from oxidation, aiding in their micellization and bioaccessibility. It has also been determined that higher protein concentration correlates positively with carotenoid

bioaccessibility (Iddir et al., 2020). However, higher protein content may also decrease the bioaccessibilities of lipophilic compounds, especially when incorporated into lipid droplets via emulsion (Zhang et al., 2024).

Protein digestibility can be influenced by food processing-mediated changes to the protein structure. For example, cooking pasta renders the gluten proteins insoluble, especially the glutenin fraction (Petitot, Abecassis, & Micard, 2009). These insoluble fractions can impact carotenoid digestibility. Well-digestible proteins could enhance carotenoid bioavailability, whereas the opposite may be true for less digestible proteins. Therefore, the digestibility of pasta proteins may be influenced by the pasta processing time, which will ultimately affect carotenoid solubilization and bioaccessibility. A clearer understanding of gluten protein structure/characteristics as influenced by food processing on the bioaccessibility and uptake of carotenoids in durum wheat is therefore needed.

2.2.2.2 Starch

Starch makes up majority of the carbohydrate content of the durum wheat grain and semolina (Wrigley et al., 2017). Amylose and amylopectin are the two polysaccharide molecules occurring in a ratio of about 1:3 by weight (Soh, Sissons, & Turner, 2006). These molecules are deposited in two types of granules, small B-type granules (average diameter of 3-5 μm) and larger A-type granules (average diameter of 13-16 μm) (Soulaka & Morrison, 1985). Two-thirds of the weight of starch is influenced by the A-granule type while the remaining one-third is affected by the B-type which accounts for about 95% of the total number of granules (Lafiandra et al., 2012). The size distribution of these granules has been shown to be variety dependant (Collado & Corke, 2003).

Even though starch accounts for most of the carbohydrates in the wheat kernel, it has been viewed in the past as having a lesser effect on pasta cooking properties, such as firmness. Amylose content and ratio of large to small starch granules on pasta quality can be significant. Compared to bread wheat, durum wheat has a slightly higher amylose content, and an increase in amylose content ultimately increases pasta firmness (Dexter & Matsuo, 1979; Vansteelandt & Delcour, 1998).

In a semolina reconstitution study conducted by Soh et al. (2006), where they added various constituents of starch, it was concluded that increasing the small-type B granules was beneficial in reducing pasta cooking loss and stickiness as these granules likely extend the interactions between the starch granules and the gluten matrix due to their large surface area. Moreover, these physical properties of starch, including amylose: amylopectin ratio, granule size, and amylose content, can influence the rate of starch hydrolysis. Small granules are digested more rapidly, presumably due to their greater surface area. Starches high in amylopectin are digested more quickly than those high in amylose due to amylose's ability to form resistant starch (Åkerberg, Liljeberg, & Björck, 1998). One such study on pasta digestibility by Aravind, Sissons, and Fellows (2011) observed that higher B-granule content showed a lower extent of starch digestion, probably due to its packing ability and density. An earlier study by Singh, Dartois, and Kaur (2010) reported the opposite, probably because the evaluation was conducted using isolated starch and not the food matrix containing starch.

During pasta processing, the protein matrix barrier reduces rapid starch hydrolysis by salivary enzymes. Moreover, other components of the food matrix including lipids and proteins can increase the content of resistant starch due to the formation of complexes during food

processing. Starch composition and its properties impacting its digestibility may affect carotenoid digestion in pasta and the extent or degree of food processing may be significant in this process.

2.2.2.3 Lipids

Lipids in durum wheat are present in small quantities (about 2-3% of the wheat kernel). About 10% of the lipid content is found in the germ with substantial amounts found in the bran, particularly the aleurone, and within the endosperm starch granules (Lafiandra et al., 2012). Comparatively, durum wheat has more lipids than soft wheat, and whole flour has more content than refined flour. Nonpolar lipids, including acylglycerols, constitute the abundant fraction, with polar lipids (phospholipids and glycolipids) making up a small proportion (Armanino, De Acutis, & Rosa Festa, 2002). The germ and aleurone fractions contain mainly the nonpolar lipids, while the endosperm predominantly contains polar lipids (Tokusoglu & Hall, 2011).

The fatty acids in these lipids are mostly unsaturated, e.g., oleic and linoleic acids, with some components of saturated fatty acids, mainly palmitic acids and stearic acid (Tokusoglu & Hall, 2011). Just like most grain components, lipids in durum wheat are influenced by grain genes and the environment such as cold weather conditions (Beleggia et al., 2013). Lipids in durum wheat have a significant effect on the quality and the texture of foods because of their ability to associate with proteins and with starch-forming inclusion complexes (Matsuo, Dexter, Boudreau, & Daun, 1986). Lipid-amylose complexes suppress starch swelling (gelatinization), reducing stickiness and cooking loss. These effects on starch gelatinization and retrogradation during food processing will influence the behavior of starch during digestion. Resistant starch formed because of increased starch retrogradation can increase carotenoid bioaccessibility due to its insoluble properties. The insoluble properties of resistant starch can reduce the viscosity of the lumen and

increase carotenoid bioaccessibility. This notwithstanding, lipids added to food can positively affect carotenoid bioaccessibility by increasing the formation of lipid droplets and micelles.

In an *in vivo* study by Gorusupudi and Baskaran (2013) involving mice, it was revealed that wheat germ oil micelles rich in both nonpolar and polar lipids significantly increased lutein absorption (88.4 ± 6 nmol/8 h/ml) than groundnut oil micelles (23.4 ± 2 nmol/8 h/ml) which mainly contained neutral oils such as oleic acids. A positive correlation was observed between linoleic acid levels and lutein bioavailability. The authors concluded that the higher bioaccessibility of lutein from the wheat germ oil group was possibly due to the presence of other minor components such as glycolipids and vitamin E abundant in wheat germ. The influence of other bioactive in whole grain durum wheat products may benefit the bioaccessibility of carotenoids beyond their lipid constituents relative to refined products.

2.2.2.4. Non-starch polysaccharides (NSP)

The non-starch polysaccharides (dietary fibre) in durum wheat are the carbohydrate polymers that are non-digestible in the small intestines and are fermentable in the colon by gut microbes producing beneficial molecules such as short chain fatty acids which mediate gut health and confer other health benefits (Shewry, Kosik, Pellny, & Lovegrove, 2020). Non-starch polysaccharides in cereal grains like durum wheat are found in the cell wall structures of the grain tissues (endosperm, aleurone, bran) consisting mainly of arabinoxylans (AX) (appr. 70%). Other polymers found in lesser quantities in these tissues include cellulose (2%), β -glucan (20%), and glucomannan (7%) (Igrejas, Ikeda, & Guzmán, 2020). AX content decreases from the bran to the endosperm tissues (Marcotuli et al., 2016).

Structurally, arabinoxylans consist of a xylose backbone linked through glycosidic linkages with arabinose residues at positions 3 or 2 or both (Shewry et al., 2020) (Fig. 2.1 b). AX varies in substitution pattern, degree of substitution, monosaccharide composition, degree of polymerization of the xylan backbone, and in terms of the presence of attached constituents, mainly ferulic acid or *p*-coumaric acid (Izydorczyk & Biliaderis, 1995). Ferulic acids may be attached to the arabinose units through ester bonds. The substitution pattern describes the pattern of substitution (mono- or di-substituted) with arabinose units. The degree of substitution is the extent to which the xylan backbone is substituted with arabinose units. It is often described as the ratio of arabinose to xylose (A/X) residues and indicates the degree of branching in the arabinoxylan structure (Izydorczyk & Biliaderis, 1995).

There are variations in substitution amongst arabinoxylans of different origins and between arabinoxylans of different grain tissues of the same origin. For example, high substitutions tend to occur in the central cells of the endosperm compared to the outer cells (Barron, Parker, Mills, Rouau, & Wilson, 2005), thus making AX content and composition highly influenced by genotype and the environment (Ciccoritti, Scalfati, Cammerata, & Sgrulletta, 2011). Due to variations in the cell wall size in grain tissues and AX distribution patterns and structure, AX content ultimately varies with milling fractions. Consequently, whole grains will have more AX content than refined flour, affecting the quality of durum wheat food products and their technological and physiological properties.

Variations in AX structure across grain tissues have been cited as responsible for AX solubility, with high ferulate esters crosslinking between AX chains resulting in reduced solubility. Thus, the solubility of AX is directly connected to health and food digestion. When consumed, AX can increase the viscosity of the small intestine content, thereby slowing down food digestion

(Marcotuli, Colasuonno, Hsieh, Fincher, & Gadaleta, 2020). Increased food viscosity in the lumen has been shown to reduce nutrient absorption.

Aside AXs influence on food digestion, its content in wheat has been shown to also affect its technological properties. Generally, wheat fibre in whole wheat pasta is detrimental to its cooking quality, i.e. by reducing pasta firmness and increasing cooking loss (Aravind, Sissons, Egan, & Fellows, 2012). Soluble fiber components in bran affect water absorption and rheological properties of pasta dough. Moreover, starch gelatinization and swelling can also be impeded by soluble fiber by limiting available water (Aravind et al., 2012). A study by Bagdi et al. (2014), however, revealed that incorporating aleurone-rich fractions of wheat (which is rich AX) into semolina did not reduce pasta firmness or increase cooking loss. They attributed this to AX ability to affect the rheological properties of wheat dough through interactions of gluten with ferulic-acid monomers (Lafiandra et al., 2012). Nonetheless, other reports indicate that antioxidants in wheat have detrimental effects on dough rheological properties and product quality (Tokusoglu & Hall, 2011).

Fiber co-passengers such as ferulic acid have been reported to inhibit starch and lipid digestive enzymes and can alter the digestibility of starch through complexation (Ferruzzi et al., 2020; Li, Ndiaye, Corbin, Foegeding, & Ferruzzi, 2020). Both interactions could potentially limit the release and micellization of carotenoids from durum wheat food matrices and influence their bioavailability. Hence, the influence of the structural distribution of AX among grain tissue will be significant in the bioaccessibility of carotenoids among the various milling fractions.

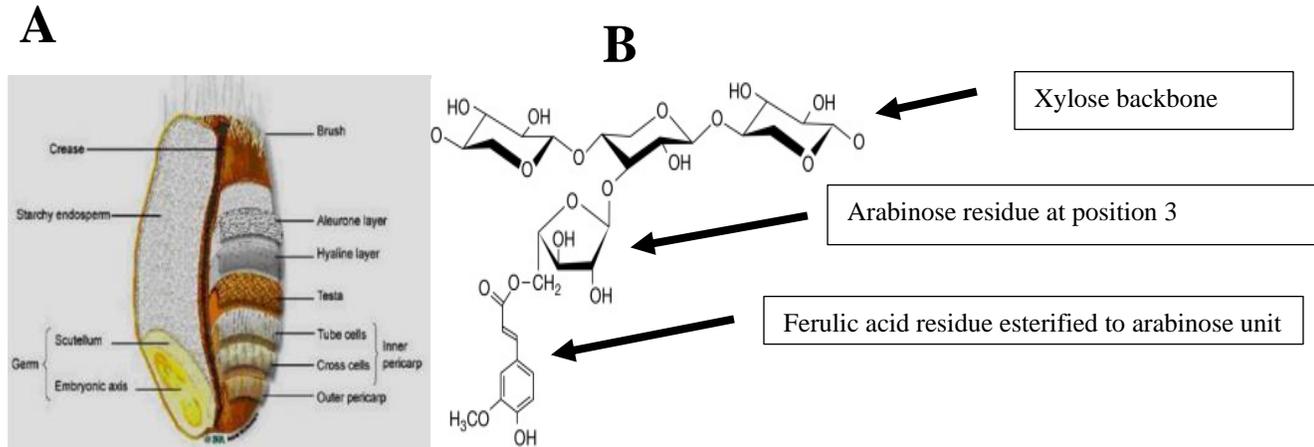


Figure 2.1. Representation of the wheat grain structure (**A**) (adapted from Grundas and Wrigley (2016) and used with permission); and arabinoxylan structure (**B**) showing a portion of (1 → 4)-β-D-xylan chain substituted at O₃-L-arabinose of one residue with a 5-O-trans-feruloyl-L-arabinofuranosyl substituent (adapted from Fincher (2016) and used with permission).

2.3. Carotenoids in durum wheat and pasta

Carotenoids are mostly C₄₀ terpenoids plant pigment compounds built from eight isoprene units bonded by tail–head bonds on the outside and tail–tail bonds on the inside, forming a linear sequence (Yan et al., 2024). They are hydrophobic in nature with a highly unsaturated polyene chain. Currently, about 1200 species of these plant pigments have been identified. Out of this, about 60 are consumed in significant amounts in human diets and about half of this number has been identified in human plasma eliciting several biological functions (Yan et al., 2024). Carotenoids commonly found in the diet include lutein, zeaxanthin, alpha and beta-carotene, and lycopene. In durum wheat, the main carotenoids are the xanthophylls, lutein, and zeaxanthin (Fig. 1.1).

Biosynthesis of these pigments in plants usually starts with forming isopentenyl diphosphate, which may be obtained by two pathways: the mevalonic pathway in the cytosol and a mevalonic-independent one in plastids. The latter is the one that is most utilized. Through a series of condensation reactions of C-20 precursors, the first colorless carotenoid phytoene is obtained. Further enzymatically catalyzed dehydration reactions lead from phytoene to the formation of lycopene. After the formation of lycopene, the carotenoid biosynthetic pathway splits into two branches, forming β -carotene and its derivatives and α -carotene and its derivatives. α -carotene and β -carotene are further hydroxylated to produce the oxygenated derivatives lutein and zeaxanthin, respectively (Cazzonelli, Nisar, Hussain, Carmody, & Pogson, 2010). Amylochromoplast plastids are the synthesizing and storage sites of these seed carotenoids. In these sites, they may be complexed with the prolamin proteins (Ferruzzi et al., 2020; Sun et al., 2018).

Lutein and zeaxanthin are naturally found in the stable all-trans configuration in durum wheat even though cis isomers can be found in processed food products such as pasta. Compared to the trans isomers, the cis compounds are present in minute concentrations. Due to steric hindrance at certain double bonds in the polyene chain, limited cis compounds are possible. Examples of some common cis-carotenoid isomers identified in durum wheat include 13-cis lutein, 13'-cis lutein, 15-cis lutein, 9-cis lutein, and 9'-cis lutein (Rodriguez-Amaya, 2016) (Fig. 1.1). These compounds are usually characterized by reduced absorbance maxima and loss of fine spectral features in addition to having a 'cis' peak (Britton, 1995). Unlike the rigid and linear trans isomers, the cis compounds are bent, which influences their solubility characteristics in solution and stability during extraction and digestion.

Aside lutein and zeaxanthin, other minor carotenoids present in some durum wheat varieties include alpha and beta-carotene and beta-cryptoxanthin (Panfili, Fratianni, & Irano, 2004).

Lutein the major xanthophyll in durum wheat accounts for more than 80% of the grain's total carotenoids and is mostly concentrated in the endosperm (Abdel-Aal et al., 2007). Its abundance in the endosperm fraction of the grain provides the unique characteristic for its utilization in the production of products such as pasta. Compared to lutein, zeaxanthin, on the other hand, is minimally present in durum but found in high concentrations in the endosperm of yellow corn, representing about 30-60% of corn's total carotenoid content (Ndolo & Beta, 2013). The trace abundance of zeaxanthin causes little to negligible concentrations in processed durum wheat foods. The low concentration of zeaxanthin has been attributed to the biosynthesis pathway involved in low and high yellow pigment genotypes. Ramachandran, Pozniak, Clarke, and Singh (2010) reported that zeaxanthin accounted for a larger proportion of the yellow pigments content in low-pigment durum genotypes and a lower percentage in high-pigment genotypes since biosynthesis of zeaxanthin is limiting in the high-pigment genotypes. The uneven distribution pattern of the xanthophylls within the grain (lutein present in all tissues, while zeaxanthin predominantly localised in the bran layers -especially the aleurone and the germ) will ultimately affect the concentrations of individual carotenoids in milling fractions used as ingredients for food production, such as pasta (Hentschel et al., 2002).

In terms of total carotenoid content (TCC) however, the germ fraction has the most concentration despite its small proportion to the kernel (Ndolo & Beta, 2013). Panfili et al. (2004) reported 1.8-fold higher TCC in the germ of durum wheat than in whole grain content.

Total carotenoid content of four Canadian durum wheat genotypes assessed earlier revealed significant differences ranging from 7.9 to 10.5 $\mu\text{g/g}$ in their semolina (Chiremba, Pozniak, & Fu, 2015). Compared to other wheat species, durum wheat contains about 2-3 times the amount of carotenoids found in bread wheat but about 2- 4 and 5 fold less the amount found in Einkorn and Tritordeum, respectively (Atienza, Ballesteros, Martín, & Hornero-Méndez, 2007). Within four tetraploids studied earlier by Abdel-Aal et al. (2007), durum wheat contained about 1.3-fold the total carotenoid content found in Emmer but was not significantly different from Kamut and Khorasan. Content and compositional differences within and between varieties observed have been attributed to genetic differences, as well as environmental factors such as temperature even though the genetic component is predominant (Hidalgo, Brandolini, Pompei, & Piscozzi, 2006; Mellado-Ortega & Hornero-Mendez, 2017).

Generally, xanthophylls including lutein and zeaxanthin are present in free forms in durum wheat, although lutein esters have been reported as common among bread, Spelt wheat species and Tritordeum (Tian et al., 2022). The ester-linked forms usually have palmitic, stearic, oleic, and linolenic fatty acids as mono or diesters. According to a recent study by Requena-Ramírez, Hornero-Méndez, Rodríguez-Suárez, and Atienza (2021), lutein esters were found in only 11 out of 156 durum wheat genotypes, indicating that these esters are variety dependant. A study by Burkhardt and Bohm (2007) also failed to find lutein esters in different durum wheat varieties from Germany. However, Atienza et al. (2007) identified 129 varieties out of 138 with lutein esters. Lutein esterification has, therefore, been suggested to increase with storage time in most wheat genotypes. The presence of these ester bonds in carotenoids affects their solubility (due to high hydrophobicity) and reduces their polarity, which ultimately affects their digestive and absorptive properties (Chitchumroonchokchai & Failla, 2006; Rodrigues et al., 2016). Nonetheless,

carotenoid esters are relatively more stable compared to their free form, even after heating (Mercadante, Rodrigues, Petry, & Mariutti, 2017). This esterification has been suggested as a mechanism by which wheat species accumulate high amounts of carotenoids. Before these esters are absorbed, however, it is believed that they are cleaved by enzymes such as cholesterol ester lipases and pancreatic lipases in the small intestines (Chitchumroonchokchai & Failla, 2006).

Although carotenoid esterification is a common phenomenon among some plant foods, it hinders the separation and identification of these compounds. Hence, saponification processes are commonly applied to carotenoid-containing food matrices, where an acidified alkaline solution extraction process eliminates lipids and chlorophyll and breaks the carotenoid-lipid esters. Although useful, to some extent, saponification can transform or denature carotenoids depending on the type of carotenoid and the food matrix (Amorim-Carrilho et al., 2014).

Due to the hydrophobic nature of carotenoids, their extraction is best achieved using nonpolar organic solvents and in consideration of the nature of the food matrix. However, for polar carotenoids such as lutein and zeaxanthin, moderate to high polar solvents, including tert-butyl methyl ether (MTBE), petroleum ether, acetone, hexane, ethanol, and their mixtures can yield higher results (Amorim-Carrilho et al., 2014). Nonetheless, the use of different solvents and methods of extraction will yield different carotenoid extract contents (Hidalgo et al., 2006; Ziegler, Schweiggert, & Carle, 2015).

Due to degradation processes, carotenoid content in raw grains or flour/ semolina does not always equate to contents in processed foods. Chiremba et al. (2015) did not find carotenes in processed pasta despite traces in the wheat grain. In pasta, a high level of yellow pigment in the semolina does not guarantee a high color in the pasta because it depends on the activity of the enzyme lipoxygenase (LOX) in the semolina during pasta processing as well as the presence of

oxygen during the extrusion process; hence the elimination of oxygen is highly recommended (Borrelli, De Leonardis, Fares, Platani, & Di Fonzo, 2003; Chiremba et al., 2015; Ficco et al., 2014). LOX levels in durum wheat are affected by genotype and environment (Fu et al., 2013). During the entire pasta production process, Hidalgo, Brandolini, and Pompei (2010) estimated a loss of carotenoids of approximately 49%. Yet another study also revealed that the whole process of pasta production resulted in carotenoid loss of 21.2–26.7% in durum genotypes with the LOX deletion in comparison with 28.9–30.3% in genotypes without the deletion (Chiremba et al., 2015). Hidalgo and colleagues therefore determined that the oxidation of carotenoids begins in the kneading phase and continues in the extrusion step. At the same time, no significant variations were observed during the drying phase. Hence, even though reductions occur, the percentage of lutein retained in the finished dried pasta product remains high.

2.4. In vitro simulated digestion, models, and bioaccessibility of xanthophylls

2.4.1. In vitro simulated digestion and models

To measure the bioaccessibility of any phytochemical, such as carotenoids, in vitro, a simulation of human upper gut digestion is usually mimicked. Human and animal in vivo studies are considered ideal. However, these methods are expensive and time-consuming. Moreover, they can be unethical, especially if infants or animals are used. On the other hand, in vitro models are cheaper, rapid, and simple to execute with no ethical constraints. (Saura-Calixto & Pérez-Jiménez, 2018). Moreover, in vitro digestion methods have been shown to correlate well with in vivo results and human bioavailability trials (Rodrigues et al., 2016).

Generally, the digestion of food starts in the mouth. Earlier studies on in vitro bioaccessibility of carotenoids from fruit- and vegetable-based meals elaborated the gastric and

intestinal phase only (Garrett, Failla, & Sarama, 1999). However, in cereals, due to the presence of significant quantities of starch, simulation of the oral phase is recommended. Hence, subsequent methods following the protocol of Garret and coworkers included the simulation of the oral phase and inclusion of salivary amylase to facilitate the digestion of starch and disintegration of the overall matrix for more accurate assessment of carotenoid bioaccessibility (Kean, Hamaker, & Ferruzzi, 2008; Thakkar, Maziya-Dixon, Dixon, & Failla, 2007).

The simulation of the oral, gastric, and intestinal phases of the digestion process is done by the addition of appropriate salt solutions and enzymes and adjusting the pH while maintaining a physiological temperature setting of 37°C. In the gastric phase, food samples are incubated at an average gastric pH of 3 in the presence of pepsin for at least 2 hrs. Pepsin is important in the bioaccessibility of carotenoids because it breaks down carotenoid-protein complexes; hence, its concentration is essential. The significance of gastric lipase has been considered marginal, so most in vitro studies do not include it. However, the digestion of lipids starts in the stomach, and the presence of lipids in food enhances the emulsification of carotenoids released from the food matrix. Gastric lipase is also essential to trigger the action of pancreatic lipase, which is vital in the lipolysis of fatty acids needed for micelle formation. Therefore, gastric lipase is essential in the digestion and bioaccessibility of carotenoids. However, commercially available gastric lipase is non-existent now for in vitro studies.

To simulate the small intestinal phase, pancreatin, and bile salts are added, and the pH is adjusted to about 7. This phase is the final phase for the hydrolysis of lipids, proteins, and carbohydrates. According to the report by Chacon-Ordonez, Carle, and Schweiggert (2019) pancreatin can be obtained as a cocktail of enzymes or added as individual enzymes: porcine pancreatic alpha-amylase, porcine trypsin, bovine chymotrypsin, porcine pancreatic lipase, and

porcine pancreatic colipase. Bile salts are important for the solubilization of carotenoids, and with pancreatin (pancreatic lipase) they aid in the formation of mixed micelles. Hence, the concentration of bile and pancreatin are equally considered important (Chacon-Ordonez et al., 2019). Low concentrations, as used in some studies, mimicking the fasting state led to reduced micelle formation. To attenuate that, increased lipolysis products are needed to swell up micelles (Reboul et al., 2006). For example, it was found in one study (Veda, Platel, & Srinivasan, 2007) that the usage of porcine pancreatin (4 mg/ ml) and porcine bile extract (25 mg/ ml) resulted in a 25-31% bioaccessibility in beta-carotene from mango compared to a bioaccessibility of 10% when pancreatin (2 mg/ml) and bile (12 mg/ml) was used for digestion of the same mango (Schweiggert, Mezger, Schimpf, Steingass, & Carle, 2012).

The termination of digestion depends on the sensitivity of the sample, the purpose of digestion, and the desired analysis to be done post-digestion. For example, due to the heat-sensitive nature of carotenoids, it would not be ideal to employ heating as a means of termination. Moreover, for the separation of the aqueous micellar fraction (which is the fraction containing carotenoids) from the food digesta, a high-speed centrifugation procedure is usually performed followed by microfiltration to separate microaggregates, crystals, and oil droplets from the filtrate that may have escaped centrifugation (Chacon-Ordonez et al., 2019). This filtration process also ensures that the carotenoids are incorporated into mixed micelles and not found in large lipid globules. However, not all in vitro methods perform this filtration process, leading to the variability of reported bioaccessibilities among various studies, even when the same food source is evaluated. Generally, for carotenoids, it is imperative to work under subdued light and oxygen as this reduces oxidation.

Due to the diversity of conditions used to simulate *in vitro* digestion in the past, such as a broad range of enzyme concentration from different sources (porcine, bovine, human origin), differences in pH adjustments and incubation periods, it limited the comparison of results across different studies. Hence a standardization of these methods was required. In 2014, Minekus and his coworkers (2014) published an international consensus model recommending enzyme concentration at each stage. Five years following this work, a position paper was published in *Nature Protocols* by the COST INFOGEST Network, providing more details and updated recommendations (Brodkorb et al., 2019). The main addition to this new protocol is that the parameters for simulating digestion are based on available physiological data of the fed state. For example, 10 mM bile concentration is recommended. Also, the oral phase of digestion is deemed mandatory regardless of the state of the food, whether solid or liquid to provide consistent dilution of the food. An advantage of this updated version is that it adapts to different objectives and types of food samples and is useful in evaluating digestion endpoints. So far, substantial studies have been conducted on the bioaccessibility of carotenoids using this method, mostly in fruits and microencapsulated food products (Brodkorb et al., 2019). Therefore, more studies using this method on cereal grains, especially wheat, are needed.

Despite the advantages of using the current harmonized method for the simulation of the digestion of various foods and the determination of a wide range of phytochemicals and food components, a tailored protocol for carotenoids may be helpful. This is because a detailed explanation on how to obtain micellar intestinal fractions, which are considered to contain bioaccessible carotenoids, is not elaborated in the current standardized method. Furthermore, foods rich in carotenoid esters require further adaptations of the model to allow the simulation of intestinal carotenoid ester cleavage by pancreatic cholesterol esterases. Aside this, a standardized method

is also needed to simulate digestion in infants and the elderly since the current method is based on the physiological conditions of a healthy adult. It is even more critical for the elderly since they have a higher predisposition to age-related macular degenerative conditions where carotenoids have been shown to be protective (Cooperstone & Schwartz, 2016).

The two main types of *in vitro* digestion models typically described in the literature are static and dynamic. The differences are that static models are immobile (requiring a constant pH and a constant food-to-enzyme ratio at each stage of digestion), and the physical processes such as mixing, hydration, and shearing are not simulated (Brodkorb et al., 2019). However, they are simple to execute and have been used extensively in most food digestion research (Cardoso et al., 2015). Unlike the static models, dynamic models mimic the gradual addition of enzymes and gastric fluid while varying the pH in addition to a gradual stomach emptying process, which is physiologically like conditions occurring *in vivo*. The downside of dynamic models is that they are complex so very few samples can be assayed at a time. In addition, they require complex maintenance, hardware and software and are overall more costly (Cardoso et al., 2015).

Semi-dynamic models are alternatives that adapt parameters based on the equivalent *in vivo* data and have the advantage of being easy to operate. Some examples of dynamic models include the TNO gastrointestinal models (TIM-1, TIM-agc), dynamic gastric model (DGM), human gastric simulator (HGS), gastrointestinal dynamic digestion system (DIDGI), and simulator of the human intestinal microbial ecosystem (SHIME) (Dima, Assadpour, Dima, & Jafari, 2020; Parada & Aguilera, 2007). Fewer studies have used the dynamic model in assessing dietary carotenoid bioaccessibility than the static model. No study was found in the literature evaluating carotenoid bioaccessibility from any wheat species using this model, warranting more investigations.

2.4.2 Bioaccessibility of lutein and zeaxanthin from durum wheat and products

Bioaccessibility is defined as the fraction of an ingested compound that is available for absorption by the enterocytes of the small intestines (Etcheverry et al., 2012; Fernandez-Garcia, Carvajal-Lerida, & Perez-Galvez, 2009). Bioaccessibility of carotenoids, in general, is low and wide-ranging. This is mainly because of the hydrophobic nature of carotenoids (Desmarchelier & Borel, 2017). Content and composition in the initially ingested food do not equate to content found after digestion or even in circulation in the human body (Rodrigues et al., 2016; Sensoy, 2014). Hence, determining factors that impact the digestion of carotenoids (bioaccessibility) is an important step in estimating their biological activity.

The bioaccessibility of carotenoids depends on numerous factors, including species and physiochemical properties of carotenoid, the matrix in which the carotenoid is found, effectors of absorption such as fat and fibre, food processing conditions, nutrient status of the host and other host genetic factors (Castenmiller & West, 1998; Cilla et al., 2018; Rodriguez-Roque et al., 2016). An in-depth discussion is provided in the following subsections.

Different bioaccessibilities have been reported for carotenoid species in the same or different foods. Generally, the relatively polar carotenoids (xanthophylls) have higher bioaccessibility than carotenes due to their orientation on the outside of lipid droplets, which facilitates their efficient incorporation into mixed micelles (Blanquet-Diot, Soufi, Rambeau, Rock, & Alric, 2009; Reboul et al., 2006). This has been demonstrated in several other studies (Chitchumroonchokchai, Schwartz, & Failla, 2004; Schweiggert et al., 2012; van het Hof et al., 1999). The high bioaccessibility of xanthophylls compared to carotenes in these studies has also been attributed to the high digestive stability of these carotenoids during digestion. Blanquet-Diot

et al. (2009) found that zeaxanthin and lutein from yellow and red tomato meals were widely stable during in vitro digestion using a dynamic digestion model. On the other hand, beta-carotene was stable for yellow tomatoes and presented losses of approximately 32% for red tomatoes. In the same study, lycopene exhibited 17-20% losses from red tomatoes. These differences in digestive stability could also have been due to the different food matrices in which these carotenoids are deposited.

Grains are largely processed before consumption. Therefore, food processing significantly affects carotenoid bioaccessibility in complex food matrices partly due to microstructural changes. Kean et al. (2008) reported that the relative bioaccessibility of lutein and zeaxanthin in maize-based foods, including extruded puff (63%) and bread (69%), was higher than in porridge (48%). Beta-carotene content in these foods was relatively lower (10-23%), although in porridge, the bioaccessibility was higher (40-63%). The authors concluded that the wet cooking process of making porridge positively influenced the bioaccessibility of the apolar carotenes. Similarly, the relative bioaccessibility of beta carotene, beta-cryptoxanthin, lutein, and zeaxanthin were 16.7, 27.7, 30.3, and 27.9%, respectively when white maize porridge supplemented with oil was digested in a study by Thakkar and Failla (2008). In a more recent study by Abdel-Aal, Rabalski, Carey, and Gamel (2023), the authors assessed the bioaccessibility of lutein and zeaxanthin from bread and muffins prepared from a composite flour containing different ratios of wheat, corn, and hairless canary seed. It was revealed that the bioaccessibility of lutein from muffins was >90%, while bread was < 80%. Moreover, the bioaccessibility of zeaxanthin among these foods was lower than lutein. The difference in lutein's bioaccessibility among the two foods was assigned to the differences in their food matrices induced by food processing.

Due to food processing, carotenoids are also prone to chemical structural changes (Nguyen et al., 2001). The application of intensive heat processing, such as cooking, has been shown to cause trans-cis isomerization of carotenoids (Udike & Schwartz, 2003). The assessment of the bioaccessibility of these transformed compounds has been deemed important, given their presence in biological tissues and organs. Although the contribution of cis compounds to the total carotenoid content in foods is minimal, they are relatively soluble compared to their trans counterparts (Britton, 1995). An assessment of lycopene isomer bioaccessibility from fried tomato revealed that the cis compound was more efficiently transferred to mixed micelles compared to the trans compound (Failla, Chitchumroonchokchai, & Ishida, 2008). Moreover, studies in humans and animal models have supported the hypothesis that cis-isomers are more efficiently micellarized (Boileau, Boileau, & Erdman, 2002; Granado-Lorencio et al., 2007; Unlu et al., 2007). The isomerization of carotenoids during digestion is also possible, and the stability of these compounds may depend on the food matrix and carotenoid species.

Compared to fruits and vegetables, relatively fewer reports on the bioaccessibility of carotenoids from wheat have been cited. For those cited, the assessments have primarily been conducted on wheat flour composites, supplemented with other food ingredients such as tuber or seed flours rich in carotenoids or even pure carotenoid compounds (Abdel-Aal et al., 2023; Armellini et al., 2019; Burešová, Paznocht, Jarošová, Doskočil, & Martinek, 2023; Read, Wright, & Abdel-Aal, 2015). Studies on carotenoid bioaccessibility from non-wheat food sources have also been cited though few (Kean, Bordenave, Ejeta, Hamaker, & Ferruzzi, 2011; Kean et al., 2008; Rošul et al., 2022; Song et al., 2021; Zhang, Ji, Zhang, Guan, & Wang, 2020). Interestingly, only one study was found on the bioaccessibility of carotenoids from durum wheat pasta (Werner & Böhm, 2011). In this study, the bioaccessibility of total carotenoids was $71.2 \pm 5.2\%$ from pure

durum wheat pasta and $57.3 \pm 1.0\%$ from egg pasta. The authors attributed the low bioaccessibility of carotenoids from egg pasta to carotenoid complexation to proteins and starch. In addition, they argued that the content of other nutrients in egg pasta, such as vitamin E and cholesterol, hindered the efficient micellization of these carotenoids. Unfortunately, the bioaccessibility of cis carotenoid isomers from these pasta samples was not evaluated, nor was their bioavailability (absorption).

To a large extent, bioaccessibility studies in cereal grains have majorly focused on phenolic compounds such as ferulic acid, flavones, and anthocyanins (Gawlik-Dziki, Dziki, Baraniak, & Lin, 2009; Mateo Anson, Havenaar, Bast, & Haenen, 2010; Monsierra, Mansilla, & Pérez, 2024; Podio, Baroni, Pérez, & Wunderlin, 2019; Tian et al., 2022). This is probably due to the relative abundance of these phenolic compounds in cereal grains and in various wheat species. In one of those recent reviews, it was revealed that currently, there was no report on lutein's bioavailability (absorption) in wheat-based foods (Tian et al., 2022). Hence, more studies are needed on the bioaccessibility and bioavailability of carotenoids from durum wheat and its food products.

2.5. Factors affecting carotenoid bioaccessibility

2.5.1. Effects of the food matrix and structure

The food matrix is described as a complex assembly of the chemical components of food, such as proteins, starch, lipids, fibre, and non-nutrients, and how they are structurally organized and interact with each other which influences the release, accessibility, digestibility, and stability of food compounds (Aguilera, 2019). The bioaccessibility of carotenoids depends on their ability to be liberated from the food matrix, withstand digestion in the gastrointestinal tract as absorbable fractions are taken up by the small intestine (Parada & Aguilera, 2007).

The food matrix has been considered a critical factor in the bioaccessibility of carotenoids. This is because plant food matrices are different. In plants, carotenoids are deposited in different cell structures or organelles depending on the food source (Schweiggert & Carle, 2017). For example, in leaves of green plants, they are mostly found complexed to proteins in chloroplast. In contrast, carotenoids in chromoplast plastids are found as lipid droplets or in a semi-crystal state associated with membranes such as those found in carrots. These differences in location and the physical form of the carotenoids can serve as barriers preventing their efficient release during digestion. In a recent study by Schweiggert et al. (2012), it was revealed that bioaccessibility of beta-carotene was in the following order: mango (10.1%) > papaya (5.3%) > tomato (3.1%) > carrot (0.5%) when carotenes from mango, papaya, tomatoes, and carrot were investigated. They argued that in mango, carotenoids are deposited in lipid-dissolved and liquid-crystalline tubular elements of mesocarp chromoplasts, hence their superior liberation and micellization compared to large solid-crystalline substructures which are found in carrot and tomato. Read et al. (2015) investigated the bioaccessibility of lutein-supplemented wheat-baked foods including, muffins, flatbread and cookies. They revealed that muffins (37.9%) and cookies (56%) had the highest and flatbread the lowest (23%) lutein bioaccessibility due to their differing fat content. Beyond this, the physical structure of the food products induced by food processing could have influenced the digestion behaviour of carotenoids, assuming the food composition was the same. Less dense foods may be easily digested compared to denser foods due to the ease with which enzymes access the food matrix, affecting the lipolysis of lipids in food, for example. Knowing the structure of the food matrix can also help understand and relate the physical state of complex processed foods to the digestion and release characteristics of nutrients and non-nutrients in foods.

The food structure and its components can easily be assessed by microscopic methods such as the scanning electron microscope (SEM) and the light microscope. However, these methods are invasive and require that the food matrix and microstructure be destroyed before analysis. Hence, non-invasive procedures such as X-ray micro-computed tomography (μ CT) can afford more advantages to understanding the structure-function relationship of food components (Schoeman, Williams, du Plessis, & Manley, 2016).

The bioaccessibility of carotenoids from food supplements or purified compounds is higher than those found in natural food matrices like fruits and vegetables (van Het Hof, West, Weststrate, & Hautvast, 2000; Yonekura & Nagao, 2007). Likewise, the bioavailability of carotenoids from animal sources is usually higher than that of plant sources, probably due to their location and physical state in these food matrices (Desmarchelier & Borel, 2017; Yan et al., 2024).

The food matrix also has various effects on the release and absorption of carotenoids in the different stages of digestion (oral phase, gastric phase, and intestinal phase) depending on the influence of digestive enzymes and fluids on the digestion behavior of macromolecules present in the food matrix (Parada & Aguilera, 2007). Carotenoids can interact with various food matrix components (water, starch, proteins, and fibre), influencing their release (Shahidi & Pan, 2022).

In cereal grains such as durum wheat, due to the uneven distribution of carotenoids in the grain, food processing such as milling will influence the content and composition of carotenoid compounds and other components of the flour, subsequently impacting the digestibility of carotenoids in foods produced from these flour ingredients. Table 2.2 outlines some studies showing the influence of food matrix characteristics on carotenoid bioaccessibility.

Table 2.2. Studies linking carotenoid bioaccessibility to specific structural matrix characteristics.

Food source	carotenoid	Matrix characteristic changes linked to carotenoid bioaccessibility	Reference
Mango, papaya, carrot, tomato	Beta-carotene	Chromoplast morphology influenced beta-carotene bioaccessibility. Decreases were observed going from mango (globular type) to papaya (tubular type), to tomato (crystalline type) and carrot (crystalline type).	(Schweiggert et al., 2012)
Mango	Beta-carotene	Qualitative and quantitative pectin changes due to mango ripening increased carotenoid accessibility by facilitating the mechanical and enzymatic disruption of the pulp during digestion.	(Ornelas-Paz et al., 2008)
Carrot	Beta-carotene	The hardness of carrot pieces due to thermal (pre)processing was inversely related to beta-carotene bioaccessibility.	Lemmens, Buggenhout, Oey, Loey, and Hendrickx (2009)
Tomato soup	Lycopene	Tomato soup products with a high viscosity only released low amounts of lycopene during digestion.	Colle, Van Buggenhout, Van Loey, and Hendrickx (2010)

2.5.2. Effects of food processing

Food processing can decrease or increase the content and availability of carotenoids through food matrix component modification (Boileau & Erdman Jr., 2004). Moreover, different food matrices can be affected differently through the same food processing techniques and vice versa (Ryan, O’connell, O’Sullivan, Aherne, & O’Brien, 2008). Palmero et al. (2014) illustrated

that the type of carotenoid constitutes an important factor determining the effect of thermal processing on their bioaccessibility. For example, lycopene is susceptible to crystallization due to its linear and rigid structure. Therefore, food processing enabling crystal break-up and dissolution may improve carotenoid release and micellization. Faulks and Southon (2005) in their review mentioned that digestion did not have any effect on intact plant cells after food preparation, indicating that food processing has a major influence on the release of bioactive compounds from the food matrix through the reduction of food particle size.

With plant food carotenoids, decreases are usually caused by oxidation degradation processes, while increases occur due to enhanced extractability of these compounds from the food matrix. It has been reported that thermal food processing improves the bioaccessibility of carotenoids (Cilla et al., 2018). Thermal food processing disrupts the food matrix and nutrient complexes, making it more digestible, facilitating an increased extraction process. Examples of some studies that reported increases in the bioaccessibility of carotenoids when thermal food processing was employed include tomatoes (Svelander et al., 2010), cassava (Thakkar et al., 2007), peppers (Victoria-Campos, Ornelas-Paz Jde, Yahia, & Failla, 2013) and spinach (O'Sullivan et al., 2010). Nonetheless, thermal food processing can also cause some losses in carotenoid bioaccessibility. For example, in red pepper, lutein bioaccessibility was decreased by boiling, grilling, microwave-cooking, and steaming (Ryan et al., 2008). The inconsistencies seen with thermal processing may be due to food matrix differences. Hence, food processing can control food digestion's disintegration and dissolution steps, influencing carotenoid bioaccessibility.

Aside from thermal food processing, some standard non-thermal food processing methods and techniques that can cause changes in food structure and consequently affect carotenoid

bioaccessibility include high-pressure homogenization, high-pressure processing, pulsed electric fields, and ultrasound (Zhang et al., 2024).

Depending on the food processing type, intensity, and duration, it may also cause chemical transformations to the molecular structure of carotenoids (Updike & Schwartz, 2003; van Het Hof et al., 2000). In carotenoids, these chemical transformations can affect their physical properties and biological activity. An assessment of lycopene bioaccessibility, revealed that increasing temperature was a significant determining factor in the efficient release and bioaccessibility of lycopene (Colle et al., 2010). Upon treatment at temperatures between 60 and 140°C, the *in vitro* bioaccessibility of all lycopene isomers studied slightly increased with increasing temperature. However, this increase was only significant after treatment at 130 and 140°C (Colle et al., 2010).

The effects of food processing also affect major food components such as proteins, starch, fiber, and lipids. Modification of these food components can serve as traps or even enhance the release and absorption of carotenoids during digestion (Svelander et al., 2010). Colle et al. (2010) observed in their study that applying homogenization pressure in tomato pulp resulted in an improved fiber network strength that decreased lycopene bioaccessibility. It was hypothesized that the fiber network entrapped lycopene, making it unavailable for digestive enzymes and bile salts. In another study by Tydeman et al. (2010), a decrease in carotenoid bioaccessibility following the heating of carrots was reported to be due to the induced separation of intact cells that encapsulate the carotenoids.

A study by Aschoff et al. (2015) illustrated that beta-cryptoxanthin bioaccessibility increased by 40% after the pasteurization of fresh orange juice compared to fresh juice. In another study by Tibäck et al. (2009), it was revealed that crushing and heating increased the lycopene

bioaccessibility from tomatoes. In that study, different crushing intensities and a subsequent heat treatment carried out as a heat shock (95 °C for 8 min), or a boiling step (100 °C for 20 min) were examined, and it was concluded that the increase in lycopene bioaccessibility was due to the reduction of pectin by the heating process.

Studies on the effect of food processing on carotenoid bioaccessibility have primarily focused on fruits, probably due to the abundance and variation of carotenoid species in these foods as well as the complexity of these food matrices (Cilla et al., 2018; Lemmens et al., 2014). Compared to fruits and vegetables (Lemmens et al., 2014; Ryan et al., 2008; Tydeman et al., 2010) relatively fewer studies have looked at the effect of food processing on carotenoid bioaccessibility in cereal grains (Kean et al., 2011; Kean et al., 2008; Zhang et al., 2020). Investigations into food processing's effect on carotenoids in durum wheat are essential, given that these foods are staples and mainly processed before consumption. Also, due to the uneven distribution of carotenoids in the durum food matrix, food processing will affect their food structure and the carotenoid bioaccessibility differently. Table 2.3 summarizes some studies that show the relationship between processing technology-induced changes on food microstructure and carotenoid bioaccessibility.

Table 2.3. Overview of various food processing methods and their effects on food microstructure and carotenoid bioaccessibility

Food processing method	Food matrix	Carotenoid	Observations	Reference
Thermal treatment	Orange juice	Beta-cryptoxanthin	Pasteurization at 90°C for 54 s, cells break down, and beta-cryptoxanthin bioaccessibility increased	Aschoff et al. (2015)
	Lettuce	Lutein Beta-Carotene	Cooking at 98 °C for 20 min, the food matrix disintegrates, and cells break down, increasing lutein and β-carotene bioaccessibility	de Oliveira et al. (2020)
	Maize	Lutein Beta-Carotene	Infrared heating at 140 °C for 6min, cell microstructure disruption and lutein and beta-carotene bioaccessibility increased.	Hossain and Jayadeep (2021)
	Tomato	Lycopene	Blanching at 60 °C /40 mins and 90 °C/ 4 mins followed by boiling, pectin breakdown in tomatoes, increased lycopene bioaccessibility	Svelander et al. (2010)
	Cassava	Beta-Carotene	Cooking for 30 mins, disruption of cells walls and organelles membrane	Thakkar et al. (2007)
	Pepper	Various carotenoids /pigments	Boiling (94°C/12.5 min) and grilling (210 °C/13.2 min) increased micellization in 75% ripened red peppers compared to raw. Decreased micellization in brown pepper, depolymerization of pectin increased, increasing pigment bioaccessibility	Claudia I. Victoria-Campos et al. (2013)
High pressure homogenization	Tomato puree	lycopene	4 °C, 1000 bar homogenized one cycle, lycopene crystals formed insoluble stable aggregates, limiting lycopene bioaccessibility	Palmero et al. (2016)

	Carrot juice	Total carotenoids	70 °C inlet temperature, 180 MPa homogenized three times, cell walls destroyed, total carotenoid bioaccessibility increased.	Liu et al. (2019)
Pulsed electric field	Tomato	beta-carotene Lycopene	Pulse electric field may induce a new structural barrier, β -carotene and lycopene bioaccessibility decreased.	Bot et al. (2018)
Ultrasound	Broccoli	Total carotenoids	24kHz/amplitude 100 mm/10-120 min, natural structural barrier disrupted, total carotenoid bioaccessibility increased.	Zhang et al. (2021)
	Tomato pulp	Lycopene	24 kHz/amplitude 100 μ m, strong fibrous network induced, lycopene bioaccessibility decreased.	Anese, Mirolo, Beraldo, and Lippe (2013)

2.5.3 Effects of lipids

Since carotenoids are lipid-soluble, they are digested and taken up from the intestine far better from a diet containing fat or oil or plant foods containing high amounts of lipids like avocado. Lipids aid the solubilization of carotenoids after their release from the food matrix. Cereals are generally low in lipids, affecting the bioaccessibility and absorption of carotenoids. However, foods such as cakes, cookies, and some snacks can contain high amounts of lipids added to their formulations during food processing, affecting the release of lipophilic compounds. Therefore, studies assessing carotenoid bioaccessibility in cereal-based foods usually add lipids or lipid-rich ingredients to the food formulation.

Lipids in foods facilitate the formation of micelles by promoting the secretion of bile salts and pancreatic lipases (Kopec & Failla, 2018). Moreover, food lipids have been shown to slow

digestion and promote chylomicron secretion, which can substantially improve carotenoid absorption (Chacon-Ordonez et al., 2019). From earlier reports, about 3-5 g of fat per meal is needed to significantly facilitate carotenoid accessibility and availability (van Het Hof et al., 2000). Nonetheless, the influence of lipids on the bioaccessibility of carotenoids may depend on the type of carotenoid (Nagao, Kotake-Nara, & Hase, 2013). It was argued earlier that due to their increased polarity, carotenoids such as lutein and zeaxanthin do not need fat and oils for bioaccessibility (Nagao et al., 2013). These carotenoids can be easily transferred to the micelles due to their surface location on lipid droplets. In contrast, apolar carotenes localized in the core of lipid droplets require lipid digestion to be solubilized into mixed micelles due to their high solubility in lipids compared to polar xanthophylls (Borel, 2003). However, Schweiggert et al. (2012) illustrated that the addition of 1-2% oil boosted the bioaccessibility of lutein (1.8-fold) and beta-cryptoxanthin (1.7-fold) from carrot and papaya test meals, respectively. Also, the in vitro digestion of goji berries in the presence of coconut oil (1%) increased the bioaccessibility of zeaxanthin by about 2-fold (Hempel et al., 2017).

The concentration of lipids influences carotenoid bioaccessibility, with increased lipid content facilitating higher bioaccessibility. For example, Yao et al. (2021), evaluated the effects of corn oil concentration (0 to 5% w/w) on the bioaccessibility of carotenoids from spinach. The results revealed that with the increase in lipid content, the bioaccessibility of lutein and β -carotene increased up to 7-fold. Another study by Liu et al. (2019) adding 10% oil increased the bioaccessibility of total carotenoids, alpha- and beta-carotene, compared to adding 2% oil.

The type of fatty acids and chain length of lipids have also been shown to mediate carotenoid bioaccessibility to different degrees. For example, monounsaturated fatty acids (which are more hydrophobic) from canola oil increased α -carotene, β -carotene, lutein, and zeaxanthin

absorption but not lycopene in humans by two-fold when added to a salad than when polyunsaturated fatty acids from soybean oil were added (Goltz, Campbell, Chitchumroonchokchai, Failla, & Ferruzzi, 2012). Peroxidation is believed to occur in the digestion of polyunsaturated fatty acids, which oxidizes and degrades these carotenoids (Nagao et al., 2013). In addition, the bioaccessibility of carotene seems to be lower when short and medium-chain-length fatty acids are digested in vitro than when long-chain fatty acids are digested. For example, Huo, Ferruzzi, Schwartz, and Failla (2007) reported an increase of 1.5- to 3-fold in micellization of carotenes from a salad puree when they substituted short- and medium-chain fatty acids with long-chain fatty acids. Nagao et al. (2013) also illustrated increased beta-carotene bioaccessibility when long-chain fatty acids were supplemented in a spinach homogenate. However, the effect was not observed for lutein and zeaxanthin.

Additionally, Yan et al. (2024) observed that monounsaturated fatty acids and long-chain triglycerides promoted the micellization of carotenes, attributing long-chain fatty acid's ability to form a sizeable hydrophobic core of mixed micelles more efficiently than short-chain fatty acids. Conversely, saturated fatty acids and medium-chain triglycerides were conducive to the micellization of xanthophylls. This was attributed to the faster digestion rates of these lipids, which produced smaller mixed micelles. There may be specific matrix-lipid-type interactions that mediate carotenoid bioaccessibility, and this may be dependent on the food source. Table 2.4 summarises some recent studies showing the effects of the type and concentration of lipids on the bioaccessibility of carotenoids.

Table 2.4. Studies on the effect of type and content of lipid on carotenoid bioaccessibility

Carotenoid	Food source	Type of lipid	Concentration of lipid	Bioaccessibility (%)	Model	Reference
Lutein	Spinach	Soybean oil, rapeseed oil, MCT and EMF	5, 10, 15 mg	Soybean oil (24-26), rapeseed oil (26-28), MCT (22-25) and EMF (24-28)	In vitro digestion	Kotake-Nara, Hase, Hoshina, Hidan, and Kobayashi (2022)
Beta-carotene, lutein and total carotenoid	Spinach	Corn oil	0, 0.2, 0.6 and 1.0 g	beta-carotene (3.0,3.5,12.5 and 16.5), lutein (4, 16.5, 16 and 18) and total carotenoid (3.1, 7, 14.4 and 18)	Rat	Yao et al. (2021)
Zeaxanthin	Goji berries	Coconut oil	0 and 1%	0% oil (6.7) and 1% oil (13.3)	In vitro digestion	Hempel et al. (2017)
Lutein	Spinach	No triglycerol (TG) Dioleoylglycerol (DG), monooleoylglycerol (MG) and oleic acid (OA)	33.3%	TG = 61; DG = 62; MG = 65; OA = 62	In vitro digestion	Nagao et al. (2013)
Alpha and beta-carotene	Raw and cooked carrots	Whey protein and corn oil	2, 4 and 8%	Raw carrot (1-2, 10-14 and 26) and boiled carrot (8-10, 25-29 and 42-45)	In vitro digestion	Zhang et al. (2015)
Beta-cryptoxanthin and lutein	Papaya (beta-cryptoxanthin) and carrot (lutein)	Sunflower oil	0,1and 2.5%	0% (papaya = 9; carrot = 16), 1% (papaya = 15; carrot = 32) and 2.5% (papaya = 15; carrot = 30)	In vitro digestion	Schweiggert et al. (2012)

MCT = medium-chain triglycerides; EMF= Emulsion formulation

2.5.4 Effects of fibre

Most studies indicate that the bioaccessibility of carotenoids is lower when digested with dietary fiber (Cervantes-Paz et al., 2017; Chacon-Ordonez et al., 2019; Desmarchelier & Borel, 2017; Kopec & Failla, 2018; Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Surprisingly, many of these studies were conducted on fruits and vegetables whose dietary fibre composition is mainly soluble pectin. Dietary fiber affects the bioaccessibility of carotenoids mainly by altering the micellization processes through interaction with bile salts and lipases (Ferruzzi et al., 2020). These interactions impede the transfer of carotenoids from lipid droplets to mixed micelles (Borel, 2003). Moreover, dietary fiber can trap carotenoids and restrict their release from the food matrix by forming aggregates after depolymerization from the digestion process. Dietary fiber can also influence the viscosity of the digestive medium, thus affecting carotenoid bioaccessibility and bioavailability through reduced enzyme activity (Tyssandier et al., 2003). Carotenoids bound to food macromolecules such as proteins and starch in the lumen of the small intestine may also be difficult to digest due to dietary fibers' negative influence on enzyme activity. Moreover, other nutrients predominantly found in the bran and germ tissues of cereal grains, such as minerals and polyphenols, may contribute to the adverse effects dietary fiber poses on carotenoid bioaccessibility. Unabsorbed carotenoids may eventually transition to the large intestines and be transformed into other absorbable metabolites or fermented by colonic bacteria.

The interaction between dietary fiber and carotenoids can be influenced by the structural properties of dietary fiber (Cervantes-Paz et al., 2017). The structure of arabinoxylans in cereals such as durum wheat has been shown to influence its solubility and water absorption. Food processing can, however, cause structural changes in dietary fiber, eventually affecting the bioaccessibility of carotenoids. For example, Wellala et al. (2022) showed that the bioaccessibility

of carotenoids was increased in blended juices containing pectin when high-pressure homogenization was applied. However, this increase was attributed to the processing treatments' effect on pectin structure, including an increase in the degree of esterification and a reduction in pectin molecular weight. In durum wheat bran, structural changes to the insoluble fiber during the extrusion process can also increase the soluble fiber content. Several structural modifications mediated by the type of food processing or its degree of application can occur with other types of soluble dietary fiber, such as arabinoxylans. Hence, more studies are needed to understand the effects of food processing on dietary fiber changes and carotenoid bioaccessibility.

2.5.5. Effects of other compounds

In the durum wheat food matrix, other compounds, such as vitamins and minerals, can also affect the bioaccessibility and absorption of carotenoids. Vitamin C and E, for example, have been shown to promote the bioaccessibility of carotenoids even though cereal grains such as durum wheat are low or negligible in vitamin C (Ferruzzi et al., 2020). However, Corte-Real et al. (2017) revealed that divalent minerals, including calcium and magnesium, negatively affected carotenoid bioaccessibility from spinach, field salad, tomato juice, apricot juice, and carrot juice even though this was food matrix and carotenoid type dependant. The bioaccessibility of β -carotene in that study was significantly higher in carrot juice than in spinach. The authors related the difference seen to the storage form of beta-carotene in plants.

High concentrations of divalent minerals are usually implicated in the adverse effects of carotenoid bioaccessibility. Higher concentrations of these minerals can combine with bile and free fatty acids, forming insoluble salts and fatty acid soaps (Ferruzzi et al., 2020). This would result in precipitation, impeding digestion and the entry of carotenoids into the mixed micelles.

Nonetheless, bile salts and pancreatic enzyme concentration can mitigate the adverse effects of divalent salts on carotenoid bioaccessibility (Corte-Real et al., 2018). In this study, it was revealed that higher bile acid concentrations (8 mM), as opposed to a lower concentration (1 mM), mitigated the negative effect of increasing concentrations of minerals on the bioaccessibility of lutein and beta-carotene. However, the impact of the concentration of pancreatin was less pronounced, and significant differences in the bioaccessibility of carotenoids between samples digested with either 100 mg/l or 990 mg/l of pancreatin were only observed when bile acid concentration was 8 mM (Corte-Real et al., 2018).

Other compounds, such as flavonoids, have also been implicated in the bioaccessibility of carotenoids. In a recent assessment of beta-carotene cellular uptake using an in vitro model by Xiao et al. (2021) it was revealed that four types of flavonoids from citrus fruits (hesperetin, hesperidin, naringenin, and naringin) increased the cellular uptake of beta-carotene. The suggested mechanism of action was identified as promoting lipid digestion and the effective solubilization of beta-carotene into micelles. However, another in vitro model using human Caco-2 TC-7 cells to assess carotenoid uptake revealed that naringenin negatively impaired lutein absorption by 10-30% (Reboul et al., 2007). Naringenin is a lipophilic polyphenol so that inhibition could have been due to competition for absorption. Table 2.5 shows some latest studies that investigated the effects of divalent mineral and flavonoids on carotenoid bioaccessibility.

Interactions between carotenoids have also been reported to mediate carotenoid bioaccessibility and absorption through competition for transport proteins. The effect of divalent minerals and other compounds such as flavonoids on the bioaccessibility of carotenoids in reported studies so far has been inconsistent and largely conducted in fruits and vegetables. Differences observed could also be due to the type of assay employed (in vitro versus in vivo) or the food

matrix type or carotenoid type, warranting more investigations, especially in cereal-based food matrices due to the abundance of polyphenols and minerals in the outer endosperm fractions of cereal grains.

Table 2.5. Studies on the effects of minerals and flavonoids on carotenoid bioaccessibility

Nutrient	Food matrix	Carotenoid	Observations	Reference
Minerals	Tomato paste	Lycopene	Dietary supplementation with calcium ions impaired lycopene bioaccessibility.	Borel et al. (2016)
	Spinach	Carotenoids	Intake of physiological concentrations of calcium ions did not affect carotenoid bioaccessibility	Corte-Real et al. (2017)
		Lutein Beta-carotene	Addition of magnesium, lutein and beta-carotene inhibited bioaccessibility	Corte-Real et al. (2018)
	Tomato juice Carrot juice	Lutein	Calcium, magnesium and zinc decreased carotenoids bioaccessibility; sodium ions increased carotenoids bioaccessibility.	Corte-Real et al. (2017)
Flavonoid	Orange-fleshed sweet potato	Beta-carotene	Naringin negatively affected beta-carotene bioaccessibility	Poulaert, Borel, Caporiccio, Gunata, and Dhuique-Mayer (2012)
		Beta-carotene	Hesperetin, hesperidin, naringenin and naringin promoted lipolysis and the mixing of beta-carotene into micelles increasing beta-carotene bioaccessibility	Xiao et al. (2021)

2.6. Absorption of xanthophylls (uptake and secretion)

For the *in vitro* intestinal simulation of the absorption process of food compounds, the human Caco-2 cell line model is widely used. Other human and non-human *in vitro* cell line culture models that are seldom utilized include the Madin-Darby canine kidney (MDCK) (originating from the canine kidney), the IEC-18 cell line (originating from rat intestine), the IPEC-J2 cell line (originating from the porcine intestine), HT29-MTX, and the less heterogenous clone of Caco-2; TC-7 (Dima et al., 2020). Moreover, tissue-based models such as the Ussing chamber model have also been used to study carotenoid absorption (Kalungwana, Marshall, Mackie, & Boesch, 2023). Though *ex vivo* in nature, this model has a similar resemblance to human conditions, they are easier and faster to employ even though tissue viability maintenance is a crucial factor to consider when using this model (Xu, Shrestha, Pr at, & Beloqui, 2021).

Caco-2 is a heterogenous parental line of the human colorectal epithelial adenocarcinoma cells. These cells can undergo spontaneous differentiation after 21 days in a standard cell culture environment, forming a monolayer of polarized cells expressing several morphological and functional characteristics of the mature enterocyte, such as tight junctions, brush borders, and microvilli (Hubatsch, Ragnarsson, & Artursson, 2007). Upon differentiation, Caco-2 cells also express several enzymes and apical transport proteins such as SR-B1, NPC1L1, CD36 and ABCA1, critical for absorbing carotenoids in these cells (During & Harrison, 2004; Yonekura & Nagao, 2007). Thus, the absorption of carotenoids is reported to occur through both passive diffusion and protein-mediated transport (Ferruzzi et al., 2020).

Typically, these transport proteins facilitate the transfer of carotenoids from the brush borders of the enterocytes to chylomicrons, where they are secreted to the blood through the lymph and further to the liver and other tissues for storage or metabolism. Essentially, after delivery

across the apical membrane by these transport proteins, carotenoids such as lutein and zeaxanthin are delivered to mitochondrial beta-carotene -9,10'-dioxygenase (BCO2) cleavage enzymes by non-specific fatty acid binding proteins where they are eccentrically cleaved generating apocarotenoids (Reboul & Borel, 2011). The cleaved carotenoids along with intact compounds and other carotenoid metabolic products such as retinyl esters, are assembled into chylomicrons and secreted across the basolateral membrane into the lymph. The carotenoid-rich chylomicrons are subsequently released into plasma, where they remain circulating until hydrolyzed into chylomicron remnants by lipoprotein lipases. The liver eventually takes up these remnants. Within the liver, some of the carotenoids are stored, and others are metabolized. The remaining fraction is transported back into the blood within very low-density lipoprotein (VLDL). Metabolism of VLDL to low-density lipoprotein (LDL) leads to the distribution of carotenoids to the various tissues (Kopec & Failla, 2018). According to von Lintig, Moon, Lee, and Ramkumar (2020), xanthophylls are predominantly found in high-density lipoprotein (HDL), while carotenes are associated with LDL in fasting circulation.

The in vitro simulation of the absorption process with Caco-2 cells is mostly in combination with the in vitro digestion model, where the apical uptake of carotenoids from mixed micelles collected after in vitro digestion is measured. Like any other in vitro model, Caco-2 model is a useful tool that allows for determining factors that may influence the absorption of carotenoids. Caco-2 cells are seeded on the porous membrane of Transwell® inserts to set up the model. The differentiated cells on these inserts separate the apical (lumen) from the basolateral compartment (submucosa), simulating the intestinal epithelium (Xu et al., 2021). The micellar fraction collected after digestion is typically diluted (e.g. 1:3; 1:4; 1:5) in cell culture growth media and provided to the apical side of the insert (Brodkorb et al., 2019). This is done to prevent cell toxicity. Before

the start of an experiment, however, the integrity of the cell monolayer is tested by measuring the transepithelial electrical resistance (TEER), which should be $> 300 \Omega \cdot \text{cm}^2$ (Hubatsch et al., 2007).

Although Caco-2 is a standard cell line, some disadvantages limit its usage. One such disadvantage is that these cell lines cannot produce mucus and some enzymes, so for a more realistic imitation of the intestinal epithelial layer, it has been proposed that it be combined with the HT29-MTX model, which is a mucus-secreting cell line (Xu et al., 2021).

Generally, the absorption of carotenoids is typically low primarily due to the hydrophobic nature of these compounds. Like its bioaccessibility, carotenoid absorption is varied and can be influenced by its hydrophobicity, interaction with other dietary ingredients, food processing, molecular transformations (isomerization) and other host genetic factors (Dima et al., 2020). Carotenoid hydrophobicity is inversely related to its bioavailability or absorption (Sy et al., 2012). Therefore, more polar carotenoids, including lutein and zeaxanthin, have been reported to have increased absorption efficiency compared to the apolar ones, such as carotenes, mainly due to their micellization efficiency and probably their ability to efficiently interact with membrane proteins for transport (Castenmiller & West, 1998). However, some reports revealed that sometimes the bioavailability of certain carotenoids may not be consistent with their bioaccessibility (Yan et al., 2024). In a recent study assessing beta-carotene and lutein bioaccessibility and bioavailability using a rat model, it was proven that although the bioaccessibility of lutein was significantly higher than that of beta-carotene, the bioavailability of beta-carotene ($150 \pm 3 \text{ ng/mL}$) was significantly higher than that of lutein which was below the detection limit (Yao et al., 2021). Yonekura and Nagao (2007) also opined that xanthophylls such as lutein have a poor cellular uptake compared to carotenes, even though xanthophylls have a higher bioaccessibility. The conflicting outcomes illustrated by these studies could potentially lie in the type (in vivo vs. in vitro) and design of the

experiment, the type of the food matrix, the digestion protocol used, or even the setup of the cell culture experiment (since these models vary in how they are designed, coupled and implemented between labs), warranting the need for standardization of the cell culture experiment.

On the other hand, some cis carotenoid compounds have been reported to have low bioavailability despite their high bioaccessibility (Desmarchelier & Borel, 2017). This may partly be attributed to the instability of these cis compounds resulting from rapid degradation (Harrison, 2012). Cis lycopene isomers have been shown to display a higher bioavailability than their all-trans isomer, due to their higher solubility in mixed micelles (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999). However, even though cis beta-carotene isomers also display a higher solubility in mixed micelles compared to all-trans beta-carotene, they showed a lower absorption efficiency from synthetic mixed micelles (During, Mahmood Hussain, Morel, & Harrison, 2002). Yang et al. (2018) also revealed in a study using synthetic micelles in a Caco-2 cell monolayer model that the bioavailability of cis-lutein was significantly lower than that of the all-trans isomer.

Indeed, discrepancies in these findings have led to several conclusions that indicate preferential uptake, re-isomerization, efficient carotenoid incorporation into chylomicrons, or selective retention of these specific isomers (Blanquet-Diot et al., 2009; Stahl & Sies, 1992). There appears to be no general agreement on the bioavailability of the trans and cis carotenoids, probably due to differences in the type of carotenoid structure. Moreover, the discrepancies in these results may also lie in the design and setup of the experiment (in vitro versus in vivo model) and the use of pure compounds rather than food-bound carotenoids.

The absorption of carotenoids in cereal grains has been minimally studied partly due to their low concentrations. Some of the studies that have assessed carotenoid absorption from wheat-

based foods have either incorporated lutein as a pure compound in the processing of these foods (Read et al., 2015) or as composite flour, sourcing ingredients from other carotenoid-rich food sources (Abdel-Aal et al., 2023). Very little information is available on the absorption efficiency of carotenoids from cereal-based foods in the literature, warranting more studies on their bioavailability, especially on durum wheat-based foods such as pasta (Tian et al., 2022).

2.7. Lutein and zeaxanthin antioxidant activity

Antioxidant activity has been described as the primary mechanism by which carotenoids confer health benefits (Krinsky & Johnson, 2005). Dietary intake and plasma concentrations of carotenoids have been associated with a decreased risk of chronic diseases, including type-2 diabetes, cancer and cardiovascular diseases (Cooperstone & Schwartz, 2016). Lutein and zeaxanthin are described as macular pigments due to their ability to protect the retina from light-induced damage (Johnson, 2004; Nwachukwu et al., 2016).

The antioxidant capacity of carotenoids is measured by their ability to scavenge free radical and singlet oxygen species. Carotenoids do this by interacting with these radicals through electron transfer, hydrogen abstraction, and addition of radical species to form carotenoid-radical adducts (Britton, 1995). The electron-rich conjugated double-bond (cdb) system of carotenoids is the main feature affecting its antioxidant activity (Britton, 1995) and this activity tends to increase with an increasing number of double bonds in the carotenoid species (Rodrigues, Mariutti, Chisté, & Mercadante, 2012). For example, zeaxanthin with an 11cdb is twice as effective in quenching singlet oxygen species, peroxy radicals and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals as lutein with a 10cdb (Edge, 1997). Nonetheless, the antioxidant activity of carotenoids measured in vitro may vary with in vivo conditions due to the different behaviors

of these compounds in membranes versus in organic solutions. For example, zeaxanthin had a higher protective ability against peroxy radical in liposomal membranes compared to beta-carotene despite equal concentration. In solution, the effect was equal (Young & Lowe, 2001).

Generally, the antioxidant capacity of carotenoids depends on the structure and concentration of carotenoid, oxygen concentration, the pH and the polarity of the solvent in the reaction mixture as well as the oxidizing agent itself (Lowe, Booth, Young, & Bilton, 1999; Young & Lowe, 2001). At high concentrations, high levels of oxidation reaction products or carotenoid aggregates can form in the presence of free radicals *in vitro*, diminishing the antioxidant activity of the carotenoids (Young & Lowe, 2001). Even in high concentrations different carotenoids can behave very differently against the same reactive oxygen species (ROS) *in vitro* due to their structural differences. For example, the ability of beta-carotene and lycopene to protect HT29 cells against DNA damage was seen only at low doses (1-2 mM). This protective effect was lost as the dose of the carotenoids was increased (4 mM) so that at the highest doses tested (10 mM) the carotenoids afforded no protection against DNA damage. Moreover, the membrane of the HT29 cells became increasingly permeabilized when high doses were administered. Conversely when zeaxanthin was employed against the same cells, a prooxidant effect was not seen (Young & Lowe, 2001).

The physical form of a carotenoid also influences its antioxidant activity. *Cis* carotenoid compounds are reported to have reduced antioxidant activity compared to their *all-trans* forms while some studies have alluded to the increased antioxidant activity of *cis* carotenoid compounds due to increased solubility when these were assessed using chemical-based assays such as against 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) (Yang et al., 2018). In a recent *in vitro* study using chemical-based assays, it was

determined that all-trans beta-carotene, 9- cis, and 13-cis beta-carotene had no significant differences in their ability to scavenge peroxy radicals generated from the thermal degradation of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) as well as the ABTS radical (Mueller & Boehm, 2011). However in a study by Bohm, Puspitasari-Nienaber, Ferruzzi, and Schwartz (2002) assessing the antioxidant activity of geometric isomers of alpha and beta carotene, zeaxanthin and lycopene using the Trolox equivalent antioxidant capacity assay (TEAC), it was revealed that all isomers of lycopene demonstrated the highest antioxidant potential with approximately twice the activity of all-trans beta-carotene. The discrepancies reported among the various studies could be due to the nature of the radical used, the nature of the test medium and the carotenoid structure.

The above-mentioned chemical-based antioxidant assays assessed spectrophotometrically require that antioxidant capacity (as determined by the reducing power of the antioxidant against generated free radicals) is measured against Trolox (the water-soluble variant of vitamin E) and reported as Trolox Equivalents (TE) (Huang et al., 2005). Since these assays differ in the mechanism of generating free radicals and their stabilities differ after generation, varying results are often reported. Hence more physiologically relevant assays, such as cellular-based assays, are preferred. Cellular-based assays model cellular biochemical processes, including uptake and metabolism, making its usage more resembling of in vivo conditions.

In assessing carotenoid antioxidant activity in food matrices, the nature and part of the food source used is important since antioxidants like carotenoids are usually unevenly distributed. In durum wheat, about 78% of total zeaxanthin and 51% of total lutein are found in the bran and germ fractions. Hence, milling fractions containing these tissues and foods prepared from them, including whole-meal foods, tend to show higher lipophilic antioxidant activities against peroxy radicals compared to fractions devoid of them (Adom, Sorrells, & Liu, 2003, 2005). Ndolo and

Beta (2013) reported higher antioxidant scavenging capacity of carotenoids from the germ and aleurone layers of various wheat varieties against DPPH radicals compared to the endosperm fractions. Fu et al. (2017) also reported that ABTS radical scavenging activity of the milling fractions of various durum wheat genotypes decreased in the order of short bran/bran > feed flour > flour/semolina in total yellow pigment extracts. Correspondingly, the total carotenoid content in these outer fractions is higher than that in the inner fraction, and their synergistic actions significantly contribute to their antioxidant abilities compared to their singular effects.

Compared to raw flour however, lower antioxidant activity is usually reported in processed foods (Hidalgo et al., 2010). This can be attributed to antioxidant degradation during food processing. The composition of antioxidant compounds formed during food processing and digestion would differ from their parent or original compounds, affecting the total carotenoid content in extracts and subsequently influencing their antioxidant capacity. More studies are needed to understand the antioxidant actions of digested carotenoid compounds, as their activities may be beneficial in the intestinal lumen. Currently, there is limited information on the antioxidant capacity of carotenoids from digested durum wheat processed foods. The antioxidant activity of carotenoids differs by the type of wheat variety, inherently stemming from genetic and environmental influences (Martini et al., 2015; Ndolo & Beta, 2013).

2.8. Summary of research gaps identified.

A review of the current literature revealed some gaps.

Firstly, there is a need for more *in vitro* digestion studies on durum wheat, including its whole and refined flour ingredients, since these represent different food matrices and their nutrient and non-nutrient components such as proteins, fibre, phenolic compounds and lipids modulate the

bioaccessibility, absorption and stability of carotenoids. Moreover, studies on different wheat varieties/genotypes will better explain the impact of the food matrix on carotenoid bioaccessibility. This will ultimately aid in selecting appropriate varieties for their carotenoid bio-functionalities.

The structure and amount of durum grain chemical components, such as proteins and dietary fibre (arabinoxylans) can modulate the bioaccessibility of carotenoids. For arabinoxylans, structural differences exist within and between wheat varieties. Food processing can induce further changes to the structures of these macromolecules, affecting how they interact with themselves and with guest molecules such as carotenoids during digestion. Hence, knowledge of structural characteristics or amounts of these macronutrients will aid in understanding carotenoid bioaccessibility.

Since durum wheat is solely cultivated for food and its products are staples, more in vitro digestion investigations are also needed into other durum wheat food products, such as couscous and bread. Due to their food processing procedures, these foods represent different food matrices/structures.

The inconsistent findings on lutein and zeaxanthin bioaccessibility in the literature are mainly due to the food matrix and the different in vitro digestion protocols used. This further highlights the need to standardize the in vitro digestion models and further investigate the effect of the food matrix and food processing.

CHAPTER 3.

Influence of cooking duration on carotenoids, physical properties and *in vitro* antioxidant capacity of pasta prepared from three Canadian durum wheat varieties

Hannah Oduro-Obeng, Bin Xiao Fu, Trust Beta

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3. Abstract

This study investigates the impact of different pasta cooking durations (al dente, fully cooked, or overcooked) on the carotenoid content and physical properties of whole wheat and refined semolina pasta prepared from three Canadian durum wheat varieties. Carotenoids were analyzed using HPLC and spectrophotometry. Generally increasing cooking duration non-significantly increased lutein, zeaxanthin, and total carotenoids, significantly increased the cis carotenoids but decreased carotenoid retention and firmness regardless of flour type or wheat variety. Despite this decrease, whole wheat pasta was significantly firmer than semolina when overcooked regardless of wheat variety. Antioxidant capacity was highly influenced by flour type but did not show a clear trend with cooking duration. Overall analysis indicates that increasing cooking duration of whole wheat or semolina pasta above 7 or 8 mins respectively allows for the maximum extraction of carotenoids with possible accessibility by digestive enzymes when consumed but this affects cooking quality including high cooking loss.

3.1. Introduction

Pasta has become a staple food among most cultures in the world especially in the West due to its ease of processing, transportation, handling, and storage accompanied by its healthy nutrient content and good sensory qualities (Sicignano, Di Monaco, Masi, & Cavella, 2015). Even though several grain and non-traditional ingredients have been evaluated for use in pasta production, durum wheat is the choice cereal due to its unique properties such as yellow colour, flavor, cooking quality, and protein content (Feillet & Dexter, 1996). This yellowness is derived from its carotenoid content, mainly the xanthophylls (lutein and zeaxanthin) which is highly influenced by wheat variety, wheat milling techniques, and pasta processing conditions (Borrelli et al., 2003).

In durum wheat, the composition and concentration of carotenoids is not homogenous within the kernel except for lutein (Panfili et al., 2004). Indeed, Borrelli and colleagues (2008) indicated a decreasing trend of yellow pigment content from the germ to the endosperm. Thus, the traditional milling of durum into refined semolina could deplete the concentration of some non-endosperm carotenoids, affecting their content in end products such as pasta (Borrelli et al., 2008; Hentschel et al., 2002). Nonetheless, semolina pasta has always had superior quality attributes with high consumer appeal compared to whole wheat pasta due to its good cooking qualities, such as low cooking loss and a firm texture (Aravind et al., 2012).

In recent times however, the consumption of whole cereal grain food products such as whole wheat pasta is widely advocated due to its healthy, natural, and high antioxidant value. Recent studies have highlighted the beneficial role antioxidant carotenoids play in diabetes and/or obesity mitigation among other non-communicable diseases (Develaraja, Reddy, Yadav, Jain, & Yadav, 2016; Maiani et al., 2009).

To be able to have any biological value, however, the influence of secondary processing methods such as cooking on the content and/or stability of carotenoids in foods such as pasta is of utmost concern as pasta is cooked before consumption. Cooking has been determined to impact not only pasta's technological properties, such as texture and cooking loss (Bornet et al., 1990; de la Peña, Wiesenborn, & Manthey, 2014) but also its chemical properties as this is linked to its structural changes influenced by the amount of water and heating to which it is subjected (Marti, Pagani, & Seetharaman, 2014). Being heat, oxygen, and light sensitive, maximizing carotenoid stability post extrusion with the aim of increased availability when consumed may depend largely on the cooking duration. Evidence showing the impact of post-harvest food processing on nutrient retention and availability has yielded some inconsistencies due to disparities in results (Cilla et al., 2018; Mellado-Ortega & Hornero-Méndez, 2015; Ryan et al., 2008). Maiani et al. (2009) in their extensive review reported decreases in carotenoid content when carotenoid-rich foods were heat processed but also suggested possible increases due to the efficient disruption of cell wall structures and/or carotenoid-protein complexes. This notwithstanding, the food matrix composition, variety variation and chemical nature of carotenoids may be necessary factors to consider.

Aside from carotenoid degradation, excessive use of heat in processing carotenoid-rich foods has also been implicated in its trans-cis transformation (Britton, 1995). Considering recent reports illustrating the presence of cis isomers in biological fluids and tissues, a lot of research interest has developed around the influence of processing on cis isomer contents in food and their subsequent bioactivity (Böhm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002).

Studies on the effect of cooking duration/time on pasta are limited, with most focusing on its cooking quality and starch digestibility (Bornet et al., 1990; Granfeldt & Björck, 1991).

Moreover, a significant portion of these studies has focused on pasta cooked to optimum (i.e. the scientific method to assess pasta cooking quality at which point the central white core of pasta disappears) or overcooked pasta to assess cooking tolerance with little or no assessment on “undercooked” pasta or pasta cooked to al dente (Manthey & Schorno, 2002; Martinez, Ribotta, Leon, & Anon, 2007; Sissons et al., 2008). Ideally, cooked pasta should be of al dente quality, firm, and resilient with little cooking loss (Dexter, Matsuo, & Macgregor, 1985). This, however, depends on the wheat variety.

There is a dearth of knowledge on the impact of different pasta cooking times on its carotenoid content and to what extent wheat genotype/variety and flour structure composition (whole wheat and refined semolina) may influence this. We hypothesize that increasing the cooking duration of pasta will significantly enhance carotenoid extractability, thereby increasing its content in extracts regardless of flour type or wheat variety. Therefore, the objective of this study was to assess the multi-effect of cooking whole wheat and refined semolina pasta prepared from three Canadian western amber durum wheat and subjected to three cooking times (*al dente*, fully cooked or overcooked) on the carotenoid content, physical properties and antioxidant capacity of carotenoid isomers of cooked pasta.

3.2. Materials and Methods

3.2.1. Chemicals

All chemicals were of analytical grade. Solvents for chromatography were of HPLC quality. All solutions were prepared by using HPLC grade water from a Milli-Q purification system (Millipore, Schwalbach, Germany). Carotenoid standards (97-99%), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Aldrich Chemical Co.

(St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were acquired from Fisher Acros Organics (Morris Plains, NJ, USA).

3.2.2. Wheat variety and sample preparation

Samples of durum wheat varieties AAC Spitfire, CDC Precision, and Transcend were provided by the Grains Research Laboratory of the Canadian Grains Commission, Canada. Wheat samples to be milled into refined semolina (RS) or whole wheat flour (WW) were cleaned and tempered to 16% and 12% moisture, respectively. Whole wheat flour (WW) was prepared by grinding 2 kg grains in an ultracentrifugal mill (ZM 200, Retsch, Germany) with a 0.5 mm screen at 35 °C. RS was prepared by milling durum wheat on a four stand Allis-Chalmers laboratory mill (Allis-Chalmers Manufacturing Co., Milwaukee, WI) in conjunction with a laboratory purifier at 21°C according to the mill flow described by Dexter, Matsuo, and Kruger (1990). All durum varieties were milled in duplicate 2 kg-batches to have a particle size distribution $>180 <600 \mu\text{m}$. Representative samples of milled wheat (blanketed in nitrogen and sealed) were stored at -40°C until further analysis. The percentage moisture and crude protein content of each sample was analyzed following approved AACC International methods 44-15.02 and 46-30.01 (AACC, 2000). The average moisture content for RS and WW flour was 14.2 and 9.9%, respectively. The protein content (dry matter basis) of RS flour was 16.7, 16.7, and 16.5% for AAC Spitfire, CDC Precision, and Transcend, while that in WW flour was 18.1, 18.2, and 18.7% for AAC Spitfire, CDC Precision, and Transcend, respectively.

3.2.3. Spaghetti preparation

3.2.3.1. Extrusion process and conditions

Spaghetti processing was conducted according to mixing and extrusion conditions described by Fu et al. (2013) with little modification. Briefly, RS or WW flour (200 g) and water (30.5 -31.5% absorption for RS) or (35.5 - 36.5% absorption for WW) were first mixed in a high-speed mixer (model DAC 400 FVZ, FlackTec, Landrum, SC) to generate uniform dough crumbs consistent with commercial requirements. Mixing was done at 800 rpm for 10 s, followed by 1200 rpm for 90 s. Preliminary experiments were conducted to determine desirable water absorption for materials from the three durum varieties.

Spaghetti was produced from RS and WW using a customized micro-extruder (Randcastle Extrusion System INC, New Jersey, USA). The internal diameter of the extruder barrel was 19 mm with a 12:1 working length-to-diameter ratio. The temperature of the barrel was set at 45 °C \pm 1. The hydrated dough was loaded into an air-eliminated hopper by operating a vacuum. This was to ensure less oxidation of carotenoids. Extrusion pressure of 100 \pm 10 Pa was monitored during the process. A four-hole 1.8 mm Teflon-coated spaghetti die was used for extrusion. The resulting fresh pasta was dried in a pilot Buhler pasta dryer (Buhler, Uzwil, Switzerland) using an 85 °C drying cycle. The average moisture content for dry RS and WW pasta was 10.4 and 10.3%, respectively. Dried extruded pasta was kept at a temperature of -40 °C until further analysis.

3.2.3.2. Cooking of spaghetti

Aliquots of pasta (10 g each) were cut into smaller pieces of 5 cm and cooked in boiling distilled water (300 ml) until cooked to al dente (A1), fully cooked (FCT), or overcooked (OC). Distilled water was used to control for the effects and variability on cooking quality and texture as

cooking water hardness has been reported to influence these properties (Dexter, Martin, & Matsuo, 1988). Depending on the flour type associated spaghetti, al dente samples (representing pasta with some presence of ungelatinized starch in the inner core) were cooked for 8 min or 7 min for RS or WW pasta, respectively. Fully cooked time (FCT) for both pasta types was assessed using AACC International Approved Method 66-51.01 (AACC, 2000). FCT corresponds to the disappearance of white in the central core of the spaghetti when pressed between two plexiglass plates at 15 s intervals during cooking. On average, FCT for WW and RS pastas was 11 min and 12 min, respectively. Pasta samples were overcooked (OC) by 6 min more than their respective FCT.

After cooking, samples were drained and rinsed with 50 ml of distilled water to determine cooking loss. An aliquot of extruded and cooked pasta was immediately analyzed in triplicate for moisture content (AACC method 44-15). The remaining samples were immediately stored at -40 °C for later carotenoid analysis within one week. The data reported refer to two different batches of each pasta sample. Cooking experiments were done on these duplicate batch samples. Unless indicated, all analysis was done in triplicate for each batch (n=6).

3.2.4. Measurement of spaghetti cooking quality

Pasta firmness, cooking loss, and water absorption were assessed as described previously by Sissons, Aravind, and Fellows (2010). Briefly, cooking loss (% total solids weight) was measured by evaporating cooking water in pre-weighed beakers to dryness overnight in a forced-air oven at 110 °C. This was calculated as a change in weight after drying and expressed as a percentage of the total dry pasta weight before cooking. Measurements were performed in triplicates for each sample. On the other hand, water absorption was calculated as a change in the weight of pasta after cooking and expressed as a percentage of the uncooked weight.

For firmness, five pasta strands (5 cm in length) were selected after cooking 10 g pasta in 300 ml distilled water to al dente, fully cooked or overcooked for measurements. Cooked samples were immediately drained and transferred into 250 ml distilled water at room temperature for 2 min to arrest cooking. Following this, strands were drained, covered with aluminum foil and analyzed immediately to minimize the total time to complete testing since resting time has been shown to influence pasta textural properties (Sissons et al., 2008). Firmness was measured using a TA-XT-Plus texture analyzer (Stable Micro Systems, Godalming, UK) by placing the pasta strands on a base plate parallel but not touching each other and cutting crosswise with a knife blade (HDPI BSK; blade set with a knife). The knife descended at 1.0 mm/sec for 9.7 mm to stop at 0.3 mm from the base plate and then returned to the start position. The base plate and knife were cleaned and dried between measurements. The distance the probe traveled into the pasta minus the set distance determined the diameter of the cooked strands. Pasta firmness was defined as the height of the peak (in grams), which is like the maximum cutting force. Each treatment was cooked once, and an average of three measurements per cooked sample was reported.

The colour of cooked pasta was determined using Minolta CM-3500d model spectrophotometer (Minolta, Osaka, Japan) and reported according to CIE color system where L* describes black to white (0-100); a* for redness (positive) - greenness (negative) and b* for yellowness (positive) - blueness (negative) following AACC International methods 14-22 (AACC, 2000). An equal volume of pasta was arranged randomly in the dish (without the black box) so that the dish bottom was completely covered and to an equal depth. The mean of three measurements was calculated for each treatment (n=6).

3.2.5. Carotenoid extraction and determination

3.2.5.1. Extraction of carotenoids

Carotenoids were extracted according to the method described by Werner and Böhm (2011) with slight modifications. Sample handling, extraction, and analysis were conducted under subdued light to minimize oxidation. Two grams of cooked pasta were weighed into 50 ml centrifuge tubes after grinding with a pestle in a porcelain mortar for 2 min. Afterward, 2 ml of ethanol, 1 ml of water, and 20 μ l of stock solution of β -apo-8-carotenal (internal standard) were added and vortexed for 30 s. Samples were again vortexed after the stepwise addition of 1 ml of ethanol, 1 ml of methyl tert-butyl ether (MTBE), and 1 ml of petroleum ether. Tubes were centrifuged at 4270 \times g for 3 mins at room temperature. The supernatant was collected and the extraction with MTBE and petroleum ether was repeated twice. Extracts were combined and rotary-evaporated using an IKA RV 10 digital evaporator (IKA Works Inc, Wilmington, NC, USA) at 35 °C. The dried extracts were mixed with 2 ml of ethanol using a Branson 5510 ultrasonic bath (Branson Ultrasonics Corporation, Mexico). Samples were transferred into 2 ml amber tubes and centrifuged for 5 min at 18800 \times g. Carotenoid extracts were filtered through a 0.45 μ m filter for HPLC and total carotenoid content (TCC) analysis.

3.2.5.2. Spectrophotometric determination of total carotenoid content (TCC)

Filtered carotenoid extracts were pipetted into 96-well flat-bottom microplates, and absorbance was read against the blank solvent. Absorbance was measured at 450 nm using a BioTek microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). Total carotenoid content was calculated using the following equation and expressed as μ g lutein equivalent/g sample. $C=(2\times A)/S\times W$ where C = lutein content in μ g/g; A = absorbance

reading, S = regression coefficient; 2 = dilution factor based on the total reconstituted volume of 2 ml and W = sample weight (g). All analysis was done in triplicate. The calibration curve was obtained by using a lutein standard with a concentration range of 0.2-5.0 $\mu\text{g/ml}$. The regression equation was $y=0.1723x-0.004$ showing a linear relationship with $R^2=0.9979$ (Abdel-Aal et al., 2007).

3.2.5.3. Determination of carotenoids using HPLC

HPLC analysis of carotenoids was accomplished with the procedure of Abdel-Aal et al. (2007) with slight modifications. The HPLC setup was a Waters model 2695 enhanced with a photodiode array detector (PAD) (Waters 996) and autosampler (Waters 717 plus) (Waters, Milford, MA, USA). The column was a 4.6 X 100 mm YMCTM carotenoid S-3 with 3 μm packing (YMC/Waters Inc., Wilmington, NC, USA). The column was operated at 35 °C. Twenty μl of the sample was injected by the autosampler and eluted with a gradient system consisting of (A) methanol/MTBE/Milli-Q water (81:15:4, v/v/v) and (B) MTBE/Methanol (90:10, v/v). The flow rate was set at 1 ml/min. The gradient was programmed as follows: 0-9 mins, 100 -75% A; 10-12 mins, 0% A; 12-13 mins, 0-100% A; and 13-15 mins, 100% A. Carotenoids were detected at 450 nm and identified by comparison with the retention times and elution sequence of all-trans lutein and zeaxanthin external standards, UV/vis spectra and those reported in the literature.

Due to lack of authentic standards, cis -lutein compounds were tentatively identified based on comparison of absorption spectra and elution profiles to similar results using S-3 column. Levels of all-trans lutein and zeaxanthin and cis-isomers of lutein in cooked RS and WW flour pasta, dry pasta, and raw wheat flours were estimated using the response curves of all-trans-lutein and all-trans zeaxanthin assessing the recovery of the internal standard (β -apo-8-carotenal). Five

concentrations in the range of 0.3-5.0 µg/ml and 0.16-2.5 µg/ml per injection of 20 µl were prepared for lutein and zeaxanthin, respectively, to generate regression equations for quantification. The regression equations ($y = 1856.6x - 264.9$ and $y = 634.39x - 3.3539$) showed a linear relationship with $R^2 = 0.999$ and $R^2 = 0.9998$ for lutein and zeaxanthin respectively. Intraday coefficient of variation (CV) for carotenoid extraction and analysis were 2.9, 6.9, 2.6, 3.1, and 2.8% for all-trans lutein, all-trans zeaxanthin, 9-cis lutein, 13-cis lutein, and 13'-cis lutein respectively.

3.2.6. Determination of antioxidant activity

The ABTS radical scavenging method was conducted according to the method described by Hwang et al., 2018 with minor modifications. Aliquots of 190 µl of ABTS working solution were mixed with 10 µl of carotenoid extracts in a 96-well flat bottom microplate. The mixtures were held for 30 min under subdued light. The absorbance was read at 750 nm against ethanol as a blank using a BioTek microplate spectrophotometer (BioTek Instruments, Inc. Winooski, USA). The analysis was done in triplicates. The standard curve was constructed using Trolox concentrations ranging from 50 to 800 µl. Antioxidant activity was calculated in mmol of Trolox equivalent per 100 g of pasta.

3.2.7. Calculation of apparent retention

Apparent retention was calculated for total carotenoid content (TCC) in cooked samples as previously described by Beta and Hwang (2018). Concentrated carotenoid extracts for both extruded and cooked pasta were reconstituted with 2 ml ethanol. Apparent retention (AR) (%) of

TCC was calculated by applying the formula: % AR = [nutrient content per g of the cooked pasta (dry basis)]/[nutrient content per g of uncooked pasta (dry basis)] × 100.

3.2.8. Statistical analysis

The experiment consisted of three durum wheat varieties (AAC Spitfire, CDC Precision, Transcend), flour type (RS and WW flour), and cooking duration (AI, FCT, OC). Durum wheat milling, spaghetti processing, and cooking were conducted in duplicate. Cooking quality was also conducted in triplicates whereas carotenoid content and colour were conducted in quadruplicate. Results were expressed as mean ± SD. Statistical analysis (ANOVA) was performed using SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC), and proc GLIMMIX was fitted to the data. A 3 x 2 x 3 factorial design for durum wheat variety, flour type, and cooking duration, respectively, was conducted to determine the effect of each factor and their interactions. Carotenoid content in flour and dry pasta was analyzed using a one-way ANOVA. Means were separated by Tukey-Kramer HSD at P-value < 0.05.

3.3. Results and Discussion

3.3.1. Carotenoid content analysis

The carotenoid content in foods has been shown to vary, being highly dependent on processing conditions, the food structure, and genotypic and environmental influences. Extruded pasta carotenoid content depends on semolina carotenoid content, carotenoid degradation by lipoxygenase (LOX), and pasta processing conditions (Fu et al., 2013). According to Hidalgo et al. (2010), about 49% of carotenoid losses occur during pasta production. In our study, total carotenoid losses after pasta extrusion ranged between 12-31% depending on the wheat variety and flour type (Table 3.1). Upon cooking, further reductions in all-trans lutein and zeaxanthin content were observed among the various wheat varieties assessed, irrespective of the flour type. Moreover, all-trans lutein was affected by a significant two-way interaction between wheat variety and cooking duration (Appendix 3-Table S3.2), as shown by results presented in Table 3.2.

Despite reductions after cooking, lutein content in all cooked pasta was similar and ranged from 4.94 - 5.91 mg/kg dry weight. Generally, extractable lutein content increased with increasing cooking duration except for AAC Spitfire, which showed a slight non-significant decrease. Unlike Transcend and Spitfire, there was a significant difference ($P > 0.05$) between Al and OC cooking durations for CDC Precision-cooked pasta. The highest and lowest lutein content was recorded among CDC Precision pasta when overcooked and cooked to al dente, respectively (Table 3.2). Extractable lutein content among the wheat varieties was as follows across all cooking durations: Spitfire > Precision > Transcend. The observed differences can be attributed to variations in their food matrix's nutrient and non-nutrient composition. An inverse relationship has been established between pasta carotenoid loss and initial semolina content (Fu et al., 2013).

As the most abundant carotenoid found in durum grains, lutein presents a homogenous distribution along the grain kernel. This may explain the lack of significance between the interaction of flour (RS or WWF) type and variety (Appendix 3-Table S3.2). This is interesting and has implications for cooking pasta in such a way that increasing the cooking time of pasta may be beneficial in the accessibility of all-trans lutein post-consumption. Our results are consistent with an earlier study by Abdel-Aal et al. (2007).

Table 3.1. Carotenoid content (mg/kg dw) in milled flour, dry pasta and cooked pasta among refined semolina and whole wheat durum varieties

Flour type	Wheat variety	All-trans Lutein	All-trans Zeaxanthin	All-trans Lutein	All-trans Zeaxanthin	All-trans Lutein	Cooking duration [#]			All-trans Zeaxanthin	
		Raw flour	Raw flour	Dry pasta	Dry pasta	Al	FCT	OC	Al	FCT	OC
Semolina	AAC Spitfire	11.22± 0.07 ^a	0.50± 0.15 ^{bcd}	7.76± 0.13 ^{cd}	0.33± 0.02 ^d	5.56± 0.19	6.17± 0.03	5.74± 0.18	0.14± 0.02	0.24± 0.03	0.22± 0.01
	CDC Precision	9.96± 0.71 ^{ab}	0.26± 0.00 ^d	8.13± 0.14 ^{bcd}	0.23± 0.02 ^d	4.76± 0.85	5.54± 0.89	5.77± 0.08	0.10± 0.02	0.16± 0.00	0.14± 0.00
	Transcend	8.45± 0.46 ^{bc}	0.40± 0.08 ^{cd}	7.34± 0.10 ^{cd}	0.20± 0.00 ^d	5.09± 0.42	5.26± 0.39	5.31± 0.28	0.13± 0.02	0.16± 0.03	0.20± 0.04
Whole wheat	AAC Spitfire	10.03± 0.68 ^{ab}	1.19± 0.27 ^a	7.49± 0.26 ^{cd}	0.92± 0.16 ^{ab}	6.01± 0.13	5.30± 0.09	5.17± 0.00	0.61± 0.02	0.56± 0.02	0.55± 0.08
	CDC Precision	8.82± 1.14 ^{bc}	0.97± 0.18 ^{ab}	7.65± 0.17 ^{cd}	0.88± 0.13 ^{abc}	5.12± 0.14	5.66± 0.15	6.06± 0.04	0.51± 0.01	0.54± 0.04	0.58± 0.01
	Transcend	8.21± 0.40 ^{bcd}	1.04± 0.02 ^a	6.35± 0.14 ^d	0.91± 0.02 ^{ab}	4.85± 0.27	4.87± 0.29	5.54± 0.31	0.56± 0.04	0.61± 0.02	0.70± 0.02

All values are means ±SD of four determinations (n=4). Data having the same lower-case superscripts within a column and carotenoid are not significantly different (p>0.05). [#]Cooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta.

Table 3.2. Significant effect of the interaction between variety and cooking duration on all-trans lutein (mg/kg dw) content in cooked pasta (averaged over milling flour type).

Wheat variety	Cooking duration ^a		
	Al	FCT	OC
AAC Spitfire	5.78±0.16 ^{ab}	5.73±0.06 ^{ab}	5.45±0.09 ^{ab}
CDC Precision	4.94±0.49 ^b	5.60±0.52 ^{ab}	5.91±0.06 ^a
Transcend	4.97±0.35 ^b	5.06±0.34 ^{ab}	5.42±0.29 ^{ab}

All values are means ± SD of three determinations. Data with the same lower-case superscripts are not significantly different ($p > 0.05$). ^aCooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta. † Note: LOD and LOQ for lutein are 0.213 µg/ml and 0.642 µg/ml respectively.

For all-trans zeaxanthin, a significant three-way interaction between wheat variety, flour type, and cooking duration was observed and is presented in Table 3.3. All-trans zeaxanthin content ranged from 0.10-0.70 mg/kg pasta dry weight after cooking, which was similar to contents reported in earlier studies that investigated different varieties of wheat (Abdel-Aal et al., 2007). Significant differences ($P < 0.05$) were observed between flour types regardless of wheat variety or cooking duration. This difference was greatly influenced by WWF pasta as these, averaged across cooking duration, were 3-, 4-, and 4-fold higher relative to the RS pasta for AAC Spitfire, CDC Precision, and Transcend, respectively ($P < 0.05$).

The highest zeaxanthin concentration was found in overcooked Transcend WW pasta, while Al-cooked Precision RS pasta recorded the lowest concentration. Overall, for both flour types, zeaxanthin increased non-significantly with increasing cooking duration except for the WW pasta samples of AAC Spitfire, which decreased slightly but non-significantly ($P > 0.05$) (Table 3.3).

All-trans zeaxanthin is reported as the second most abundant xanthophyll in many wheat species and varieties investigated so far (Abdel-Aal et al., 2007). As an antioxidant carotenoid, it is mainly located in the outer endosperm layers of wheat grains (Hentschel et al., 2002). Recent reports from our lab showed its abundance in the aleurone layers of some wheat samples (Ndolo & Beta, 2013). Our results show that its abundance in WWF samples compared to RS bears credence to its uneven distribution pattern within the grain and between varieties.

Unlike the endosperm, which represents the largest and most stable proportion of fractions among wheat varieties, the bran and germ fractions vary widely (Gómez, Gutkoski, & Bravo-Núñez, 2020). Thus, the concentration of carotenoids in germ and bran may differ among wheat varieties.

Table 3.3. Significant effect of the interaction between wheat variety, flour type and cooking duration on all-trans zeaxanthin (mg/kg dw) content in cooked pasta.

Wheat variety/flour type ^b	Cooking duration ^a		
	Al	FCT	OC
AAC Spitfire SL	0.14±0.02 ^{cd}	0.24±0.03 ^c	0.22±0.01 ^{cd}
AAC Spitfire WW	0.61±0.02 ^{ab}	0.56±0.02 ^b	0.55±0.08 ^b
CDC Precision SL	0.10±0.02 ^d	0.16±0.00 ^{cd}	0.14±0.00 ^{cd}
CDC Precision WW	0.51±0.01 ^b	0.54±0.04 ^b	0.58±0.01 ^{ab}
Transcend SL	0.13±0.02 ^{cd}	0.16±0.03 ^{cd}	0.20±0.04 ^{cd}
Transcend WW	0.56±0.04 ^b	0.61±0.02 ^{ab}	0.70±0.02 ^a

All values are means ± SD of three determinations. Data with the same lower-case superscripts are not significantly different ($P>0.05$). ^aCooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta. ^b flour type: SL, refined semolina; WW, whole wheat flour. † Note: LOD and LOQ for zeaxanthin are 0.041 µg/ml and 0.124 µg/ml respectively.

Unlike the two major carotenoids (all-trans lutein and zeaxanthin), cis-carotenoid compounds have been mainly identified in processed food samples. These geometrical isomers occur because of exposure to excessive heat and/or oxygen and light during food processing. Due to their significance in processed carotenoid-rich foods and biological samples, a lot of interest has been generated around their accessibility and possible bioactivity. Results on the investigated variable effects on 9-cis lutein content in cooked pasta are presented in Table 3.4. There were significant main effects of flour type and cooking duration on 9-cis lutein content (Appendix 3-Table S3.2).

9-Cis lutein content was significantly higher in pasta produced from RS (0.71 ± 0.02 mg/kg dry weight) relative to their WWF (0.66 ± 0.01 mg/kg dry weight) flour samples ($P < 0.05$). This highlights its high preponderance in grain endosperm. As expected, the content of this isomer increased as cooking time increased. On the contrary, there was no significant difference ($P > 0.05$) in 9-cis lutein content between raw flour types and dry pasta products, meaning the conventional pasta-making process was not enough to cause a significant increase in the content of this cis-isomer (Appendix 2-Table S3.1). Compared to uncooked pasta samples (dry pasta), cooking reduced the content of this carotenoid isomer slightly, possibly due to its degradation into apocarotenoids, which were not detectable by the HPLC method employed.

A significant main effect of cooking duration was also observed for both 13-cis and 13'-cis lutein isomers (Appendix 3-Table S3.2). The content of these isomers ranged from 0.80-1.01 and 0.60-0.84 mg/kg dry weight for 13-cis and 13'-cis lutein respectively and these contents also increased with increasing cooking duration (Table 3.4). Unlike 9-cis lutein, these contents in cooked samples were similar to contents in uncooked dry pasta samples (Appendix 2-Table S3.1), meaning minimal degradation of these carotenoid isomers occurred, if any. Britton (1995) indicated that these stereoisomers were easily formed and relatively stable due to their smaller steric hindrance compared to other double bonds in some positions. Moreover, unlike the 9-cis isomers, the 13-cis isomers were not flour-type dependent.

Table 3.4. Significant main effects of cooking duration on 9-, 13-, and 13'-cis lutein content (mg/kg dw) in cooked pasta

Cooking duration ^a	Cis-lutein isomers		
	9-cis lutein	13-cis lutein	13'-cis lutein
Al	0.61±0.04 ^c	0.80±0.01 ^c	0.60±0.01 ^c
FCT	0.67±0.04 ^b	0.91±0.01 ^b	0.71±0.01 ^b
OC	0.77±0.02 ^a	1.01±0.01 ^a	0.84±0.00 ^a

All values are means ± SD of three determinations. Data with the same lower-case superscripts are not significantly different at $P > 0.05$. ^aCooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta.

The total of all the carotenoids assessed (total carotenoids) was significantly influenced by a two-way interaction between wheat variety and cooking duration (Appendix 4-Table S3.3). Being the summation of individual carotenoids analyzed by HPLC, a similar trend as all-trans lutein was observed. Previous reports have shown a positive association between total carotenoids analysed by HPLC and lutein content (Abdel-Aal et al., 2007). Total carotenoids (TC) averaged over flour type were similar and ranged from 7.17- 8.92 mg/kg dry weight. Increasing cooking duration increased TC in pasta extracts like the other parameters assessed. This increase was significant in overcooked CDC Precision and Transcend samples relative to their al dente samples ($P < 0.05$). There was no significant difference between cooking duration for AAC Spitfire-produced pasta ($P > 0.05$).

For carotenoids to have any health benefits, their stability or retention during food processing, such as cooking, is important. In this study, the stability of individual carotenoids and total carotenoids as assessed by HPLC and TCC as assessed photometrically are presented in

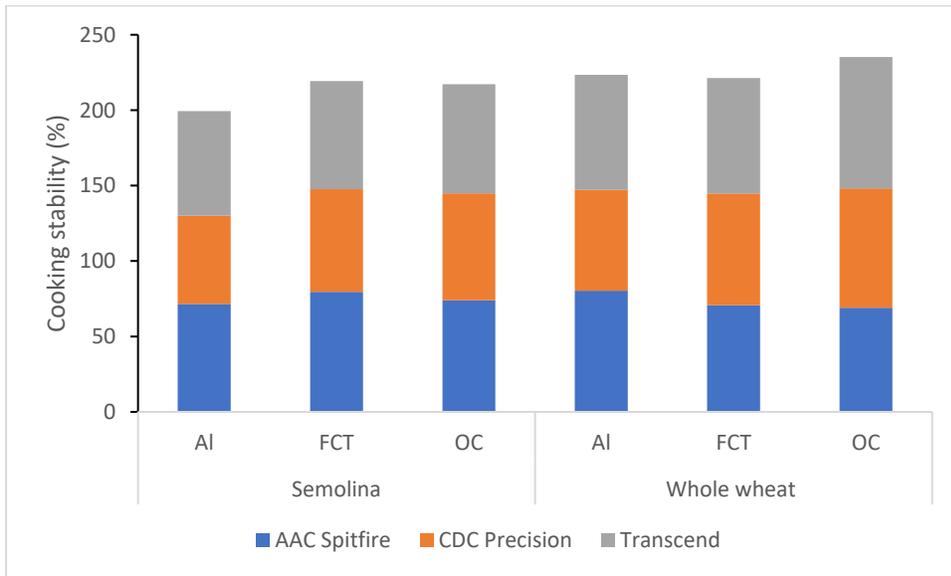
Figures 3.1, Figure 3.2, and in Appendix 6-Figure S3.1. Despite losses after cooking, more than 40% of all-trans lutein and zeaxanthin were generally retained among the wheat varieties regardless of the cooking duration (Figure 3.1). Increasing cooking duration increased the relative stability of individual carotenoids and total carotenoids among both flour-type samples due to the increased extractability of these carotenoids. Retention of lutein and zeaxanthin among the wheat varieties followed the order; Transcend>Spitfire>Precision irrespective of the flour type or cooking duration.

Conversely, TCC decreased with the increase in cooking duration among wheat varieties. Despite this, the retention of carotenoids was greater (>60%) than the content assessed using HPLC (> 40%). On average, Precision (86.9%) recorded the highest stability, while Spitfire (75.5%) had the lowest across the flour types and cooking durations. The order was as follows: Precision>Transcend> Spitfire. Additionally, TCC stability was slightly higher in whole wheat flour samples than in refined semolina samples, eliciting a three-way interaction effect (Appendix 3-Table S3.2).

The reduction in retention as cooking duration increased could be due to reflectance characteristics. The conjugated double bonds (cdb) in carotenoids are responsible for their colour properties (Ngamwonglumlert & Devahastin, 2019). More cis compounds and/or apocarotenoids were formed in OC samples. These compounds, under the modification of the c.d.bs and general structural properties, may have promoted the reduction in color intensity as the cooking duration increased. Moreover, TCC, as assessed photometrically, has been reported to overestimate the carotenoid contents in grain extracts due to the presence of other non-carotenoid yellow pigments, including chlorophyll-like compounds, flavonoids most likely found in the seed coat of grains (Burkhardt & Bohm, 2007). Precision may contain higher contents of these non-carotenoid pigments. This could have accounted for the higher pigment retention assessed using this

parameter than that estimated using HPLC. Fu et al. (2018) earlier reported a significant pigment loss among RS samples produced from lower vitreous kernels due to finer granulation.

(a)



(b)

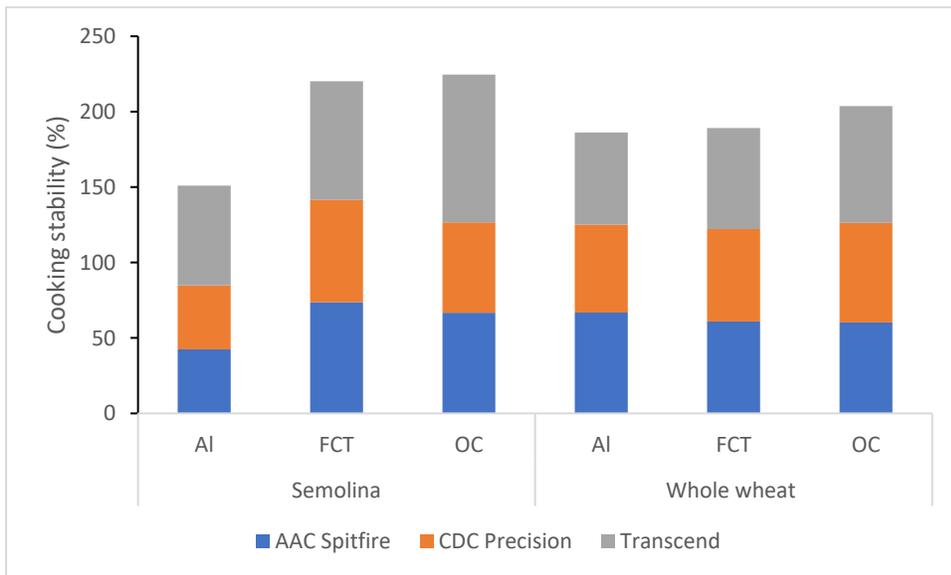


Figure 3.1. Influence of cooking duration and wheat variety on cooking stability of **A**; all-trans lutein and **B**; all-trans zeaxanthin. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta.

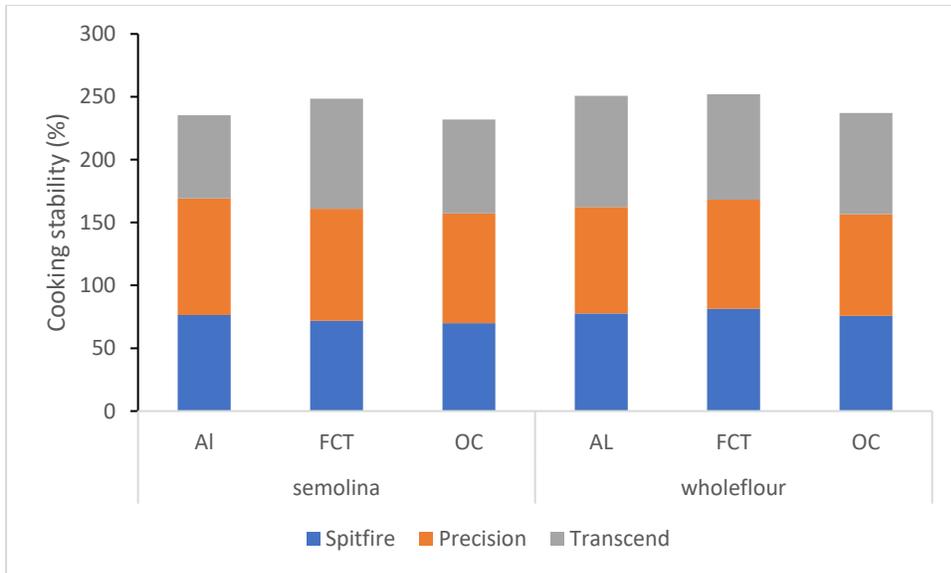


Figure 3.2. Effect of wheat variety and cooking duration on the cooking stability (%) of total carotenoid content (TCC) in refined semolina and whole wheat pasta. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta.

3.3.2. Cooking quality analysis of pasta

A significant three-way interaction between wheat variety, flour type and cooking duration for water absorption was also observed (Appendix 3-Table S3.2), as shown in Table 3.5. Water absorption ranged from 228.0% -325.3%. Increasing cooking duration significantly increased water absorption for RS and WW flour pasta regardless of the wheat variety ($P < 0.05$). No significant difference ($P > 0.05$) in water absorption was observed between wheat varieties for RS

or WWF when pasta was cooked to Al, FCT or OC, except in WW flour Spitfire and Precision pasta when overcooked ($P < 0.05$). On average water absorption among RS pasta was 1.1-fold higher than in whole wheat flour pasta. In earlier studies, bran-substituted RS or WW flour pasta was reported to have low water absorption and swelling index (data not shown) due to bran's competition with starch for the same amount of water (Aravind et al., 2012; Manthey & Schorno, 2002).

Unlike water absorption, a two-way interaction between flour type and cooking duration influenced cooking loss. Cooking loss ranged from a low of 4.3% in RS to a high of 8.8% in WWF flour pasta ($P < 0.05$) (Appendix 5-Table S3.4). Our results are in agreement with those of others who reported cooking loss between 7% and 5.3% for WWF and RS pasta, respectively (Manthey & Schorno, 2002). Whole wheat flour pasta significantly leached out greater amounts of total organic matter in the cooking water than RS pasta, regardless of the cooking duration. There was a significant effect of cooking duration for both flours, with pasta cooked to Al recording the lowest cooking losses. Dziki and Laskowski (2005) indicated a positive correlation between cooking duration and loss. Manthey and Schorno (2002) also indicated that soluble non-starch polysaccharides in bran fractions cause higher cooking loss. Moreover, a dilution of the gluten matrix by bran-germ particles could have exposed starch granules to excessive swelling and rupture compared to RS pasta.

High pasta firmness has been related to good cooking quality. This characteristic is attributed to the specific structural organisation of starch and proteins when pasta is cooked, which are affected by both processing conditions and the wheat protein profile (Marti et al., 2014). In this study, pasta firmness was a function of wheat variety, flour type and cooking duration (Appendix 3-Table S3.2). As cooking duration progressed, pasta absorbed more water and became less firm

regardless of flour type or wheat variety (Table 3.5). Nonetheless, whole wheat flour pasta, irrespective of the wheat variety, was relatively firmer than RS pasta when overcooked. Consequently, on average, WW flour pasta was 1.1-fold firmer than RS pasta. Significant differences were observed between wheat varieties depending on the cooking duration and flour type. Generally, pasta produced from Precision and Transcend appeared firmer than from Spitfire, regardless of the flour type or cooking duration. This could have accounted for the ease of all-trans lutein extraction from Spitfire, which underscores the influence of the food matrix. Evidently, Transcend-produced WWF pasta cooked to AI was the firmest, and OC Spitfire-produced RS pasta was the least firm. The effect of kernel vitreous-ness is positively correlated to pasta firmness (Fu et al., 2018). High vitreous kernels are variety dependant; hence, the differences seen between varieties.

As expected, results seen for firmness depicted an opposite trend of water absorption. Sissons and colleagues (2008) indicated an inverse relationship between pasta firmness and water absorption. Contrary to other reports in the literature, our results and those of Vignola, Bustos, and Pérez (2018) revealed that when pasta samples were overcooked, WW samples were significantly firmer than their RS relatives. The likely explanations could be due to the high flour protein (refer to materials and methods subsection 3.2.2.) and the lipid content in WWF compared to RS, which may have extended protein polymerisation during overcooking to the detriment of starch gelatinisation. The high lipid content in WWF flour pasta can bind to starch granules and reduce disruption, ensuring a firmer product. Another reason for the high firmness among WW flour pasta could be due to the extrusion effect on insoluble fibre content in WW pasta. Soluble fibre content is reported to increase during extrusion of wheat flour products (Björck & Asp, 1983). Soluble fibre could have contributed to the pasta structure strength by forming a network around starch

granules, leading to a stronger cohesiveness between starch and protein, which was possibly enhanced by an increased moisture content and cooking duration.

A general relation exists between wheat and pasta protein content and pasta firmness (Wood, Batey, Hare, & Sissons, 2001). However, Grzybowski and Donnelly (1979) indicated no change in pasta firmness as cooking time increased for high-protein wheat varieties. Despite its relatively high RS flour protein content, Spitfire-produced pasta was less firm when overcooked (Table 3.5). This means the effect on firmness when pasta is overcooked may not be attributable to just flour protein content alone but also gluten strength.

Pasta brightness and yellowness are attractive quality attributes that influence consumer perception. Hence, WWF or bran-substituted RS pasta is usually considered inferior to RS or conventional pasta and does not usually have great consumer appeal. Our study had a significant wheat variety, flour type and cooking duration influence on pasta's yellow colour, as described by a positive b^* (Table 3.5). Increasing cooking duration significantly decreased pasta's yellow colour regardless of the flour type or wheat variety ($P < 0.05$) except for CDC Precision-produced WWF pasta in which samples cooked to AI were not significantly different from their OC samples ($P > 0.05$). This observation might be due to the leaching of yellow pigment content and or chemical degradation or isomerisation of carotenoids.

Between flour types, pasta made from RS significantly recorded higher b^* values than WWF samples regardless of cooking duration or variety ($P < 0.05$). WWF pasta appeared brownish due to bran content, which significantly accounted for reduced (b^*). Within each cooking duration for RS pasta, wheat variety had a significant effect; AAC Spitfire recorded the highest value for b^* , followed by CDC Precision and Transcend depicting the high lutein content in both cooked and uncooked Spitfire samples. For WWF pasta, however, the trend depended on the cooking

duration employed, and it followed: AI-cooked samples (Transcend> Spitfire> Precision); FCT and OC (Spitfire> Transcend> Precision).

Trans-cis isomerisation has been shown to reduce the colour concentration of food samples containing carotenoids (Ngamwonglumlert, Devahastin, Chiewchan, & Raghavan, 2020). Moreover, changes in pasta reflectance characteristics after cooking and/or interaction with other molecules resulting in structural changes could have accounted for these results (Wood et al., 2001). Pasta b* has been shown to be related to particle size, which is also dependent on wheat variety vitreous-ness (Fu et al., 2018). Bran and increased protein content among WW pasta, on the other hand, are responsible for a more reddish colour, leading to a reduced b* (Fu et al., 2018; Manthey & Schorno, 2002).

Table 3.5. Significant interaction effect of flour type, wheat variety and cooking duration on water absorption, firmness, and yellow colour index of pasta.

Milling flour type ^b	Wheat variety	Cooking duration ^a	Water absorption (%)	Firmness (g)	Pasta yellow colour (b*)
	<i>AAC Spitfire</i>	AI	236.38 ^{gh}	337.78 ^{cb}	35.23 ^a
		FCT	288.64 ^{cd}	260.47 ^{fg}	32.14 ^d
		OC	323.46 ^a	184.55 ^j	30.09 ^g
Semolina	<i>CDC Precision</i>	AI	251.80 ^{fg}	401.71 ^a	34.17 ^b
		FCT	278.48 ^{cde}	266.29 ^f	31.65 ^e
		OC	320.95 ^a	215.51 ^{hi}	29.11 ^h

		AI	237.76 ^{gh}	394.83 ^a	32.70 ^c
	<i>Transcend</i>	FCT	275.63 ^{de}	271.51 ^{ef}	30.82 ^f
		OC	325.32 ^a	194.18 ^{ij}	28.54 ⁱ
		AI	236.06 ^{gh}	307.73 ^{cd}	27.39 ^j
	<i>AAC Spitfire</i>	FCT	269.17 ^{ef}	245.76 ^{fgh}	27.03 ^k
		OC	314.27 ^a	217.88 ^{hi}	26.59 ^l
		AI	229.11 ^h	359.68 ^b	24.83 ⁿ
WW flour	<i>CDC Precision</i>	FCT	262.06 ^{ef}	300.48 ^{de}	25.47 ^m
		OC	294.37 ^{bc}	251.27 ^{fg}	24.59 ⁿ
		AI	228.02 ^h	423.60 ^a	28.52 ⁱ
	<i>Transcend</i>	FCT	270.75 ^{de}	330.35 ^{bcd}	26.32 ^l
		OC	307.17 ^{ab}	234.75 ^{gh}	25.37 ^m

All values are means \pm SD of three determinations. Values with the same lower-case superscripts within a column are not significantly different ($P>0.05$). ^aCooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. ^b flour type: WW, whole wheat flour

3.3.3. Changes in antioxidant capacity

The antioxidant capacity of WWF and RS pasta prepared from three wheat varieties cooked to achieve al dente, fully cooked or overcooked, is presented in Figure 3.3 by a significant three-way interaction. Antioxidant capacity ranged from 46.46 - 132.29 mmol TE/100 g pasta sample dry weight.

Generally, regardless of the wheat variety or cooking duration, antioxidant carotenoids in WWF flour pasta significantly scavenged the ABTS radical relative to its RS samples. To put this in perspective, averaged across the cooking duration, antioxidant capacity in WWF flour pasta was 1-, 2- and 3-fold significantly higher than RS pasta for AAC Spitfire, CDC Precision and Transcend, respectively. According to Beta and Hwang (2018), the scavenging capacity of antioxidants from food samples was a function of its concentration, which was significantly influenced by wheat variety and flour type in our study (Appendix 3-Table S3.2). An earlier study reported a decrease in total carotenoid content in durum wheat with milling (Borrelli et al., 2008). Evidently, the highest scavenging capacity against the non-physiological ABTS radical was determined for Transcend-produced WWF pasta cooked to Al, and the lowest was determined for OC Transcend-produced RS pasta, indicating that antioxidant composition was essential in its activity.

RS pasta devoid of germ and bran fractions was significantly low in zeaxanthin, a predominant carotenoid in durum wheat (Table 3.1). The significant presence of this carotenoid in WWF flour samples, especially in the Transcend variety, accounted for the higher antioxidant activity of its WWF flour pasta. Indications in the literature point to the fact that the antioxidant properties of carotenoids are related to their molecular structure, such as chain length, terminal groups, and oxygen substituents. In the case of zeaxanthin, it is represented by an extra conjugated

double bond in its polyene chain, which appears to serve as a stronger antioxidant against free radicals (Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996).

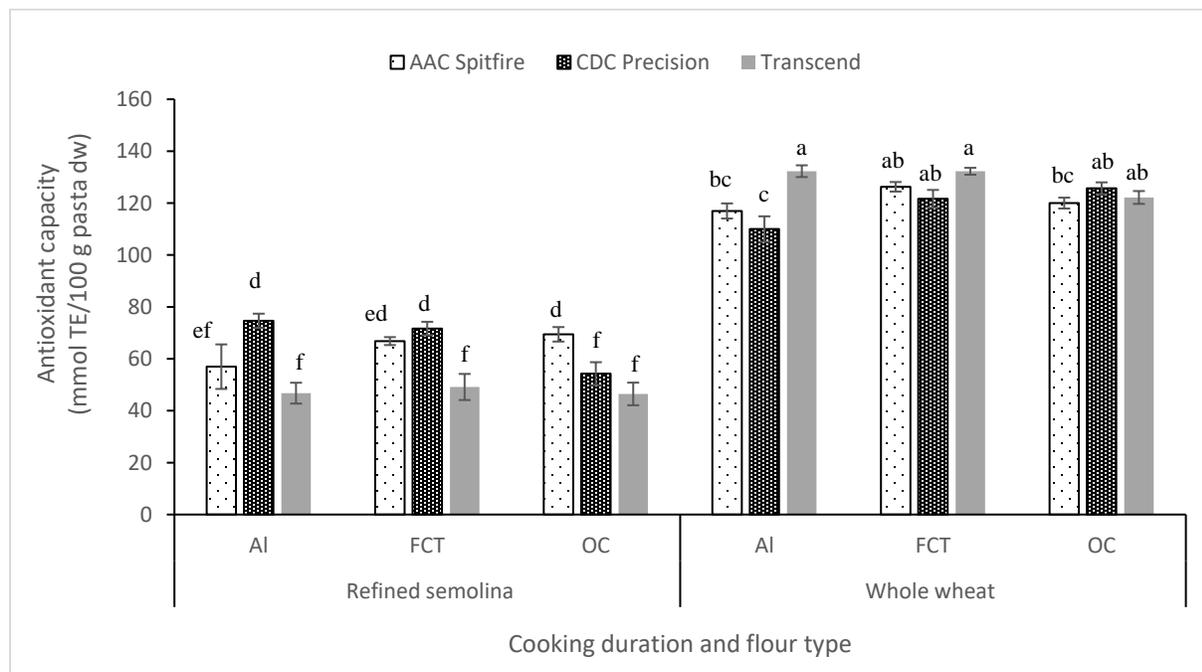


Figure 3.3. Effect of wheat variety and cooking duration on pasta antioxidant capacity (mmol TE/100g sample in dry weight) in refined semolina and whole wheat pasta. Values are means \pm SD;(n=3). Bars with the same lower-case superscripts are not significantly different (P>0.05). Cooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta.

3.4. Conclusions

The carotenoid content and retention, cooking quality, and ABTS radical scavenging capacity of durum wheat pasta produced from different wheat varieties milled to WWF flour and RS and cooked to Al, FCT and OC were studied. Generally, the extractable content and stability of all-trans lutein, all-trans zeaxanthin, total carotenoids and the cis isomers of lutein as assessed

by HPLC increased as pasta cooking duration increased. Contrary to this observation however, the retention of total carotenoids (TCC), as assessed photometrically, decreased overall with increasing pasta cooking time. This observation rejects our initial hypothesis and highlights the discriminatory outcome between the two analysis methods employed in carotenoid quantification. Increased cooking duration significantly increased water absorption, cooking loss and decreased firmness and b* value. Despite the influence of cooking duration on both carotenoid content and physical properties studied, changes were also dependent on wheat variety and, more likely, on nutrient distribution in wheat grain and the different structural changes in RS and WWF pasta that occurred during cooking.

To the best of our knowledge, this is the first in-depth study focusing on the influence of different pasta cooking durations on the nutritional, cooking quality and antioxidant capacity of durum wheat pasta. In furtherance to this, current studies are focusing on the influence of pasta cooking duration on carotenoid availability upon digestion in the gut.

Connections between Chapters 3 and 4

The results from Chapter 3 highlighted the factors that influenced the content release and stability of pasta carotenoids, including their cooking quality using three durum wheat varieties. The results showed that, generally, increasing cooking duration increased extractable carotenoids and, by this, increased their apparent retention. Although all-trans lutein content extracted from cooked Spitfire pasta was the highest among the wheat varieties, its retention was lower than Transcend but higher than Precision. In terms of physical properties, increasing the cooking duration of pasta also modified the pasta food structure, rendering it less firm. The next chapter, Chapter 4, investigates the bioaccessibility of the carotenoids from the three wheat varieties produced- whole wheat and refined semolina pasta cooked to al dente, fully cooked and overcooked, similarly examining factors that influence carotenoid bioaccessibility, including the food matrix composition. The recovery of the carotenoids was also monitored at each stage of digestion to determine their stability. This enabled the understanding of the release pattern and transformation/degradation of carotenoids as affected by both digestive and non-digestive factors.

CHAPTER 4.

Effect of cooking duration on carotenoid content, digestion and potential absorption efficiencies among refined semolina and whole wheat pasta products.

Hannah Oduro-Obeng, Franklin B. Apea-Bah, Kun Wang, Bin Xiao Fu, Trust Beta

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4. Abstract

Bioaccessibility of carotenoids varies among different foods due to factors such as food matrix composition and type or extent of processing. Hence it is important to understand the extent to which these factors influence carotenoid bioaccessibility after consumption and digestion of food. This study evaluated the carotenoid content, micellization efficiency, digestive stability, antioxidant activity, and bioaccessibility of carotenoids as impacted by wheat variety and cooking duration among whole wheat flour (WWF) and refined semolina (RS) pasta. WWF and RS pasta were processed from three durum wheat varieties (AAC Spitfire, CDC Precision, Transcend) and cooked to al dente (Al), fully cooked (FCT) or overcooked (OC). The study showed that wheat variety and cooking duration were significant functions of bioaccessible lutein in RS samples while only variety influenced the bioaccessibility of zeaxanthin and lutein in WWF samples. In both WWF and RS, the effect of variety on the bioaccessibility of lutein and zeaxanthin were similar and was as follows: Transcend > CDC Precision > AAC Spitfire. Cooking to Al significantly caused an increment in bioaccessible lutein in RS samples regardless of wheat variety. This influence of cooking duration (Al > FCT > OC) was inversely related to the lutein concentrations in undigested pasta (OC = FCT > Al). An assessment of the relationship between food matrix components of Transcend and its carotenoid bioaccessibility revealed that in addition to its contents, changes to the fractional composition of the matrix as induced by food processing may be partly responsible for its high bioaccessible carotenoid content. DPPH scavenging activity among WWF samples was about 2-fold greater or more than RS samples regardless of variety or cooking duration before and after digestion. Our data suggests that the effect of wheat food matrix and cooking duration modulates the bioaccessibility and antioxidant activity of RS and WWF pasta products.

4.1. Introduction

Carotenoids are a varied group of naturally occurring liposoluble plant pigments synthesized in both photosynthetic plants and animals and some non-photosynthetic bacteria and fungi with both nutritional and biological functions (Britton, 1995). Generally, plasma concentrations of these phytochemicals in humans and animals are attained exclusively through food consumption (Al-Yafeai & Bohm, 2018). Several nutritional epidemiological studies have drawn an inverse relationship between the high consumption of carotenoids and reduced non-communicable disease risk (Maiani et al., 2009; Xavier & Mercadante, 2019). In nature, most carotenoids occur in their trans isomers; however, food processing may cause trans-cis transformations, increasing the cis isomer content in processed foods (Schieber & Carle, 2005).

Grain food products are an excellent avenue for the intake of carotenoids since many populations rely on them as staples. However, carotenoid concentrations in grain-based food products are minimal compared to quantities in fruits and vegetables (Panfili et al., 2004). Durum wheat and its food products, such as pasta, are, however, rich sources of the oxygen-containing carotenoids (lutein and zeaxanthin) when compared to other cereal grains (Ndolo & Beta, 2013). Their distribution within the grain matrix is, however, heterogeneous with the exception of probably lutein (Panfili et al., 2004). These differences in distribution may affect the intake and subsequent functionality of these carotenoids when cereal grain such as durum wheat is milled, processed, or digested. Moreover, carotenoid properties have been cited as a factor affecting concentration in processed food as well as their bioaccessibility (Al-Yafeai & Bohm, 2018).

As the potential health benefits of carotenoids become more apparent, there has been intense interest in determining their bioaccessibility from many foods, including pasta. Bioaccessibility is the fraction of an ingested compound made available for intestinal absorption (Etcheverry et al.,

2012). Due to the high cost associated with most human intervention studies, static models mimicking the human digestion system have been developed with high resemblance to the numerous parameters related to *in vivo* digestion with high predictability (Brodkorb et al., 2019; Etcheverry et al., 2012). Bioaccessibility of carotenoids from natural sources has been remarked as very low compared to other phytochemicals (Xavier & Mercadante, 2019). Factors including the food matrix (carotenoid location and cell wall structures) and/ or food source, food processing such as cooking, and carotenoid type are considered the main determinants of carotenoid bioaccessibility (Borel, 2003; Cilla et al., 2018; Etcheverry et al., 2012). Bioaccessibility can differ between different carotenoid sources for the same carotenoid and between different carotenoids within the same source.

As such, in this study, we evaluate the effect of wheat variety and cooking duration on the bioaccessibility and other absorption potentials of carotenoids in pasta. Simple food processing methods such as cooking may negatively or positively impact carotenoid bioaccessibility as its intensity may affect carotenoid bioaccessibility (Rodriguez-Roque et al., 2016). Pasta cooking parameters have not been explored much to increase pasta carotenoids' content extractability and bioaccessibility. Moreover, the effects of cooking time on pasta have focused mainly on the cooking quality and starch digestibility (Bornet et al., 1990). Also, there is a dearth of information on the bioaccessibility of carotenoids in whole wheat flour pasta as most of the few studies focusing on pasta have concentrated on refined or conventional semolina products (Marchetti et al., 2018; Werner & Böhm, 2011).

Therefore, this study investigated for the first time the effect of food matrix and food processing on pasta carotenoid bioaccessibility. We envisaged that increased cooking time would enhance carotenoid extractability, leading to better bioaccessibility regardless of wheat variety in

refined semolina and whole wheat pasta. Accordingly, our objectives were to assess the main influence of wheat variety and cooking duration and their interactions on carotenoid digestive stability and bioaccessibility using the static *in vitro* digestion model developed by INFOGEST in refined semolina and whole wheat flour pasta. Following this we also assessed the DPPH antiradical scavenging activity of digested and undigested carotenoids to determine their effectiveness in mitigating reactive oxygen species (ROS) as affected by the different food matrix and processing conditions. Information from this study will help identify functional ingredients/foods from durum wheat, not only in content but also to find out to which extent carotenoids from such products can be used by the body when affected by food processing.

4.2. Materials and Methods

4.2.1. Chemicals

All solutions were prepared using ultrapure water generated by a Milli-Q purification system (Millipore, Schwalbach, Germany). Simulated digestive fluids (400 ml) for oral, gastric, and intestinal digestion were prepared using salt stock solutions and milli-Q water according to the protocol of Brodkorb *et al.* (Brodkorb *et al.*, 2019). All salts were HPLC or ACS certified. KCl; potassium chloride (cat.no. P330), KH₂PO₄; potassium phosphate monobasic (cat. no. P285), NaHCO₃; sodium bicarbonate (cat. no. S233), (NH₄)₂CO₃; ammonium carbonate (cat. no. A651), CaCl₂(H₂O)₂; calcium chloride (cat. no. C70), NaOH; sodium hydroxide (cat. no. S318) and HCl (cat. no.SA56) were purchased from Fisher-Scientific Company (Ottawa, ON, Canada); NaCl; sodium chloride (cat. no. 7581) from Mallinckrodt Specialty Chemical Co. Paris, Kentucky, USA and MgCl₂(H₂O)₆; magnesium chloride hexahydrate (cat. no. M2670) from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Bile extracts porcine (cat. no. B8631) was obtained

from the Sigma-Aldrich Chemical Company along with the digestive enzymes: α -Amylase from porcine pancreas (A3176, 10U/mg), pepsin from porcine gastric mucosa (P7125, 679U/ mg protein) and pancreatin from porcine pancreas (P7545, 8 X USP). The 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were also purchased from Sigma-Aldrich (St Louis, MO, USA). All solvents for carotenoid extraction and chromatography were of HPLC grade and were obtained from Fisher Scientific. Lutein reference standard and trans- β apo- 8- carotenal internal standard were purchased from Sigma-Aldrich and zeaxanthin from Cayman Chemical Company, Michigan, USA.

4.2.2. Wheat variety and sample preparation

Samples of durum wheat varieties AAC Spitfire, CDC Precision, and Transcend were provided by the Grains Research Laboratory of the Canadian Grains Commission, Canada. Before milling into refined semolina (RS) or whole wheat flour (WWF), wheat samples were cleaned and tempered to 16% and 12% moisture, respectively. Whole wheat flour (WWF) was prepared by grinding 2 kg grains in an ultracentrifugal mill (ZM 200, Retsch, Germany) equipped with a 0.5 mm screen at 35°C. RS was prepared by milling durum wheat on a four-stand Allis-Chalmers laboratory mill (Allis-Chambers Manufacturing Co., Milwaukee, WI, USA) in conjunction with a laboratory purifier at 21°C according to the mill flow described by Dexter et al. (1990). Durum wheat samples were milled in duplicate 2 kg batches to have a particle size distribution $>180 <600 \mu\text{m}$.

4.2.3. Spaghetti preparation

4.2.3.1. Extrusion process and conditions

Spaghetti processing was conducted according to mixing and extrusion conditions described by Fu et al. (2013) with little modifications. Briefly, RS or WWF (200 g) and cold water (30.5 -31.5% absorption for RS) or (35.5 - 36.5% absorption for WWF) were first mixed in a high-speed asymmetric centrifugal mixer (model DAC 400 FVZ, FlackTec, Landrum, SC) to generate uniform dough crumbs consistent with commercial requirements. Mixing was done at 800 rpm for 10 s followed by 1200 rpm for 90s after preliminary experiments were conducted to establish desirable water absorption for materials from the three durum varieties.

Spaghetti was produced from RS and WWF using a customized micro-extruder (Randcastle Extrusion System INC, New Jersey, USA). The internal diameter of the extruder barrel was 19 mm with a 12:1 working length-to-diameter ratio. The temperature of the barrel was set at $45^{\circ}\text{C} \pm 1$. The hydrated dough was loaded into an air-eliminated hopper by operating a vacuum. This was to eliminate air bubbles and ensure less oxidation of carotenoids. Extrusion pressure of 100 ± 10 Pa was monitored during the process. A four-hole 1.8 mm Teflon coated spaghetti die was used for the extrusion process. The resulting fresh pasta was dried in a pilot Buhler pasta dryer (Buhler, Uzwil, Switzerland) using an 85°C drying cycle. After drying, the average moisture content for dry RS and WWF pasta was 10.39 and 10.33%, respectively. Dried extruded pasta was kept at a temperature of -40°C until further analysis.

4.2.3.2. Cooking of spaghetti

Aliquots of pasta (10 g each) were cut into 5 cm pieces and cooked in 300 ml boiling distilled water in a 600 ml Pyrex glass beaker at 400 °C on an Isotemp hot plate (Fisherbrand, Thermo Fisher Scientific, China) until either cooked to *al dente* (Al), fully cooked (FCT) or overcooked (OC). Distilled water was used to control for the effects and variability on cooking quality and texture, as cooking water hardness has been reported to influence these properties (Dexter et al., 1988). Depending on the flour type associated spaghetti, *al dente* samples (representing pasta with some presence of ungelatinized starch in the inner core) were cooked uncovered for 8 min or 7 min for RS or WWF pasta, respectively. Fully cooked time (FCT) for both pasta types was assessed using AACC International Approved Method 66-51.01 (AACC, 2000). FCT corresponds to the disappearance of white in the central core of the spaghetti when pressed between two plexiglass plates at 15 s intervals during cooking. On average, FCT for WWF and RS pasta was 11 min and 12 min, respectively. Pasta samples were overcooked (OC) by 6 min beyond their respective FCT. The beakers containing FCT and OC samples, however, were covered with perforated aluminum foil during the cooking process. After cooking, samples were drained and rinsed with 50 ml of distilled water to arrest cooking. Samples were then frozen, freeze-dried, and milled using a Proctor Silex coffee grinder (Hamilton Beach Brands, Inc., Glen Allen, VA 23060, Model 80301C) to pass through a 500 µm sieve. Milled pasta samples were stored in airtight dark plastic containers at -40 °C for *in vitro* digestion. Cooking experiments were done in duplicates for each cooking duration on two batches of refined and whole wheat flour pasta (n=4).

4.2.4. Chemical composition

The proximate composition analysis was performed in duplicate according to official methods for moisture (method 44-15.02), crude protein (method 46-30.01), and crude fat (method 30-25.01) contents (AACC, 2000). The enzymatic determination of total starch content (method 76-13.01- total starch assay procedure, amyloglucosidase/ α -amylase method, Megazyme, Ireland) and total, insoluble and soluble dietary fibre contents (method 32-07.01-Megazyme assay) was carried out following the manufacturer's instructions.

4.2.5. Simulation of the upper gastrointestinal digestion of pasta

This procedure was performed according to the methodology described earlier by Brodkorb et al. (2019) with several modifications to enable us to assess carotenoid bioaccessibility in a starch-rich food product like pasta. Additionally, standard units of enzymes and bile concentrations were evaluated following the various procedures outlined in the protocol.

4.2.5.1. Preparation of digestive, enzyme, and bile solutions

Simulated digestive fluids, including Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF), were prepared to a volume of 400 ml (1.25x) from stock salt solutions according to Brodkorb et al. (2019). Modifications were also made to the amount of bile extract in SIF. All solutions were stored at -20°C in aliquots and thawed as needed for each batch of digestion. The bile extract (Sigma-Aldrich Chemical Co., B8631) dissolved in SIF was prepared to result in 0.2% w/v of the total digestive solution (i.e., 0.045 g bile extract per gram of food, given that the average molecular weight of bile salt is 500 g/mol). Enzymes obtained from Sigma-Aldrich Chemical Co., including α -amylase (A3176), pepsin (P7125), and pancreatin

(P7545, 8 X USP), were prepared into fresh solutions for each batch of digestion and kept on ice. Cold solutions of 1500 U/ml of α -amylase in water, 25000 U/ml pepsin in SGF, and 800 U/ml of pancreatin in SIF were prepared. Prior to digestion, the enclosed shaking water bath (VWR, Goshen Parkway, PA, USA) and digestive solutions were warmed up to 37 °C.

4.2.5.2 *In vitro* upper gut digestion procedure

Three stages were performed: oral, gastric, and intestinal phases were accomplished followed by separation of the aqueous phase containing mixed micelles (filtrate) through filtration. The corresponding quantities of digestive fluids, enzyme solutions, $\text{CaCl}_2 (\text{H}_2\text{O})_2$ and water were added according to the individual stages below. The analysis was carried out in quadruplicate.

Oral phase: Approximately two grams of milled freeze-dried cooked pasta (particle size <500 μm) was weighed into a 125 mL Erlenmeyer flask covered with aluminum foil and digested in the dark. To this, 3 mL of ultra-pure water was added to achieve a consumable and/ or swallowable paste-like consistency at the end of the oral phase. Following this, 3.5 ml of SSF, 0.5 ml α -amylase in water (to achieve 75 U/ml in the final mixture in the oral stage), 25 μl of calcium chloride (0.3 M), and double distilled water were added to reach a final volume of 10 ml. The flasks were immediately incubated at 37 °C, agitating vigorously in a shaking water bath at 150 rpm for 2 mins.

Gastric phase: To the oral bolus, 7.5 ml of SGF was added, and the pH was adjusted to 3 using a predefined volume of 1 M HCl. Five microlitres of calcium chloride (0.3 M) was added, followed by 1.6 ml pepsin in water (to achieve 2000 U/ml in the final mixture). The pH of the samples was verified again, and the value approximated to 3. The pH slightly increased above 3 during digestion, but this was not a problem because a recent report by Werner and Bohn

determined that an average pH value of 4 was ideal for the bioaccessibility of lipophilic compounds such as carotenoids (Werner & Böhm, 2011). The final volume of 20 ml was reached by topping up with double-distilled water. The flasks were quickly blanketed with nitrogen gas, covered with aluminum foil, and incubated at 37 °C for 2 hr at a mixing speed of 150 rpm using the shaking water bath.

Intestinal phase: To the 20 ml gastric chyme, 11 ml of SIF was added, and the pH was adjusted to 7 using 1 M NaOH, reflecting close to physiological conditions in the duodenum where carotenoids are absorbed. Following this, 2.5 ml of bile solution in SIF (to reach a final concentration of 10 mM at the end of digestion), 40 µl of calcium chloride (0.3 M), 5 ml of pancreatin in SIF (to reach 100 U/ml in the final mixture) and double distilled water were added to reach a total final volume of 40 ml. The reaction vessel was again blanketed with nitrogen and incubated at 37 °C for 2 h amidst continuous shaking in the water bath.

4.2.5.3. Isolation of micellar fraction

At the end of the simulated digestion, the final digesta was quickly transferred into 50 ml centrifuge tubes and brought to a volume of 44 ml by washing the Erlenmeyer flask with an appropriate amount of water while on ice. The aqueous micellar fraction was separated from the digesta residue by centrifuging at 4270×g for 20 min at 4 °C followed by filtration of the supernatant through a 0.22 µm pore size syringe filter. Aliquots of undigested pasta, final digesta, micellar fraction (herein filtered supernatant or filtrate as used in Table titles), and solid residue were immediately snapped with nitrogen gas and freeze-dried for further analysis.

4.2.5.4. Determination of carotenoid digestive stability in the different digestion steps

This evaluation was considered to investigate possible alterations (transformations and/or degradations) that the xanthophylls and their isomers from pasta could undergo when subjected to each phase of the digestive procedure separately (i.e., Oral phase, Oral + Gastric phase and Oral +Gastric + Intestinal phase). The “digesta” (not centrifuged) from each phase was determined for recovery.

4.2.6. Carotenoid analysis

4.2.6.1. Extraction of carotenoids

Carotenoids were extracted from approximately 2 g of undigested pasta, digesta, filtered supernatants, and 0.5 g of the residue using the method of Werner and Böhm (2011) with several modifications under subdued light. Briefly, samples were weighed into 50 ml centrifuge tubes, after which 3 ml of water and 2 ml of ethanol were added to the solid samples and vortexed vigorously. This was to ensure an extensive quantitative extraction. Following this, 3 μ l from a 50-ppm stock solution of trans β -apo-8-carotenal was added as an internal standard to both digested and undigested samples for quantification. One millilitre each of ethanol, methyl tert-butyl ether (MTBE), and petroleum ether were added in that order, after which samples were vortex for about a minute. Ethanol has been determined to denature the carotenoid-protein complexes, allowing petroleum ether to extract the nonpolar carotenoids. The tubes were centrifuged at 4270 \times g at 4 °C for 3 min. The organic nonpolar upper phase was collected using disposable transfer pipettes, and the extraction with MTBE and petroleum ether was repeated until a clear appearance was observed with the extraction solvents. Combined extracts were rotary

evaporated at 34 °C. The dried extracts were reconstituted in 2 ml ethanol using an ultrasonic water bath and stored at -20 °C overnight before further analysis.

4.2.6.2. Separation, identification, and quantification of carotenoids by HPLC

Resolubilized dried extracts were centrifuged using a Sorvall Legend Micro 21 Centrifuge (Thermo Scientific, Am Kalkberg, Germany) at 18,800×g for 5 mins and filtered through a 0.45 µm filter in preparation for HPLC. Four hundred microlitres were transferred into an HPLC amber vial (equipped with a glass insert). HPLC separation of carotenoids was carried out using the accomplished procedure of Abdel-Aal et al. (2007) with slight modifications. The setup was a Waters model 2695 enhanced with a photodiode array detector (PAD) (Waters 996) and autosampler (Waters 717 plus) (Waters, Milford, MA, USA). The column was a 4.6×100 mm YMCTM carotenoid S-3 with 3 µm packing (YMC/Waters Inc., Wilmington, NC, USA) operated at 35 °C. Twenty microlitres of the sample were injected by the autosampler and eluted with a gradient system consisting of (A) methanol/MTBE/Milli-Q water (81:15:4, v/v/v) and (B) MTBE/Methanol (90:10, v/v). The flow rate was set at 1 ml/min, and the total run time was 15 mins. The gradient was programmed as follows: 0-9 mins, 100 -75% A; 10-12 mins, 0% A; 12-13 mins, 0-100% A; and 13-15 mins, 100% A.

Carotenoids were detected at 450 nm. Peaks were identified by their retention times, followed by a comparison of the absorption spectra of the eluted compounds with the elution sequence to the all-trans lutein and zeaxanthin reference standards, their UV/Vis spectra, and those reported in the literature. Considering that the amounts of cis isomers were close to the limit of quantification (LOQ), tentative identification was carried out based on a comparison of elution order and the peaks in the absorbance spectrum with the literature using a similar protocol. The λ

max of cis isomers is reported to be slightly lower than their corresponding all-trans isomers and is characterized by the presence of a “cis” peak at about 142 nm below the longest-wavelength maximum of the all-trans compound (Britton, 1995).

Carotenoids were quantified using a six-point analytical curve of all-trans lutein (0.3-10.0 µg/ml, $R^2=0.997$) and all-trans zeaxanthin (0.08-2.5 µg/ml, $R^2=0.996$) representing the regression equations $y= 32986x-2901.3$ and $y= 65215x- 6076.8$ respectively. The cis isomers of lutein were estimated using the curve of its corresponding all-trans lutein isomer. All chromatograms were processed using Empower Pro, build number 1154 (Waters, Milford, MA, USA). The repeatability of the carotenoid concentration was checked by injecting one standard each day when samples were running. Precision was evaluated by the relative coefficient of variability, which was 4.9, 6.8, 6.5, 9.8, 9.8, 4.3 and 5.9% for all-trans lutein, all-trans zeaxanthin, 15-cis lutein, 13-cis lutein, 13'-cis lutein, 9-cis lutein and 9'-cis lutein respectively. Limits of detection (LOD) and quantification (LOQ) were determined using parameters of the analytical curve. The values of LOD and LOQ were 1.04 µg/ml and 3.14 µg/ml, respectively, for all-trans lutein and 0.31 µg/ml and 0.93 µg/ml for all-trans zeaxanthin. Quadruplicate analyses were performed.

4.2.6.3. Spectrophotometric measurements

Filtered carotenoid extracts were pipetted into 96-well flat-bottom microplates, and absorbance was read against the blank solvent (ethanol). Absorbance was measured at 450 nm using a BioTek microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). Total carotenoid content was measured and calculated according to an earlier protocol (Abdel-Aal et al., 2007) using the following equation and expressed as µg lutein equivalent/g sample. $C=(2 \times A)/S \times W$ where C = lutein content in µg/g; A = absorbance reading, S = regression

coefficient; 2 = dilution factor based on the total reconstituted volume of 2 ml and W = sample weight (g). All analysis was done in triplicate. The calibration curve was obtained by using lutein standard with concentration range of 0.08-10.0 $\mu\text{g/ml}$.

4.2.6.4. Bioaccessibility calculations and equations

The percentage of carotenoid micellized was used as a measure of bioaccessibility. That is the percentage of carotenoid transferred to the aqueous micellar fraction after digestion in relation to its content in the undigested samples (as indicated in Eq. (1)). Efficiency of micellization was defined as the concentration (%) of carotenoids that was transferred from the digesta to the aqueous micellar fraction (Eq. (2)). At the same time, digestive stability was calculated by the contents of carotenoids in the digesta in relation to undigested food samples (Eq. (3)).

$$\% \text{ Bioaccessibility} = \frac{\text{Carotenoid content}_{\text{micellar fraction}}}{\text{Carotenoid content}_{\text{undigested pasta}}} \times 100 \quad (1)$$

$$\% \text{ Efficiency of micellization} = \frac{\text{Carotenoid content}_{\text{micellar fraction}}}{\text{Carotenoid content}_{\text{digesta}}} \times 100 \quad (2)$$

$$\% \text{ Recovery} = \frac{\text{Carotenoid content}_{\text{digesta}}}{\text{Carotenoid content}_{\text{undigested pasta}}} \times 100 \quad (3)$$

4.2.7. Determination of *in vitro* chemical antioxidant activity

The antioxidant activity of undigested and digested carotenoid extracts was evaluated using the colorimetric method reported by Brand-Williams, Cuvelier, and Berset (1995) which is based on the 1,1-diphenyl- 2-picrylhydrazyl (DPPH•) assay with some adaptations. Aliquots of 195 μ l of ethanolic DPPH (60 μ m/ L) working solution were mixed with 5 μ l of carotenoid extracts (in ethanol without internal standard) or Trolox working solutions in a 96-well flat bottom microplate and the mixtures held for 30 mins under subdued light. Absorbance was read at 515 nm against ethanol as a blank using a BioTek microplate spectrophotometer (BioTek Instruments, Inc. Winooski, USA). The analysis was done in triplicates. The standard curve was constructed using Trolox concentrations ranging from 50 to 800 μ l. Antioxidant activity was calculated in mmol of Trolox equivalent per 100 g of pasta.

4.2.8. Statistical analysis

The experiment consisted of three durum wheat varieties (AAC Spitfire, CDC Precision, Transcend), flour type (RS and WWF flour), and cooking duration (Al, FCT, OC). Two-way analysis of variance (ANOVA) was carried out to determine significant differences in concentration and bioaccessibility of carotenoids from pasta in relation to the factors: wheat variety and cooking duration for each flour type. ANOVA assessing separately their main and interaction effects were evaluated using SAS statistical software version 9.4 (SAS Institute Inc., Cary, NC) with proc GLIMMIX fitted to the data. Simple main effects of the significant interactions were determined for the two independent variables. The relationship between macronutrient content and carotenoid bioaccessibility was performed using Pearson's correlation. The strength of the correlation was classified according to Statology.com where: an absolute value of the Pearson

correlation coefficient, $r < 0.25$ = “no relationship”; $0.25 < r < 0.5$ = “weak”; $0.5 < r < 0.75$ = “moderate”; $r > 0.75$ = “strong”. Means were separated by Tukey-Kramer HSD and significance was defined at 5% ($P < 0.05$). The results were expressed as mean \pm SD. All experiments were conducted in triplicate unless indicated otherwise.

4.3. Results and Discussion

4.3.1. Carotenoids in cooked refined semolina and whole wheat flour pasta.

The concentration of carotenoids in the three durum wheat varieties- processed semolina or whole wheat pasta, which were cooked to al dente, fully cooked or overcooked is presented in Appendices 8 and 9 (Tables S4.1, S4.2) for refined semolina pasta and Appendices 10 and 11 (Tables S4.3, S4.4) for whole wheat pasta. ANOVA results are presented in Tables 4.1 and 4.2, respectively. The qualitative profile of carotenoids between the wheat varieties was similar except for all-trans zeaxanthin in the refined semolina pasta samples, which were below the LOD. The average concentration for all-trans lutein across cooking duration ranged from 0.77 to 0.87 mg/100 g and 0.88 to 0.96 mg/ 100 g dry-weight pasta for refined semolina and whole wheat samples, respectively (Appendix 8-Table S4.1 and Appendix 10-Table S4.3). As expected, cis carotenoid (lutein) geometrical isomers were determined in the cooked samples. As minor and less stable components of most dietary carotenoid food sources, reports suggest that thermal food processing causes their formation (Britton, 1995). Despite being in lower concentrations compared to their all-trans isomers, their recent identification and relevance in biological samples warrants more investigations into their molecular transformations and/or degradation before and after consumption.

From the ANOVA tables (Tables 4.1 and 4.2), most of the carotenoids were significantly affected by the effect of cooking and their concentrations were significantly different among the three levels of cooking duration (i.e., A1, FCT, OC). Specifically, cooking durations above 8 mins (FCT or OC) resulted in higher extractable carotenoids, possibly due to cell wall depolymerization. This was to be expected since increased cooking duration may have facilitated the breakdown of polysaccharide cell walls and carotenoid-protein complexes, allowing for the efficient extractability of carotenoids from the plastids of the wheat grain storage proteins. Several reports in the literature, including our most recent study (Oduro-Obeng, Fu, & Beta, 2021) have discussed the relevant role food processing, including thermal processing, can play in the efficient release of phytochemicals, including carotenoids (Barba et al., 2017; Bohm, 2018; Kopec & Failla, 2018).

For WWF samples, however, neither significant main nor interaction significant effects were observed for all carotenoid species except for 13'-cis lutein. This isomer showed significant main effect differences ($P < 0.05$) in its concentration among the three levels of cooking duration (OC=FCT>A1) (Table 4.2).

All-trans lutein and zeaxanthin are the major carotenoid species in durum wheat (Burkhardt & Bohm, 2007), and their distribution within and between grains is not homogenous except for lutein (Panfili et al., 2004). The similar concentration range reported for cooked pasta samples between RS and WWF for all-trans lutein corroborates with a recent report from our lab indicating that there was no influence of flour type on all-trans lutein content in cooked pasta samples (Oduro-Obeng et al., 2021). Zeaxanthin, on the other hand, is reported to be mainly located in the outer endosperm layers of wheat grains (Ndolo & Beta, 2013). Hence, during conventional wheat milling, removing the bran-aleurone-associated layers can deplete the concentrations of this

compound in their refined flour and products, thus affecting its intake and subsequent functionality.

Table 4.1. Significant main and interaction effect of the independent variables (wheat variety and cooking duration) on the carotenoid content in undigested RS pasta and their corresponding bioaccessibility.

Carotenoids in RS pasta

Variable	ANOVA	Concentrations in undigested pasta (mg/100g dry weight)							Bioaccessibility (%)						
		All-trans lutein	All-trans zeaxanthin	15-cis lutein	13-cis lutein	13'-cis lutein	9-cis lutein	9'-cis lutein	All-trans Lutein	All-trans zeaxanthin	15-cis lutein	13-cis lutein	13'-cis lutein	9-cis lutein	9'-cis lutein
	<i>Main effect</i>														
Wheat Variety	AAC Spitfire	NS	ND	NS	NS	NS	NS	NS	124.45b*	ND	NS	NS	NS		
	CDC Precision	NS	ND	NS	NS	NS	NS	NS	153.48a*	ND	NS	NS	NS		
	Transcend	NS	ND	NS	NS	NS	NS	NS	156.94a*	ND	NS	NS	NS		
#Cooking duration	AI	0.674b	ND	NS	0.069b	0.041b	0.056b		187.72a	ND	159.06a	145.82a	165.09a		
	FCT	0.832a	ND	NS	0.085a	0.050a	0.065a		144.99b	ND	108.86b	123.55b	139.51b		
	OC	0.925a	ND	NS	0.089a	0.055a	0.069a		102.17c	ND	84.35b	95.63c	114.69c		
	<i>Interaction effect</i>														
AAC Spitfire	AI	NS	ND	NS	NS	NS	NS	0.041bB	NS	ND	NS	NS	NS	118.64aA	124.81aB
	FCT	NS	ND	NS	NS	NS	NS	0.059aAB	NS	ND	NS	NS	NS	87.60aB	90.18bB
	OC	NS	ND	NS	NS	NS	NS	0.057aB	NS	ND	NS	NS	NS	96.38aA	94.51bA
CDC Precision	AI	NS	ND	NS	NS	NS	NS	0.052bA	NS	ND	NS	NS	NS	146.18aA	161.72aA
	FCT	NS	ND	NS	NS	NS	NS	0.062aA	NS	ND	NS	NS	NS	138.34aA	148.17aA
	OC	NS	ND	NS	NS	NS	NS	0.064aAB	NS	ND	NS	NS	NS	84.71bA	87.52bA
Transcend	AI	NS	ND	NS	NS	NS	NS	0.052bA	NS	ND	NS	NS	NS	136.85aA	149.75aAB
	FCT	NS	ND	NS	NS	NS	NS	0.052bB	NS	ND	NS	NS	NS	139.84aA	155.69aA
	OC	NS	ND	NS	NS	NS	NS	0.068aA	NS	ND	NS	NS	NS	85.02bA	86.93bA

Different capital letters in the same column and cooking duration (AI, FCT, OC) represent significant differences ($p < 0.05$) within wheat variety (AAC Spitfire, CDC Precision, Transcend), while different lower-case letters in the same column for each wheat variety show significant difference within cooking duration ($p < 0.05$) for the interaction effect. NS; not significant as determined by ANOVA; ND, not detected by HPLC-PDA ($< \text{LOD}$). #Cooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. RS; Refined semolina pasta. ANOVA; Analysis of variance. * Significantly different from WWF pasta; All-trans lutein (LOD:1.04 $\mu\text{g/ml}$, LOQ: 3.14 $\mu\text{g/ml}$), all-trans zeaxanthin (LOD:0.31 $\mu\text{g/ml}$, LOQ: 0.93 $\mu\text{g/ml}$).

Table 4.2. Significant main and interaction effect of the independent variables (wheat variety and cooking duration) on the carotenoid content (mg/100g dw) in undigested WWF pasta and their corresponding bioaccessibility.

		Carotenoids in WWF pasta													
Variable	ANOVA	Concentrations in undigested pasta (mg/100g dw)							Bioaccessibility (%)						
		All-trans lutein	All-trans zeaxanthin	15-cis lutein	13-cis lutein	13'-cis lutein	9-cis lutein	9'-cis lutein	All-trans lutein	All-trans zeaxanthin	15-cis lutein	13-cis lutein	13'-cis lutein	9-cis lutein	9'-cis lutein
<i>Main effect</i>															
Wheat Variety	AAC Spitfire	NS	NS	NS	NS	NS	NS	NS	104.03b	121.34b	90.10b	99.04b	107.78b	96.16b	98.17b
	CDC Precision	NS	NS	NS	NS	NS	NS	NS	101.13b	130.93b	86.78b	100.68ab	104.61b	95.14b	102.48b
	Transcend	NS	NS	NS	NS	NS	NS	NS	139.06a	186.41a	120.15a	137.91a	148.34a	141.62a	156.92a
#Cooking duration	AI	NS	NS	NS	NS	0.049b	NS	NS	NS	NS	NS	NS	NS	NS	NS
	FCT	NS	NS	NS	NS	0.058ab	NS	NS	NS	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	0.064a	NS	NS	NS	NS	NS	NS	NS	NS	NS
<i>Interaction effect</i>															
AAC Spitfire	AI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	FCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CDC Precision	AI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	FCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Transcend	AI	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	FCT	ND	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are means of four determinations (n=4). Different letters in the same column are significantly different (p<0.05). NS; not significant as determined by ANOVA; ND; not detected by HPLC-PDA (<LOD). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. WWF; whole wheat pasta. ANOVA; Analysis of variance. All-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

4.3.2. Carotenoid bioaccessibility

To the best of our knowledge, no previous study has investigated the effect of food matrix and food processing on the bioaccessibility of carotenoids in durum wheat pasta. Tables 4.1 and 4.2 show the significant main effects and interactions of these variables on the bioaccessibility of carotenoids from cooked RS and WWF pasta, respectively. After *in vitro* digestion, bioaccessibility was determined as the percent ratio of the filtered supernatant (aqueous micellar fraction) after centrifuging to the original carotenoid content in undigested samples. The aqueous phase containing mixed micelles (herein the filtrate) was labeled as the bioaccessible fraction.

The average bioaccessibility of all-trans lutein and all-trans zeaxanthin across cooking duration for RS and WWF pasta ranged from 124.46 to 156.94% and <LOD for RS and 101.13 to 139.06% and 121.34 to 186.41% for WWF pasta respectively (Appendix 8-Tables S4.1. and Appendix 10- Table S4.3). Bioaccessibility of lutein in WWF pasta was significantly lower than in RS pasta, indicative of the restrictive nature of cell wall components in releasing carotenoids. Moreover, other bran components in WWF, including divalent minerals, can negatively affect its carotenoid bioaccessibility (Table 4.1 and 4.2). Bioaccessibility above 100% suggests that cooking was able to disintegrate cell walls and /or protein-pigment complexes, allowing for the efficient action of digestive enzymes along the digestive tract. There were significant differences ($P < 0.05$) in the bioaccessibility of all-trans lutein among the three different wheat varieties and cooking durations, with Transcend being the most bioaccessible for both RS and WWF pasta and AI cooked samples having the highest bioaccessible lutein content (Table 4.1). Also, for zeaxanthin, the main effect of wheat variety was significant only in the WWF flour (Table 4.2). Interestingly, our most recent report assessing the effect of cooking time using these same varieties revealed that Transcend contained relatively lower concentrations of lutein in both RS and WW flour pasta

regardless of cooking time. In contrast, AAC spitfire contained the highest (Oduro-Obeng et al., 2021). However, the cooking stability of all-trans lutein in Transcend (75.6%) was slightly higher compared to AAC spitfire (74.2%) irrespective of the flour type and cooking duration (Oduro-Obeng et al., 2021). This may have accounted for its high lutein bioaccessibility. Petry and Mercadante (2017) indicated that the higher the concentration of carotenoids, the higher the tendency to aggregate and/or the lower the micellization efficiency, reducing bioaccessibility. O'Connell et al. (2007) also indicated that when the carotenoid content of digested samples is high, their transfer to mixed micelles is generally low, possibly due to interactions with other food matrix components.

The chemical composition of the food matrix, including protein, fiber, starch, and lipids, can modulate the release and digestion of carotenoids through interactions that may be protective or inhibitory depending on digestive conditions and food processing conditions. Food processing can influence the content and properties of these nutrients, thereby affecting their digestibility and the digestibility of carotenoids. To evaluate matrix factors that influenced the release of carotenoids in the wheat varieties, correlation analysis was performed. From the correlation matrix table (Table 4.4), all macronutrients were positively correlated with the content of bioaccessible carotenoids (all-trans lutein and all-trans zeaxanthin). However, the strengths of these correlations differed depending on the wheat variety and flour type suggesting nutrient compositional differences between these wheat varieties as impacted by food processing. In the RS samples, fat, protein, and starch contents were not associated ($r < 0.25$) with lutein digestibility in the Transcend wheat variety, which had the highest bioaccessible lutein content (Table 4.1). However, total dietary fiber was strongly associated ($r=0.98$). Precision, on the other hand, which was not significantly different from Transcend in terms of bioaccessible lutein in RS samples, was strongly

correlated with its fat ($r=0.96$) and dietary fiber ($r=0.96$) content, moderately correlated with total starch ($r=0.66$), and not correlated with its protein ($r=0.11$) content (Table 4.4). Spitfire, which showed the lowest content of bioaccessible lutein was weakly correlated with total dietary fiber ($r=0.42$) and total starch ($r=0.54$) but strongly correlated with its fat ($r=0.75$) and protein ($r=0.78$) content. Similarly, within WWF flour, the strength of the correlations varied as the content of bioaccessible lutein significantly decreased from Transcend to Spitfire (Table 4.2).

Proteins have emulsifying properties like bile salts due to their amphiphilic properties, which can enhance the release of liposoluble compounds such as carotenoids in aqueous media and promote micellization (G. Zhang et al., 2024). Iddir et al. (2020), in their recent study, revealed a positive correlation between higher protein concentration and carotenoid bioaccessibility. In our study, this was valid, and its contents were positively and strongly associated with the high bioaccessible lutein content of Transcend in the WW flour but not RS. Proteins can adsorb onto the surfaces of lipid droplets, forming protective layers that prevent the aggregations of these lipid droplets. By this, proteins indirectly improve carotenoid digestibility (Iddir et al., 2020). Currently, the influence of proteins on the bioaccessibility of carotenoids is unclear and may be positive or negative depending on its digestibility, digestive conditions, and even the carotenoid type (Iddir et al., 2022). From our results, it can also be inferred that this influence was wheat flour type dependant due to their different protein content. Food processing, such as cooking, can also alter the microstructure or properties of gluten (the main proteins in wheat flour), affecting its digestibility. Hence in addition to its contents, protein quality (gluten composition) may have accounted for the high lutein bioaccessibility in RS Transcend and helps explain the lack of association between protein content and bioaccessible lutein in RS Transcend (Table 4.3).

Lipids, on the other hand, aid the solubilization of carotenoids after their release from the food matrix, and higher concentrations have been shown to be beneficial in this process. Moreover, lipids in foods facilitate the formation of micelles by promoting the secretion of bile salts and pancreatic lipases (Desmarchelier & Borel, 2017; Kopec & Failla, 2018). Our study showed a strong and positive association between lipid content and the significantly high content of bioaccessible lutein in Precision RS and the significantly low bioaccessible lutein content in Spitfire RS and WWF (Tables 4.1 and 4.2). The lack of correlation found in Precision WWF and Transcend RS and WWF samples also meant that in addition to fatty acid contents in these varieties, their composition might be a better predictor of their digested carotenoid contents.

Although a negative association is reported between high fiber content (mainly soluble dietary fiber) in food and carotenoid bioaccessibility (Braulio Cervantes-Paz et al., 2017), a positively strong correlation between the significantly high bioaccessible lutein content in Transcend and Precision and total dietary fiber content in RS pasta was observed. Similarly, a positive association was observed in Transcend WWF pasta, but this was moderate, suggesting that alternative mechanisms were involved in dietary fiber's role in the digestibility of lutein. This observation was unexpected. However, a recent study assessing carotenoid bioaccessibility in maize reported a positive association between lutein digestibility and dietary fiber content (Zurak et al., 2024). In this study, the authors suggested that the positive correlation was due to the neutral detergent-insoluble fiber's (mainly hemicellulose) content and its ability to increase digesta viscosity, thereby inhibiting droplet aggregation in the gastric phase (Zurak et al., 2024). In our study, the insoluble dietary fibre content was significantly higher than soluble dietary fibre content (Table 4.3) irrespective of the wheat variety or wheat flour type suggesting that the positive association observed may be due to the high contribution of insoluble dietary fibre to the total

dietary fibre content in the pasta samples. Fares et al. (2008) earlier indicated that cooking pasta resulted in an increase in the insoluble dietary fibre content compared to uncooked samples. As much as the above hypothesis may be valid, in-soluble dietary fiber can also reduce the viscosity of the food digesta, allowing for increased accessibility of digestive enzymes to digest lipid droplets into micelles and increase carotenoid bioaccessibility.

Transcend RS and WWF bioaccessible lutein content was not correlated with its total starch content, suggesting that beyond its contents, the fractional composition of starch may have influenced its bioaccessibility, presumably resistant starch (retrograded starch). Resistant starch was reported to have increased in cooked pasta samples compared to uncooked samples due to the reorganization of amylose after cooling the pasta in an earlier study (Fares et al., 2008). The degree and quantity of resistant starch in food depends on starch properties including amylose content, amylose: amylopectin ratio, and starch granular size, food processing factors, such as time and temperature, and additional food components, including proteins, lipids, and fiber content (Fares, Platani, Baiano, & Menga, 2010; Scott & Awika, 2023). Though not quantified in this study, resistant starch can lower the viscosity of the lumen due to its insoluble properties and prevent lipid droplets from aggregating in the stomach, thereby increasing the accessibility of lipase to the lipid phase (McClements et al., 2015). Currently, no report can be found on the effect of resistant starch on carotenoid bioaccessibility in the literature, warranting further studies to understand the mechanism involved. The differences in the relationship observed among the wheat varieties and flour types can be attributed to the differences in starch properties of the wheat varieties which may have resulted in different starch compositions upon food processing (Artavia, Cortés-Herrera, & Granados-Chinchilla, 2020).

Ultimately, variations in the grain's macronutrient content and composition as a function of cooking influenced macronutrient digestibility and the digestibility of carotenoids. It can be concluded from the above observations that Transcend, which had the highest bioaccessible lutein content, was partly influenced by its nutrient composition, such as fat composition, protein quality, and probably its starch fractional composition altered by food processing, necessitating further studies into grain matrix properties and their influence on the release of digested carotenoids.

Table 4.3. Macronutrient composition of refined semolina and whole wheat cooked pasta (g/100g dwb)

Variety	Cooking duration#	Refined semolina pasta					Whole wheat pasta								
		Moisture	Crude fat	Crude Protein	Total Starch	Total dietary fibre	IDF	SDF	Moisture	Crude fat	Crude Protein	Total Starch	Total dietary fibre	IDF	SDF
Spitfire	AI	13.58	0.03	18.45	83.28	4.38	4.38	0.00	13.33	1.26	19.96	66.92	11.49	11.49	0.00
	FCT	13.02	0.13	18.68	77.18	4.36	4.36	0.00	13.17	1.24	20.61	69.07	11.43	11.43	0.00
	OC	13.01	0.06	18.53	83.08	5.03	5.03	0.00	13.12	1.56	20.16	67.81	12.29	12.29	0.00
	Mean	13.20	0.07	18.55	81.18	4.59	4.59		13.21	1.35	20.24	67.93	11.74	11.74	
Precision	AI	13.77	0.18	18.88	80.62	4.56	4.56	0.00	13.42	1.19	20.10	66.59	12.57	11.87	0.69
	FCT	13.91	0.09	18.73	77.52	4.56	4.56	0.00	13.12	0.98	20.37	66.36	12.73	12.73	0.00
	OC	13.67	0.02	18.83	83.45	4.94	4.94	0.00	13.26	1.00	20.37	67.32	13.09	13.09	0.00
	Mean	13.78	0.10	18.81	80.53	4.69	4.69		13.27	1.06	20.28	66.76	12.80	12.56	
Transcend	AI	9.28	0.03	18.65	77.53	4.96	4.96	0.00	10.21	0.92	20.46	63.71	12.20	12.09	0.11
	FCT	12.38	0.34	19.44	82.22	5.20	5.20	0.00	10.50	0.87	20.15	66.26	12.01	12.01	0.00
	OC	8.53	0.10	18.79	77.44	6.54	5.75	0.79	10.04	0.97	20.29	62.79	13.99	13.72	0.27
	Mean	10.06	0.16	18.96	79.06	5.56	5.30		10.25	0.92	20.30	64.25	12.73	12.61	

Values are means of three determinations (n=3). Different letters within a column are significantly different ($p < 0.05$). # Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta. (dwb)= dry weight basis; IDF=insoluble dietary fibre; SDF= soluble dietary fibre.

Table 4.4. Correlation matrix between bioaccessible lutein and zeaxanthin and pasta macronutrient content

		RS				WWF			
Spitfire	All-trans lutein	$r=0.75$ $p=0.463$	$r=0.78$ $p=0.429$	$r=0.54$ $p=0.633$	$r=0.46$ $p=0.698$	$r=0.99$ $p=0.042$	$r=0.33$ $p=0.784$	$r=0.22$ $p=0.859$	$r=0.99$ $p=0.037$
	All-trans zeaxanthin	ND	ND	ND	ND	$r=0.99$ $p=0.010$	$r=0.26$ $p=0.835$	$r=0.14$ $p=0.912$	$r=0.99$ $p=0.014$
Precision	All-trans lutein	$r=0.96$ $p=0.191$	$r=0.11$ $p=0.933$	$r=0.66$ $p=0.538$	$r=0.96$ $p=0.188$	$r=0.14$ $p=0.913$	$r=0.22$ $p=0.858$	$r=0.99$ $p=0.044$	$r=0.82$ $p=0.385$
	All-trans zeaxanthin	ND	ND	ND	ND	$r=0.79$ $P=0.422$	$r=0.84$ $P=0.367$	$r=0.76$ $P=0.583$	$r=0.99$ $P=0.105$
Transcend	All-trans lutein	$r=0.16$ $p=0.898$	$r=0.11$ $p=0.930$	$r=0.07$ $p=0.953$	$r=0.95$ $p=0.207$	$r=0.13$ $p=0.919$	$r=0.83$ $p=0.378$	$r=0.14$ $p=0.912$	$r=0.53$ $p=0.642$
	All-trans zeaxanthin	ND	ND	ND	ND	$r=0.99$ $p=0.103$	$r=0.59$ $p=0.599$	$r=0.99$ $p=0.065$	$r=0.83$ $p=0.381$
		CF	CP	TS	TDF/IDF	CF	CP	TS	TDF/IDF

RS- Refined semolina; WWF- Whole wheat flour; CF- Crude fat; CP- Crude protein; TS- Total starch; TDF-Total dietary fibre; IDF- insoluble dietary fibre ND- Not determined. Significance was set at $p<0.05$.

In addition to the main effect of wheat variety in the RS samples, the main effect of cooking duration was also a significant function in determining bioaccessible lutein ($P < 0.05$). Firstly, increasing pasta cooking duration could have simply caused an increase in carotenoid degradation/oxidation during digestion due to an increase in its contents in the lumen (as depicted by decreases in their aqueous micelles (filtrate) and micellization efficiency (Fig. 4.1)) reducing bioaccessibility. Moreover, cooking above the 8-minute cooking duration (FCT and OC) could have induced irreversible modifications to the food matrix components, partly influencing the digestion behavior of carotenoids. For example, with excessive cooking, proteins may have formed aggregates, reduced their digestibility and emulsification properties, or formed complexes with the released carotenoids. Amylose content in pasta could have been reduced due to leaching into cooked water as the cooking duration increased. Reduced amylose content meant less retrograded starch (resistant starch) was formed. As resistant starch has insoluble properties, it can reduce the viscosity of the lumen, increasing the digestibility of carotenoids. Hence, with an increase in viscosity relative to content in pasta cooked to AI or FCT, carotenoid digestion was reduced. Furthermore, the released carotenoids could have also bound to the digestive enzymes, hindering their solubilization into lipid droplets. Proteins, including enzymes, have been reported to negatively affect carotenoid digestion (Iddir et al., 2022). For dietary fibre, increasing cooking duration increased its contents. These non-starch polysaccharides could have bounded to bile acids reducing their emulsification properties.

Like all-trans lutein, a significant wheat variety effect on the bioaccessibility of all trans-zeaxanthin was observed in WWF pasta samples, again suggesting the influence of their different food matrices on the digestibility of zeaxanthin (Table 4.3). Gómez et al. (2020), in an earlier report, cited that the bran and germ biological layers of wheat grain, which are the rich portions of

zeaxanthin, vary widely among the grains. Moreover, grain vitreousness was reported as a function of higher carotenoid release in some commercial maize genotypes studied earlier, with zeaxanthin relating positively to vitreousness (Saenz, Borrás, & Gerde, 2021). The significantly high bioaccessibility observed for zeaxanthin compared to lutein in WWF pasta samples could be attributed to their different chemical properties affecting their stability throughout digestion. Moreover, zeaxanthins' location in the grain tissue amidst lipid matrices could have facilitated their efficient digestion compared to lutein. To our knowledge, this is the first report on durum wheat semolina pasta carotenoids showing that enhanced extractability and /or inherent content may not necessarily translate to better bioaccessibility.

Regarding the food matrix component effect on bioaccessible zeaxanthin, these also correlated positively to the low or high bioaccessibility of zeaxanthin in the wheat varieties identified in the whole wheat flour pasta samples (Table 4.4). Moreover, these positive associations were strong except for protein and starch content, which showed no correlations with bioaccessible zeaxanthin in Spitfire. For Transcend, all nutrients were strongly associated with bioaccessible zeaxanthin except protein content, which was moderate. This association was less strong compared to that for lutein. The influence of protein on carotenoid bioaccessibility has been determined to be affected by the carotenoid type and could be the reason for the difference in the strength of the associations between zeaxanthin and lutein (Table 4.4) (Iddir et al., 2022).

These results are in tandem with information on the efficiency of micellization in Fig. 4.1, which also shows the effectiveness of cooking to AI or FCT in increasing the transferability of all-trans lutein to the filtrate-containing mixed micelles compared to OC. Micellization efficiency is suggested as a useful measure for the comparative assessment of bioaccessibility (Kean et al., 2008). In WWF pasta samples (Fig. 4.2), however, micellization efficiency was not influenced by

the cooking duration ($P>0.05$) as mirrored in results from Table 2 (bioaccessibility of carotenoids in WWF pasta).

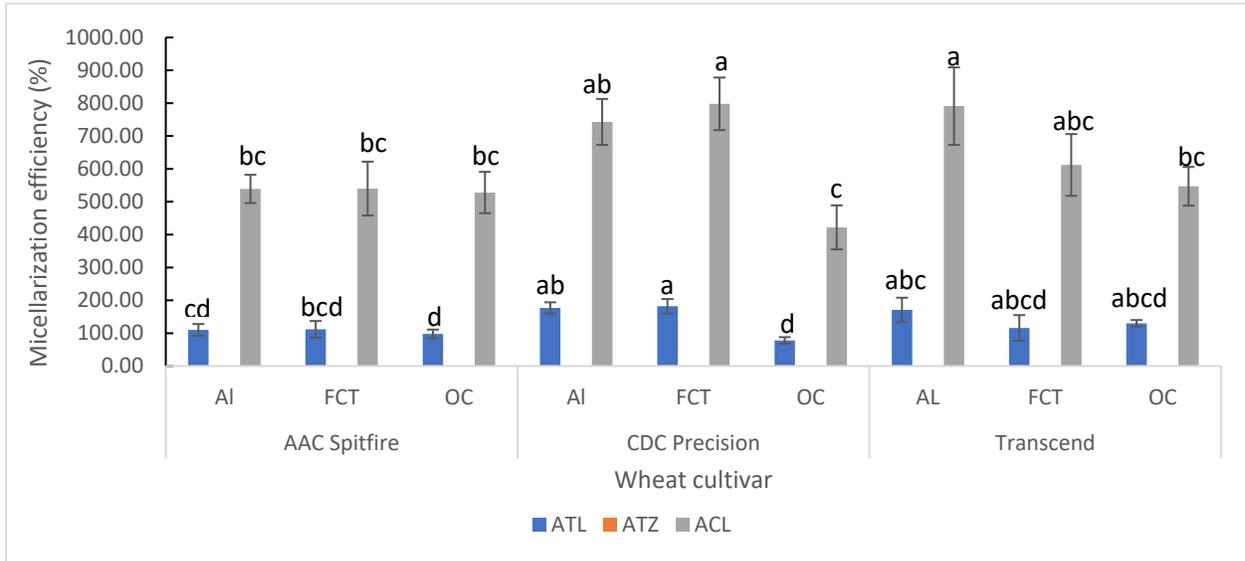


Figure 4.1. Micellization Efficiency of individual carotenoids across cooking duration and wheat variety combination effect in refined semolina pasta. Values are means \pm SD for three observations. Different lower-case letters above bars indicate significant differences in micellization efficiency between the independent variables (wheat variety/cooking duration effect) within a carotenoid species ($P<0.05$). Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta. Carotenoid species: ATL, all- trans lutein; ATZ, all-trans zeaxanthin; ACL, all-cis lutein.

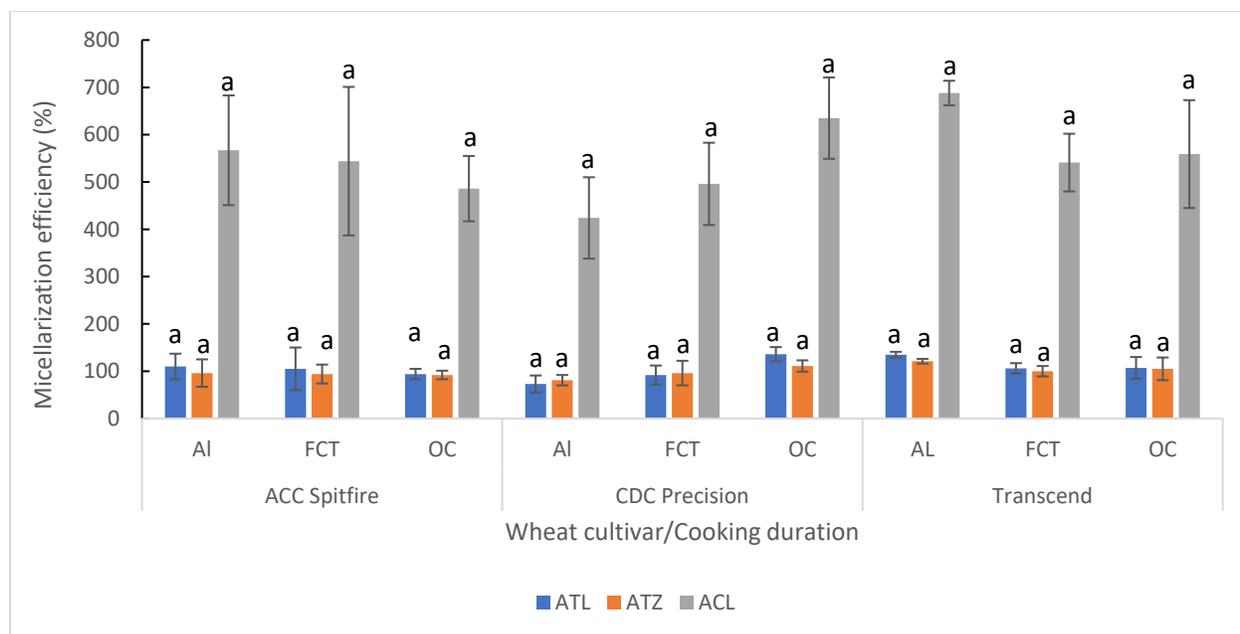


Figure 4.2. Micellization efficiency of individual carotenoids across cooking duration and wheat variety combination effect in whole wheat flour pasta. Values are means \pm SD for three observations. Same lower-case letters above bars indicate non-significant differences in micellization efficiency between the independent variables (wheat variety/cooking duration effect) within a carotenoid species ($P>0.05$). Cooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta. Carotenoid species: ATL, all- trans lutein; ATZ, all-trans zeaxanthin; ACL, all-cis lutein.

Like their all-trans isomers, the bioaccessibility of some cis lutein compounds was also significantly influenced by the effect of cooking duration among RS samples ($P<0.05$). Specifically, 15-, 13- and 13'-cis lutein were more bioaccessible when cooked to Al (Al>FCT>OC) (Table 4.1). Bioaccessibility above 100 % for some of these isomers may suggest the isomerization of their all-trans- form during digestion and/ or their high micellization efficiency as depicted in Fig. 4.1. Due to their formation during food processing, it is not surprising that the main effect of cooking had the most influence on their bioaccessibility. Furthermore, the variety

differences in bioaccessibilities seen at the cooking levels of FCT and AI, for 9- and 9'-cis lutein suggest that increased cooking duration of RS pasta beyond 12 mins impacted negatively on the availability of these carotenoids after digestion just as their all-trans compounds.

Summed together, all-cis lutein efficiency of micellization was significantly greater ($P < 0.05$) than their all-trans isomer (Fig. 4.1). This meant that carotenoid properties (molecular and chemical properties) were a determinant of carotenoid transfer to micelles from lipid droplets and hence bioaccessibility. Ferruzzi, Lumpkin, Schwartz, and Failla (2006), earlier indicated that the structure of cis carotenoid promoted a more stable isomer devoid of aggregation and crystallization among lipid droplets during digestion facilitating their efficient micellization. The unique structural characteristics of the carotenoid molecules could influence their absorption and their association with other macromolecules.

Among WWF samples, however, the bioaccessibility of all cis lutein isomers was significantly influenced by the main effect of wheat variety only ($P < 0.05$). Like their all-trans lutein counterparts, we propose that the influence of wheat variety on the bioaccessibility of all-cis lutein merely indicates the food matrix differences (e.g., fibre, protein, and fat content components) and the ability of digestive enzymes to breakdown cell wall matrices. On average, Transcend was 78 and 64 % more efficient in micellization than CDC Precision and AAC Spitfire, respectively, regardless of cooking duration (Fig. 4.2).

4.3.3. Carotenoid stability during *in vitro* digestion

To better understand the carotenoid content and possible alterations and/or transformations carotenoids may undergo at the various stages of *in vitro* digestion and how this may have influenced their bioaccessibility, aliquots of RS-digested pasta were collected at the end of each

phase (Oral, Oral + Gastric, and Oral + Gastric + Intestinal). Results are presented in Table 4.5 (ANOVA) and Appendix 12-Table S4.5.

Generally, reductions in carotenoid content were recorded after the oral and gastric phases of digestion (Appendix 8-Table S4.1 and Appendix 12-Table S4.5). These reductions or losses were more pronounced in the gastric phase. Losses ranging from 11-42 and 35-45% for FCT and OC, respectively, as opposed to 11-29% for AI, were observed after gastric digestion. These significant losses between FCT and OC during gastric digestion could have contributed to the overall reduction in carotenoid bioaccessibility at these cooking levels. Similar to our results, Xavier and Mercadante (Xavier & Mercadante, 2019) reported that carotenoid losses after *in vitro* digestion are usually 8-40%. A closer look at Appendix 12-Table S4.5, however, revealed that, on average, the content of total carotenoids (summation of all carotenoids assessed by HPLC) liberated in the oral phase for RS pasta was slightly lower than the duodenal phase but higher than the gastric phase irrespective of wheat variety or cooking duration. This dip in the gastric phase could be attributed to the acid environment. Carotenoids are reported to degrade quickly in low pH (Britton, 1995). Moreover, it has been reported that carotenoid oxidative metabolites, including anhydroluteins, can be formed during gastric digestion (Khachik, Englert, Beecher, & Cecil Smith, 1995). These dehydration products have been found in human plasma and are identified as enzymatic dehydration metabolites occurring in the presence of hydrochloric acid during gastrointestinal digestion (Khachik, Beecher, Goli, & Lusby, 1991). These undetected biotransformations and /or oxidative degradation along the gastrointestinal tract, including the formation of apo-carotenoids, could have accounted for the dip in total carotenoid stability. However, compared to total carotenoids, TCC, as assessed photometrically, was generally low regardless of wheat variety or cooking duration across all stages of *in vitro* digestion (Appendix

12-Tables S4.5). The quantification of TCC in grains and grain products is reported to overestimate their contents as opposed to contents measured by the HPLC method, ostensibly due to the presence of other non-carotenoid pigments, including flavonoids, especially in WWF (Burkhardt & Bohm, 2007). Focusing on the various stages of digestion, in the gastric phase, the relatively low TCC compared to the duodenal phase could be explained by a possible masked effect of α -amylase, pepsin, and the salts contained in the simulated digestive solutions. Color pigments in bile extract, on the other hand, may have contributed to the seemingly increased TCC in the duodenal phase. We therefore propose that some caution should be taken when interpreting TCC from digestion data.

Table 4.5. Significant main and interaction effect of wheat variety and cooking duration on the carotenoid content (mg/100g dry weight pasta) in digested RS pasta samples after each phase of in vitro digestion.

Carotenoid content (mg/100g dry weight) in RS pasta																
Variable	ANOVA	Oral Phase					Oral + Gastric phase					Oral + Gastric + Intestinal phase				
		All-trans lutein	All-trans zeaxanthin	Total cis lutein	Total carotenoids	TCC	All-trans lutein	All-trans zeaxanthin	Total cis lutein	Total carotenoids	TCC	All-trans lutein	All-trans zeaxanthin	Total cis lutein	Total carotenoids	TCC
<i>Main effect</i>																
Wheat Variety	AAC Spitfire		ND				NS	ND	NS	NS		NS	ND	NS	NS	
	CDC Precision		ND				NS	ND	NS	NS		NS	ND	NS	NS	
	Transcend		ND				NS	ND	NS	NS		NS	ND	NS	NS	
#Cooking duration	AI		ND				NS	ND	NS	NS		NS	ND	NS	NS	
	FCT		ND				NS	ND	NS	NS		NS	ND	NS	NS	
	OC		ND				NS	ND	NS	NS		NS	ND	NS	NS	
<i>Interaction effect</i>																
AAC Spitfire	AI	0.395bB	ND	0.194bB	0.589bB	0.382cC	NS	ND	NS	NS	0.267bA	NS	ND	NS	NS	0.722aA
	FCT	0.483abB	ND	0.220abB	0.703abB	0.416bB	NS	ND	NS	NS	0.332aA	NS	ND	NS	NS	0.713aA
	OC	0.659aAB	ND	0.275aA	0.934aAB	0.566aA	NS	ND	NS	NS	0.251bB	NS	ND	NS	NS	0.735aA
CDC Precision	AI	0.661aA	ND	0.280aA	0.941aA	0.544aA	NS	ND	NS	NS	0.191cB	NS	ND	NS	NS	0.653bB
	FCT	0.677aAB	ND	0.282aAB	0.959aAB	0.509bA	NS	ND	NS	NS	0.215cB	NS	ND	NS	NS	0.683abA
	OC	0.726aA	ND	0.291aA	1.017aA	0.405cB	NS	ND	NS	NS	0.261aAB	NS	ND	NS	NS	0.722aA
Transcend	AI	0.775aA	ND	0.316aA	1.091aA	0.467aB	NS	ND	NS	NS	0.120cC	NS	ND	NS	NS	0.526cC
	FCT	0.718aA	ND	0.291aA	1.009aA	0.423bB	NS	ND	NS	NS	0.202bB	NS	ND	NS	NS	0.621bB
	OC	0.470bB	ND	0.205bB	0.676bB	0.307cC	NS	ND	NS	NS	0.279aA	NS	ND	NS	NS	0.759aA

Values are means of four determinations (n=4). Different capital letters in the same column and cooking duration (AI, FCT, OC)

represent significant differences (p<0.05) within wheat varieties (AAC Spitfire, CDC Precision, Transcend), while different lower-case

letters in the same column for each wheat variety show significant differences within cooking duration ($p < 0.05$) for the interaction effect. NS; not significant as determined by ANOVA; ND; not detected by HPLC-PDA ($< \text{LOD}$). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. RS; Refined semolina pasta. TCC; Total carotenoid content assessed spectrophotometrically. ANOVA; Analysis of variance. All-trans lutein (LOD: $1.04 \mu\text{g/ml}$, LOQ: $3.14 \mu\text{g/ml}$), all-trans zeaxanthin (LOD: $0.31 \mu\text{g/ml}$, LOQ: $0.93 \mu\text{g/ml}$).

Table 4.6. Significant main and interaction effect of wheat variety and cooking duration on the carotenoid content (mg/100g dry weight pasta) in digested WWF pasta samples after each phase of in vitro digestion

Carotenoid content (mg/100g dry weight) in WWF pasta																
Variable	ANOVA	Oral Phase					Oral + Gastric phase					Oral + Gastric + Intestinal phase				
		All-trans lutein	All-trans zeaxanthin	Total cis lutein	Total carotenoids	TCC	All-trans lutein	All-trans zeaxanthin	Total cis lutein	Total carotenoids	TCC	All-trans lutein	All-trans zeaxanthin	Total cis lutein	Total carotenoids	TCC
<i>Main effect</i>																
Wheat Variety	AAC Spitfire	NS	0.020ab	0.259ab	NS	1.078a	NS	NS	NS	NS		NS	0.028b	NS	NS	
	CDC Precision	NS	0.017b	0.228b	NS	0.971b	NS	NS	NS	NS		NS	0.025b	NS	NS	
	Transcend	NS	0.021a	0.276a	NS	0.911c	NS	NS	NS	NS		NS	0.031a	NS	NS	
#Cooking duration	AI	0.993a	0.020a	0.262ab	1.275a	0.952b	NS	NS	NS	NS		NS	NS	0.279b	NS	
	FCT	0.757b	0.017b	0.223b	0.997b	0.986b	NS	NS	NS	NS		NS	NS	0.319a	NS	
	OC	1.009a	0.020a	0.279a	1.308a	1.023a	NS	NS	NS	NS		NS	NS	0.339a	NS	
<i>Interaction effect</i>																
AAC Spitfire	AI	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.150aA	NS	NS	NS	NS	0.653aA
	FCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.087aA	NS	NS	NS	NS	0.676aA
	OC	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.094aA	NS	NS	NS	NS	0.655aA
CDC Precision	AI	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.016bB	NS	NS	NS	NS	0.638aA
	FCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.163aA	NS	NS	NS	NS	0.620aAB
	OC	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.975bA	NS	NS	NS	NS	0.640aA
Transcend	AI	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.001aB	NS	NS	NS	NS	0.516bB
	FCT	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.073aA	NS	NS	NS	NS	0.575aB
	OC	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.059aA	NS	NS	NS	NS	0.621aA

Values are means of four determinations (n=4). Different capital letters in the same column and cooking duration (Al, FCT, OC) represent significant differences (p<0.05) within wheat varieties (AAC Spitfire, CDC Precision, Transcend), while different lower-case letters in the same column for each wheat variety show significant difference within cooking duration (p<0.05) for the interaction effect. NS; not significant as determined by ANOVA; ND; not detected by HPLC-PDA (<LOD). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. WWF; whole wheat flour pasta. TCC; Total carotenoid content assessed spectrophotometrically. ANOVA; Analysis of variance. All-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

Similarly, for WWF samples, TCC was relatively lower than total carotenoids across the digestion stages (Table 4.6-ANOVA and Appendix 13-Table S4.6). Quite opposite to RS, however, TCC and total carotenoids in WWF pasta appeared to increase after gastric digestion. This increment after the gastric phase could be due to increased isomerization of the trans compounds, as depicted by a relatively high all-cis lutein content among WWF samples compared to RS (Appendix 13-Table S4.6). From these observations, it can be reasoned that although low pH can facilitate carotenoid isomerization, this is highly food matrix dependent. Proteins have buffering properties (McClements et al., 2015). Hence, the high protein content in WWF pasta might have increased the pH in the gastric phase, stabilizing the digested carotenoids.

These subtle differences observed in carotenoid contents assessed within each stage of digestion were mainly reflective of the food matrix differences and/or flour-type pasta. These ultimately accounted for differences in the bioaccessibility of carotenoids between RS and WWF pasta.

Based on their stabilities, the recovery of the main carotenoids in pasta was calculated as the fraction found in the digesta relative to content in undigested pasta (Eq. 3), and results are presented in Table 4.7- ANOVA and Appendix 8-Table S4.1 for RS pasta and Table 4.8-ANOVA and Appendix 10-Table S4.3 for WWF pasta. All-trans lutein recovery in the oral phase for RS samples (Table 4.7) was significantly affected by the interaction effect of wheat variety and cooking duration, and the effect ranged from 49.45 -114.51%. Transcend was the most recovered when its pasta was cooked to Al or FCT, while Spitfire was the most recovered when cooked to OC. This high recovery of Transcend resulted in its high bioaccessibility as shown in Table 4.1. No new compounds were detected under the wavelength employed; hence, recoveries above 100% could be due to low molecular weight degradation compounds formed during digestion, which

were overlooked under the current HPLC method. Kean et al. (2008) indicated that recoveries above 100% were most likely due to variations in extraction recoveries from digesta and undigested samples. Nonetheless, recovery of the internal standard was >95% in our study. By the end of duodenal phase of digestion, the percentage recovery of all-trans lutein was 1.04 to 2.21-fold greater than in the oral phase regardless of cooking duration or wheat variety.

Table 4.7. Significant main and interaction effect on the recovery (%) of the major carotenoids after each phase of the in vitro digestion for refined semolina pasta

Variable	ANOVA	Recovery (%) in RS pasta					
		Oral Phase		Oral+ Gastric phase		Oral +Gastric + Intestinal	
		All-trans lutein	All-trans zeaxanthin	All-trans lutein	All-trans zeaxanthin	All-trans lutein	All-trans zeaxanthin
<i>Main effect</i>							
	AAC Spitfire		ND	NS	ND		ND
Wheat variety	CDC Precision		ND	NS	ND		ND
	Transcend		ND	NS	ND		ND
	AI		ND	NS	ND		ND
#Cooking duration	FCT		ND	NS	ND		ND
	OC		ND	NS	ND		ND
<i>Interaction effect</i>							
AAC Spitfire	AI	66.64aB	ND	NS	ND	147.11aA	ND
	FCT	59.57aB	ND	NS	ND	89.97bA	ND
	OC	79.61aA	ND	NS	ND	107.43abA	ND
CDC Precision	AI	89.97aAB	ND	NS	ND	98.62aA	ND
	FCT	75.44aAB	ND	NS	ND	93.19aA	ND
	OC	74.55aAB	ND	NS	ND	111.91aA	ND
Transcend	AI	114.51aA	ND	NS	ND	119.07abA	ND
	FCT	99.35aA	ND	NS	ND	137.46aA	ND

OC 49.42bB ND NS ND 75.11bA ND

Values are means of four determinations (n=4). Different capital letters in the same column and cooking duration (AI, FCT, OC) represents significant differences (p<0.05) within wheat varieties (AAC Spitfire, CDC Precision, Transcend) while different lower-case letters in the same column for each wheat variety show significant difference within cooking duration (p<0.05) for the interaction effect. NS; not significant as determined by ANOVA; ND; not detected by HPLC-PDA (<LOD). #Cooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. RS; refined semolina pasta. ANOVA; Analysis of variance.

For the WWF samples, however, cooking was the main factor affecting the recoveries of all-trans lutein, and this was only significant in the oral phase. In contrast, the main effect of wheat variety affected the zeaxanthin content across the three phases of digestion. Specifically, in the oral stage, cooking to AI resulted in more stable lutein (Table 4.8) regardless of the wheat variety. At the same time, zeaxanthin was more stable among the Transcend wheat variety regardless of cooking duration. In summary, the carotenoid recoveries in this study after in vitro digestion were related to the factors of carotenoid type, food matrix, and cooking duration mirroring their influence on carotenoid bioaccessibility.

Table 4.8. Significant main and interaction effect on the recovery (%) of the main carotenoids after each phase of the in vitro digestion for whole wheat flour pasta.

Variable	ANOVA	Recovery (%) in WWF pasta					
		Oral Phase		Oral+ Gastric phase		Oral +Gastric + Intestinal	
		All-trans lutein	All-trans zeaxanthin	All-trans lutein	All-trans zeaxanthin	All-trans lutein	All-trans zeaxanthin
<i>Main effect</i>							
Wheat variety	AAC Spitfire	NS	101.10ab	NS	107.83b	NS	143.67b
	CDC Precision	NS	96.65b	NS	138.26ab	NS	139.61b
	Transcend	NS	119.16a	NS	154.05a	NS	172.21a
#Cooking duration	AI	116.95a	NS	NS	NS	NS	NS
	FCT	82.77b	NS	NS	NS	NS	NS
	OC	109.18a	NS	NS	NS	NS	NS
<i>Interaction effect</i>							
AAC Spitfire	AI	NS	NS	NS	NS	NS	NS
	FCT	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	NS	NS
CDC Precision	AI	NS	NS	NS	NS	NS	NS
	FCT	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	NS	NS
Transcend	AI	NS	NS	NS	NS	NS	NS
	FCT	NS	NS	NS	NS	NS	NS
	OC	NS	NS	NS	NS	NS	NS

Values are means of four determinations (n=4). Different lower-case letters in the same column for each main effect show significant differences (p<0.05). NS; not significant as determined by ANOVA. #Cooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. WWF; whole wheat flour pasta. ANOVA; Analysis of variance.

4.3.4. DPPH scavenging activity of carotenoids in processed durum wheat pasta

The antioxidant activity of carotenoids was tested for undigested and digested RS and WWF pasta samples to determine the ability of these antioxidant carotenoid extracts to scavenge the non-physiological DPPH radical along the upper digestive tract. Generally, the DPPH radical scavenging ability of the carotenoids was about 2-fold higher among WWF pasta samples compared to RS regardless of wheat variety, cooking duration, or stage of digestion (Fig. 4.3 and Fig.4.4). Higher conjugated double bonds (cdb) of carotenoids have been cited as the main feature responsible for their singlet oxygen quenching ability in organic solvents (Edge & Truscott, 2018). Hence, the presence of zeaxanthin (11 cdb) versus lutein (10 cdb) in WWF pasta samples accounted for this variation. Moreover, other phytochemicals, including phenolic compounds in WWF, could have contributed to the high antiradical scavenging capacity. However, this was not evaluated in this study.

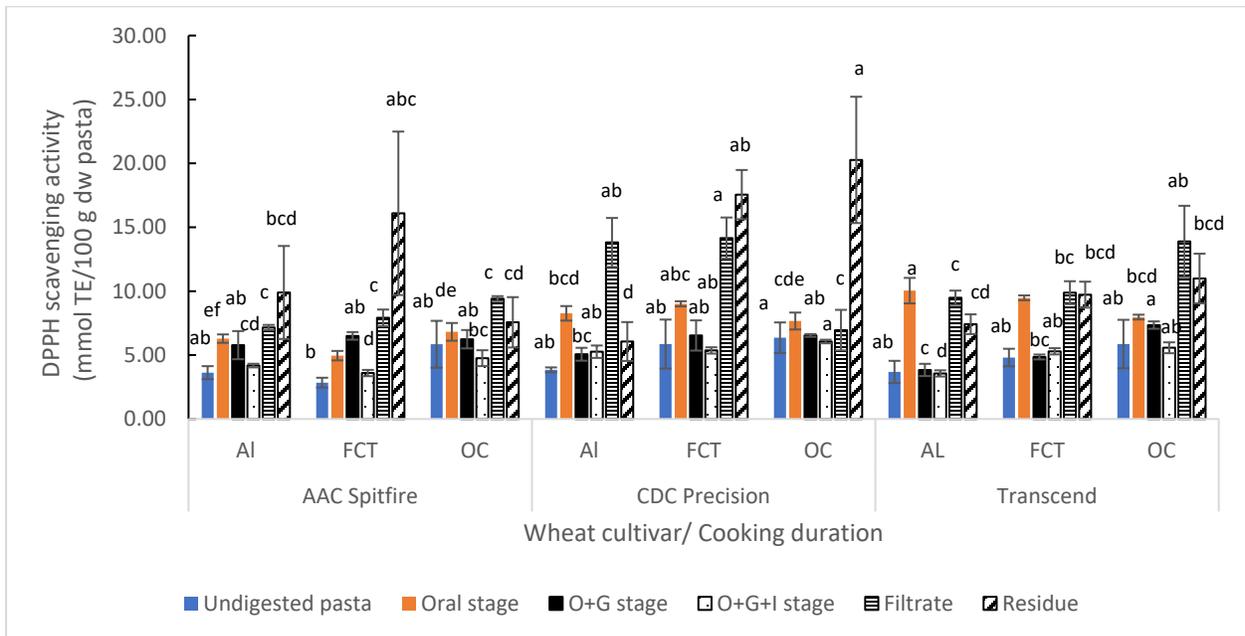


Figure 4.3. DPPH scavenging activity in undigested and digested pasta between cooking duration and wheat variety combination effect in refined semolina pasta. Values are means± SD for three observations. Different lower-case letters above bars indicate significant differences in carotenoid antioxidant activity in scavenging the DPPH radical between the independent variables (wheat variety/cooking duration effect) within a digestion stage ($P<0.05$). Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta. Digestion stage: O, oral; G, gastric; I, intestinal stages.

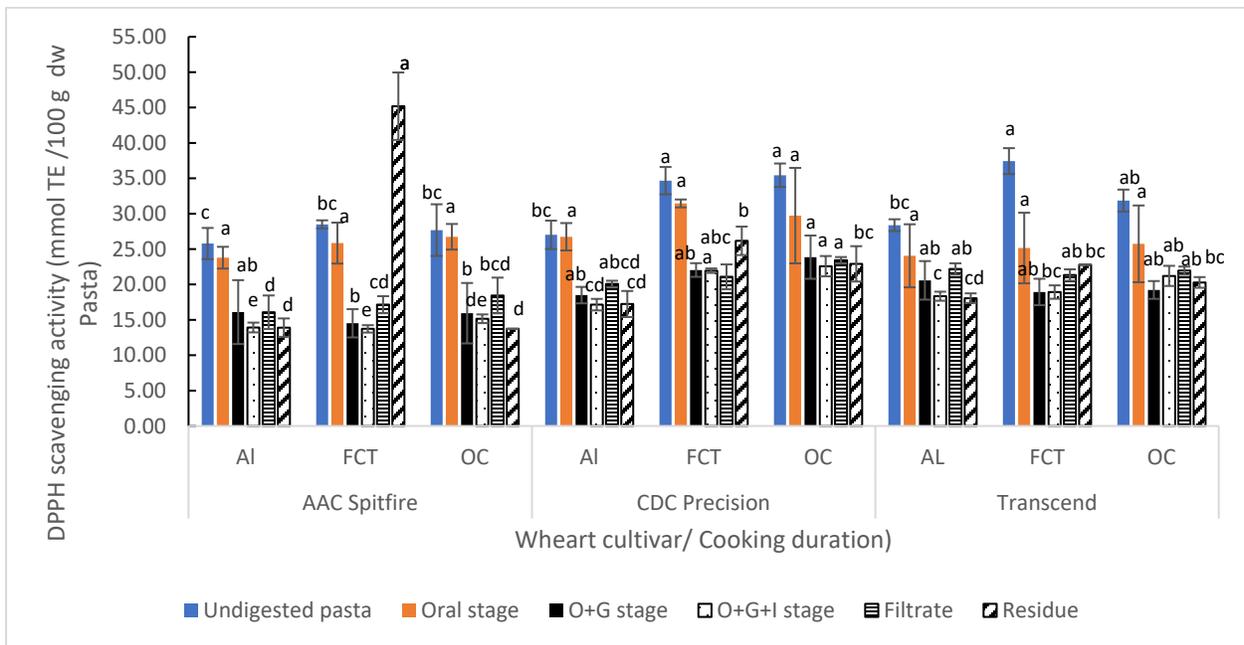


Figure 4.4. DPPH scavenging activity in undigested and digested pasta between cooking duration and wheat variety combination effect in whole wheat flour pasta. Values are means± SD for three observations. Different lower-case letters above bars indicate significant differences in carotenoid antioxidant activity in scavenging the DPPH radical between the independent variables (wheat variety/cooking duration effect) within a digestion stage ($P<0.05$). Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta. Digestion stage: O, oral; G, gastric; I, intestinal stages.

Undigested samples generally showed a higher scavenging capacity than digested samples among the WWF pasta (Fig. 4.4). Moreover, when not digested, cooking WWF pasta beyond 7 mins significantly increased DPPH radical inhibition for CDC Precision and Transcend pasta (Fig. 4.4), suggesting an increased extractability of the antioxidant compounds when cooked to FCT or OC and an increase in the content of these carotenoids in Precision and Transcend due to their low degradation after cooking. In the digested samples, the antioxidant capacity of the carotenoids decreased from the oral stage to the duodenal stage regardless of the wheat variety or cooking duration. The reduction in antioxidant capacity among digested samples may be due to the production of oxidative products and metabolites that have reduced antioxidant activity (Kotake-Nara & Nagao, 2011). Nonetheless, unlike the oral and gastric phases, Precision and Transcend cooked to OC significantly showed higher antioxidant activity compared to AI-cooked samples in the intestinal phase ($p < 0.05$). Carotenoids are much more stable in neutral and high pH. The high protein content in WWF samples may have facilitated their higher antioxidant activity by increasing carotenoid stability and solubility in the extracts at high pH. At high pH, proteins unfold, exposing more hydrophobic regions for the attachment of carotenoids (Zhang et al., 2024). The differences observed within the digested samples among the cooking durations for each wheat variety may simply reflect the total carotenoid contents released. According to Beta and Hwang (2018), the scavenging capacity of antioxidant compounds such as carotenoids was a function of its content.

Like WWF, undigested RS samples increased with cooking duration, though non-significant (Fig.4.3). The antioxidant capacity of the carotenoids also decreased from the oral stage to the duodenal stage. However, after the intestinal phase of digestion, the antioxidant capacity

appeared similar or higher than that of undigested samples suggesting that enzymes involved in the intestinal digestion of food including pancreatin could release bound carotenoids from the pasta matrix. The even higher antioxidant capacity of the carotenoids in the micellar fraction (filtrate extracts) suggests that bioaccessible carotenoids having antioxidant properties and are capable of attenuating free radical species in the intestinal lumen were significantly released.

In principle, all unabsorbed carotenoids reach the colon. Carotenoids in food digestion residues are important as they transit to the lower gut, where they could exert beneficial effects through fermentation processes (Xavier & Mercadante, 2019). Residues from digested FCT or OC cooked samples compared to AI appeared to largely quench the DPPH radical regardless of wheat variety, suggesting that these cooking durations influenced the food matrix in such a way that they entrapped digested carotenoids, preventing their efficient release in the upper gut and allowing for their fermentation in the lower gut which can be made available for absorption. Comparing the digestion phases across whole wheat and refined semolina past, on average, the oral phase ranked the highest in quenching the DPPH radical (26.58 mmol TE/ 100g pasta) followed by the residue (22.25 mmol TE/ 100g pasta), filtrate (20.19 mmol TE/100g dw pasta), O+ G (18.86 mmol TE/100g pasta) and O+G+I (18.11 mmol TE/100g dw pasta) in that order compared to undigested samples (30.75 mmol TE/ 100g pasta). The higher scavenging ability of WWF pasta residue could be due to remnants of zeaxanthin in the bran residue, as earlier explained (Oduro-Obeng et al., 2021).

4.4. Conclusions

Wheat variety and food processing (herein cooking duration) exerted significant influences singly or in combination on the bioaccessibility and antioxidant activity of carotenoids in digested pasta as well as on their concentrations in undigested pasta. The main influence of cooking duration and wheat variety impacted the bioaccessibility of all-trans lutein in RS pasta while only wheat variety affected lutein and zeaxanthin in WWF samples. Specifically, bioaccessible lutein in RS samples increased by the following cooking order: Al>FCT>OC while wheat variety affected lutein in the order of Transcend = CDC Precision >AAC Spitfire. Similarly, for WWF, the highest bioaccessibility of lutein and zeaxanthin was impacted by Transcend and the lowest by AAC Spitfire. Our data suggests that varieties with relatively lower concentrations of all-trans lutein and zeaxanthin appear to have higher bioaccessibility of these carotenoids. Moreover, an assessment of the relationship between matrix component content and carotenoid bioaccessibility in the wheat varieties showed positive correlations in both RS and WWF flour pasta. Transcend, the variety with the highest bioaccessible lutein and zeaxanthin was not correlated with fat, protein, and starch contents in RS pasta suggesting that differences observed in carotenoid bioaccessibility among the wheat varieties and flour types were due to process-induced modifications to the food matrix that affected its content and fractional composition. This provides greater insight into pasta matrix properties associated with improved digestion of carotenoids.

Contrary to the effect of cooking duration seen for bioaccessible lutein in RS samples, concentrations in undigested RS samples were influenced by the following order: OC=FCT>Al. Comparing the two flour types, our data indicates that bioaccessible lutein in RS pasta was 1.2, 1.5, and 1.1-fold higher than WWF pasta for AAC Spitfire, CDC Precision, and Transcend, respectively. To the best of our knowledge, this is the very first report comparing the

bioaccessibility of carotenoids in pasta between refined semolina and whole-grain flour, and our results reveal that high carotenoid extractability may not necessarily translate to high bioaccessibility, at least when RS pasta is cooked to different cooking times.

Overall, the main carotenoids in durum wheat (lutein and zeaxanthin) were very stable regardless of digestion stage; with the highest recoveries found in the duodenal phase with a value of 185.42%. By the end of digestion, all-trans lutein was impacted by an interaction effect of variety and cooking duration among RS samples while the main effect of wheat variety influenced all-trans zeaxanthin among the WWF samples. Our data also indicates the possible trans-cis isomerization and/ or degradation of carotenoids during the gastric phase of digestion, and these transformations may have been affected by the flour type.

Although antioxidant activity against the DPPH radical was 2-fold greater in WWF samples than in RS, CDC Precision and Transcend provided the highest antioxidant activity when overcooked (OC), and this was observed at the end of the duodenal digestion.

We conclude that the effect of wheat variety and cooking duration modulates the bioaccessibility and antioxidant activity of RS and WWF pasta products. These results provide a better understanding of the effects of wheat matrix structure on carotenoid release during digestion. Hence, attention should be given to the identification and selection of wheat varieties to produce functional foods employing the appropriate processing techniques that will enhance the overall absorption potential of individual and total carotenoids as used by the human body since these techniques can affect carotenoid digestibility. To these further studies are warranted toward the *in vivo* elucidation of the effect of wheat variety and cooking duration on the carotenoids in pasta.

Connections between Chapters 4 and 5

In chapters 3 and 4, the extractable contents, retention, bioaccessibility, and digestive stability of individual and total carotenoids were examined as functions of three durum wheat food matrices and food processing (cooking duration). The next chapter, Chapter 5, evaluates the in vitro absorption potential of these digested carotenoids to further understand the food matrix/structure and food processing properties that may mediate carotenoid uptake using human Caco-2 intestinal cells. This evaluation was done by assessing whole wheat and refined semolina pasta of the wheat variety Transcend due to its significantly higher carotenoid digestibility and bioaccessibility in both refined semolina flour and whole wheat flour pasta compared to the other wheat varieties (Chapter 4). Moreover, pasta microstructure was examined for a more in-depth understanding of the relationship between cooked pasta food structure and carotenoid bioaccessibility and absorption in Transcend. Finally, the antioxidant properties of the carotenoids to attenuate oxidative stress in Caco-2 cells were evaluated.

CHAPTER 5.

Carotenoid cellular uptake and antioxidant activity from differently cooked semolina and whole wheat pasta

Hannah Oduro-Obeng, Muhammad Mudassir Arif Chaudhry, Daniel Zogona, Jitendra Paliwal,

Bin Xiao Fu, Trust Beta

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5. Abstract

In this study, we evaluated the impact of the food matrix and cooking duration on the cellular uptake of lutein from refined semolina (RS) and whole wheat flour (WWF) durum wheat pasta cooked to al dente (Al), fully cooked (FCT), and overcooked (OC). Cellular antioxidant actions of carotenoid from digested and cooked pasta samples against 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress in human Caco-2 cells were also assessed. Results revealed that the percentage of lutein cellular uptake was below 10%. Cooking duration was non-significant in the cellular uptake of lutein. There were no significant differences in bioaccessibility and uptake efficiency between RS and WWF pasta. Antioxidant actions of carotenoid against AAPH-induced oxidative stress showed an efficient cytoprotective effect and intracellular oxidative stress attenuation. This was, however, flour type and concentration dependent. Intricate physical and chemical structural changes in the pasta matrix might have accounted for the observed differences. Hence, carotenoid absorption and protection against oxidants are mediated by pasta cooking and the overall food matrix, as they affect the digestive outcome of carotenoids. Understanding the effects of food processing can help improve the functionality of pasta carotenoids and enhance their ability to reach target organs and promote optimum health.

5.1. Introduction

Carotenoids are natural lipophilic bioactive compounds suggested to exert their bioactivity mainly through their antioxidative properties and regulation of inflammatory response mechanisms. These properties enable the scavenging of lipid peroxy radicals and singlet oxygen species, which contribute to the etiology of several nutrition-related non-communicable diseases (Kopec & Failla, 2018). During digestion, carotenoids released from various food matrices can scavenge and prevent the generation of reactive oxygen species (ROS) in the gastrointestinal tract.

The biological activity of carotenoids depends on their bioaccessibility and intestinal absorption mechanisms. Essentially, the potential of a food bioactive such as carotenoids to exert any bioactivity depends on their release from the food matrix and changes during digestion and absorption (Cilla et al., 2018). Hence, the food matrix and food processing are critical factors that mediate carotenoid bioaccessibility and absorption.

Generally, in plant foods, the food matrix that contains carotenoids is complex and varies with the source of food (Schweiggert & Carle, 2017). Thus, the food matrix and linkages at molecular levels might explain the high variability of micellization and bioaccessibility of the same carotenoid in different foods or different carotenoids in the same food type (Cervantes-Paz et al., 2017). These matrices present as barriers to the effective release of carotenoid compounds. Therefore, appropriate food processing methods and /or techniques are required to break these barriers without inducing further restrictions.

Bioaccessibility is the fraction of a compound released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (Etcheverry et al., 2012). Conversely, bioavailability can be defined as the proportion of a dietary nutrient (or its

metabolites) that is ultimately available for utilization or storage by target tissues following digestion, absorption, and distribution without consideration of bioactivity.

As a result of their relatively higher polarity, xanthophylls, such as lutein, have been reported to be readily absorbed compared to carotenes due to the orientation of their hydroxyl groups at the surface of mixed micelles (Zaripheh & Erdman, 2002). On the other hand, *in vitro* studies of beta carotene isomers using Caco-2 cells showed a preferential cellular uptake of the all-trans isomers relative to the cis compounds (During et al., 2002). Conversely, in the case of lycopene, cis carotenoid isomers have been found in higher concentrations in human plasma compared to their trans counterparts (Ross et al., 2011). These findings have led to several conclusions that indicate preferential uptake, efficient carotenoid incorporation into chylomicrons, or selective retention of these specific isomers (Blanquet-Diot et al., 2009; Stahl & Sies, 1992). Moreover, others have also implied isomerization or degradation during digestion or intestinal absorption (Harrison, 2012).

Carotenoids in durum wheat are a minor component compared to fruits and vegetables. However, processing can potentially facilitate the release of substantial amounts of these lipophilic bioactive compounds (van Het Hof et al., 2000). Therefore, food products such as pasta processed from durum wheat may present a significant source of dietary carotenoids to many cultures worldwide due to their high and regular consumption. Food processing steps that precede the consumption of this product can contribute to significant structural changes at the micro and macro levels.

In our previous study, we found that cooking duration had a significant influence on the solubility and micellization of carotenoid isomers at the pre-absorptive stage (Oduro-Obeng et al., 2022). However, the fate of these carotenoids during intestinal uptake is yet to be determined. To

the best of our knowledge, no study has reported on the absorption of dietary carotenoids following the digestion of different durum wheat pasta products. Most studies so far have focused on non-cereal sources or supplements of carotenoids (Chitchumroonchokchai & Failla, 2006; Chitchumroonchokchai et al., 2004) or non-xanthophylls (Ferruzzi et al., 2006) while others too have focused on pure compounds of non-dietary origin (Yang et al., 2017). Therefore, this study addresses the knowledge gap on the uptake potential of carotenoids from differently processed durum wheat products.

The objectives of this study were to evaluate the uptake efficiency of carotenoids from durum wheat pasta products cooked to al dente, fully cooked, and overcooked using differentiated Caco-2 cell monolayers after digestion using the standardized INFOGEST protocol. Additionally, we assessed the cellular protective effects of these carotenoids against AAPH-induced toxicity and oxidative damage in Caco-2 cells. We hypothesize that when cooked differently, lutein, the primary carotenoid in durum wheat pasta products, will have different absorption efficiencies irrespective of the food matrix and neutralize intracellular free radicals.

5.2. Materials and Methods

5.2.1. Chemicals and reagents

All solutions were prepared using ultrapure water generated by a milli-Q purification system (Millipore, Schwalbach, Germany). All salts (KCl; potassium chloride (cat. No. P330), KH_2PO_4 ; potassium phosphate monobasic (cat. No. P285), NaHCO_3 ; sodium bicarbonate (cat. No. S233), $(\text{NH}_4)_2\text{CO}_3$; ammonium carbonate (cat. No. A651), $\text{CaCl}_2(\text{H}_2\text{O})_2$; calcium chloride (cat. No. C70), NaOH; sodium hydroxide (cat. No. S318) and HCl; hydrochloric acid (cat. No. SA56)) from Fisher Scientific (Ottawa, ON, Canada); NaCl; sodium chloride (cat. No. 7581) from

Mallinckrodt Specialty Chemical Co. (Paris, KY, USA), and $\text{MgCl}_2(\text{H}_2\text{O})_6$; magnesium chloride hexahydrate (cat. No. AC41341) from Acros Organics (Logan, UT, USA) were HPLC or ACS certified. Extraction solvents and HPLC solvents were all chromatographic grade and obtained from Fisher Scientific. Bile extract from porcine (SC-214601) was from Chem Cruz (Santa Cruz Biotechnology, Inc, Dallas, TX, USA.) while the digestive enzymes: porcine α -amylase (A3176, 10 U/mg), pepsin from porcine gastric mucosa (P7125, 679 U/mg protein) and pancreatin from porcine pancreas (P7545, 8 x USP) were from Sigma-Aldrich (St. Louis, MO, USA). Lutein and zeaxanthin reference standards were from Cayman Chemical Company (Ann Arbor, MI, USA), while trans- β apo-8-carotenal (internal standard), 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH, 97 %) and 3,5- di-tert-4 butylhydroxytoluene (BHT, $\geq 99\%$) were also purchased from Sigma-Aldrich. The fluorescent dye, 5-(and-6)-chloromethyl-2',7'- dichlorofluorescein diacetate (CM-H₂DCFDA) was acquired from Invitrogen (Eugene, OR, USA). Dimethyl sulfoxide (DMSO) was purchased from Tocris Bioscience (Toronto, ON, Canada) while 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), trypsin-EDTA (0.25 %), the cell culture medium, Dulbecco's modified eagle's medium (DMEM), low glucose, GlutamaxTM supplement, pyruvate; Dulbecco's phosphate buffer solution (DPBS) no calcium, no magnesium; penicillin-streptomycin (10,000 U/ml), hank's balanced salts solution (HBSS), as well as the 75 cm² polystyrene culture flask and the 96-well flat bottom polystyrene microplate, were all acquired from Thermo Fisher Scientific (Logan, UT, USA). Six-well plates and permeable inserts were from Nest Biotechnology (Wuxi Nest Biotechnology Co. Ltd; Jiangsu, China), fetal bovine serum (FBS) was from Cytiva (Marlborough, MA, USA), and Caco-2 cells were acquired from American Type Culture Collection (ATCC) (Manassas, VA, USA).

5.2.2. Durum wheat grain and flour milling

The Grain Research Laboratory of the Canadian Grain Commission, Canada, kindly provided the durum wheat variety Transcend. Milling into refined semolina (RS) or whole wheat flour (WWF), samples followed previously reported methodology by Dexter et al. (1990). Briefly, wheat grains were cleaned and tempered to 16% and 12% moisture content for RS and WWF, respectively. Whole wheat flour (WWF) was prepared by milling grains in an ultra-centrifugal mill (ZM 200; Retsch, Haan, Germany) with a 0.5 mm screen. In contrast, refined semolina (RS) was prepared by milling grains on a four-stand Allis-Chalmers laboratory mill (Allis-Chambers Manufacturing Co., Milwaukee, WI, USA) with a laboratory purifier.

5.2.3. Preparation of extruded and cooked pasta

Spaghetti was extruded according to mixing and extrusion conditions described in an earlier study conducted by Fu et al. (2013). Refined semolina (RS) or whole wheat flour (WWF) and water were initially mixed in a high-speed asymmetric centrifugal mixer (model DAC 400 FVZ, SpeedMixer; FlackTec, Landrum, SC, USA) at 800 rpm for 10 s followed by 1200 rpm for 90 s to generate uniform dough crumps. This was immediately followed by extrusion, which was achieved using a customized micro-extruder (Randcastle Extrusion System Inc., Ceder Grove, NJ, USA). The extruder barrel diameter was 19 mm with a 12:1 working length-to-diameter ratio, and the temperature was set at $45\text{ }^{\circ}\text{C} \pm 1$ with an extrusion pressure of 100 ± 10 pa. A four-hole 1.8 mm Teflon-coated spaghetti die served the extrusion process. Finally, the resulting fresh pasta was dried in a pilot Buhler pasta dryer (Buhler, Uzwil, Switzerland) using a 325-min drying cycle at $85\text{ }^{\circ}\text{C}$.

Cooking pasta to Al, FCT and OC was done by boiling at least 10 g of pasta (cut into 5 cm pieces) in 300 ml of boiling distilled water in a 600 ml Pyrex glass beaker at 100 °C on an Isotemp hot plate (Fisherbrand, Thermo Fisher Scientific, Ottawa, ON, Canada). Al dente samples (representing pasta with some ungelatinized starch in the inner core) were cooked uncovered for 8 min or 7 min for RS or WWF pasta, respectively. Fully cooked time for both RS and WWF pasta types was assessed using AACC International Approved Method 66–51.01.17(AACC, 2000), while overcooked samples were evaluated 6 min beyond their respective fully cooked time. Following the cooking process, samples were drained and rinsed with 50 ml of distilled water to arrest cooking, frozen, freeze dried, and milled using a proctor silex coffee grinder (Model 80301C, Hamilton Beach Brands, Inc., Glen Allen, VA, USA) to pass through a 500 µm sieve. These samples were stored in airtight dark plastic containers at –40 °C until further analysis. All analyses were performed in triplicate.

5.2.4. In vitro simulation of upper gastrointestinal digestion

Simulated digestive fluids (400 ml) for oral, gastric, and intestinal digestion were prepared using salt stock solutions and milli-Q water according to the protocol of Brodkorb et al. (2019). Additionally, all details of the simulated human digestion process were performed according to our previous publication (Oduro-Obeng et al., 2022). Following the intestinal phase of digestion, samples were immediately put on ice to stop the digestion process and centrifuged at 4270×g for 1 h to isolate the micellar (aqueous) fraction from digesta residue. The resulting supernatant was collected with a syringe and filter-sterilized using a surfactant-free cellulose acetate filter (0.22 µm; GVS North America Inc., Sanford, ME, USA) to remove microcrystalline aggregates. These physical separation processes were done to remove insoluble digesta, digestive enzymes, and bile

salts to minimize their toxic effect on Caco-2 cells and maintain monolayer integrity. Carotenoids were further extracted from aliquots of micelles using the methods of Werner and Böhm (2011) and cited by Oduro-Obeng et al. (2022). A blank control without the pasta samples was incubated under the same conditions to correct for interferences from buffers and digestive enzymes. Carotenoids were not detected in this control (data not shown). Bioaccessibility was determined as the percentage fraction of carotenoids in the undigested samples found in the mixed micelles (filtered aqueous fraction).

5.2.5. Cell culture using the Caco-2 cell model

The parent cell line of Caco-2 cells (HTB-37™) purchased from American type culture collection (ATCC) (Manassas, VA, USA) were kindly provided by Dr. Chengbo Yang (University of Manitoba, Winnipeg, Canada) at passage 5. The cells were routinely maintained in a 75 cm² polystyrene culture chamber flask (Corning Costar, New York City, NY, USA) at 37 °C in a humidified atmosphere of 5% CO₂ and 95% O₂. The cells were grown in a complete growth medium containing Dulbecco's modified eagle's medium (DMEM) with 1g/L D-glucose content, supplemented with 10% fetal bovine serum (FBS; Cytiva), penicillin-streptomycin (10,000 U/ml), Glutamax, and sodium pyruvate (110 mg/L). Culture media was replenished every 2-3 days. Cells were sub-cultured after reaching 70-80% confluence using trypsin-EDTA (0.25%). Cells were washed twice with Dulbecco's phosphate buffer solution (DPBS) before adding trypsin for 3-5 mins. Detached cells were diluted with complete growth media to stop trypsin activity. Cells were counted with the hemocytometer to determine their density. For the different experiments described in the following sections below, cells were seeded into 96-well flat bottom tissue culture-

treated polystyrene plates (Fisher Scientific) or 6-well semi-permeable membrane Transwell inserts (Wuxi Nest Biotechnology, Co. Ltd, Jiangsu, China).

5.2.6. Effect of carotenoid extracts on cell viability via MTT assay

Stock solutions of carotenoid extracts of digested and undigested pasta samples were prepared by reconstituting concentrated extracts to 100 mg/ml using ethanol. Stock solutions were diluted with serum-free basal media to obtain nine working concentrations (5, 10, 25, 50, 100, 250, 500, 1000 and 2000 µg/ml). The total carotenoid content in these concentrations was equivalent to 0.09-5.78 µg/ml for the undigested and 0.06-1.04 µg/ml for the digested samples. The final concentrations of ethanol in cells ranged between 0.005-2% v/v.

To determine the effect of carotenoid on Caco-2 cell viability, the MTT [3-(4, 5- dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay was employed. In viable cells, mitochondrial succinate dehydrogenase converts the MTT (yellow color) into a purple formazan derivative. Caco-2 cells were seeded (100 µl/well) at a density of 2×10^4 cells/well in a 96-well plate and allowed to attach for 24 h in the incubator. After attachment, the medium from each well was carefully aspirated, and cells were washed twice with PBS, then treated with 100 µl of serum-free DMEM containing the prepared carotenoid working solutions (as indicated in section 5.2.6) for 24 h. Following this, the treatment solutions were aspirated, and cells were further incubated with 100 µl of MTT solution (0.5 mg/ml) for 3 h without light. The media containing the MTT (including the control) was discarded, and the produced formazan was solubilized in DMSO (100 µl) by incubating the plates for 15 mins at 37 °C. Absorbance was read in a Synergy H4 Hybrid multi-mode microplate reader (BioTek, Winooski, VT, USA) at 570 nm, and the cell viability was calculated by subtracting the blank from the treated and control wells to correct for the background

resistance value. Measurements were done in triplicates, and cell viability was calculated as a percentage of the control (untreated cells).

$$\text{Cell viability (\%)} = \frac{\text{absorption in treated cells}}{\text{absorption in untreated cells}} \times 100 \quad (1)$$

From the cell viability determination assay results (Fig. 5.4a and b), the effects of cooking duration appeared to be non-significant after the dose of 500 $\mu\text{g/ml}$; hence, we decided to continue our investigations on the carotenoid antioxidant protecting effects using concentrations of 5, 25, 100, 500 and 1000 $\mu\text{g/ml}$.

5.2.7. Determination of AAPH concentration used in the assays.

To determine the optimal concentration of AAPH to induce cellular oxidative stress in Caco-2, cells, we constructed dose dependent curves. AAPH concentrations of 0, 1, 3, 5, 7, 10, 15, 20, 25 mM diluted in PBS from a stock of 250 mM were incubated with confluent cells (cell density 2×10^4 cells/well) in 96 well plates for 4 h. Cell death by AAPH-induced oxidation was determined by MTT assay (final concentration of 0.5 mg/ml) and expressed as cell viability (%). After 4 h of treatment with AAPH, cells were aspirated of all media, washed with PBS, and treated with 100 μl of MTT solution for 3 h. The media containing the MTT (including the control) was discarded, and the produced formazan was solubilized in DMSO (100 μl) by incubating the plates for 15 mins at 37°C . Absorbance (570 nm) was read in a microplate reader (BioTek, Winooski, VT, USA) and the cell viability calculated by subtracting the blank from treated and control wells to correct for the background resistance value. Measurements were done in triplicates.

5.2.8. Protective effect of carotenoid extracts against AAPH-induced cytotoxicity

The cytoprotective activity of extracts of undigested and digested RS and WWF pasta against AAPH-induced oxidation was determined by MTT assay according to Gliwa, Gunenc, Ames, Willmore, and Hosseinian (2011) with some modifications. Caco-2 cells (100 μ l; 2×10^5 cells/ml) were seeded in 96-well plates and maintained until confluent. Following cell confluence, the medium from all wells was aspirated and washed twice with PBS. Then 100 μ l of sample extracts (5, 25, 100, 500, and 1000 μ g/ml) were added to appropriate sample wells, and 100 μ l of serum-free DMEM was added to control wells and incubated at 37 °C, 5 % CO₂ for 2 h. Blank wells contained only DMEM. After 2 h of pretreatment, 10 μ l of 3 mM AAPH was added to the sample and AAPH-positive control wells. Cells were incubated for another 4 h, after which the media was removed, and 100 μ l of the MTT reagent (0.5 mg/ml) was added to all wells except the blank wells. The plates were incubated for another 3 h until the intracellular punctate purple color was visible under the microscope. Detergent reagent was then added to all wells except the blank wells. The plates were swirled gently, covered with aluminum foil, and left in the dark for another 15 min at room temperature. The plate covers were removed afterward and measured for absorbance at 570 nm using a Synergy H4 Hybrid multi-mode microplate reader (BioTek, Winooski, VT, USA). Cytotoxicity level (%) was then calculated by transforming the results to the percentage cell viability of unchallenged cells (control, set at 100 % viability). Analysis was done in quadruplicates.

5.2.9. Intracellular antioxidant activity (CAA) assay

The cellular antioxidant activities of undigested and digested carotenoid extracts were determined according to the methods reported by Wolfe and Liu (2007) with some modifications. Caco-2 cells were cultured at 2×10^5 cells/ml density on 96-well microplates for 24 h at 37 °C and 5% CO₂ to reach confluence. The cells were first washed twice with PBS. Triplicate wells were pretreated with 50 µl of the working concentrations of the carotenoid extracts (1000, 500, 100, 25, 5 µg/ml) dissolved in ethanol and diluted in serum-free DMEM for 30 mins. Afterward, 50 µl of 5 µM 5-(and-6)-chloromethyl-2',7'- dichlorofluorescein diacetate (CM-H₂DCFDA dissolved in ethanol and diluted in serum-free DMEM) was added before incubation for another 30 mins. After 1 h of incubation, all culture media was removed before treatment with 100 µl of AAPH (100 µM) dissolved in PBS. Cells treated with CM-H₂DCFDA and AAPH were set as positive control. Cells treated with only CM-H₂DCFDA were set as blank wells, and the negative control wells (sample background) contained samples and CM-H₂DCFDA without AAPH. The 96-well plates containing cells were placed in a fluorescence plate reader (BioTek, Winooski, VT, USA) at 37 °C with excitation and emission wavelengths 485 and 538, respectively. The fluorescence was collected every 15 min for 1 h. The CAA value was calculated based on the following equation:

$$\text{CAA unit (\%)} = 100 - [(\int \text{SA} - \int \text{BAs}) / (\int \text{CA} - \int \text{BAc}) \times 100] \quad (2)$$

$\int \text{SA}$ is the integrated area from the sample curve, $\int \text{BAs}$ is the integrated area from the sample background curve, $\int \text{CA}$ is the integrated area from the control curve, and $\int \text{BAc}$ is the integrated area from the control background curve.

5.2.10. Cellular uptake of carotenoids by Caco-2 cells

Caco-2 intestinal cells were seeded at a density of 2×10^4 cells/well in flat bottom 6-well plates with trans-well permeable support inserts of polyethylene (PET) membrane (0.4 μm pore size; Wuxi Nest Biotechnology, Jiangsu, China). Caco-2 cells at passages 17-28 were used for experiments between 21-29 days post confluence because cultures of Caco-2 cells exhibit maximum differentiation at this age as assessed by the activities of alkaline phosphatase and sucrase (Garrett, Failla, Sarama, & Craft, 1999). The cells were maintained according to the previously established protocol (Hubatsch et al., 2007), and the culture medium (1 ml and 2 ml for apical and basolateral sides, respectively) was changed every 72 h. Transepithelial electrical resistance (TEER) measurements were taken twice weekly using a milli-cell electrical resistance system (ESR-2) (Millipore-sigma, Burlington, MA, USA) to ensure the integrity of the monolayer was intact. TEER, a non-destructive method, is a widely accepted quantitative technique used to measure the tight junction dynamics in cell culture models for epithelial monolayers (Kalungwana et al., 2023). TEER values above $300 \Omega \times \text{cm}^2$ following subtraction of the background electrical resistance on differentiated monolayers were deemed satisfactory (Hubatsch et al., 2007).

At the beginning of the experiment, monolayers were washed twice with 1 ml Hank's balanced salts solution (HBSS) before adding 1 ml carotenoid-enriched media (containing 0.75 ml DMEM and 0.25 ml aqueous filtrate) to each insert in triplicate and incubated at 37°C for 4 h. This dilution was necessary to counter the toxic effect enzymes and salts from the simulated *in vitro* digestion process could pose on the integrity of cell monolayers during cellular absorption. The MTT assay's preliminary work showed that the micelle-enriched medium was not toxic to the cells (data not shown). Moreover, the integrity of the cells was microscopically assessed during the incubation period by comparing monolayers exposed and not exposed to test media. DMEM media

without FBS (2 ml) was added in the basolateral compartment to determine transport efficiency. After incubation, the media from both sides of the inserts were collected, and monolayers were washed twice with ice-cold PBS. Cells were then scraped into 15 ml tubes in 1 ml of ice-cold phosphate-buffered saline containing 10% (v/v) ethanol and 45 $\mu\text{mol/L}$ BHT and stored at $-80\text{ }^{\circ}\text{C}$ until carotenoid extraction and HPLC analysis. Media containing carotenoid micelles was also added to a set of wells without cells to monitor the stability of the carotenoids during incubation. A blank treatment was also conducted under the same conditions to correct the interference from the media. Intestinal cellular uptake was calculated as the fraction (%) of the carotenoids recovered in Caco-2 cells relative to the amount initially added on the apical sides of the cell monolayers.

5.2.11. Extraction and analysis of carotenoids

Carotenoids were extracted from culture media samples before incubation and after (media from the apical and basolateral compartments) as well as from cell suspensions according to the method of Rošul et al. (2022) with some modifications while working under subdued light to minimize oxidation. For aqueous samples (samples from cell experiments), a volume of 1 ml was used. Cells were initially sonicated on ice for 1 min, adding 1 ml of ethanol and 2 ml of n-hexane. Samples were then homogenized on a vortex (10000 rpm, 10 min) and centrifuged ($1400\times g$, 10 min, $4\text{ }^{\circ}\text{C}$). The upper phase from the tube was collected, and the lower phase was extracted in the same way as hexane. The two upper phases were merged in the same tube and dried under nitrogen. Residues were dissolved in 400 μl of ethanol and centrifuged using a Sorvall Legend Micro 21 centrifuge (Thermo Scientific, Karlberg, Germany) at $18\ 800\times g$ for 5 min. Samples were then filtered through a 0.45 μm syringe filter, and four hundred microlitres were transferred into an HPLC amber vial (equipped with a glass insert). HPLC separation of carotenoids was carried

out using the accomplished procedure of Abdel-Aal et al. (2007) with slight modifications. The setup was a Waters model 2695 enhanced with a photodiode array detector (PAD) (Waters 996) and autosampler (Waters 717 plus) (Waters, Milford, MA, USA). The column was a 4.6× 100 mm YMC™ carotenoid S-3 with 3µm packing (YMC/Waters Inc., Wilmington, NC, USA), which was operated at 35 °C. Twenty microliters of the sample were injected by the autosampler and eluted with a gradient system consisting of (A) methanol/MTBE/milli-Q water (70 : 25: 5, v/v/v) and (B) MTBE/methanol (90: 10, v/v). The flow rate was set at 1 ml/ min, and the total run time was 15 min. The gradient was programmed as follows: 0–9 min, 100–75% A; 10–12 min, 0% A; 12–13 min, 0–100% A; and 13–15 min, 100% A. The eluent was continuously monitored at 450 nm. Peaks were also observed at 360 nm for potential oxidation products or derivatives before and after *in vitro* digestion and cellular transport. Peaks were identified by their retention times, followed by a comparison of the absorption spectra of the eluted compounds with the elution sequence to the all-trans lutein and zeaxanthin authentic reference standards, their UV/vis spectra, and those previously reported in the literature using polymeric C₃₀ column (Abdel-Aal et al., 2007). Considering that the amounts of cis isomers were close to the limit of quantification (LOQ), tentative identification was carried out based on the comparison of elution order and the peaks in the absorbance spectrum of the samples with those achieved from the iodine photomutation mixture of lutein standard and extracts (Nguyen et al., 2001) as well as that from the literature using a similar protocol. The λ max of cis isomers is reported to be slightly lower (2-6 nm) than their corresponding all-trans isomers. It is characterized by the presence of a “cis” peak at about 142 nm below the longest-wavelength maximum of the all-trans compound (Britton, 1995). For method validation, carotenoids were quantified using a ten-point analytical curve for all-trans lutein (0.02–10.0 µg/ml, R² =0.9946) and all-trans zeaxanthin (0.005–2.5 µg/ml, R² =0.9898)

showing good linearity of the calibration curves and representing the linear regression equations $y = 210136x - 10413$ and $y = 50142x - 986.15$ respectively. The content of cis isomers of lutein was estimated using the curve of its corresponding all-trans lutein isomer. These extrapolations from the pure standards were done after correction for extraction efficiency based on the recovery of the internal standard. Recovery of carotenoids was monitored by the addition of trans β -apo-8-carotenal (1 μ l) to test samples, and determinations during extraction and chromatography procedures were found to be greater than 95%. Carotenoids were not detected in cells maintained under standard tissue culture conditions without exposure to carotenoid extracts (data not shown). Limits of detection (LOD) and quantification (LOQ) were calculated as a signal-to-noise ratio of 3 and 10, respectively, and expressed in μ g/ml. The values of LOD and LOQ were 1.00 μ g/ml and 3.02 μ g/ml, respectively, for all-trans lutein and 0.34 μ g/ml and 1.04 μ g/ml for all-trans zeaxanthin. All chromatograms were collected and processed using Empower Pro, build number 1154 (Waters, Milford, MA, USA). Quadruplicate analyses were performed.

5.2.12. Pasta microstructure determination using X-ray micro-computed tomography

Quantitative and qualitative 3D analysis of pasta microstructure was done using an X-ray micro-CT (Skyscan 1275, Bruker, Belgium) capable of operating in the power range of 20-100 kV as previously published by (Ramachandran, Erkinbaev, Thakur, & Paliwal, 2021) with some modifications.

Scanning: Cooked and uncooked (control) pasta strands (3 cm in length) were each mounted on a cylindrical rod using transparent wax to avoid any vibrations during sample rotation while scanning. The optimal settings for scanning were set at a source voltage of 40 kV and current of 180 μ a with a rotational angle of 0.2° over 180°. Samples were scanned for 15 min to obtain high-

resolution images. In total, 1072 image projections (1671 x 1382 pixels) were acquired. Scanning was done in triplicates for each treatment and control (uncooked).

Image reconstruction and analysis: The 2D images acquired earlier at each rotational angle were reconstructed to form 3D volumes to highlight the external and internal geometries using the NRecon software (Version 1.6.10.2, Bruker, Kontich, Belgium). The reconstructed images were corrected for contrast, ring artifacts, misalignment, and beam hardening. A moderate value of ring artifacts reduction (10) and misalignment compensation (10) were applied to eliminate acquisition problems. A region of interest (ROI) was selected from the original image during reconstruction to reduce computational time. The large dataset of 3D images was further processed using a graphics processing unit (GPU)-enabled computer (Precision T7810, RAM 64 GB, 3.2 GHZ, Dell Corp. Ltd., Round Rock, TX, USA) by developing a task list that included tasks in the following order (thresholding, despeckle, saving bitmaps, 3D analysis). The 3D image analysis and volume rendering were done using the CTAn software package (Bruker, Kontich, Belgium) by applying a custom-written pre-processing algorithm. Qualitatively, analysis was conducted to visualize the internal structure of the pasta strand. Two-dimensional cross-section images and virtual sections of 3D images were obtained to observe different views for the treatment and the control. The 3D models of the pasta samples were developed using CTVox software (Bruker, Kontich, Belgium). Quantitatively, measurements extracted from 3D volume included closed porosity, open porosity (void fraction), and total porosity.

5.2.13. Statistical analysis

SAS (9.4) (SAS Institute Inc., Cary, NC, USA) was used for data analysis. A two-way analysis of variance (ANOVA) using the proc GLIMMIX command was employed to evaluate the null hypothesis. The slice command was employed to determine the simple main effects. When the null hypothesis was rejected, the mean differences were separated by Tukey's Kramer HSD test. In addition, three-way ANOVA was performed to assess the effects of cooking duration, flour type, and carotenoid concentration among digested and undigested samples on response variables. A two-tailed paired t-test was performed to determine differences in absorption efficiencies and bioaccessibility of lutein between whole wheat flour and refined semolina flour pasta samples. All data are presented as the mean value \pm standard deviation (SD) of three or more independent experiments considering a significance level of 5%.

5.3. Results and Discussion

5.3.1. Cellular uptake efficiency of lutein from digested pasta samples

There is limited information on the cellular uptake of carotenoids from durum wheat pasta. To our knowledge, this is the first report on the uptake of lutein from different durum wheat pasta products using differentiated human intestinal epithelial cells (Caco-2). Even though *cis* lutein compounds and all-*trans* zeaxanthin were bioaccessible as reported in our previous study (Oduro-Obeng et al., 2022), these compounds were not detected in the cells or the basolateral media (data not shown) after 4 h of incubation, irrespective of the food matrix or cooking duration. Chitchumroonchokchai et al. (2004) also did not detect *cis*-lutein isomers in Caco-2 cells incubated with micelle-enriched media from the digestion of spinach. Due to their instability, these *cis*-isomers and their metabolites could have rapidly degraded before or during incubation.

Additionally, they could have become undetectable under the current HPLC protocol due to extremely low (<LOD) concentrations. Moreover, it is also possible that a fraction of these compounds bounded non-specifically to the Transwell plates during incubation. The fact they were not detected in the cells also meant that trans-cis isomerization intracellularly, if any, was negligible. The presence of these cis isomers in human plasma has been thought to emanate from the digestion process rather than during absorption (Yang et al., 2018), as in the case of cis lycopene (Ross et al., 2011). Generally, the absorption of cis carotenoid compounds has been reported to be low compared to their all-trans compounds despite their efficient micellization process (Desmarchelier & Borel, 2017).

We monitored the stability of the carotenoid isomers using cell-free wells and discovered that the recovery of all-trans lutein in carotenoid-enriched media after 4 h of incubation ranged from 92.2 ± 0.4 to $101.3 \pm 0.6\%$. However, the total cis lutein recovered ranged from 0 to $1.3 \pm 0.2\%$, indicating that the cis isomers were unstable or undetectable. Tan et al. (2014) reported that the bent structure of cis-lutein prevented their efficient absorption compared to the rigid and linear polar all-trans isomer in a study using human Caco-2 cells. It must be stated, however, that some of these studies that reported the presence of cis-lutein compounds in the cells or basolateral side using the Caco-2 model employed artificial micelles or pure compounds (Chitchumroonchokchai et al., 2004; Yang et al., 2018). Carotenoids presented as pure compounds are better absorbed *in vitro* because they do not have a restrictive barrier in the form of the food matrix.

Although all-trans lutein was detected in the cells in minute concentrations, it was not recovered in the basolateral medium; hence, the transport mechanisms of lutein in this study could not be ascertained. This was not surprising as it is well known that Caco-2 does not secrete any chylomicrons under standard cell culture conditions, which aid in the transport of carotenoids to

the serosal (basolateral) side of the lumen for onward delivery to the liver through the lymph (Fernández-García et al., 2012). Our results agree with a recent study by Kalungwana et al. (2023), who, despite employing a longer incubation time of 16 h, were unable to detect lutein isomers in the serosal side from carotenoid enriched micelles of digested, orange-fleshed sweet potato using the Caco-2 model. In contrast to our work, however, O'Sullivan, Ryan, and O'Brien (2007) indicated the presence of carotenoids in the basolateral compartment of the Transwell plates using the Caco-2 model. The differences observed could be related to the membrane pore size used, the matrix containing carotenoids (a synthetic emulsion was used in their study), and the presence or absence of stimulation of lipoprotein secretion, which is required for carotenoid transport using oleic acid and taurocholate (During & Harrison, 2004). Using a larger membrane pore size of 3 μm , as depicted in the work of During et al. (2002), as opposed to 0.4 μm in our study, could facilitate the influx of cells or apical content to the basolateral side of the Transwell plates. Even though stimulating compounds might have aided the secretion of chylomicrons and promoted the transport of carotenoids across the intestinal epithelial cells, as reported in the above studies, their physiological relevance has been questioned. An ideal cell culture model, a direct substitute for the intestinal microenvironment, should simulate the complex physiological environment and reflect natural responses.

In this study, cellular uptake of lutein was generally less than 10% (Fig. 5.1). Specifically, lutein uptake ranged from 3.37-7.95 % for RS samples and 3.65-5.24 % for WWF samples. Though non-significant in the two flour types (RS and WWF), pasta cooked to AI was 1.6 and 2.4-fold greater than pasta cooked to FCT and OC, respectively, in RS and 1.4 and 1.4-fold greater than pasta cooked to both FCT and OC, respectively, in WWF. Overall, cooking to AI resulted in a nearly 2-fold increase in lutein cellular uptake compared to FCT and OC. A paired t-test conducted

did not also show significant differences ($P > 0.05$) in the uptake efficiency of lutein between RS and WWF pasta samples irrespective of the cooking duration. Our results are in tandem with the previous works conducted by During et al. (2002) and O'Sullivan et al. (2007).

The relatively poor uptake of these lipophilic compounds has been attributed to their weak solubility in the lumen's aqueous environment. Thus, evidence shows that receptor-mediated epithelial protein transporters such as scavenger receptor class B type 1 (SR-B1) and NPC1-like intracellular cholesterol transporter 1 (NPC1L1) facilitate their uptake across the epithelial cells for packaging into chylomicrons (Desmarchelier & Borel, 2017). Yonekura and Nagao (2007) earlier reported that xanthophylls such as lutein have a poor cellular uptake as opposed to carotenes, even though xanthophylls have a higher bioaccessibility. Indeed, the cell membrane comprises lipid bilayers, which may facilitate the absorption of higher lipophilic compounds such as beta carotene than lutein, a more polar carotenoid. This notwithstanding, other studies (de Oliveira et al., 2020; Reboul et al., 2006) have conflicted with this observation. The lack of consensus between the various *in vitro* studies assessing carotenoid bioavailability using human Caco-2 cells could be due to the different food matrices and/or the effect of food processing on matrix components.

Food processing-mediated matrix changes have been shown to affect the bioaccessibility of carotenoids in carotenoid-containing foods that need processing such as heating before consumption (Zhang et al., 2024). In this study, this may have mediated lutein uptake by the Caco-2 epithelial cells because micellization efficiency (relative bioaccessibility) has been shown to correlate positively with the absorption efficiency (bioavailability) of carotenoids (Reboul et al., 2006). Indeed, the micellization efficiency of lutein was higher in AI and lowest in OC-cooked RS pasta in an earlier report from our lab (Oduro-Obeng et al., 2022).

The next section (section 5.3.2) evaluates pasta cooking-induced structural and/or matrix component changes that may have mediated lutein uptake using X-ray tomography.

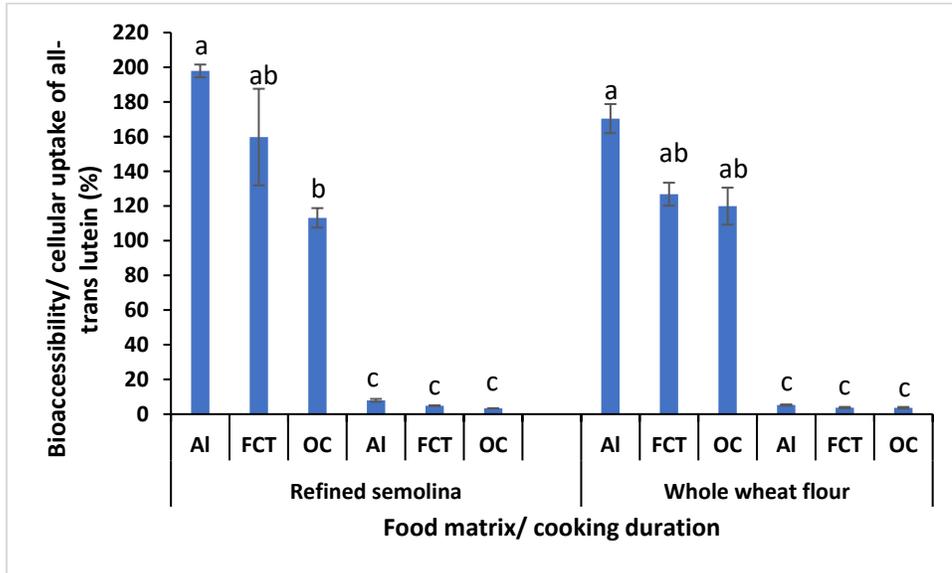


Figure 5.1. In vitro bioaccessibility and cellular uptake of all-trans lutein from refined semolina and whole wheat pasta digest cooked to al dente, fully cooked and overcooked. The data are presented as means \pm SD of three replicates. Bars with different lower-case letters represent significant differences ($P < 0.05$) within each food matrix. A paired t-test did not show significant differences ($P > 0.05$) between refined semolina and whole wheat pasta for the response variable. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked; flour type pasta: RS, refined semolina; WWF, whole wheat flour.

5.3.2. Effect of cooking duration on pasta microstructure

X-ray micro-CT has recently seen extensive usage in the food research sector, leveraging its application to characterizing structures three-dimensionally and allowing the evaluation of their internal morphology or the food matrix (Schoeman et al., 2016). In this study, the effect of food processing (cooking duration) on pasta microstructure was assessed to determine the structural changes occurring due to the different cooking durations and how this relates to the digestion of pasta carotenoids and, subsequently, its bioaccessibility and potential absorption mechanisms. Cooked pasta samples (RS and WWF) were immediately frozen after cooking and freeze-dried before analysis.

Porosity was quantified using the open and closed pore morphological parameters to show the precise microstructural changes during food processing and quality evaluation. The initial total porosity was $0.096 \pm 0.05\%$ for RS and $0.54 \pm 0.03\%$ for WWF before cooking (UC; control samples) and probably consisted of air pockets trapped within the pasta strands during the extrusion process. The interference of bran and germ in whole wheat pasta weakened its gluten matrix, resulting in a less cohesive and weakened structure during the drying process of pasta.

According to Fig. 5.2, increasing the cooking duration significantly increased ($P < 0.05$) the total porosity in both RS and WWF pasta. This effect appeared greater among the RS samples than in WWF pasta. The prolonged cooking duration may have increased moisture content in the core of the pasta strands, leading to greater starch gelatinization and larger cell sizes compared to Al-cooked samples, where a shorter cooking time may have allowed gelatinization of only the outer layer biopolymers (Petitot et al., 2009). As such, starch granules expanded and disrupted with increased cooking time, leaching out their contents due to a weakened protein matrix, forming pores, and reducing the density of the samples. Moreover, an increase in cooking duration could

have increased the internal temperature of the pasta strands well enough to cause a rise in internal pressure forming pores (Jayasinghe, Jeddi, Paziuk, & Erkinbaev, 2023).

WWF pasta's influence of the outer endosperm constituents and germ could have accounted for its relatively reduced total porosity due to reduced water absorption of starch. Bran has been reported to interfere with gluten formation, while lipids in germ form complexes with starch, reducing uniform hydration and gelatinization of starch granules through reduced water absorption efficiency (Aravind et al., 2012). A reduction in internal moisture content among WWF pasta may have attenuated the internal pressure during the prolonged cooking time (OC), resulting in fewer opened pores than RS pasta. A similar phenomenon could happen with AI samples due to their low internal moisture content and ungelatinized starch granules, which results from their shorter cooking time.

Dexter, Dronzek, and Matsuo (1978) investigated the microstructure of cooked pasta and stated that the formation of an open structure was a typical phenomenon that occurred concomitantly with starch gelatinization. Process- structural changes observed ultimately affected pasta carotenoid biochemical properties. Increased pasta porosity meant that samples were less dense, which enhanced carotenoid extractability using polar solvents. Moreover, increased temperature due to prolonged cooking duration also resulted in carotenoid chemical transformations (isomerization), as earlier reported (Oduro-Obeng et al., 2022; Oduro-Obeng et al., 2021) increasing their content. This transformation affected carotenoid solubility and its ability to be bioaccessible.

From our previous study, we determined that carotenoids from OC samples (more porous), were significantly less bioaccessible compared to AI-cooked samples (less porous) (Oduro-Obeng et al., 2022). From the same study, the digestive stability of OC pasta was lower than AI by the

end of digestion in RS pasta, meaning lutein rapidly degraded, possibly due to its high content in the lumen leading to its low transfer to the micelles. Moreover, it is possible that modifications to pasta macromolecules induced by the cooking process affected their digestibility and that of lutein. For example, the protein matrix around the starch granules in OC samples could have depolymerized/ denatured extensively after OC. Following the gastric phase of digestion, macromolecular structural alterations, including protein aggregation, may have occurred in the gut with these samples. Subsequently, these aggregates could have been physically bound to lipid droplets, preventing their access to lipases. Moreover, they could have complex gastric and intestinal digestive enzymes such as pepsin and pancreatin, reducing their activity and subsequent carotenoid bioaccessibility. These aggregates could also form insoluble or irreversible complexes with the released carotenoids in the digesta, reducing bioaccessibility. Justifiably, proteins have been reported to reduce carotenoid digestion by inhibiting the activity of digestive enzymes such as gastric lipase (Iddir et al., 2022). Al-cooked pasta, on the other hand, could have caused a reduction in its protein digestibility, increasing its emulsification properties and, subsequently, carotenoid bioaccessibility.

For the WWF pasta samples, a similar observation was found, where Al (less porous) cooked samples were more bioaccessible compared to OC (more porous) cooked samples though non-significant ($P>0.05$; Fig. 5.1). This non-significance suggested that reduced carotenoid degradation occurred by the end of digestion even among the OC samples due to their low porosity or compact structure compared to the OC in RS pasta (this observation is qualitatively confirmed in Fig. 5.3 among the pasta images where overcooked WWF pasta (**A**) looks more compact than overcooked RS pasta (**E**)).

The identified trends from quantified porosity values conform with the qualitative results presented in Fig. 5.3, where the less dense spaces (occupied by air pockets) are identified by black/grey color, and the solid matter (occupied by denatured proteins) are identified by white. The present work enabled a better understanding of the process-structure and how this structure may have influenced carotenoid bioaccessibility and absorption. The hydro-thermal treatments employed in this study are bound to alter the pasta products' microstructure, affecting both the final product's physical and chemical characteristics.

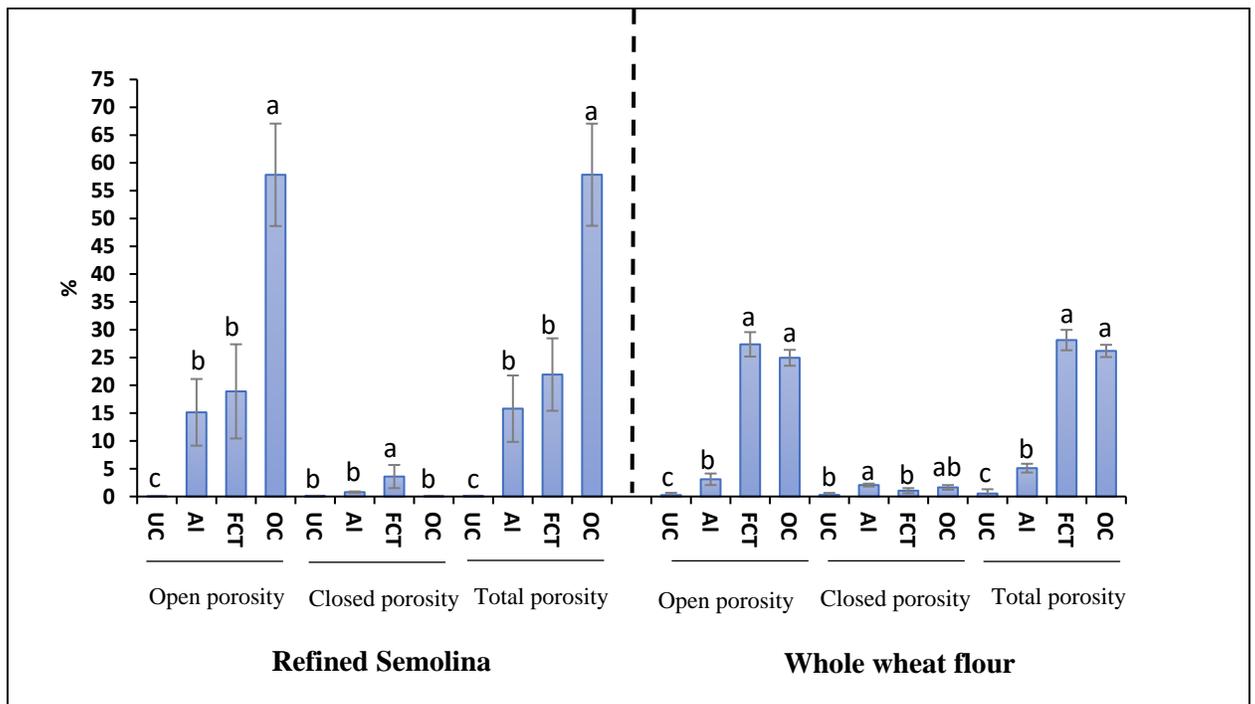


Figure 5.2. X-ray microtomography analysis of the porosity of uncooked (control) and cooked refined semolina and whole wheat pasta samples. The data are presented as means \pm SD of three replicates. Bars with different lower-case letters represent significant differences ($P < 0.05$) with each porosity parameter among both refined semolina and whole wheat flour. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked; UC, uncooked pasta.

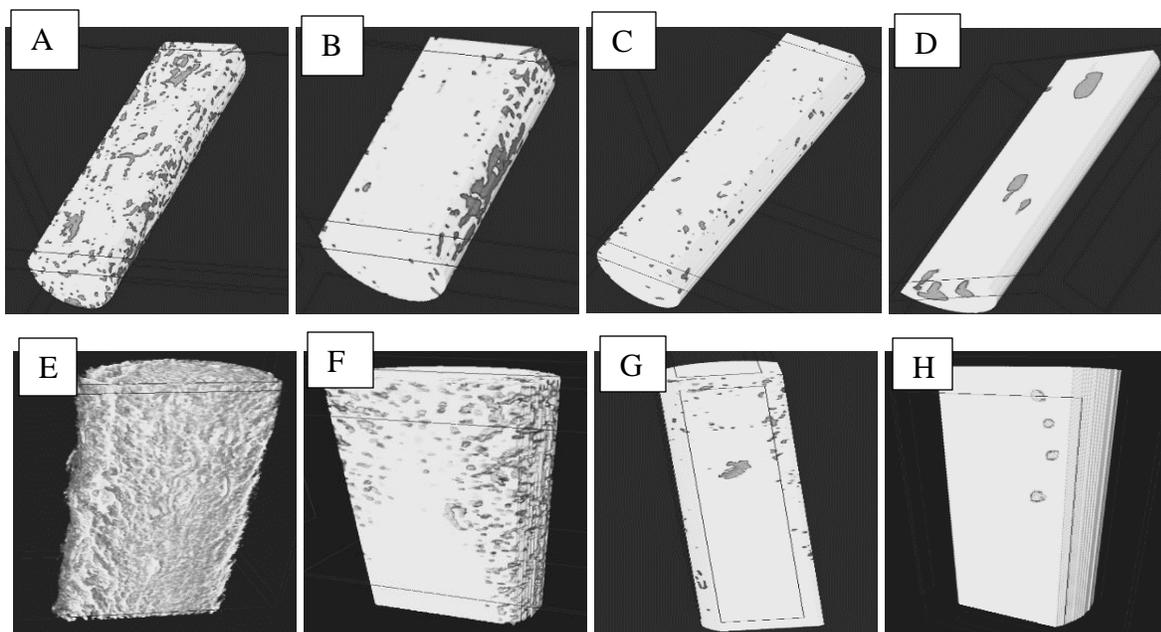


Figure 5.3. Sample 3D images of whole wheat flour pasta (**A**; overcooked, **B**; fully cooked, **C**; al dente cooked, **D**; uncooked) and refined semolina pasta (**E**; overcooked, **F**; fully cooked, **G**; al dente cooked, **H**; uncooked).

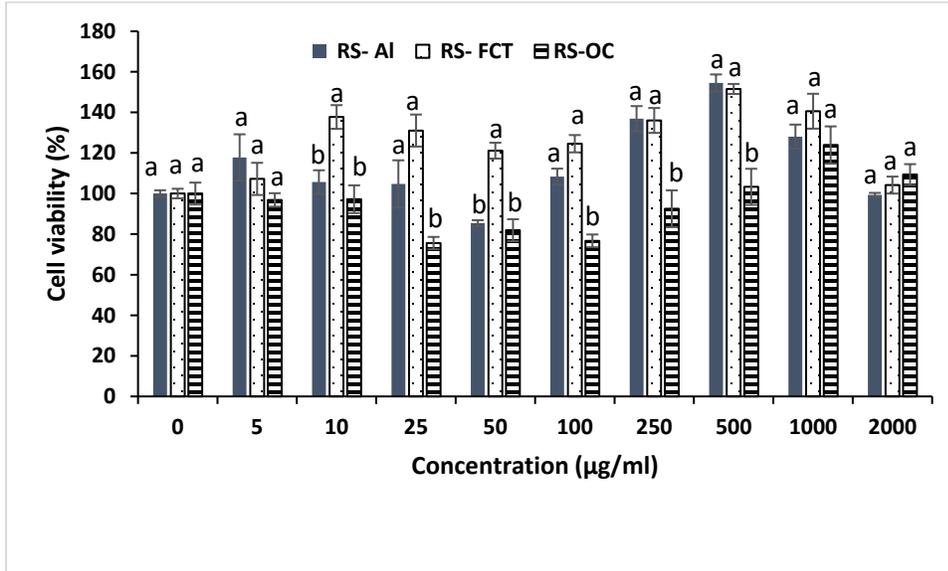
5.3.3. Cytotoxic effect of ethanolic carotenoid extracts

In this study, the MTT assay was performed to determine the concentrations of carotenoid extracts that are not detrimental to the survival of the cells. Since ethanol served as a vehicle for the extracts, preliminary results indicated that a concentration of 1% or less was non-toxic to the cells (data not shown). Hence, the highest concentration of the undigested extracts used in this study was 2000 $\mu\text{g/ml}$. In living cells, the MTT could be transformed by mitochondria into formazan, whose amount is positively related to living cell numbers (Sabouri-Rad, Oskuee, Mahmoodi, Gholami, & Malaekheh-Nikouei, 2017). The dose-response effect of the ethanolic extracts on Caco-2 cells is presented in Fig. 5.4a and b.

Cell viability following treatment with these extracts was above 80% regardless of the cooking duration or carotenoid concentration in both RS and WWF pasta food matrices, and the extract's effects on cells were deemed non-toxic. Nonetheless, comparatively, cell viability was slightly higher in the RS samples than the WWF samples (Fig. 5.4) possibly due to cell wall components such as fibre and minerals in WWF that hindered the absorption of carotenoids (Corte-Real et al., 2017). The cell viability appeared to significantly increase among the Al or FCT samples within concentrations below 1000 µg/ml in RS pasta compared to the overcooked samples. There was no significant difference ($P>0.05$) among the extracts of the cooked pasta samples when the concentration was above 1000 µg/ml for this food matrix. Even though cell viability was also reduced in the highest concentration (2000 µg/ml) of the extracts used in this study, this did not cause toxicity to the cells as this was comparable to the control. The relative reduction in cell viability at this concentration (2000 µg/ml) and among the overcooked samples could be related to reduced absorption. Moreover, high concentrations could have reduced the basal media pH from a normal pH range of 7.0-7.6, which was not tolerated by human cells (Cilla, González-Sarriás, Tomás-Barberán, Espín, & Barberá, 2009). The reduction in cell viability among overcooked extracts in RS pasta samples could also be attributed to a high cis-isomer saturation effect among these extracts. Carotenoids in overcooked samples were readily extractable and contained more cis compounds than their al dente or fully cooked pasta samples (Oduro-Obeng et al., 2021). Although more soluble, these cis compounds are less stable and could degrade rapidly, hindering cell growth. Moreover, the degradation products in these oxygen-sufficient cells could be toxic to the growth of the cells. Additionally, their bent structure could hinder their penetration in the cells, accumulate in the solvent medium, and starve the cells of nutrients, causing a reduction in cell growth.

On the other hand, in whole wheat pasta (WWF), overcooked samples appeared to markedly promote cell growth compared to their al dente or fully cooked pasta also in concentrations below 1000 µg/ml (Fig. 5.4b). We found this outcome interesting since it represented the opposite phenomenon in the refined semolina pasta samples. Whole wheat pasta samples, by their rich phytochemical and nutrient composition of the outer endosperm layers and germ, could enhance cell growth, especially when these bioactives are easily extractable using polar solvents. For example, lipophilic polyphenols such as flavonoids suggested as contributing to the total pigment content in durum wheat when assessed spectrophotometrically (Burkhardt & Bohm, 2007), was shown to improve cell viability and inhibited oxidative stress-induced cytotoxicity in HepG2 cells in an earlier study (Chen, Chu, Chyau, Chu, & Duh, 2012). Although not assessed in this study, phenolic compounds and carotenoids found in abundance in the outer endosperm layers and germ in durum wheat (Ndolo & Beta, 2013), may have prevented cell apoptosis and enhanced cell growth. Hence, these results indicate that the food matrix and the cooking duration are strong determinants of the effects of pasta carotenoids on the viability of Caco-2 cells (Table 1).

(a)



(b)

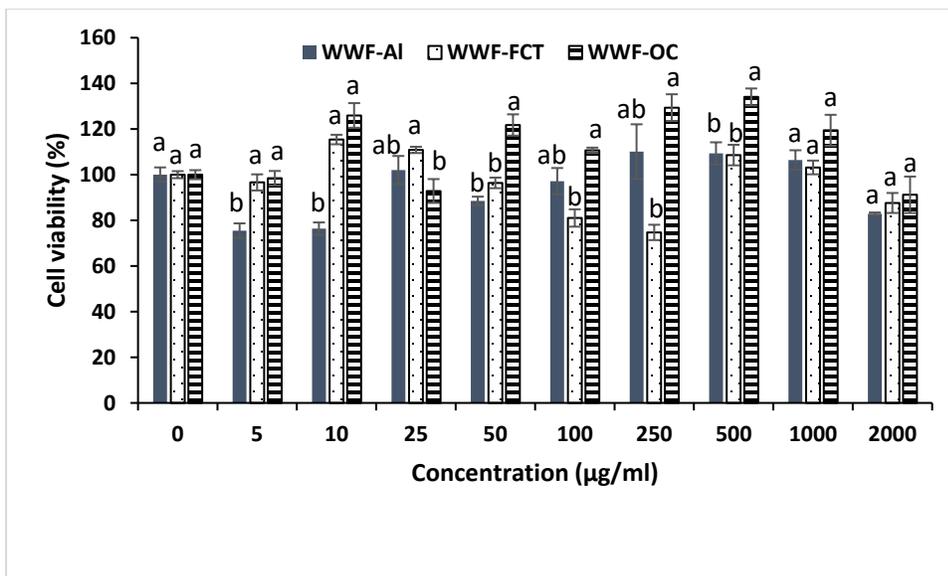


Figure 5.4. Effects of undigested carotenoid extracts from refined semolina (a) and whole wheat flour (b) cooked pasta samples and their concentrations on the viability of Caco-2 cells in comparison to the untreated cells (control). The data are presented as means \pm SD of three biological and three technical replicates. Bars with different lower-case letters represent significant differences ($P < 0.05$) among the extracts at the same concentration. Cooking duration: AI, al dente;

FCT, fully cooked time; OC, overcooked; flour type pasta: RS, refined semolina; WWF, whole wheat flour.

Table 5.1. Three-way ANOVA evaluation for sample cytotoxicity, cytoprotective and intracellular antioxidant activity (CAA)

	Cytotoxicity	Cytoprotective	Cytoprotective	CAA	CAA
	Undigested	Undigested	Digested	Undigested	Digested
	P-value				
Cooking duration (CD)	<.0001	<.0001	0.0005	<.0001	0.0336
Flour type (FL)	<.0001	<.0001	0.2511	0.0033	0.8390
Concentration (Conc)	<.0001	0.2276	<.0001	<.0001	<.0001
CD*FL	<.0001	<.0001	0.0014	<.0001	<.0001
CD*Conc	<.0001	0.0006	0.3815	0.0002	<.0001
FL*Conc	0.0003	<.0001	<.0001	<.0001	<.0001
CD*FL*Conc	<.0001	0.0325	0.3181	<.0001	<.0001

ANOVA- Analysis of variance

To further explore the cytoprotective and intracellular antioxidant activity of the carotenoid extracts, an optimal AAPH concentration was determined for the induction of cellular oxidative stress in the Caco-2 cells. This was done by constructing a dose-dependent curve using concentrations of 0, 1, 3, 5, 10, 15, 20, 25 mM of AAPH as described in the materials and methods section 5.2.7. An escalating concentration of AAPH from 0 to 25 mM resulted in a decrease in cell viability (Fig.5.5). Based on the results, the LD₅₀ (the concentration of a substance that is lethal to 50% of the organisms exposed to it in a toxic test) obtained from concentration-response curves was determined to be 3 mM. This was used to stimulate sufficient levels of oxidation in the subsequent assays.

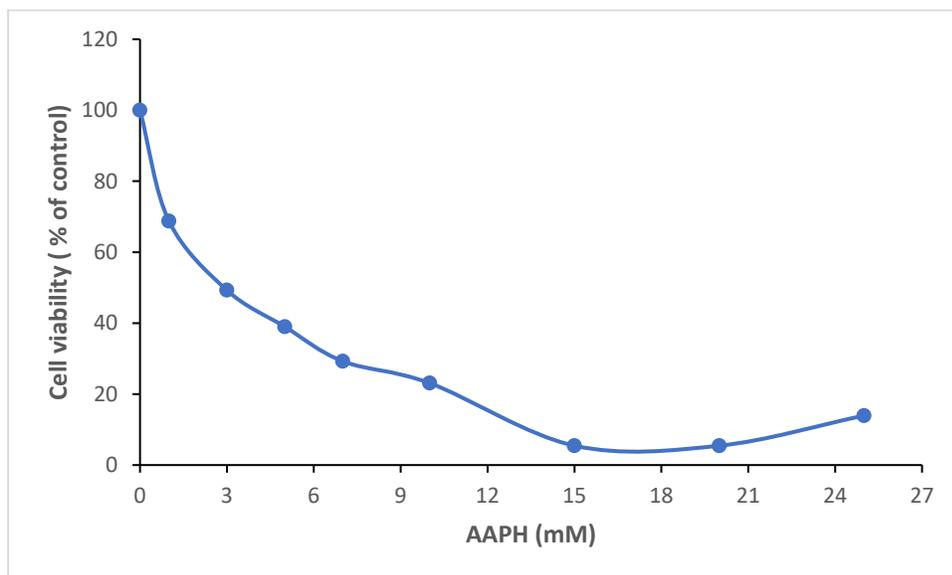


Figure 5.5. Cytotoxicity of AAPH peroxy radical generated at 0 to 25 mM in Caco-2 cells measured by MTT assay.

5.3.4. Cytoprotective of carotenoids from cooked RS and WWF pasta

Everyday life activities generate large quantities of reactive oxygen species (ROS), which mediate oxidative stress-related pathological conditions. Hence, a decent balance between pro-oxidants and antioxidants must generally be achieved to maintain redox homeostasis. The two major systems for controlling ROS generation and countering oxidative damage are the synergistic actions of both enzymatic and non-enzymatic antioxidants (Wang et al., 2018). Therefore, finding excellent food sources of antioxidants for disease prevention has become an exciting and urgent topic for researchers.

Carotenoids are excellent quenchers and scavengers of ROS. The cytoprotective effects of dietary antioxidants such as carotenoids are believed to occur before absorption, protecting the

gastrointestinal epithelial layer from oxidative stress and possibly reducing bowel inflammatory conditions (Lauer, Mieres-Castro, Schmalle, Frank, & Jiménez-Aspee, 2024).

In this study, oxidative stress was induced using the water-soluble azo-compound, AAPH, and the protective effect of carotenoids (measured as percentage cell viability) from digested and undigested RS and WWF pasta samples against the cytotoxic effect of AAPH was assessed. It has been determined that under aerobic conditions, AAPH primary radicals are converted into peroxy radicals at a constant rate, which induces the oxidation of polyunsaturated lipids, causing lipid peroxidation of biological membranes and oxidation of other substrates (Naparło, Soszyński, Bartosz, & Sadowska-Bartos, 2020).

After 2 h of pre-treatment of Caco-2 cells with undigested (Figs. 5.6a and b) and digested (Figs. 5.7a and b) carotenoids from RS and WWF pasta samples, cells were challenged with 3 mM AAPH for 4 h, and their survival was assessed. The viability of cells treated with only AAPH was significantly reduced, with a survival rate of $47.54 \pm 0.6\%$. However, when cells were pretreated with the carotenoid extracts from the undigested RS and WWF samples before exposure to oxidative stress induced by AAPH, they showed significant cytoprotection ($P < 0.05$), and this was greatest among the RS samples compared to WWF, mirroring observations from the cytotoxicity assay (section 5.3.3). The maximum cytoprotection was observed from extracts of pasta cooked to al dente (AI) ($167.18 \pm 18\%$) for the RS samples and extracts from overcooked (OC) samples ($102.57 \pm 11\%$) for the WWF pasta. Averaged over the cooking duration, the inhibition of cytotoxicity by the carotenoid extracts revealed a 14.26% increase (79.38% to 93.64%) in RS and an 8.70% decrease (71.05% to 62.36%) in WWF as concentrations applied to the cells increased from 5 to 1000 $\mu\text{g/ml}$. Globally, extracts from AI cooked and OC samples from the RS and WWF pasta, respectively, appeared to improve cell viability much better than the FCT, regardless of the

extract concentration. Cis carotenoid compounds and degradation products such as apocarotenoids have been shown to have antioxidant activity effect against peroxy radicals (Böhm et al., 2002; Mueller & Boehm, 2011) as the concentration of these compounds was found to have increased with prolonged cooking in our earlier study (Oduro-Obeng et al., 2021).

Similarly, cytoprotection was greatest among cooked samples from the RS food matrix compared to WWF for the digested samples, though not significant (Table 1; $p=0.2511$), but generally lower than the undigested samples (Fig. 5.6). Data presented in section 5.2.6 indicates that approximately, between 33-82% of the carotenoid content in the undigested samples were lost after digestion. This may have caused the lower cytoprotection observed among digested samples compared to undigested ones. Our results agree with Cilla et al. (2009), who reported a 47% loss in the content of phenolic compounds during the digestion of fruit beverages from their original content in these samples. All extracts from WWF digested pasta samples were also able to protect Caco-2 cells following free-radical generation significantly ($P<0.05$). However, significant differences were not seen between cooking duration (Al, FCT, OC) ($P>0.05$). This observation is consistent with the results of the uptake efficiency of lutein, the primary carotenoid in these crude extracts (Fig. 5.1). Unlike WWF, a prooxidant effect was observed among the RS samples when cells were incubated with higher doses ($> 100 \mu\text{g/ml}$) of the extracts. This observation was not seen with the undigested samples, which meant that the digestion process probably induced some physicochemical changes (including enzyme interactions) among the RS-cooked samples, affecting their total carotenoid content and/or composition/properties. Nonetheless, there was no difference in carotenoid profile between the undigested and digested pasta samples (data not shown). However, the formation of oxidative degradation products/ metabolites of lutein during gastric digestion could have been possible even though these were undetected in this study under

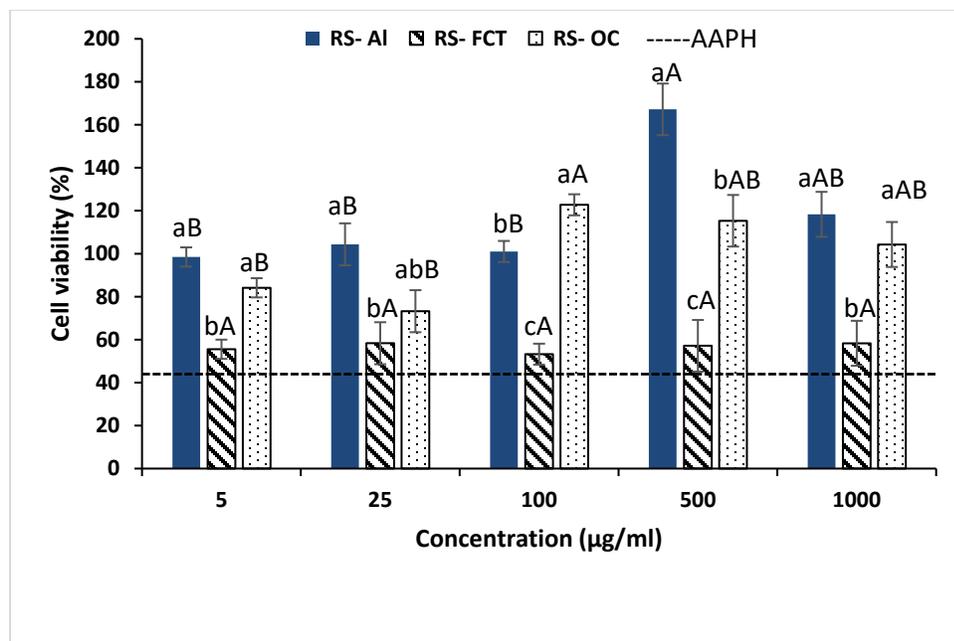
the current HPLC setup. Kotake-Nara and Nagao (2011) earlier opined that dehydration products of lutein, i.e., 3-hydroxy-3',4'-didehydro- β - γ -carotene and 3-hydroxy-2',3'-didehydro- β - ϵ -carotene formed non-enzymatically under the acidic condition of the stomach could have decreased antioxidative properties and consequently lower the antioxidant capacity of digested carotenoids. Conversely, the protective effect of WWF samples at these same concentrations (500 and 100 $\mu\text{g/ml}$) could be ascribed to the phytochemical and nutrient-rich component of the cell wall and germ fractions, which are deliberately absent from RS pasta samples. Zeaxanthin, a primary carotenoid in durum wheat, is chiefly found in the aleurone and germ fractions (Ndolo & Beta, 2013) and has been shown to scavenge peroxy radicals by hydrogen donation mechanisms (Rodrigues, Mariutti, Chiste, & Mercadante, 2012). Moreover, carotenoid structure has been reported as a factor that influences its antioxidant capacity (Young & Lowe, 2001). Generally, the Caco-2 cells were better protected from oxidative stress when lower concentrations of the extracts were used. A significant steep decrease of 60.67% in RS (89.56% to 28.88%) and a slight decrease of 16.52% in WWF (74.51% to 57.99%) (Table 1; $p < .0001$) were observed as concentrations applied to the cells increased from 5 to 1000 $\mu\text{g/ml}$, suggesting the protective effect of carotenoid extracts did not appear to follow a dose-dependant manner at least for the digested samples.

The reduction in cytoprotection observed at higher concentrations among digested RS pasta samples could be attributed to higher aggregation or crystallization of the carotenoid isomers in the extracts, causing insolubility, oxidation, or cytotoxicity in the cells. Moreover, the cell membrane could have been rendered permeable with these high extract doses, causing leakage (El-Agamey et al., 2004). Carotenoid aggregation has been determined to be highly influenced by the structure of the carotenoid but also by other factors, such as the pH of their containing solvents and isomerization (Hempel, Schädle, Leptihn, Carle, & Schweiggert, 2016). Lutein having two

terminal hydroxyl groups in its structure, has been shown to form weakly coupled J-aggregates from H-aggregates in polar solvents through intermolecular hydrogen bonding upon an increase in solvent pH, (presumably because of deprotonation of the -OH groups) (Wang, Berg, Hsu, Merrill, & Tauber, 2012). In this study, it can be speculated that digested samples had a higher pH than undigested samples due to the addition of sodium hydroxide at the intestinal phase, which resulted in the formation of J-aggregates in the digested samples. This change in aggregates due to an increase in pH could have influenced the antioxidative capacity of the carotenoids at higher concentrations in the digested RS samples due to poor solubility, instability, and an increase in fewer electron transfers. Moreover, the change in aggregates could have been a decisive factor resulting in the lower antioxidant activity in the digested compared to undigested samples. On the other hand, the non-prooxidant effect of the carotenoid extracts observed among the WWF samples suggests that the carotenoid structure played a role in the antioxidant activity of the extracts, even at higher concentrations, by increasing their solubility in their carrier medium. Despite the prooxidant effect at these high concentrations, the cooking duration effect was significant ($P < 0.05$), with AI samples showing considerable protection compared to FCT or OC. Food processing has been shown to induce the formation of cis-carotenoid isomers, and their concentration is enhanced by processing intensity (Updike & Schwartz, 2003). By the nature of their bent structures, these cis isomers are more soluble than their linear all-trans counterparts. Moreover, cis isomerization has been shown to prevent an ordered aggregation of carotenoid molecules (Hempel et al., 2016). AI dente (AI) cooked samples having relatively low amounts of cis carotenoids may have promoted aggregates that could contribute to higher electron transfer, leading to a higher antioxidant activity within these high doses. Palmero et al. (2014) determined that the structure of carotenoids affects the nature of aggregates formed upon food processing,

impacting their solubility. Thus, we hypothesize that the cytoprotection of carotenoids from cooked pasta samples is probably related to their structure-activity relationship, and the differences between these cooking durations may only be observed when higher concentrations of the extracts are provided to Caco-2 cells. Naparło et al. (2020) opined that under conditions that can be physiologically achieved, the prooxidant effects of bioactive compounds are most probably of negligible significance. Our results conform to those of Young and Lowe (2001) where β -carotene was seen to play a prooxidant role at concentrations between 4-10 μ M and a protective role at concentrations between 2-3 μ M when applied to H_2O_2 - induced HT-29 cells.

(a)



(b)

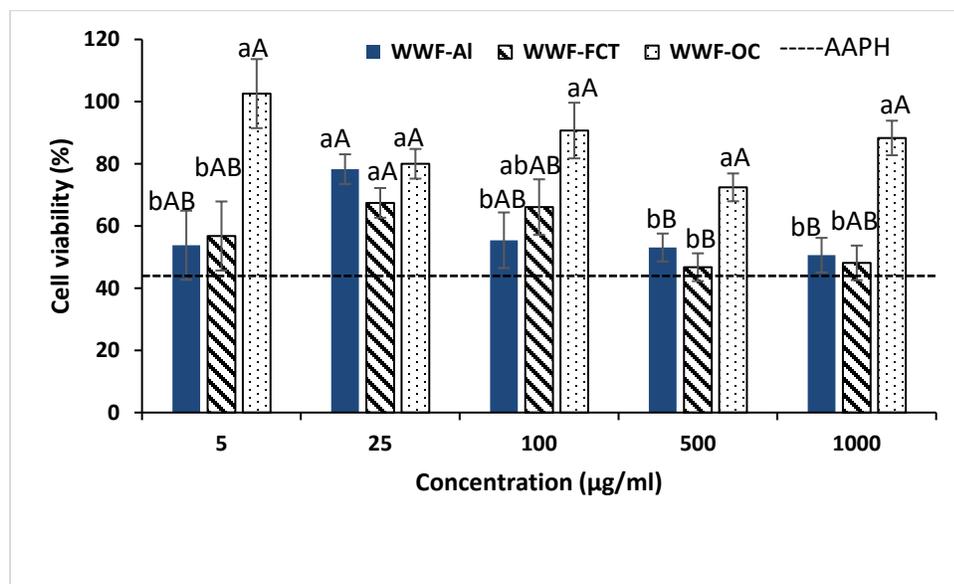
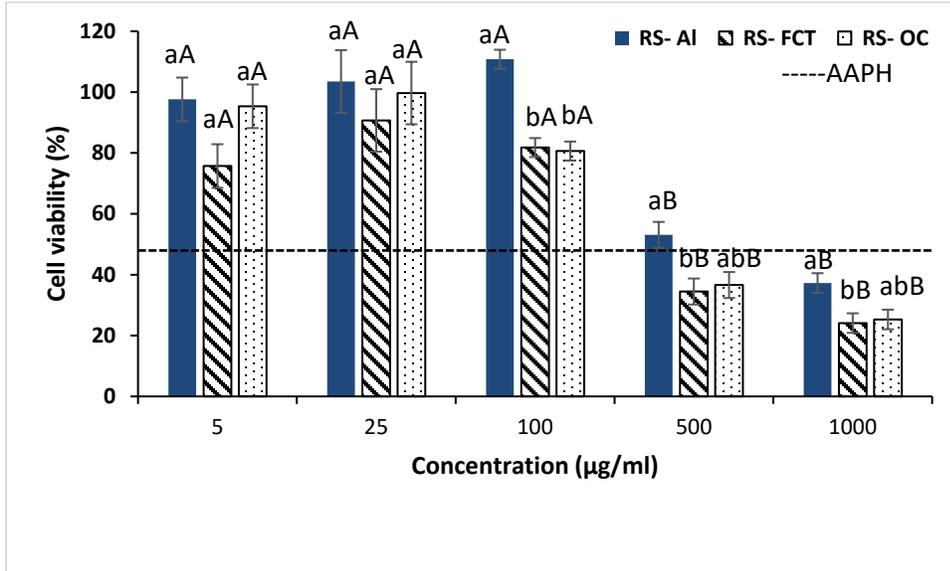


Figure 5.6. The protective effects of carotenoid extracts from undigested refined semolina (a) and whole wheat flour (b) pasta samples and their concentrations on the AAPH-induced oxidation in Caco-2 cells. The data are presented as means \pm SD of three biological and three technical replicates. Bars with different lower-case letters represent significant differences ($P < 0.05$) among the different cooking durations of the same concentration. The different capital letters indicate significant differences ($P < 0.05$) between the different concentrations at the same cooking duration. These are compared to the 3 mM AAPH oxidative stress control. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked; flour type pasta: RS, refined semolina; WWF, whole wheat flour.

(a)



(b)

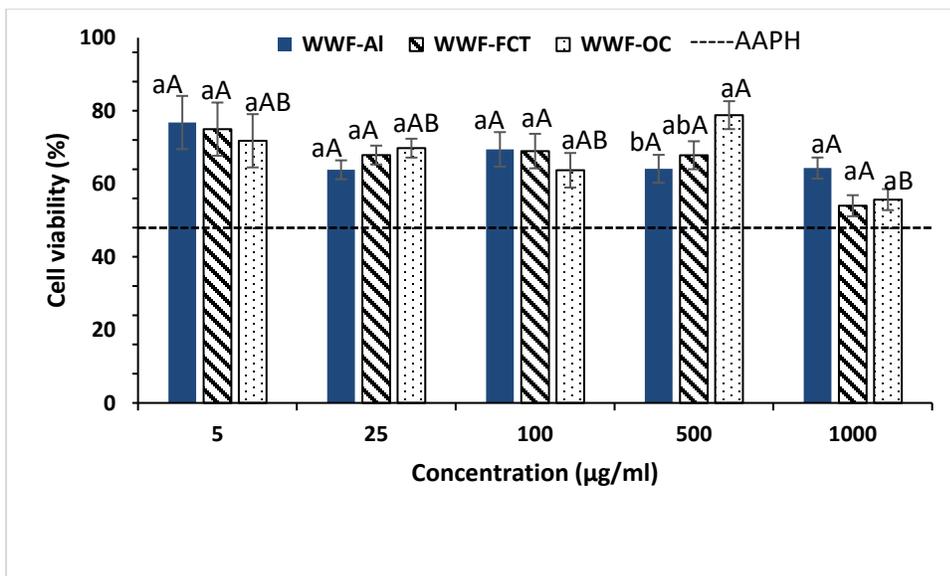


Figure 5.7. The protective effects of carotenoid extracts from digested refined semolina (a) and whole wheat flour (b) pasta samples and their concentrations on the AAPH-induced oxidation of confluent Caco-2 cells. The data are presented as means \pm SD of three biological and three technical replicates. Bars with different lower-case letters represent significant differences ($P < 0.05$) among the different cooking durations of the same concentration. The different capital

letters indicate significant differences ($P < 0.05$) between the different concentrations at the same cooking duration. These are compared to the 3mM AAPH oxidative stress control. Cooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked; flour type pasta: RS, refined semolina; WWF, whole wheat flour.

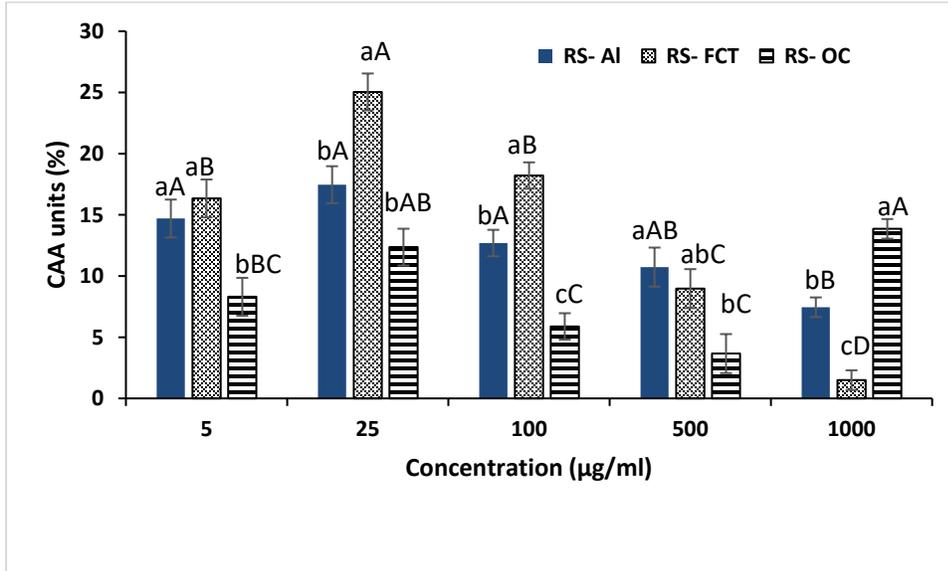
5.3.5. Intracellular antioxidant activity of carotenoid extracts against AAPH oxidation

The intracellular antioxidant activity (CAA) assay is regarded as a more biologically relevant assay than commonly used chemical antioxidant activity assays (Song et al., 2010). The fluorescein-labelled dye DCFH-DA can cross the plasma membrane into the cytoplasm and become hydrolysed by non-specific esterases to non-fluorescent DCFH. In the presence of an oxidant such as AAPH, DCFH is oxidized to DCF, a highly fluorescent compound. Therefore, the presence of antioxidants such as carotenoids could inhibit DCFH oxidation and the resulting increase in fluorescence thus exerting its antioxidant activity (Wolfe et al., 2008). Hence, CAA was calculated based on the fluorescence intensity of carotenoid extracts from the cooked pasta samples, which will have a higher fluorescence intensity than the negative control, and the AAPH-induced cells, which will have a higher fluorescence intensity than the carotenoid-treated cells. Results of the intracellular antioxidant protection of the cooked pasta extracts against the oxidant are shown in Figs. 5.8 and 5.9.

CAA units (%) ranged from $1.49 \pm 0.80 - 25.04 \pm 1.5\%$ and $2.46 \pm 1.2 - 32.72 \pm 3. \%$ for undigested RS and WWF, respectively, and $3.00 \pm 0.6 - 35.86 \pm 2.2\%$ and $9.96 \pm 1.5 - 28.66 \pm 3.1\%$ for digested RS and WWF respectively. On average, Al (12.61%) and FCT (14.01%) had similar but higher CAA than OC (8.81%) among the undigested RS pasta, while for the undigested WWF samples, Al (5.37 %) and FCT (5.10 %) were markedly lower than OC (18.78 %). For the

digested samples, AI (12.19%), FCT (17.97%) and OC (14.33 %) cooked-RS pasta and AI (20.29 %), FCT (13.61%) and OC (13.90%) cooked-WWF pasta had similar intracellular antioxidant activity. Essentially, RS pasta cooked to AI or FCT and WWF pasta cooked to OC showed higher CAA for the undigested samples (Fig. 5.8), mirroring observations reported earlier for carotenoid cytoprotection (Fig.5.6). Moreover, a steep reduction of 77.60% in CAA was observed as the concentration of carotenoids increased from 5-1000 $\mu\text{g/ml}$ for digested RS pasta, while a slight reduction of 22.06% was observed for digested WWF pasta, like results obtained for the cytoprotection of digested RS and WWF pasta (Fig. 5.7). Nonetheless, generally, the CAA of RS samples did not significantly differ from WWF samples among the digested (Table 1; $P=0.8390$) which is consistent with lutein cellular uptake as evaluated by a paired t-test. Differences in cooking duration for the digested samples (RS and WWF) appeared to depend on the extract concentration, as depicted by a significant cooking duration*concentration interaction (Table 1). However, like the cytoprotective effect, the intracellular antioxidant effect of the carotenoid extracts against AAPH-induced ROS formation at higher doses ($>100 \mu\text{g/ml}$) diminished in the digested RS samples. Reasonably, the low bioactivity of antioxidants at high concentrations might be nature's way of preventing cytotoxicity *in vivo* as membrane transporters can efficiently regulate the excessive influx of bioactive compounds such as carotenoid into cells, hence their apparent low bioavailability (Naparło et al., 2020). The intracellular antioxidant mechanism of carotenoids is believed to be through the modulation of the Nrf2/Keap1/ARE pathway, upregulating the gene expression levels of antioxidant enzymes in Caco-2 cells and helping them to withstand the oxidative stress induced by AAPH (Darawsha, Trachtenberg, Levy, & Sharoni, 2021). Hence, in this study, we conclude that the carotenoid extracts from the different cooking durations probably affected this pathway differently, and it depended on the concentration of the carotenoid extract.

(a)



(b)

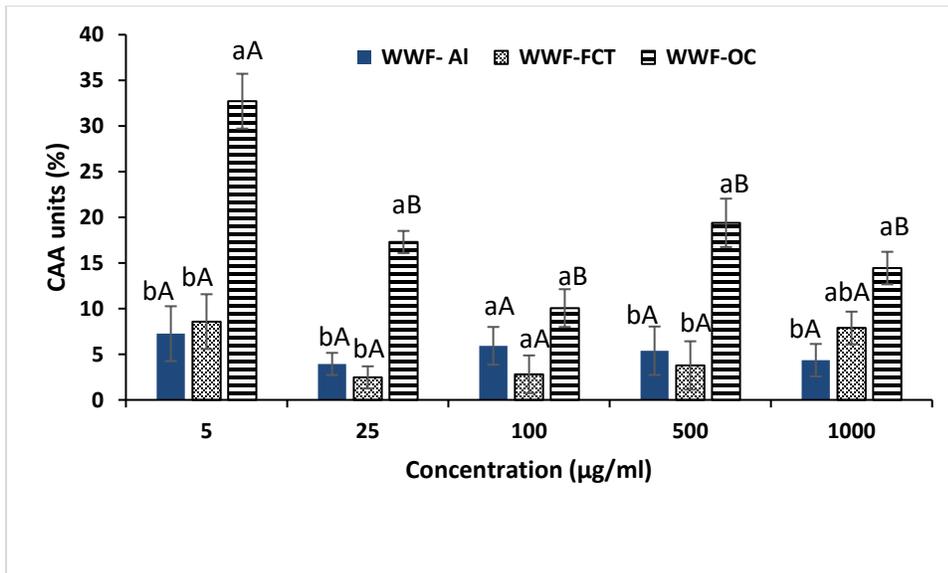
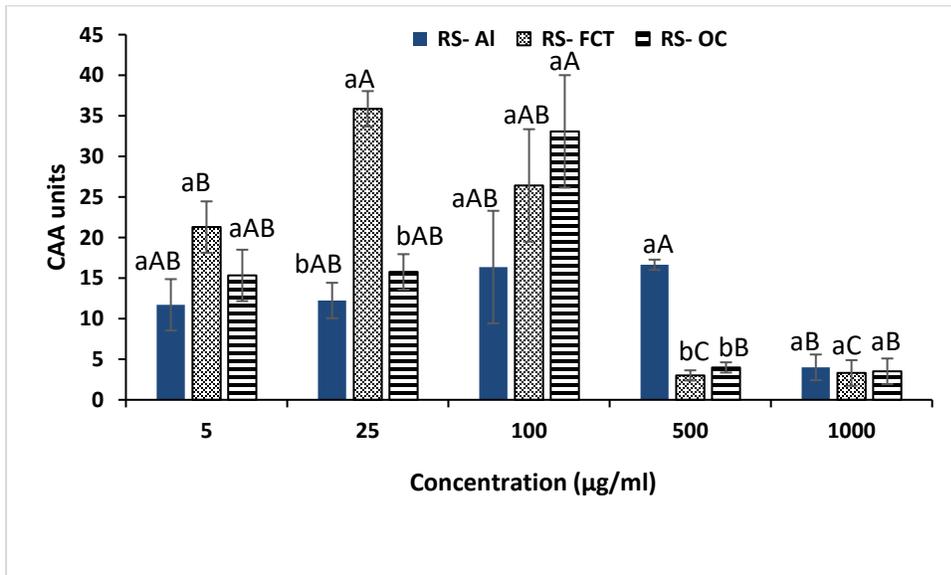


Figure 5.8. Cellular antioxidant activities of carotenoid extracts from undigested refined semolina (a) and whole wheat flour (b) pasta samples at different concentrations (5, 25, 100, 500 and 1000 µg/ml) against AAPH-induced oxidation in Caco-2 cells. The data are presented as means ± SD of three biological and three technical replicates. Bars with different lower-case letters represent significant differences (P < 0.05) among the different cooking durations of the same concentration.

The different capital letters indicate significant differences ($P < 0.05$) between the different concentrations at the same cooking duration. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked; flour type pasta: RS, refined semolina; WWF, whole wheat flour.

(a)



(b)

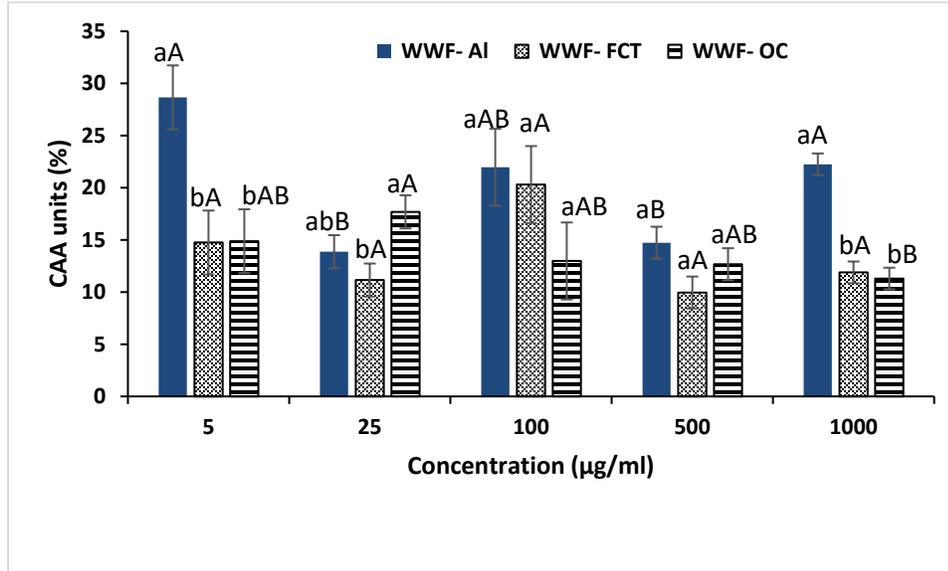


Figure 5.9. Cellular antioxidant activity of carotenoid extracts from digested refined semolina (a) and whole wheat flour (b) pasta samples at different concentrations (5, 25, 100, 500 and 1000 µg/ml) against intracellular AAPH-induced oxidation. The data are presented as means \pm SD of three biological and three technical replicates. Bars with different lower-case letters represent significant differences ($P < 0.05$) among the different cooking durations of the same concentration. The different capital letters indicate significant differences ($P < 0.05$) between the different concentrations at the same cooking duration. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked; flour type pasta: RS, refined semolina; WWF, whole wheat flour.

5.4. Conclusions

With the increasing consumer interest in health-promoting functional foods, understanding how dietary antioxidants like carotenoids are affected by food processing and their fate after digestion and absorption is crucial for maximizing their benefits. As a complex cellular structure,

the food matrix mediates the restricted release of food bioactives, including carotenoids. Hence, it has become imperative to find strategies to overcome these barriers and enhance dietary antioxidant availability for efficient bioactivity. In this study, we showed that despite the efficient bioaccessibility of lutein in durum wheat pasta, its uptake by Caco-2 cells was minimal, showing non-significant differences between cooked pasta samples irrespective of the wheat flour type. Moreover, despite their high solubility and micellization efficiency, cis-lutein isomers were undetectable in the Caco-2 cells. Although pasta carotenoids had low cellular uptake, they showed efficient antioxidant activity, especially at low concentrations, against peroxy radical-mediated cytotoxicity and intracellular stress. Carotenoid structure appeared to influence antioxidant activity at high concentrations of the extracts. Differences observed among cooking durations on the antioxidant activity of the digested (RS and WWF) carotenoids depended on the extract concentration. Pasta microstructural determination revealed that food processing (cooking duration) modulated the pasta samples' internal morphology, affecting their physical and chemical properties and further carotenoid extractability and bioaccessibility.

Further studies are needed to understand the influence of these food-related factors on carotenoid absorption under *in vivo* conditions and the molecular signaling pathways involved in the antioxidant activity of carotenoids from durum wheat.

CHAPTER 6.

General Discussion, Summary, and Conclusions

6.1. General Discussion

Although currently, consumers are aware of the health benefits of phytochemicals in plant-based foods, including carotenoids, only a small proportion will be absorbed, metabolized, and transported to target tissues where they exert their biological function, usually in the range of 1-2 μM (Eroglu, Wang, Crook, & Bohn, 2024). To elicit any benefits, however, bioactive compounds must be accessible after digestion. The bioaccessibility of carotenoids largely depends on their release from the food matrix, food processing effects on the food matrix, digestion conditions including enzymes and pH, carotenoid physicochemical properties, and other host-related factors, including single nucleotide polymorphism in genes encoding enzymes that mediate carotenoid digestion and absorption among others (von Lintig et al., 2020).

Due to the uneven distribution of carotenoids in cereals, including their physical state in the grain tissues and the presence of other food components such as proteins, starch, lipids, water, and fiber, efficient food processing methods are needed to alter the food structure/matrix enhancing carotenoid digestibility. Indeed, the structure of processed food, even with the same chemical composition, can influence the intake and bioaccessibility of nutrients and other bioactive components, leading to significant differences in biological impact. Generally, thermal food methods, including cooking, have improved carotenoid bioaccessibility by facilitating the disruption of the food matrix and nutrient complexes, making it more digestible (Cilla et al., 2018). However, the duration or intensity of food processing can control food digestion's disintegration and dissolution steps through several modifications to matrix components, leading to molecular interactions between the released carotenoids (Zhang et al., 2024).

Hence, this research has shown that different cooking durations significantly impacted carotenoid bioaccessibility, especially among refined semolina food products (Oduro-Obeng et al.,

2022). The cooking duration highly influenced the extent to which pasta macromolecules such as starch, fibre and the protein matrix surrounding the starch granules disintegrated. For example, it is presumed that starch granules found only in the external region of al dente cooked pasta were largely deformed and gelatinized, surrounded by thin protein films (Petitot et al., 2009) (as illustrated by a schematic diagram in Appendix 1). This processed-induced transformation may have reduced the release of organic matter (mostly amylose), as evidenced by a lower cooking loss (Oduro-Obeng et al., 2021). Higher amylose retention in the pasta samples meant increased retrograded starch content due to amylose recrystallization during pasta cooling and storage, increasing its resistant starch composition and reducing luminal viscosity, further enhancing carotenoid digestion after ingestion. A directly opposite phenomenon could have occurred with the overcooked pasta samples where starch granules in the pasta strand's external, intermediate, and central parts were fully swollen and erupted, embedded in a less dense protein network (Zou, Sissons, Gidley, Gilbert, & Warren, 2015). This may have leached out significant amounts of amylose, reducing its ability to increase its resistant starch fraction during pasta storage. Also, the digestibility of the modified proteins positively and negatively impacted carotenoid digestion in the al dente cooked and overcooked pasta samples, respectively. Proteins have emulsifying properties due to their amphipathic nature, which can enhance the emulsification of carotenoids in lipid droplets (Zhang et al., 2024). Conversely, proteins can also adsorb to lipid membranes, hindering the activity of digestive enzymes such as lipases (Iddir et al., 2022). Currently, the influence of protein on carotenoid bioaccessibility is unclear. It may also depend on its content, protein type, or carotenoid type (Iddir et al., 2020), requiring more studies into their structure-functional properties regarding carotenoid bioaccessibility.

These differences in carotenoid digestion mediated by food processing-induced matrix modifications also influenced carotenoid (lutein) uptake efficiency, although this was non-significant (Oduro-Obeng et al., 2024).

In addition to dietary factors that impact carotenoid absorption, other non-dietary factors, including gut disease conditions (e.g., celiac disease, crohn's disease, ulcerative colitis), the consumption of alcohol, ethnicity, sex, and age of an individual, among other factors, have been implicated in this process in vivo, further presenting additional challenges to the uptake of these lipophilic compounds (Desmarchelier & Borel, 2017). Nonetheless, the results of carotenoid digestion and absorption in vitro are well corroborated in vivo, even though complete in vivo conditions may not be fully mimicked in vitro. Justifiably, these in vitro models serve as simple predictive tools capable of establishing mechanisms involved in the digestion of bioactive compounds such as carotenoids. Moreover, the relationship established between matrix components and carotenoid bioaccessibility further provides insights for selecting targeted agronomic traits that will not only be important for breeding programs but also enhance the digestibility of carotenoids.

Although dietary carotenoids are generally considered non-essential (since they are not nutrients but bioactive compounds and their absence from the diet causes no specific deficiencies), their consumption among vegetarians (vegans) may be essential since it is the only avenue through which they acquire vitamin A. Moreover, carotenoids may be “essential” for the aging population in preventing or lowering the risk of age-related macular degeneration (ARMD) progression. It can also be calculated that carotenoids may be “essential” for persons not consuming foods with preformed vitamin A due to unavailability, as in many developing countries. Currently, there are no dietary reference intakes by the United States Department of Agriculture (USDA) due to the

numerous factors that influence bioaccessibility and bioavailability (Böhm et al., 2021), emphasizing the need for more empirical data from research from a variety of food sources to provide evidence for establishing recommendations. Nonetheless recent research indicates that in the absence of preformed vitamin A, a recommendation of 3.9 and 4.5 mg/day of beta-carotene for females and males is beneficial. This approximates about 0.65 and 0.75 mg of vitamin A (retinol equivalent) intake recommendation, given a conversion factor of 1:6 of beta-carotene to retinol (Eroglu et al., 2024). This is slightly below the recommended daily allowance (RDA) for vitamin A (900 µg) for healthy adult man. Yet another study revealed that a higher approximation of 10.8 mg/day beta-carotene or 21.6 mg/day alpha-carotene (or other provitamin A carotenoids) is needed to achieve the same RDA. These discrepancies are probably due to the use of different equivalency factors (Böhm et al., 2021).

For lutein and zeaxanthin, which are deemed macular carotenoids owing to their antioxidative actions in the eye, current intakes are also considered low even from carotenoid-rich sources such as fruits and vegetables. Alves-Rodrigues and Shao (2004) revealed that Americans consumed only 1.7 mg/day of lutein, far less than the amount of 3.8 mg/day intended to be achieved from consuming 3-5 servings of vegetables daily. This underscores the importance of consuming other food sources relevant in these bioactive compounds, especially staples such as durum wheat, complementing dietary intakes from fruits and vegetables. Clinically, to improve visual acuity and reduce the risk of ARMD and cataracts, dietary supplements of 10 mg (equivalent to 100 g of spinach/day) and 2 mg/day of lutein and zeaxanthin, respectively, are usually prescribed (Böhm et al., 2021; Eroglu et al., 2024).

6.2. Research Summary and General Conclusions

This thesis aimed to evaluate the bioaccessibility of carotenoids in three Canadian durum wheat varieties, which are ranked among the top four durum varieties grown in western Canada in terms of protein and carotenoid content, investigating the influence of some food-related factors, i.e., the food matrix and food processing, on the digestion behavior of carotenoids. The three durum wheat varieties (AAC Spitfire, CDC Precision, and Transcend) were milled and processed into pasta, which served as a model food for the study. Subsequently, the pasta samples were cooked to three cooking durations, i.e., al dente cooked (Al), fully cooked (FCT), and overcooked (OC). The investigation of these factors enabled us to determine pasta food structure and matrix composition in these wheat varieties related to the digestion of carotenoids as functions of the different cooking durations.

The main themes of the research included 1) characterizing the wheat varieties in terms of their carotenoid contents in milled flour, dry pasta, and cooked pasta, 2) evaluating the effects of cooking duration on the retention and chemical transformation of carotenoids, 3) evaluating the effect of food processing and the food matrix on carotenoid bioaccessibility, digestive stability and absorption efficiency, further examining matrix components associated with carotenoid digestion and release (bioaccessibility), 4) determining the antioxidant capacity of carotenoids as influenced by the food matrix and pasta cooking duration before and after digestion.

The results in Chapter 3 showed that the wheat varieties differed in their initial carotenoid contents, with Spitfire having higher lutein and zeaxanthin content than Transcend and Precision. Lutein was the predominant compound in refined semolina (RS), whole wheat flour (WWF), and processed pasta. Moreover, lutein content was slightly higher in RS compared to WWF; the

opposite was true for zeaxanthin, confirming the uneven distribution of these carotenoids in the wheat grain. Upon pasta extrusion, carotenoid contents decreased, and pasta cooking induced further decreases. Nonetheless, OC pasta samples generally contained higher amounts of extractable carotenoids than AI and FCT due to a weakened and porous structure caused by the prolonged cooking duration. Cooking to OC also affected the cooking quality of pasta as it induced a less firm pasta structure with an increase in cooking loss and water absorption. On the other hand, pasta cooked to AI was firmer due to reduced cooking time. Moreover, WWF pasta with high protein content was firmer compared to RS. Transcend-produced RS pasta, regardless of its relatively low protein content, was firmer than Spitfire, suggesting that in addition to protein content, its gluten quality influenced the integrity or properties of its protein matrix around starch granules.

Despite the decreases in lutein and zeaxanthin content, cooking increased the cis-isomer concentration of these compounds from their milled flour contents, and this increase was significantly high in the OC samples compared to the other cooking durations. These differences in carotenoid content between the wheat varieties and the cooking durations caused significant differences in their cooking stability, with Transcend being more stable after cooking. This subsequently influenced carotenoid bioaccessibility among the wheat varieties.

The results in Chapter 4 provide information on the bioaccessibility of the carotenoids, their digestive stability at the various phases of digestion, micellization efficiency, and antioxidant scavenging capacity using the DPPH radical assay. This chapter also investigates the matrix properties of the wheat varieties associated with the digestion of carotenoids. The results showed that Transcend had the highest lutein and zeaxanthin bioaccessibility, and Spitfire had the lowest in both RS and WWF flour pasta. Moreover, cooking duration also influenced carotenoid

bioaccessibility, including cis-lutein isomers, with pasta cooked to AI having the greatest and OC having the lowest bioaccessibility of these carotenoids in RS pasta. Cooking duration was not a main factor that affected carotenoid bioaccessibility in WWF pasta. Hence, the cis-lutein isomer bioaccessibility in WWF pasta was also influenced by the main effects of the durum wheat varieties. Additionally, the bioaccessibility of lutein and zeaxanthin was positively associated with the contents of its pasta macronutrients, including lipids, starch, protein, and fiber. Transcend, the variety with the highest bioaccessible lutein and zeaxanthin was not correlated with fat, protein, and starch contents in RS pasta, suggesting that differences observed in carotenoid bioaccessibility among the wheat varieties and flour types were probably due to process-induced modifications of the food matrix that affected its content and fractional composition. Therefore, further bioaccessibility studies are needed to ascertain the relationship between carotenoid bioaccessibility and cooked pasta matrix properties, employing more wheat varieties with different nutrient and carotenoid content.

Generally, lutein bioaccessibility was above 100 %, especially among the RS pasta samples, meaning enzymatic digestion could break down matrix-induced barriers, resulting in their efficient release and solubilization. The relatively low level of bioaccessible lutein in WWF pasta compared to RS indicates the restrictive nature of cell wall components in releasing carotenoids. Moreover, other bran components in WWF, including divalent minerals, can negatively affect its carotenoid bioaccessibility.

Specifically, the highest release mainly occurred at the intestinal phase and the lowest in the gastric phase for all-trans lutein in the RS pasta samples. On the contrary, the highest release of lutein in the WWF flour pasta mainly occurred in the gastric phase of digestion, suggesting that digestive conditions, especially pH, strongly influenced carotenoid release and stability during

digestion. Carotenoids are generally stable in high and neutral pH, indicating that some components of the WWF pasta matrix may have buffered the low pH in the gastric phase, making it more basic, like the intestinal phase. A similar release trend was mirrored by the cis geometric isomers of lutein in both RS and WWF pasta, meaning that these carotenoids were equally stabilized in a more basic environment. Although carotenoids in RS pasta were better released at the end of digestion, pasta cooked to AI was more stable, efficiently micellarized, and bioaccessible than OC pasta, suggesting that carotenoids in OC pasta might have been rapidly degraded upon its release in the lumen compared to AI or formed complexes with modified structures of macromolecules induced by the cooking process of OC during digestion. The antioxidant capacity of the carotenoids was higher in WWF pasta than in RS, confirming carotenoid compositional differences among grain milling fractions in their capacity to scavenge free radicals. Moreover, decreases were observed from the oral to the intestinal phase in both RS and WWF pasta when pasta was digested. This finding suggests that carotenoid cleavage products or derivatives with lower antioxidant activity were significantly produced by the end of the intestinal phase of digestion. However, higher antioxidant activity was observed among the aqueous micellar fraction (bioaccessible fraction) suggesting that antioxidants with scavenging properties were significantly released and micellarized. Residues after digestion showed an efficient antioxidant capacity, indicating that unabsorbed carotenoids can potentially elicit further protection upon reaching the colon.

Chapter 5 examined the uptake efficiency of carotenoids in the Transcend wheat variety to determine the variations in their absorption between RS and WWF pasta when cooked to AI, FCT and OC using human Caco-2 cells. Transcend was chosen due to its significantly high micellization efficiency and bioaccessibility among the three wheat varieties. Moreover, micellization efficiency

has been shown to correlate positively with the absorption efficiency of carotenoids (Reboul et al., 2006), which is why we chose Transcend. The cellular antioxidant capacity of the digested and undigested carotenoids was also evaluated to gain better insight into their inhibitory properties using a biological model, the Caco-2 cell model that was incubated with a widely used pro-oxidant (AAPH) to generate free radicals under thermal decomposition. Compared to the other in vitro antioxidant assays (DPPH and ABTS) evaluated in chapters 3 and 4, this model afforded us the benefit of assessing the antioxidant capacity of the carotenoids against a physiologically relevant radical (peroxyl radical), which contrasted the above spectrophotometrical assays in their use of non-physiological radicals. Cellular-based assays have been reported to model cellular biochemical processes, including uptake and metabolism, making their usage more resembling of in vivo conditions (Kellett, Greenspan, & Pegg, 2018). The effectiveness or capacity of antioxidants such as carotenoids depends on the type of oxidant. In as much as chemical antioxidant assays may be good screening tools for assessing the antioxidant capacity of bioactives such as carotenoids, they may be poor predictors of their cellular actions. Moreover, the total carotenoid composition can be modified due to digestion, leading to the formation of metabolites or oxidative degradation products that have lower or higher antioxidant activity (Kotake-Nara & Nagao, 2011). Therefore, it may be necessary to evaluate the antioxidant activities of dietary carotenoids by employing different assays to gain more insight into their properties and possible mechanisms of action as functions of their food matrices or food processing, for example, before and after digestion. The microstructure of cooked pasta samples was also assessed to determine its influence on the release of digested carotenoids using X-ray micro-CT. This is a non-invasive imaging technique widely used in the medical field. However, it shows promise as a tool that can allow the structure of food, such as pasta, to be characterized three-dimensionally (Schoeman et

al., 2016). This study showed that the cellular uptake of all-trans lutein was low (<10 %). Zeaxanthin and the cis isomers of lutein were not detected in the cells, and none of the carotenoid species was secreted into the basolateral side of the Transwell® inserts, which simulates the intestinal epithelium. Digested and undigested carotenoids were found to be efficient free radical scavengers. However, after digestion, the protective capacity of the carotenoids slightly reduced presumably from an altered composition after digestion. Unlike the undigested samples, digested RS and WWF were not significantly different in attenuating oxidative stress induced by AAPH. At higher concentrations of the digested RS extracts only, carotenoids exhibited prooxidant activity, suggesting that the antioxidant activity of carotenoids was influenced by its structure or flour carotenoid composition, even at higher concentrations. Pasta microstructural determination revealed that food processing (cooking duration) modulated pasta internal morphology, affecting the extractability, digestive stability, and bioaccessibility of carotenoids.

In summary, this research provided insight into the effects of pasta cooking duration and its matrix on the contents and retention of carotenoids, their stability during digestion, micellization efficiency, bioaccessibility, uptake efficiency, and antioxidant activities, further examining matrix components associated with carotenoid digestion and release (bioaccessibility). Our data generally concludes that durum wheat is a relevant source of bioaccessible carotenoids. The magnitude of carotenoid bioaccessibility will, however, depend on the effects of food processing on the wheat food matrix, which will ultimately influence carotenoid stability and its bioaccessibility after digestion. In other words, durum wheat chemical composition influences the effects of pasta cooking duration on the bioaccessibility of its carotenoids. When processed into a food product like pasta, the impact of cooking may be significant in the bioaccessibility of carotenoids in refined semolina products but not whole wheat pasta.

6.3. Summary of Contributions

The main contributions of the work presented in this thesis are as follows:

- Determined the bioaccessibility of the major and minor carotenoid compounds in durum wheat, assessing the effect of matrix and food processing factors on their release, digestive stability at the various stages of digestion, and absorption.
- Provided insight into the influence of different cooking durations on the nutritional and physical properties of three Canadian durum wheat pasta products
- Determined the antioxidant capacity of the bioaccessible carotenoids as influenced by the different food processing methods employing various chemical and cellular-based assays
- Demonstrated the novel application of X-ray micro-CT imaging to non-destructively assess the internal morphological features of processed durum wheat pasta products, further revealing the influence of processed-induced matrix changes on carotenoid digestion

6.4. Recommendations for Future Research

The findings from this research showed that the bioaccessibility of carotenoids in durum wheat differs among varieties due to matrix composition differences, which can be impacted by food processing when the wheat grains are processed into a food product like pasta. Additionally, the antioxidant activity of carotenoids in the processed pasta depended on its contents and stability as influenced by food processing. It also depended on its structure and presumably its total composition as affected by digestion. However, further studies are needed to:

- 1) Evaluate the bioaccessibility and bioavailability (absorption) of these carotenoids in vivo using animal models or human subjects to understand better the release mechanisms and

the factors that affect their digestion and absorption. Although our results show that the wheat varieties are a good source of bioaccessible carotenoids, carotenoid digestibility in humans is also affected by other non dietary factors such as gender, age, ethnicity, and underlying health conditions, including Celiac and Crohn's disease. Nonetheless, in vitro bioaccessibility assessment has been shown to correlate positively with in vivo results.

- 2) Understand other food matrix chemical properties and characteristics, such as resistant starch and amylose content, grain physical properties, and other non-nutrient components, and their relationship with durum wheat carotenoid bioaccessibility, employing more sample types (wheat varieties) and sample sizes.
- 3) Understand and establish the relationship between pasta carotenoid bioaccessibility and its technological properties if any. Preliminary findings suggest that carotenoid bioaccessibility is greatest in firmer pasta products and lowest in less firm products.
- 4) Assess the occurrence of carotenoid esters in cereal grains to evaluate their effects on bioaccessibility and bioavailability since they are stable even after food processing compared to their free counterparts.
- 5) Assess carotenoid bioaccessibility in other durum wheat food products, including bread and couscous. This will lend more understanding to the influence of the food matrix and food processing on the digestion of durum wheat carotenoids. This may also help optimize efficient processing methods that will ultimately enhance the digestibility of carotenoids in these food matrices.
- 6) Assess the molecular mechanisms by which processed carotenoids prevent free radical-mediated cell death

7) Determine the antioxidant activity of these carotenoids in vivo, employing preclinical studies to confirm results obtained in the ex vivo cellular assay.

As with all studies, this research had its limitations. It was limited by using an in vitro model to assess carotenoid bioaccessibility. While other in vitro models, such as the semi-dynamic and dynamic models, exist and may better represent in vivo conditions, they are expensive and difficult to operate. However, as earlier indicated, the results of in vitro models correlate well with those performed in vivo. Therefore, a confirmation study using in vivo conditions is recommended, as earlier ascertained.

For the ex vivo absorption of most bioactive compounds, including carotenoids, the human Caco-2 cell model has been widely used and considered the gold standard. However, these cell lines are not mucus-producing cell lines, which does not represent a realistic imitation of the intestinal epithelial layer. Therefore, future research could consider using Caco-2 cells co-cultured with HT29-MTX, a mucin-secreting cell line from goblet cells.

REFERENCES

- AACC. (2000). *Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 14-22, 44-16, 46-30 and 66-51*. The Association: St. Paul, MN. AACC International.
- Abdel-Aal, E.-S. M., Rabalski, I., Carey, C., & Gamel, T. H. (2023). Bioaccessibility and cellular uptake of lutein, zeaxanthin and ferulic acid from muffins and breads made from hairless canary seed, wheat and corn blends. *Foods*, *12*(6), 1307. doi:10.3390/foods12061307
- Abdel-Aal, E.-S. M., Young, J. C., Rabalski, I., Hucl, P., & Fregeau-Reid, J. (2007). Identification and quantification of seed carotenoids in selected wheat species. *Journal of Agricultural and Food Chemistry*, *55*(3), 787-794. doi:10.1021/jf062764p
- Abdel-Aal el, S. M., Young, J. C., Akhtar, H., & Rabalski, I. (2010). Stability of lutein in wholegrain bakery products naturally high in lutein or fortified with free lutein. *Journal of Agricultural and Food Chemistry*, *58*(18), 10109-10117. doi:10.1021/jf102400t
- Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry*, *51*(26), 7825-7834. doi:10.1021/jf030404l
- Adom, K. K., Sorrells, M. E., & Liu, R. H. (2005). Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. *Journal of Agricultural and Food Chemistry*, *53*(6), 2297-2306. doi:10.1021/jf048456d
- Aguilera, J. M. (2019). The food matrix: implications in processing, nutrition and health. *Critical Reviews in Food Science and Nutrition*, *59*(22), 3612-3629. doi:10.1080/10408398.2018.1502743

- Åkerberg, A., Liljeberg, H., & Björck, I. (1998). Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. *Journal of Cereal Science*, *28(1)*, 71-80. doi:<https://doi.org/10.1006/jcers.1997.0173>
- Al-Yafeai, A., & Bohm, V. (2018). In vitro bioaccessibility of carotenoids and vitamin E in rosehip products and tomato paste as affected by pectin contents and food processing. *Journal of Agricultural and Food Chemistry*, *66(15)*, 3801-3809. doi:10.1021/acs.jafc.7b05855
- Alves-Rodrigues, A., & Shao, A. (2004). The science behind lutein. *Toxicology Letters*, *150(1)*, 57-83. doi:10.1016/j.toxlet.2003.10.031
- Amorim-Carrilho, K. T., Cepeda, A., Fente, C., & Regal, P. (2014). Review of methods for analysis of carotenoids. *TrAC Trends in Analytical Chemistry*, *56*, 49-73. doi:10.1016/j.trac.2013.12.011
- Anese, M., Mirolo, G., Beraldo, P., & Lippe, G. (2013). Effect of ultrasound treatments of tomato pulp on microstructure and lycopene in vitro bioaccessibility. *Food Chemistry*, *136(2)*, 458-463. doi:10.1016/j.foodchem.2012.08.013
- Apea-Bah, F. B., & Beta, T. (2021). Advances in understanding the nutritional value of antioxidants in wheat. In T. Beta (Ed.), *Improving the Nutritional and Nutraceutical Properties of Wheat and other Cereals* (1st ed., pp. 29-65). Cambridge, UK: Burleigh Dodds Science Publishing Limited.
- Aravind, N., Sissons, M., Egan, N., & Fellows, C. (2012). Effect of insoluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. *Food Chemistry*, *130(2)*, 299-309.

- Armanino, C., De Acutis, R., & Rosa Festa, M. (2002). Wheat lipids to discriminate species, varieties, geographical origins and crop years. *Analytica Chimica Acta*, 454(2), 315-326. doi:[https://doi.org/10.1016/S0003-2670\(01\)01548-3](https://doi.org/10.1016/S0003-2670(01)01548-3)
- Armellini, R., Peinado, I., Asensio-Grau, A., Pittia, P., Scampicchio, M., Heredia, A., & Andres, A. (2019). In vitro starch digestibility and fate of crocins in pasta enriched with saffron extract. *Food Chemistry*, 283, 155-163. doi:10.1016/j.foodchem.2019.01.041
- Artavia, G., Cortés-Herrera, C., & Granados-Chinchilla, F. (2020). Total and resistant starch from foodstuff for animal and human consumption in Costa Rica. *Current Research in Food Science*, 3, 275-283. doi:10.1016/j.crfs.2020.11.001
- Aschoff, J. K., Rolke, C. L., Breusing, N., Bosy-Westphal, A., Högel, J., Carle, R., & Schweiggert, R. M. (2015). Bioavailability of β -cryptoxanthin is greater from pasteurized orange juice than from fresh oranges – a randomized cross-over study. *Molecular Nutrition and Food Research*, 59(10), 1896-1904. doi:<https://doi.org/10.1002/mnfr.201500327>
- Atienza, S. G., Ballesteros, J., Martín, A., & Hornero-Méndez, D. (2007). Genetic variability of carotenoid concentration and degree of esterification among Tritordeum (\times Tritordeum Ascherson et Graebner) and durum wheat accessions. *Journal of Agricultural and Food Chemistry*, 55(10), 4244-4251. doi:10.1021/jf070342p
- Autio, K., & Salmenkallio-Marttila, M. (2003). Understanding microstructural changes in biopolymers using light and electron microscopy. In G. Kaletunc, & K. J. Breslauer (Eds.), *Characterization of Cereals and Flours* (Vol. 124). New York: Marcel Dekker, Inc.

- Bagdi, A., Szabó, F., Gere, A., Kókai, Z., Sipos, L., & Tömösközi, S. (2014). Effect of aleurone-rich flour on composition, cooking, textural, and sensory properties of pasta. *LWT - Food Science and Technology*, 59 (2, Part1), 996-1002. doi:<https://doi.org/10.1016/j.lwt.2014.07.001>
- Barba, F. J., Mariutti, L. R., Bragagnolo, N., Mercadante, A. Z., Barbosa-Canovas, G. V., & Orlie, V. (2017). Bioaccessibility of bioactive compounds from fruits and vegetables after thermal and nonthermal processing. *Trends in Food Science and Technology*, 67, 195-206.
- Barron, C., Parker, M. L., Mills, E. N. C., Rouau, X., & Wilson, R. H. (2005). FTIR imaging of wheat endosperm cell walls in situ reveals compositional and architectural heterogeneity related to grain hardness. *Planta*, 220(5), 667-677. doi:10.1007/s00425-004-1383-6
- Barron, C., Surget, A., & Rouau, X. (2007). Relative amounts of tissues in mature wheat (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science*, 45(1), 88-96.
- Beleggia, R., Platani, C., Nigro, F., De Vita, P., Cattivelli, L., & Papa, R. (2013). Effect of genotype, environment and genotype-by-environment interaction on metabolite profiling in durum wheat (*Triticum durum* Desf.) grain. *Journal of Cereal Science*, 57(2), 183-192. doi:10.1016/j.jcs.2012.09.004
- Beta, T., & Hwang, T. (2018). Influence of heat and moisture treatment on carotenoids, phenolic content, and antioxidant capacity of orange maize flour. *Food Chemistry*, 246, 58-64. doi:10.1016/j.foodchem.2017.10.150
- Björck, I., & Asp, N. G. (1983). The effects of extrusion cooking on nutritional value -A literature review. *Journal of Food Engineering*, 2(4), 281-308. doi:10.1016/0260-8774(83)90016-x

- Blanquet-Diot, S., Soufi, M., Rambeau, M., Rock, E., & Alric, M. (2009). Digestive stability of xanthophylls exceeds that of carotenes as studied in a dynamic in vitro gastrointestinal system. *Journal of Nutrition*, *139*(5), 876-883. doi:10.3945/jn.108.103655
- Böhm, V., Lietz, G., Olmedilla-Alonso, B., Phelan, D., Reboul, E., Bánati, D., . . . Bohn, T. (2021). From carotenoid intake to carotenoid blood and tissue concentrations – Implications for dietary intake recommendations. *Nutrition Reviews*, *79*(5), 544-573. doi:10.1093/nutrit/nuaa008
- Bohm, V., Puspitasari-Nienaber, N. L., Ferruzzi, M. G., & Schwartz, S. J. (2002). Trolox equivalent antioxidant capacity of different geometrical isomers of alpha-carotene, beta-carotene, lycopene, and zeaxanthin. *Journal of Agricultural and Food Chemistry*, *50*(1), 221-226. doi:10.1021/jf010888q
- Bohn, T. (2018). Metabolic fate of bioaccessible and non-bioaccessible carotenoids. In F. Saura-Calixto & J. P.-. Jimenez (Eds.), *Non-extractable Polyphenols and Carotenoids* (5th ed., pp. 165-200). UK: The Royal Society of Chemistry.
- Boileau, A. C., & Erdman Jr., J. W. (2004). Impact of food processing on content and bioavailability of carotenoids. In N. I. Krinsky, S. T. Mayne, & H. Sies (Eds.), *Carotenoids in Health and Disease* (pp. 228-247). United Kingdom: CRC Press.
- Boileau, A. C., Merchen, N. R., Wasson, K., Atkinson, C. A., & Erdman, J. W., Jr. (1999). Cis-lycopene is more bioavailable than trans-lycopene in vitro and in vivo in lymph-cannulated ferrets. *Journal of Nutrition*, *129*(6), 1176-1181. doi:10.1093/jn/129.6.1176
- Boileau, T. W., Boileau, A. C., & Erdman, J. W., Jr. (2002). Bioavailability of all-trans and cis-isomers of lycopene. *Experimental Biology and Medicine* (Maywood, N.J.), *227*(10), 914-919. doi:10.1177/153537020222701012

- Borel, P. (2003). Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clinical Chemistry and Laboratory Medicine*, *41*(8), 979-994. doi:10.1515/CCLM.2003.151
- Borel, P., Desmarchelier, C., Dumont, U., Halimi, C., Lairon, D., Page, D., . . . Rémond, D. (2016). Dietary calcium impairs tomato lycopene bioavailability in healthy humans. *British Journal of Nutrition*, *116*(12), 2091-2096. doi:10.1017/S0007114516004335
- Bornet, F. R., Cloarec, D., Barry, J. L., Colonna, P., Guillaud, S., Laval, J. D., & Galmiche, J. P. (1990). Pasta cooking time: Influence on starch digestion and plasma glucose and insulin responses in healthy subjects. *American Journal of Clinical Nutrition*, *51*(3), 421-427. doi:10.1093/ajcn/51.3.421
- Borrelli, G., De Leonardis, A., Fares, C., Platani, C., & Di Fonzo, N. (2003). Effects of modified processing conditions on oxidative properties of semolina dough and pasta. *Cereal Chemistry*, *80*(2), 225-231.
- Borrelli, G. M., De Leonardis, A. M., Platani, C., & Troccoli, A. (2008). Distribution along durum wheat kernel of the components involved in semolina colour. *Journal of Cereal Science*, *48*(2), 494-502.
- Bot, F., Verkerk, R., Mastwijk, H., Anese, M., Fogliano, V., & Capuano, E. (2018). The effect of pulsed electric fields on carotenoids bioaccessibility: The role of tomato matrix. *Food Chemistry*, *240*, 415-421. doi:10.1016/j.foodchem.2017.07.102
- Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25-30.
- Britton, G. (1995). Structure and properties of carotenoids in relation to function. *FASEB Journal*, *9*(15), 1551-1558.

- Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assuncao, R., Ballance, S., . . . Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991-1014. doi:10.1038/s41596-018-0119-1
- Brouns, F. (2012). Wheat aleurone: Separation, composition, health aspects, and potential food use. *Critical Reviews in Food Science and Nutrition.*, *52*(6), 553-568.
- Burešová, B., Paznocht, L., Jarošová, V., Doskočil, I., & Martinek, P. (2023). The effect of boiling and in vitro digestion on the carotenoid content of colored-grain wheat. *Journal of Food Composition and Analysis*, *115*, 105002. doi:https://doi.org/10.1016/j.jfca.2022.105002
- Burkhardt, S., & Bohm, V. (2007). Development of a new method for the complete extraction of carotenoids from cereals with special reference to durum wheat (*Triticum durum* Desf.). *Journal of Agricultural and Food Chemistry*, *55*(21), 8295-8301. doi:10.1021/jf0712853
- Canadian Grain Commission. (2023). *Quality of Canada Western Amber Durum*. Retrieved from <https://grainscanada.gc.ca/en/grain-research/grain-harvest-export-quality/wheat/2023/canada-western-amber-durum/>
- Cardoso, C., Afonso, C., Lourenço, H., Costa, S., & Nunes, M. L. (2015). Bioaccessibility assessment methodologies and their consequences for the risk–benefit evaluation of food. *Trends in Food Science and Technology*, *41*(1), 5-23. doi:10.1016/j.tifs.2014.08.008
- Castenmiller, J. J., & West, C. E. (1998). Bioavailability and bioconversion of carotenoids. *Annual Review of Nutrition*, *18*(1), 19-38. doi:10.1146/annurev.nutr.18.1.19
- Cazzonelli, C. I., Nisar, N., Hussain, D., Carmody, M. E., & Pogson, B. J. (2010). Biosynthesis and regulation of carotenoids in plants-Micronutrients, vitamins and health benefits. In E. C. Pua & M. R. Davey (Eds.), *Plant Developmental Biology - Biotechnological Perspectives* (pp. 117-137). Berlin, Heidelberg: Springer Berlin Heidelberg.

- Cereals Canada. (2023). *Canada's 2023 New wheat Crop Report*. Retrieved from <https://cerealscanada.ca/2023-wheat-crop/#in-review-shipping>
- Cervantes-Paz, B., Ornelas-Paz, J. J., Ruiz-Cruz, S., Rios-Velasco, C., Ibarra-Junquera, V., Yahia, E. M., & Gardea-Bejar, A. A. (2017). Effects of pectin on lipid digestion and possible implications for carotenoid bioavailability during pre-absorptive stages: A review. *Food Research International (Ottawa, Ont.)*, *99(Pt 2)*, 917-927. doi:10.1016/j.foodres.2017.02.012
- Chacon-Ordonez, T., Carle, R., & Schweiggert, R. (2019). Bioaccessibility of carotenoids from plant and animal foods. *Journal of the Science of Food and Agriculture*, *99(7)*, 3220-3239. doi:10.1002/jsfa.9525
- Chen, Z.-T., Chu, H.-L., Chyau, C.-C., Chu, C.-C., & Duh, P.-D. (2012). Protective effects of sweet orange (*Citrus sinensis*) peel and their bioactive compounds on oxidative stress. *Food Chemistry*, *135(4)*, 2119-2127. doi:<https://doi.org/10.1016/j.foodchem.2012.07.041>
- Chiremba, C., Pozniak, C. J., & Fu, B. X. (2015). Changes in semolina yellow pigment content and carotenoid composition during pasta processing. *Cereal Chemistry*, *92(6)*, 551-556. doi:<https://doi.org/10.1094/CCHEM-04-15-0065-R>
- Chitchumroonchokchai, C., & Failla, M. L. (2006). Hydrolysis of zeaxanthin esters by carboxyl ester lipase during digestion facilitates micellarization and uptake of the xanthophyll by Caco-2 human intestinal cells. *Journal of Nutrition*, *136(3)*, 588-594. doi:10.1093/jn/136.3.588
- Chitchumroonchokchai, C., Schwartz, S. J., & Failla, M. L. (2004). Assessment of lutein bioavailability from meals and a supplement using simulated digestion and Caco-2 human intestinal cells. *Journal of Nutrition*, *134(9)*, 2280-2286. doi:10.1093/jn/134.9.2280

- Ciccoritti, R., Scalfati, G., Cammerata, A., & Sgrulletta, D. (2011). Variations in content and extractability of durum wheat (*Triticum turgidum* L. var durum) arabinoxylans associated with genetic and environmental factors. *International Journal of Molecular Sciences*, *12*(7), 4536-4549. doi:10.3390/ijms12074536
- Cilla, A., Bosch, L., Barberá, R., & Alegría, A. (2018). Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols. *Journal of Food Composition and Analysis*, *68*, 3-15. doi:10.1016/j.jfca.2017.01.009
- Cilla, A., González-Sarrías, A., Tomás-Barberán, F. A., Espín, J. C., & Barberá, R. (2009). Availability of polyphenols in fruit beverages subjected to in vitro gastrointestinal digestion and their effects on proliferation, cell-cycle and apoptosis in human colon cancer Caco-2 cells. *Food Chemistry*, *114*(3), 813-820. doi:https://doi.org/10.1016/j.foodchem.2008.10.019
- Collado, L. S., & Corke, H. (2003). Starch properties and functionality. In G. Kaletunç & K. Breslauer (Eds.), *Characterization of Cereals and Flours: Properties, Analysis, and Applications* (Vol. 124). New York: Marcel Dekker Inc.
- Colle, I., Lemmens, L., Van Buggenhout, S., Van Loey, A., & Hendrickx, M. (2010). Effect of thermal processing on the degradation, isomerization, and bioaccessibility of lycopene in tomato pulp. *Journal of Food Science*, *75*(9), C753-759. doi:10.1111/j.1750-3841.2010.01862.x
- Colle, I., Van Buggenhout, S., Van Loey, A., & Hendrickx, M. (2010). High pressure homogenization followed by thermal processing of tomato pulp: Influence on

- microstructure and lycopene in vitro bioaccessibility. *Food Research International*, 43(8), 2193-2200. doi:<https://doi.org/10.1016/j.foodres.2010.07.029>
- Cooperstone, J. L., & Schwartz, S. J. (2016). Recent insights into health benefits of carotenoids. In R. Carle & R. M. Schweiggert (Eds.), *Handbook on Natural Pigments in Food and Beverages* (pp. 473-497). UK: Elsevier.
- Corte-Real, J., Bertucci, M., Soukoulis, C., Desmarchelier, C., Borel, P., Richling, E., . . . Bohn, T. (2017). Negative effects of divalent mineral cations on the bioaccessibility of carotenoids from plant food matrices and related physical properties of gastro-intestinal fluids. *Food and Function*, 8(3), 1008-1019. doi:10.1039/c6fo01708h
- Corte-Real, J., Desmarchelier, C., Borel, P., Richling, E., Hoffmann, L., & Bohn, T. (2018). Magnesium affects spinach carotenoid bioaccessibility in vitro depending on intestinal bile and pancreatic enzyme concentrations. *Food Chemistry*, 239, 751-759. doi:10.1016/j.foodchem.2017.06.147
- Darawsha, A., Trachtenberg, A., Levy, J., & Sharoni, Y. (2021). The protective effect of carotenoids, polyphenols, and estradiol on dermal fibroblasts under oxidative stress. *Antioxidants (Basel)*, 10(12), 2023. doi:10.3390/antiox10122023
- de la Peña, E., Wiesenborn, D. P., & Manthey, F. A. (2014). Pasta-to-water ratio, hotplate surface temperature, and beaker material affect pasta cooking time and cooked quality. *Cereal Chemistry*, 91(5), 489-495.
- de Oliveira, C. L., Brychkova, G., Esteves-Ferreira, A. A., McKeown, P., de Souza Gomes, M., Maluf, W. R., . . . Spillane, C. (2020). Thermal disruption of the food matrix of biofortified lettuce varieties modifies absorption of carotenoids by Caco-2 cells. *Food Chemistry*, 308, 125443-125443. doi:10.1016/j.foodchem.2019.125443

- De Santis, M. A., Giuliani, M. M., Giuzio, L., De Vita, P., Lovegrove, A., Shewry, P. R., & Flagella, Z. (2017). Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *European Journal of Agronomy*, *87*, 19-29. doi:<https://doi.org/10.1016/j.eja.2017.04.003>
- Desmarchelier, C., & Borel, P. (2017). Overview of carotenoid bioavailability determinants: From dietary factors to host genetic variations. *Trends in Food Science and Technology*, *69*, 270-280. doi:10.1016/j.tifs.2017.03.002
- Develaraja, S., Reddy, A., Yadav, M., Jain, S., & Yadav, H. (2016). Whole grains in amelioration of metabolic derangements. *Journal of Nutritional Health and Food Science*, *4(4)*, 1-11. doi:10.15226/jnhfs.2016.00173
- Dexter, J., Dronzek, B., & Matsuo, R. (1978). Scanning electron microscopy of cooked spaghetti. *Cereal Chemistry*, *55(1)*, 23-30.
- Dexter, J., Martin, D., & Matsuo, R. (1988). The effect of roll flute orientation on durum wheat experimental milling performance and semolina quality. *Canadian Institute of Food Science and Technology Journal*, *21(2)*, 187-193.
- Dexter, J., Matsuo, R., & Kruger, J. (1990). The spaghetti-making quality of commercial durum wheat samples with variable α -amylase activity. *Cereal Chemistry*, *67(405)*, 91-116.
- Dexter, J. E., & Matsuo, R. R. (1979). Effect of starch on pasta dough rheology and spaghetti cooking quality [Cereals]. *Cereal chemistry*, *56(3)*.
- Dexter, J. E., Matsuo, R. R., & Macgregor, A. W. (1985). Relationship of instrumental assessment of spaghetti cooking quality to the type and the amount of material rinsed from cooked spaghetti. *Journal of Cereal Science*, *3(1)*, 39-53. doi:[https://doi.org/10.1016/S0733-5210\(85\)80032-1](https://doi.org/10.1016/S0733-5210(85)80032-1)

- Dima, C., Assadpour, E., Dima, S., & Jafari, S. M. (2020). Bioavailability and bioaccessibility of food bioactive compounds; overview and assessment by in vitro methods. *Comprehensive Reviews in Food Science and Food Safety*, 19(6), 2862-2884. doi:<https://doi.org/10.1111/1541-4337.12623>
- Dupont, D., Alric, M., Blanquet-Diot, S., Bornhorst, G., Cueva, C., Deglaire, A., . . . Van den Abbeele, P. (2019). Can dynamic in vitro digestion systems mimic the physiological reality? *Critical Reviews in Food Science and Nutrition*, 59(10), 1546-1562. doi:10.1080/10408398.2017.1421900
- During, A., & Harrison, E. H. (2004). Intestinal absorption and metabolism of carotenoids: Insights from cell culture. *Archives of Biochemistry and Biophysics*, 430(1), 77-88. doi:10.1016/j.abb.2004.03.024
- During, A., Mahmood Hussain, M., Morel, D. W., & Harrison, E. H. (2002). Carotenoid uptake and secretion by Caco-2 cells: β -Carotene isomer selectivity and carotenoid interactions. *Journal of Lipid Research*, 43(7), 1086-1095. doi:10.1194/jlr.M200068-JLR200
- Dziki, D., & Laskowski, J. (2005). Evaluation of the cooking quality of spaghetti. *Polish Journal of Food and Nutrition Sciences*, 14(2), 153-157.
- Edge, R. (1997). The carotenoids as antioxidants - A review. *Journal of Photochemistry and Photobiology.*, 41(3), 189-200.
- Edge, R., & Truscott, T. G. (2018). Singlet oxygen and free radical reactions of retinoids and carotenoids-A review. *Antioxidants*, 7(1), 5.
- El-Agamey, A., Lowe, G. M., McGarvey, D. J., Mortensen, A., Phillip, D. M., Truscott, T. G., & Young, A. J. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties.

- Archives of Biochemistry and Biophysics*, 430(1), 37-48.
doi:<https://doi.org/10.1016/j.abb.2004.03.007>
- Eroglu, A., Al'Abri, I. S., Kopec, R. E., Crook, N., & Bohn, T. (2023). Carotenoids and their health benefits as derived via their interactions with gut microbiota. *Advances in Nutrition*, 14(2), 238-255. doi:10.1016/j.advnut.2022.10.007
- Eroglu, A., Wang, G., Crook, N., & Bohn, T. (2024). Carotenoids. *Advances in Nutrition (Bethesda, Md.)*, 15(11), 100304. doi:10.1016/j.advnut.2024.100304
- Etcheverry, P., Grusak, M. A., & Fleige, L. E. (2012). Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B(6), B(12), D, and E. *Frontiers in Physiology*, 3, 317-317. doi:10.3389/fphys.2012.00317
- Evers, A. D., O'Brien, L., & Blakeney, A. B. (1999). Cereal structure and composition. *Australian Journal of Agricultural Research*, 50(5), 629-650. doi:10.1071/ar98158
- Evers, T., & Millar, S. (2002). Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science*, 36(3), 261-284. doi:<https://doi.org/10.1006/jcrs.2002.0435>
- Failla, M. L., Chitchumroonchokchai, C., & Ishida, B. K. (2008). In vitro micellarization and intestinal cell uptake of cis isomers of lycopene exceed those of all-trans lycopene¹². *The Journal of Nutrition*, 138(3), 482-486. doi:<https://doi.org/10.1093/jn/138.3.482>
- Fares, C., Codianni, P., Nigro, F., Platani, C., Scazzina, F., & Pellegrini, N. (2008). Processing and cooking effects on chemical, nutritional and functional properties of pasta obtained from selected emmer genotypes. *Journal of the Science of Food and Agriculture*, 88(14), 2435-2444. doi:10.1002/jsfa.3350

- Fares, C., Platani, C., Baiano, A., & Menga, V. (2010). Effect of processing and cooking on phenolic acid profile and antioxidant capacity of durum wheat pasta enriched with debranning fractions of wheat. *Food Chemistry*, *119*(3), 1023-1029. doi:10.1016/j.foodchem.2009.08.006
- Faulks, R. M., & Southon, S. (2005). Challenges to understanding and measuring carotenoid bioavailability. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1740*(2), 95-100. doi:10.1016/j.bbadis.2004.11.012
- Feillet, P., & Dexter, J. (1996). Quality requirements of durum wheat for semolina milling and pasta production. In J. E. Kruger, R. B. Matsuo, & J. W. Dick (Eds.), *Pasta and Noodle Technology* (pp. 95-131). St. Paul, MN, USA: AACC-American Association of Cereal Chemists.
- Fernández-García, E., Carvajal-Lérida, I., Jarén-Galán, M., Garrido-Fernández, J., Pérez-Gálvez, A., & Hornero-Méndez, D. (2012). Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. *Food Research International (Ottawa, Ont.)*, *46*(2), 438-450. doi:https://doi.org/10.1016/j.foodres.2011.06.007
- Fernandez-Garcia, E., Carvajal-Lerida, I., & Perez-Galvez, A. (2009). In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research*, *29*(11), 751-760. doi:10.1016/j.nutres.2009.09.016
- Ferruzzi, M. G., Kruger, J., Mohamedshah, Z., Debelo, H., & Taylor, J. R. N. (2020). Insights from in vitro exploration of factors influencing iron, zinc and provitamin A carotenoid bioaccessibility and intestinal absorption from cereals. *Journal of Cereal Science*, *96*, 103126. doi:https://doi.org/10.1016/j.jcs.2020.103126

- Ferruzzi, M. G., Lumpkin, J. L., Schwartz, S. J., & Failla, M. (2006). Digestive stability, micellarization, and uptake of β -carotene isomers by Caco-2 human intestinal cells. *Journal of Agricultural and Food Chemistry*, *54*(7), 2780-2785. doi:10.1021/jf0530603
- Ficco, D. B. M., Mastrangelo, A. M., Trono, D., Borrelli, G. M., De Vita, P., Fares, C., . . . Papa, R. (2014). The colours of durum wheat: A review. *Crop and Pasture Science*, *65*(1), 1-15. doi:<https://doi.org/10.1071/CP13293>
- Ficco, D. B. M., Prandi, B., Amaretti, A., Anfelli, I., Leonardi, A., Raimondi, S., . . . Rossi, M. (2019). Comparison of gluten peptides and potential prebiotic carbohydrates in old and modern *Triticum turgidum* ssp. genotypes. *Food Research International*, *120*, 568-576. doi:<https://doi.org/10.1016/j.foodres.2018.11.007>
- Fincher, G. B. (2016). Cereals: Chemistry and physicochemistry of non-starchy polysaccharides. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains* (Second Edition) (pp. 208-223). Oxford: Academic Press.
- Fu, B.X., Schlichting, L., Pozniak, C., & Singh, A. (2013). Pigment loss from semolina to dough: Rapid measurement and relationship with pasta colour. *Journal of Cereal Science*, *57*(3), 560-566.
- Fu, B. X., Chiremba, C., Pozniak, C. J., Wang, K., & Nam, S. (2017). Total phenolic and yellow pigment contents and antioxidant activities of durum wheat milling fractions. *Antioxidants*, *6*(4), 78. doi:10.3390/antiox6040078
- Fu, B. X., Wang, K., Dupuis, B., Taylor, D., & Nam, S. (2018). Kernel vitreousness and protein content: Relationship, interaction and synergistic effects on durum wheat quality. *Journal of Cereal Science*, *79*, 210-217. doi:<https://doi.org/10.1016/j.jcs.2017.09.003>

- Garrett, D. A., Failla, M. L., & Sarama, R. J. (1999). Development of an in vitro digestion method to assess carotenoid bioavailability from meals. *Journal of Agricultural and Food Chemistry*, *47*(10), 4301-4309.
- Garrett, D. A., Failla, M. L., Sarama, R. J., & Craft, N. (1999). Accumulation and retention of micellar β -carotene and lutein by Caco-2 human intestinal cells. *The Journal of Nutritional Biochemistry*, *10*(10), 573-581.
- Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Lin, R. (2009). The effect of simulated digestion in vitro on bioactivity of wheat bread with Tartary buckwheat flavones addition. *LWT - Food Science and Technology*, *42*(1), 137-143. doi:<https://doi.org/10.1016/j.lwt.2008.06.009>
- Gliwa, J., Gunenc, A., Ames, N., Willmore, W. G., & Hosseinian, F. S. (2011). Antioxidant activity of alkylresorcinols from rye bran and their protective effects on cell viability of PC-12 AC Cells. *Journal of Agricultural and Food Chemistry*, *59*(21), 11473-11482. doi:10.1021/jf2023353
- Goltz, S. R., Campbell, W. W., Chitchumroonchokchai, C., Failla, M. L., & Ferruzzi, M. G. (2012). Meal triacylglycerol profile modulates postprandial absorption of carotenoids in humans. *Molecular Nutrition and Food Research*, *56*(6), 866-877. doi:10.1002/mnfr.201100687
- Gómez, M., Gutkoski, L. C., & Bravo-Núñez, Á. (2020). Understanding whole-wheat flour and its effect in breads: A review. *Comprehensive Reviews in Food Science and Food Safety*, 1-25. doi:DOI: 10.1111/1541-4337.12625
- Gorusupudi, A., & Baskaran, V. (2013). Wheat germ oil: A potential facilitator to improve lutein bioavailability in mice. *Nutrition*, *29*(5), 790-795. doi:<https://doi.org/10.1016/j.nut.2012.11.003>

- Granado-Lorencio, F., Blanco-Navarro, I., Pérez-Sacristán, B., & Hernández-Álvarez, E. (2017). Biomarkers of carotenoid bioavailability. *Food Research International*, 99, 902-916. doi:<https://doi.org/10.1016/j.foodres.2017.03.036>
- Granado-Lorencio, F., Olmedilla-Alonso, B., Herrero-Barbudo, C., Pérez-Sacristán, B., Blanco-Navarro, I., & Blázquez-García, S. (2007). Comparative in vitro bioaccessibility of carotenoids from relevant contributors to carotenoid intake. *Journal of Agricultural and Food Chemistry*, 55(15), 6387-6394. doi:10.1021/jf070301t
- Granfeldt, Y., & Björck, I. (1991). Glycemic response to starch in pasta: A study of mechanisms of limited enzyme availability. *Journal of Cereal Science*, 14(1), 47-61.
- Grundas, S., & Wrigley, C. W. (2016). Ultrastructure of the wheat grain, flour, and dough. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains* (Second Edition) (pp. 384-395). Oxford: Academic Press.
- Harrison, E. H. (2012). Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1821(1), 70-77. doi:10.1016/j.bbalip.2011.06.002
- Hempel, J., Schädle, C. N., Leptihn, S., Carle, R., & Schweiggert, R. M. (2016). Structure related aggregation behavior of carotenoids and carotenoid esters. *Journal of Photochemistry and Photobiology A: Chemistry*, 317, 161-174. doi:<https://doi.org/10.1016/j.jphotochem.2015.10.024>
- Hempel, J., Schädle, C. N., Sprenger, J., Heller, A., Carle, R., & Schweiggert, R. M. (2017). Ultrastructural deposition forms and bioaccessibility of carotenoids and carotenoid esters from goji berries (*Lycium barbarum* L.). *Food Chemistry*, 218, 525-533. doi:10.1016/j.foodchem.2016.09.065

- Hentschel, V., Kranl, K., Hollmann, J., Lindhauer, M. G., Böhm, V., & Bitsch, R. (2002). Spectrophotometric determination of yellow pigment content and evaluation of carotenoids by high-performance liquid chromatography in durum wheat grain. *Journal of Agricultural and Food Chemistry*, *50*(23), 6663-6668. doi:10.1021/jf025701p
- Hidalgo, A., Brandolini, A., & Pompei, C. (2010). Carotenoids evolution during pasta, bread and water biscuit preparation from wheat flours. *Food Chemistry*, *121*(3), 746-751. doi:https://doi.org/10.1016/j.foodchem.2010.01.034
- Hidalgo, A., Brandolini, A., Pompei, C., & Piscozzi, R. (2006). Carotenoids and tocopherols of einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.). *Journal of Cereal Science*, *44*(2), 182-193. doi:https://doi.org/10.1016/j.jcs.2006.06.002
- Hossain, A., & Jayadeep, A. (2021). Infrared heating induced improvement of certain phytochemicals, their bioaccessible contents and bioaccessibility in maize. *Food Science and Technology*, *142*, 110912. doi:10.1016/j.lwt.2021.110912
- Hubatsch, I., Ragnarsson, E. G. E., & Artursson, P. (2007). Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nature Protocols*, *2*, 2111+.
- Huo, T., Ferruzzi, M. G., Schwartz, S. J., & Failla, M. L. (2007). Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids. *Journal of Agricultural and Food Chemistry*, *55*(22), 8950-8957. doi:10.1021/jf071687a
- Iddir, M., Dingemans, G., Porras Yaruro, J. F., Hammaz, F., Borel, P., Schlee, T., . . . Bohn, T. (2020). Influence of soy and whey protein, gelatin and sodium caseinate on carotenoid bioaccessibility. *Food and Function*, *11*(6), 5446-5459. doi:10.1039/d0fo00888e

- Iddir, M., Pittois, D., Guignard, C., Weber, B., Gantenbein, M., Larondelle, Y., & Bohn, T. (2021). Whey- and soy protein isolates added to a carrot-tomato juice alter carotenoid bioavailability in healthy adults. *Antioxidants*, *10(11)*, 1748. doi:10.3390/antiox10111748
- Iddir, M., Vahid, F., Merten, D., Larondelle, Y., & Bohn, T. (2022). Influence of proteins on the absorption of lipophilic vitamins, carotenoids and curcumin – A Review. *Molecular Nutrition and Food Research*, *66(13)*, 2200076. doi:https://doi.org/10.1002/mnfr.202200076
- Igrejas, G., Ikeda, T. M., & Guzmán, C. (2020). Wheat quality for improving processing and human health (1st 2020. ed.). Cham: Springer International Publishing.
- Izydorczyk, M. S., & Biliaderis, C. G. (1995). Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydrate Polymers*, *28(1)*, 33-48. doi:https://doi.org/10.1016/0144-8617(95)00077-1
- Jayasinghe, M., Jeddi, D., Paziuk, B., & Erkinbaev, C. (2023). Effect of infrared treatment on chemical and structural quality of soybean: Quantitative three-dimensional characterization using x-ray microtomography. *Food and Bioprocess Technology*, *17*, 2830-2841. doi:10.1007/s11947-023-03289-6
- Johnson, E. J. (2004). A biological role of lutein. *Food Reviews International*, *20(1)*, 1-16. doi:10.1081/FRI-120028826
- Kadkol, G. P., & Sissons, M. (2016). Durum wheat: Overview. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains* (Second ed., pp. 117-124). Oxford: Academic Press.
- Kalungwana, N. A., Marshall, L., Mackie, A., & Boesch, C. (2023). An ex vivo intestinal absorption model is more effective than an in vitro cell model to characterise absorption of

- dietary carotenoids following simulated gastrointestinal digestion. *Food Research International (Ottawa, Ont.)*, 166, 112558-112558. doi:10.1016/j.foodres.2023.112558
- Kaplan Evlice, A. (2022). The effect of durum wheat genotypes on cooking quality of pasta. *European Food Research and Technology*, 248(3), 815-824. doi:10.1007/s00217-021-03899-4
- Kean, E. G., Bordenave, N., Ejeta, G., Hamaker, B. R., & Ferruzzi, M. G. (2011). Carotenoid bioaccessibility from whole grain and decorticated yellow endosperm sorghum porridge. *Journal of Cereal Science*, 54(3), 450-459. doi:https://doi.org/10.1016/j.jcs.2011.08.010
- Kean, E. G., Hamaker, B. R., & Ferruzzi, M. G. (2008). Carotenoid bioaccessibility from whole grain and degermed maize meal products. *Journal of Agricultural and Food Chemistry*, 56(21), 9918-9926. doi:10.1021/jf8018613
- Kellett, M. E., Greenspan, P., & Pegg, R. B. (2018). Modification of the cellular antioxidant activity (CAA) assay to study phenolic antioxidants in a Caco-2 cell line. *Food Chemistry*, 244, 359-363. doi:https://doi.org/10.1016/j.foodchem.2017.10.035
- Khachik, F., Beecher, G. R., Goli, M. B., & Lusby, W. R. (1991). Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *Pure and Applied Chemistry*, 63(1), 71-80. doi:10.1351/pac199163010071
- Khachik, F., Englert, G., Beecher, G. R., & Cecil Smith, J. (1995). Isolation, structural elucidation, and partial synthesis of lutein dehydration products in extracts from human plasma. *Journal of Chromatography B: Biomedical Sciences and Applications*, 670(2), 219-233. doi:https://doi.org/10.1016/0378-4347(95)00169-7

- Kopec, R. E., & Failla, M. L. (2018). Recent advances in the bioaccessibility and bioavailability of carotenoids and effects of other dietary lipophiles. *Journal of Food Composition and Analysis*, *68*, 16-30. doi:<https://doi.org/10.1016/j.jfca.2017.06.008>
- Kotake-Nara, E., Hase, M., Hoshina, R., Hidan, M., & Kobayashi, H. (2022). Effect of an emulsified formulation on vegetable carotenoid bioaccessibility. *Journal of Oleo Science*, *71(1)*, 135-140. doi:10.5650/jos.ess21265
- Kotake-Nara, E., & Nagao, A. (2011). Absorption and metabolism of xanthophylls. *Marine Drugs*, *9(6)*, 1024-1037. doi:10.3390/md9061024
- Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, *26(6)*, 459-516. doi:10.1016/j.mam.2005.10.001
- Lafiandra, D., Masci, S., Sissons, M., Dornez, E., Delcour, J. A., Courtin, C. M., & Caboni, M. F. (2012). Kernel components of technological value. In M. Sissons, J. Abecassis, B. Marchylo, & M. Carcea (Eds.), *Durum Wheat : Chemistry and Technology* (Second ed., pp. 91-98). St. Paul, MN: American Assoc. of Cereal Chemists.
- Lauer, L. A., Mieres-Castro, D., Schmalle, V., Frank, J., & Jiménez-Aspee, F. (2024). Cytoprotective effects of iridoids from *Gaultheria* spp. berries in gastric and intestinal epithelial cells after in vitro digestion. *Food Bioscience*, *57*, 103587. doi:<https://doi.org/10.1016/j.fbio.2024.103587>
- Lemmens, L., Buggenhout, S. V., Oey, I., Loey, A. V., & Hendrickx, M. (2009). Towards a better understanding of the relationship between the β -carotene in vitro bioaccessibility and pectin structural changes: A case study on carrots. *Food Research International*, *42(9)*, 1323-1330. doi:10.1016/j.foodres.2009.04.006

- Lemmens, L., Colle, I., Van Buggenhout, S., Palmero, P., Van Loey, A., & Hendrickx, M. (2014). Carotenoid bioaccessibility in fruit-and vegetable-based food products as affected by product (micro) structural characteristics and the presence of lipids: A review. *Trends in Food Science and Technology*, *38*(2), 125-135.
- Li, L., Niu, Y., Ruan, Y., DePauw, R. M., Singh, A. K., & Gan, Y. (2018). Agronomic advancement in tillage, crop rotation, soil health, and genetic gain in durum wheat cultivation: A 17-Year Canadian Story. *Agronomy*, *8*(9). doi:10.3390/agronomy8090193
- Li, M., Ndiaye, C., Corbin, S., Foegeding, E. A., & Ferruzzi, M. G. (2020). Starch-phenolic complexes are built on physical CH- π interactions and can persist after hydrothermal treatments altering hydrodynamic radius and digestibility of model starch-based foods. *Food Chemistry*, *308*, 125577. doi:https://doi.org/10.1016/j.foodchem.2019.125577
- Liu, C. Y., Shepherd, K. W., & Rathjen, A. J. (1996). Improvement of durum wheat pastamaking and breadmaking qualities. *Cereal Chemistry*, *73*(2), 155-166.
- Liu, J., Bi, J., Liu, X., Zhang, B., Wu, X., Wellala, C. K. D., & Zhang, B. (2019). Effects of high pressure homogenization and addition of oil on the carotenoid bioaccessibility of carrot juice. *Food and Function*, *10*(1), 458-468. doi:10.1039/C8FO01925H
- Lowe, G. M., Booth, L. A., Young, A. J., & Bilton, R. F. (1999). Lycopene and β -carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses. *Free Radical Research*, *30*(2), 141-151. doi:10.1080/10715769900300151
- Maiani, G., Periago Castón, M. J., Catasta, G., Toti, E., Cambrodón, I. G., Bysted, A., . . . Valoti, M. (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition and Food Research*, *53*(S2), S194-S218.

- Manthey, F. A., & Schorno, A. L. (2002). Physical and cooking quality of spaghetti made from whole wheat durum. *Cereal Chemistry*, 79(4), 504-510.
- Marchetti, N., Bonetti, G., Brandolini, V., Cavazzini, A., Maietti, A., Meca, G., & Mañes, J. (2018). Stinging nettle (*Urtica dioica* L.) as a functional food additive in egg pasta: Enrichment and bioaccessibility of lutein and β -carotene. *Journal of Functional Foods*, 47, 547-553. doi:10.1016/j.jff.2018.05.062
- Marchylo, B. A., & Dexter, J. E. (2001). Pasta production. In O. Gavin (Ed.), *Cereals Processing Technology* (pp. 109-130). Cambridge, England: Woodhead Publishing Limited.
- Marcotuli, I., Colasuonno, P., Hsieh, Y. S. Y., Fincher, G. B., & Gadaleta, A. (2020). Non-starch polysaccharides in durum wheat: A review. *International Journal of Molecular Sciences*, 21(8). doi:10.3390/ijms21082933
- Marcotuli, I., Hsieh, Y. S. Y., Lahnstein, J., Yap, K., Burton, R. A., Blanco, A., . . . Gadaleta, A. (2016). Structural variation and content of arabinoxylans in endosperm and bran of durum wheat (*Triticum turgidum* L.). *Journal of Agricultural and Food Chemistry*, 64(14), 2883-2892. doi:10.1021/acs.jafc.6b00103
- Marti, A., Pagani, M. A., & Seetharaman, K. (2014). Textural attributes of wheat and gluten free pasta. *Food Texture Design and Optimization*, 4, 222-224.
- Martinez, C. S., Ribotta, P. D., Leon, A. E., & Anon, M. C. (2007). Physical, sensory and chemical evaluation of cooked spaghetti. *Journal of Texture Studies*, 38(6), 666-683.
- Martini, D., Taddei, F., Ciccoritti, R., Pasquini, M., Nicoletti, I., Corradini, D., & D'Egidio, M. G. (2015). Variation of total antioxidant activity and of phenolic acid, total phenolics and yellow coloured pigments in durum wheat (*Triticum turgidum* L. var. durum) as a function

- of genotype, crop year and growing area. *Journal of Cereal Science*, 65, 175-185.
doi:<https://doi.org/10.1016/j.jcs.2015.06.012>
- Mateo Anson, N., Havenaar, R., Bast, A., & Haenen, G. R. M. M. (2010). Antioxidant and anti-inflammatory capacity of bioaccessible compounds from wheat fractions after gastrointestinal digestion. *Journal of Cereal Science*, 51(1), 110-114.
doi:<https://doi.org/10.1016/j.jcs.2009.10.005>
- Matsuo, R. R., Dexter, J. E., Boudreau, A., & Daun, J. K. (1986). The role of lipids in determining spaghetti cooking quality. *Cereal Chemistry*, 63(6), 484-489.
- McClements, D. J., Zou, L., Zhang, R., Salvia-Trujillo, L., Kumosani, T., & Xiao, H. (2015). Enhancing nutraceutical performance using excipient foods: Designing food structures and compositions to increase bioavailability. *Comprehensive Reviews in Food Science and Food Safety*, 14(6), 824-847. doi:<https://doi.org/10.1111/1541-4337.12170>
- Mellado-Ortega, E., & Hornero-Mendez, D. (2017). Lutein esterification in wheat flour increases the carotenoid retention and is induced by storage temperatures. *Foods*, 6(12), 111.
doi:10.3390/foods6120111
- Mellado-Ortega, E., & Hornero-Méndez, D. (2015). Carotenoids in cereals: An ancient resource with present and future applications. *Phytochemistry Reviews*, 14(6), 873-890.
- Mercadante, A. Z., Rodrigues, D. B., Petry, F. C., & Mariutti, L. R. B. (2017). Carotenoid esters in foods - A review and practical directions on analysis and occurrence. *Food Research International*, 99(Pt 2), 830-850. doi:10.1016/j.foodres.2016.12.018
- Miller, N. J., Sampson, J., Candeias, L. P., Bramley, P. M., & Rice-Evans, C. A. (1996). Antioxidant activities of carotenes and xanthophylls. *FEBS Letters*, 384(3), 240-242.

- Minekus, M., Alming, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., . . . Dupont, D. (2014). A standardised static in vitro digestion method suitable for food—An international consensus. *Food and Function*, *5*(6), 1113-1124.
- Monsierra, L., Mansilla, P. S., & Pérez, G. T. (2024). Whole flour of purple maize as a functional ingredient of gluten-free bread: Effect of in vitro digestion on starch and bioaccessibility of bioactive compounds. *Foods*, *13*(2). doi:10.3390/foods13020194
- Moran, N. E., Mohn, E. S., Hason, N., Erdman, J. W., & Johnson, E. J. (2018). Intrinsic and extrinsic factors impacting absorption, metabolism, and health effects of dietary carotenoids. *Advances in Nutrition (Bethesda, Md.)*, *9*(4), 465-492. doi:10.1093/advances/nmy025
- Mueller, L., & Boehm, V. (2011). Antioxidant activity of β -carotene compounds in different in vitro assays. *Molecules (Basel, Switzerland)*, *16*(2), 1055-1069. doi:10.3390/molecules16021055
- Nagao, A., Kotake-Nara, E., & Hase, M. (2013). Effects of fats and oils on the bioaccessibility of carotenoids and vitamin E in vegetables. *Bioscience Biotechnology and Biochemistry*, *77*(5), 1055-1060. doi:10.1271/bbb.130025
- Naparło, K., Soszyński, M., Bartosz, G., & Sadowska-Bartos, I. (2020). Comparison of antioxidants: The limited correlation between various assays of antioxidant activity. *Molecules (Basel, Switzerland)*, *25*(14), 3244. doi:10.3390/molecules25143244
- Ndolo, V. U., & Beta, T. (2013). Distribution of carotenoids in endosperm, germ, and aleurone fractions of cereal grain kernels. *Food Chemistry*, *139*(1-4), 663-671. doi:10.1016/j.foodchem.2013.01.014

- Ngamwonglumlert, L., & Devahastin, S. (2019). Carotenoids. In *Encyclopedia of Food Chemistry* (pp. 40-52).
- Ngamwonglumlert, L., Devahastin, S., Chiewchan, N., & Raghavan, V. (2020). Plant carotenoids evolution during cultivation, postharvest storage, and food processing: A review. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1561-1604. doi:10.1111/1541-4337.12564
- Nguyen, M., Francis, D., & Schwartz, S. (2001). Thermal isomerisation susceptibility of carotenoids in different tomato varieties. *Journal of the Science of Food and Agriculture*, 81(9), 910-917. doi:10.1002/jsfa.911
- Nwachukwu, I. D., Udenigwe, C. C., & Aluko, R. E. (2016). Lutein and zeaxanthin: Production technology, bioavailability, mechanisms of action, visual function, and health claim status. *Trends in Food Science and Technology*, 49, 74-84.
- O'Brien, N. M. M. (2008). Micellarisation of carotenoids from raw and cooked vegetables. *Plant Foods for Human Nutrition*, 63(3), 127-133.
- O'Connell, O. F., Ryan, L., & O'Brien, N. M. (2007). Xanthophyll carotenoids are more bioaccessible from fruits than dark green vegetables. *Nutrition Research (New York, N.Y.)*, 27(5), 258-264. doi:10.1016/j.nutres.2007.04.002
- O'Sullivan, L., Ryan, L., & O'Brien, N. (2007). Comparison of the uptake and secretion of carotene and xanthophyll carotenoids by Caco-2 intestinal cells. *British Journal of Nutrition*, 98(1), 38-44. doi:10.1017/S000711450769446X
- O'Hearn, M., Lara-Castor, L., Cudhea, F., Miller, V., Reedy, J., Shi, P., . . . Global Dietary, D. (2023). Incident type 2 diabetes attributable to suboptimal diet in 184 countries. *Nature Medicine*, 29(4), 982-995. doi:10.1038/s41591-023-02278-8

- O'Sullivan, L., Galvin, K., Aisling Aherne, S., & O'Brien, N. M. (2010). Effects of cooking on the profile and micellarization of 9- cis-, 13- cis- and all- trans- β -carotene in green vegetables. *Food Research International*, 43(4), 1130-1135. doi:10.1016/j.foodres.2010.02.012
- Oduro-Obeng, H., Apea-Bah, F. B., Wang, K., Fu, B. X., & Beta, T. (2022). Effect of cooking duration on carotenoid content, digestion and potential absorption efficiencies among refined semolina and whole wheat pasta products. *Food and Function*, 13(11), 5953-5970. doi:10.1039/D2FO00611A
- Oduro-Obeng, H., Chaudhry, M. M. A., Zogona, D., Paliwal, J., Fu, B. X., & Beta, T. (2024). Carotenoid cellular uptake and antioxidant activity from differently cooked semolina and whole wheat pasta. *Journal of Cereal Science*, 120, 104037. doi:10.1016/j.jcs.2024.104037
- Oduro-Obeng, H., Fu, B. X., & Beta, T. (2021). Influence of cooking duration on carotenoids, physical properties and in vitro antioxidant capacity of pasta prepared from three Canadian durum wheat cultivars. *Food Chemistry*, 363, 130016.
- Ornelas-Paz, J. D. J., Failla, M. L., Yahia, E. M., & Gardea-Bejar, A. (2008). Impact of the stage of ripening and dietary fat on in vitro bioaccessibility of β -carotene in 'Ataulfo' mango. *Journal of Agricultural and Food Chemistry*, 56(4), 1511-1516. doi:10.1021/jf072751r
- Palafox-Carlos, H., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science*, 76(1), R6-R15. doi:https://doi.org/10.1111/j.1750-3841.2010.01957.x
- Palmero, P., Colle, I., Lemmens, L., Panozzo, A., Nguyen, T. T. M., Hendrickx, M., & Van Loey, A. (2016). Enzymatic cell wall degradation of high-pressure-homogenized tomato puree

- and its effect on lycopene bioaccessibility. *Journal of the Science of Food and Agriculture*, 96(1), 254-261. doi:10.1002/jsfa.7088
- Palmero, P., Lemmens, L., Hendrickx, M., & Van Loey, A. (2014). Role of carotenoid type on the effect of thermal processing on bioaccessibility. *Food Chemistry*, 157, 275-282. doi:10.1016/j.foodchem.2014.02.055
- Panfili, G., Fratianni, A., & Irano, M. (2004). Improved normal-phase high-performance liquid chromatography procedure for the determination of carotenoids in cereals. *Journal of Agricultural and Food Chemistry*, 52(21), 6373-6377.
- Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. *Journal of Food Science*, 72(2), R21-R32. doi:https://doi.org/10.1111/j.1750-3841.2007.00274.x
- Payne, A. N., Zihler, A., Chassard, C., & Lacroix, C. (2012). Advances and perspectives in in vitro human gut fermentation modeling. *Trends in Biotechnology (Regular ed.)*, 30(1), 17-25. doi:10.1016/j.tibtech.2011.06.011
- Petitot, M., Abecassis, J., & Micard, V. (2009). Structuring of pasta components during processing: Impact on starch and protein digestibility and allergenicity. *Trends in Food Science and Technology*, 20(11-12), 521-532. doi:10.1016/j.tifs.2009.06.005
- Petry, F. C., & Mercadante, A. Z. (2017). Impact of in vitro digestion phases on the stability and bioaccessibility of carotenoids and their esters in mandarin pulps. *Food and Function*, 8(11), 3951-3963. doi:10.1039/C7FO01075C
- Podio, N. S., Baroni, M. V., Pérez, G. T., & Wunderlin, D. A. (2019). Assessment of bioactive compounds and their in vitro bioaccessibility in whole-wheat flour pasta. *Food Chemistry*, 293, 408-417. doi:https://doi.org/10.1016/j.foodchem.2019.04.117

- Poulaert, M., Borel, P., Caporiccio, B., Gunata, Z., & Dhuique-Mayer, C. (2012). Grapefruit juices impair the bioaccessibility of β -carotene from orange-fleshed sweet potato but not its intestinal uptake by Caco-2 cells. *Journal of Agricultural and Food Chemistry*, *60*(2), 685-691. doi:10.1021/jf204004c
- Pozniak, C., & Clarke, J. (2016). CDC Precision durum wheat. *Canadian Journal of Plant Science*, *97*(2), 344-348.
- Pronin, D., Börner, A., Weber, H., & Scherf, K. A. (2020). Wheat (*Triticum aestivum* L.) breeding from 1891 to 2010 contributed to increasing yield and glutenin contents but decreasing protein and gliadin contents. *Journal of Agricultural and Food Chemistry*, *68*(46), 13247-13256. doi:10.1021/acs.jafc.0c02815
- Ramachandran, A., Pozniak, C. J., Clarke, J. M., & Singh, A. K. (2010). Carotenoid accumulation during grain development in durum wheat. *Journal of Cereal Science*, *52*(1), 30-38. doi:10.1016/j.jcs.2010.02.014
- Ramachandran, R. P., Erkinbaev, C., Thakur, S., & Paliwal, J. (2021). Three dimensional characterization of micronized soybean seeds using x-ray microtomography. *Food and Bioproducts Processing*, *127*, 388-397. doi:10.1016/j.fbp.2021.03.007
- Read, A., Wright, A., & Abdel-Aal, E.-S. M. (2015). In vitro bioaccessibility and monolayer uptake of lutein from wholegrain baked foods. *Food Chemistry*, *174*, 263-269. doi:https://doi.org/10.1016/j.foodchem.2014.11.074
- Reboul, E., & Borel, P. (2011). Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. *Progress in Lipid Research*, *50*(4), 388-402. doi:10.1016/j.plipres.2011.07.001

- Reboul, E., Richelle, M., Perrot, E., Desmoulins-Malezet, C., Pirisi, V., & Borel, P. (2006). Bioaccessibility of carotenoids and vitamin E from their main dietary sources. *Journal of Agricultural and Food Chemistry*, *54*(23), 8749-8755. doi:10.1021/jf061818s
- Reboul, E., Thap, S., Tourniaire, F., André, M., Juhel, C., Morange, S., . . . Borel, P. (2007). Differential effect of dietary antioxidant classes (carotenoids, polyphenols, vitamins C and E) on lutein absorption. *British Journal of Nutrition*, *97*(3), 440-446. doi:10.1017/S0007114507352604
- Requena-Ramírez, M. D., Hornero-Méndez, D., Rodríguez-Suárez, C., & Atienza, S. G. (2021). Durum Wheat (*Triticum durum* L.) landraces reveal potential for the improvement of grain carotenoid esterification in breeding programs. *Foods*, *10*(4), 757. doi:10.3390/foods10040757
- Rodrigues, D. B., Mariutti, L. R. B., & Mercadante, A. Z. (2016). An in vitro digestion method adapted for carotenoids and carotenoid esters: Moving forward towards standardization. *Food and Function*, *7*(12), 4992-5001.
- Rodrigues, E., Mariutti, L. R., Chiste, R. C., & Mercadante, A. Z. (2012). Development of a novel micro-assay for evaluation of peroxy radical scavenger capacity: Application to carotenoids and structure-activity relationship. *Food Chemistry*, *135*(3), 2103-2111. doi:10.1016/j.foodchem.2012.06.074
- Rodriguez-Amaya, D. B. (2016). Food carotenoids: Chemistry, biology, and technology. Chichester, West Sussex, UK ;: John Wiley and Sons, Ltd.
- Rodriguez-Roque, M. J., de Ancos, B., Sanchez-Vega, R., Sanchez-Moreno, C., Cano, M. P., Elez-Martinez, P., & Martin-Belloso, O. (2016). Food matrix and processing influence on

- carotenoid bioaccessibility and lipophilic antioxidant activity of fruit juice-based beverages. *Food and Function*, 7(1), 380-389. doi:10.1039/c5fo01060h
- Ross, A. B., Vuong, L. T., Ruckle, J., Synal, H. A., Schulze-König, T., Wertz, K., . . . Williamson, G. (2011). Lycopene bioavailability and metabolism in humans: An accelerator mass spectrometry study. *American Journal of Clinical Nutrition*, 93(6), 1263-1273. doi:10.3945/ajcn.110.008375
- Rošul, M., Đerić, N., Mišan, A., Pojić, M., Šimurina, O., Halimi, C., . . . Reboul, E. (2022). Bioaccessibility and uptake by Caco-2 cells of carotenoids from cereal-based products enriched with butternut squash (*Cucurbita moschata* L.). *Food Chemistry*, 385, 132595. doi:https://doi.org/10.1016/j.foodchem.2022.132595
- Ryan, L., O'connell, O., O'Sullivan, L., Aherne, S., & O'Brien, N. M. (2008). Micellarisation of carotenoids from raw and cooked vegetables. *Plant Foods for Human Nutrition*, 63(3), 127-133.
- Sabouri-Rad, S., Oskuee, R. K., Mahmoodi, A., Gholami, L., & Malaekheh-Nikouei, B. (2017). The effect of cell penetrating peptides on transfection activity and cytotoxicity of polyallylamine. *BioImpacts : BI*, 7(3), 139-145. doi:10.15171/bi.2017.17
- Saenz, E., Borrás, L., & Gerde, J. A. (2021). Carotenoid profiles in maize genotypes with contrasting kernel hardness. *Journal of Cereal Science*, 99, 103206.
- Saura-Calixto, F., & Pérez-Jiménez, J. (2018). Non-extractable polyphenols and carotenoids: Importance in human nutrition and health (1st ed. Vol. 5). La Vergne: Royal Society of Chemistry.

- Schieber, A., & Carle, R. (2005). Occurrence of carotenoid cis-isomers in food: Technological, analytical, and nutritional implications. *Trends in Food Science and Technology*, *16*(9), 416-422. doi:<https://doi.org/10.1016/j.tifs.2005.03.018>
- Schoeman, L., Williams, P., du Plessis, A., & Manley, M. (2016). X-ray micro-computed tomography (μ CT) for non-destructive characterisation of food microstructure. *Trends in Food Science and Technology* *47*, 10-24. doi:10.1016/j.tifs.2015.10.016
- Schweiggert, R. M., & Carle, R. (2017). Carotenoid deposition in plant and animal foods and its impact on bioavailability. *Critical Reviews in Food Science and Nutrition*, *57*(9), 1807-1830. doi:10.1080/10408398.2015.1012756
- Schweiggert, R. M., Mezger, D., Schimpf, F., Steingass, C. B., & Carle, R. (2012). Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato. *Food Chemistry*, *135*(4), 2736-2742. doi:<https://doi.org/10.1016/j.foodchem.2012.07.035>
- Scott, G., & Awika, J. M. (2023). Effect of protein–starch interactions on starch retrogradation and implications for food product quality. *Comprehensive Reviews in Food Science and Food Safety*, *22*(3), 2081-2111. doi:10.1111/1541-4337.13141
- Sensoy, I. (2014). A Review on the relationship between food structure, processing, and bioavailability. *Critical Reviews in Food Science and Nutrition*, *54*(7), 902-909. doi:10.1080/10408398.2011.619016
- Shahidi, F., & Pan, Y. (2022). Influence of food matrix and food processing on the chemical interaction and bioaccessibility of dietary phytochemicals: A review. *Critical Reviews in Food Science and Nutrition*, *62*(23), 6421-6445. doi:10.1080/10408398.2021.1901650

- Shewry, P., Kosik, O., Pellny, T., & Lovegrove, A. (2020). Wheat cell wall polysaccharides (dietary fibre). In G. Igrejas, T. M. Ikeda, & C. Guzmán (Eds.), *Wheat Quality for Improving Processing and Human Health* (pp. 255-272). Cham: Springer International Publishing.
- Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, *60*(6), 1537-1553.
doi:10.1093/jxb/erp058
- Shewry, P. R. (2024). Can we increase the use of wheat and other cereals as sources of protein? *Journal of Cereal Science*, *117*, 103899. doi:https://doi.org/10.1016/j.jcs.2024.103899
- Sicignano, A., Di Monaco, R., Masi, P., & Cavella, S. (2015). From raw material to dish: Pasta quality step by step. *Journal of the Science of Food and Agriculture*, *95*(13), 2579-2587.
doi:10.1002/jsfa.7176
- Singh, A. K., Clarke, J. M., Knox, R. E., DePauw, R. M., McCaig, T. N., Fernandez, M. R., & Clarke, F. R. (2012). Transcend durum wheat. *Canadian Journal of Plant Science*, *92*(4), 809-813.
- Singh, A. K., DePauw, R. M., Knox, R., Clarke, J. M., McCaig, T. N., Cuthbert, R. D., & Ruan, Y. (2016). AAC Spitfire durum wheat. *Canadian Journal of Plant Science*, *97*(1), 157-164.
- Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: A review. *Trends in Food Science and Technology*, *21*(4), 168-180.
doi:https://doi.org/10.1016/j.tifs.2009.12.001
- Sissons, M., Aravind, N., & Fellows, C. M. (2010). Quality of fiber-enriched spaghetti containing microbial transglutaminase. *Cereal Chemistry*, *87*(1), 57-64.

- Sissons, M., Schlichting, L., Egan, N., Aarts, W., Harden, S., & Marchylo, B. (2008). A standardized method for the instrumental determination of cooked spaghetti firmness. *Cereal Chemistry*, *85*(3), 440-444.
- Sissons, M. J., Soh, H. N., & Turner, M. A. (2007). Role of gluten and its components in influencing durum wheat dough properties and spaghetti cooking quality. *Journal of the Science of Food and Agriculture*, *87*(10), 1874-1885. doi:<https://doi.org/10.1002/jsfa.2915>
- Soh, H. N., Sissons, M. J., & Turner, M. A. (2006). Effect of starch granule size distribution and elevated amylose content on durum dough rheology and spaghetti cooking quality. *Cereal Chemistry*, *83*(5), 513-519. doi:10.1094/CC-83-0513
- Song, W., Derito, C. M., Liu, M. K., He, X., Dong, M., & Liu, R. H. (2010). Cellular antioxidant activity of common vegetables. *Journal of Agricultural and Food Chemistry*, *58*(11), 6621-6629. doi:10.1021/jf9035832
- Song, Y., Ndolo, V., Fu, B. X., Ames, N., Katundu, M., & Beta, T. (2021). Effect of processing on bioaccessibility of carotenoids from orange maize products. *International Journal of Food Science & Technology*, *56*(7), 3299-3310. doi:10.1111/ijfs.15038
- Soulaka, A. B., & Morrison, W. R. (1985). The amylose and lipid contents, dimensions, and gelatinisation characteristics of some wheat starches and their A-and B-granule fractions. *Journal of the Science of Food and Agriculture*, *36*(8), 709-718.
- Stahl, W., & Sies, H. (1992). Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *Journal of Nutrition*, *122*(11), 2161-2166.

- Sun, T., Yuan, H., Cao, H., Yazdani, M., Tadmor, Y., & Li, L. (2018). Carotenoid metabolism in plants: The role of plastids. *Molecular Plant*, *11*(1), 58-74. doi:<https://doi.org/10.1016/j.molp.2017.09.010>
- Svelander, C. A., Tibäck, E. A., Ahrné, L. M., Langton, M. I. B. C., Svanberg, U. S. O., & Alminger, M. A. G. (2010). Processing of tomato: Impact on in vitro bioaccessibility of lycopene and textural properties. *Journal of the Science of Food and Agriculture*, *90*(10), 1665-1672. doi:<https://doi.org/10.1002/jsfa.4000>
- Sy, C., Gleize, B., Dangles, O., Landrier, J.-F., Veyrat, C. C., & Borel, P. (2012). Effects of physicochemical properties of carotenoids on their bioaccessibility, intestinal cell uptake, and blood and tissue concentrations. *Molecular Nutrition and Food Research*, *56*(9), 1385-1397. doi:<https://doi.org/10.1002/mnfr.201200041>
- Tan, C., Xue, J., Lou, X., Abbas, S., Guan, Y., Feng, B., . . . Xia, S. (2014). Liposomes as delivery systems for carotenoids: Comparative studies of loading ability, storage stability and in vitro release. *Food and Function* *5*(6), 1232-1240. doi:[10.1039/c3fo60498e](https://doi.org/10.1039/c3fo60498e)
- Thakkar, S. K., & Failla, M. L. (2008). Bioaccessibility of pro-vitamin A carotenoids is minimally affected by non pro-vitamin A xanthophylls in maize (*Zea mays* sp.). *Journal of Agricultural and Food Chemistry*, *56*(23), 11441-11446. doi:[10.1021/jf802430u](https://doi.org/10.1021/jf802430u)
- Thakkar, S. K., Maziya-Dixon, B., Dixon, A. G. O., & Failla, M. L. (2007). β -Carotene micellarization during in vitro digestion and uptake by Caco-2 cells is directly proportional to β -carotene content in different genotypes of cassava. *The Journal of Nutrition*, *137*(10), 2229-2233.

- Thakur, N., Raigond, P., Singh, Y., Mishra, T., Singh, B., Lal, M. K., & Dutt, S. (2020). Recent updates on bioaccessibility of phytonutrients. *Trends in Food Science and Technology*, *97*, 366-380. doi:<https://doi.org/10.1016/j.tifs.2020.01.019>
- Tian, W., Zheng, Y., Wang, W., Wang, D., Tilley, M., Zhang, G., . . . Li, Y. (2022). A comprehensive review of wheat phytochemicals: From farm to fork and beyond. *Comprehensive Reviews in Food Science and Food Safety*, *21*(3), 2274-2308. doi:<https://doi.org/10.1111/1541-4337.12960>
- Tibäck, E. A., Svelander, C. A., Colle, I. J. P., Altskär, A. I., Alminger, M. A. G., Hendrickx, M. E. G., . . . Langton, M. I. B. C. (2009). Mechanical and thermal pretreatments of crushed tomatoes: Effects on consistency and in vitro accessibility of lycopene. *Journal of Food Science*, *74*(7), E386-E395. doi:<https://doi.org/10.1111/j.1750-3841.2009.01255.x>
- Tokusoglu, O., & Hall, C. (2011). *Fruit and cereal bioactives : Sources, chemistry, and applications* (1st ed.). Boca Raton: CRC Press.
- Tydeman, E. A., Parker, M. L., Wickham, M. S. J., Rich, G. T., Faulks, R. M., Gidley, M. J., . . . Waldron, K. W. (2010). Effect of carrot (*Daucus carota*) microstructure on carotene bioaccessibility in the upper gastrointestinal tract. 1. In vitro simulations of carrot digestion. *Journal of Agricultural and Food Chemistry*, *58*(17), 9847-9854. doi:10.1021/jf101034a
- Tyssandier, V., Reboul, E., Dumas, J. F., Bouteloup-Demange, C., Armand, M., Marcand, J., . . . Borel, P. (2003). Processing of vegetable-borne carotenoids in the human stomach and duodenum. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *284*(6), G913-923. doi:10.1152/ajpgi.00410.2002
- Unlu, N. Z., Bohn, T., Francis, D. M., Nagaraja, H. N., Clinton, S. K., & Schwartz, S. J. (2007). Lycopene from heat-induced cis-isomer-rich tomato sauce is more bioavailable than from

- all-trans-rich tomato sauce in human subjects. *British Journal of Nutrition*, 98(1), 140-146.
doi:10.1017/S0007114507685201
- Updike, A. A., & Schwartz, S. J. (2003). Thermal processing of vegetables increases cis isomers of lutein and zeaxanthin. *Journal of Agricultural and Food Chemistry*, 51(21), 6184-6190.
- van het Hof, K. H., Brouwer, I. A., West, C. E., Haddeman, E., Steegers-Theunissen, R. P. M., van Dusseldorp, M., . . . Hautvast, J. (1999). Bioavailability of lutein from vegetables is 5 times higher than that of β -carotene. *American Journal of Clinical Nutrition*, 70(2), 261-268.
- van Het Hof, K. H., West, C. E., Weststrate, J. A., & Hautvast, J. G. (2000). Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition*, 130(3), 503-506.
doi:10.1093/jn/130.3.503
- Vansteelandt, J., & Delcour, J. A. (1998). Physical behavior of durum wheat starch (*Triticum durum*) during industrial pasta processing. *Journal of Agricultural and Food Chemistry*, 46(7), 2499-2503. doi:10.1021/jf9710692
- Veda, S., Platel, K., & Srinivasan, K. (2007). Varietal differences in the bioaccessibility of β -carotene from mango (*Mangifera indica*) and papaya (*Carica papaya*) fruits. *Journal of Agricultural and Food Chemistry*, 55(19), 7931-7935. doi:10.1021/jf0712604
- Victoria-Campos, C. I., Ornelas-Paz Jde, J., Yahia, E. M., & Failla, M. L. (2013). Effect of the interaction of heat-processing style and fat type on the micellarization of lipid-soluble pigments from green and red pungent peppers (*Capsicum annum*). *Journal of Agricultural and Food Chemistry*, 61(15), 3642-3653. doi:10.1021/jf3054559
- Victoria-Campos, C. I., Ornelas-Paz, J. d. J., Yahia, E. M., Jiménez-Castro, J. A., Cervantes-Paz, B., Ibarra-Junquera, V., . . . Escalante-Minakata, P. (2013). Effect of ripening, heat

- processing, and fat type on the micellarization of pigments from jalapeño peppers. *Journal of Agricultural and Food Chemistry*, 61(41), 9938-9949. doi:10.1021/jf4032124
- Vignola, M. B., Bustos, M. C., & Pérez, G. T. (2018). Comparison of quality attributes of refined and whole wheat extruded pasta. *LWT*, 89, 329-335.
- von Lintig, J., Moon, J., Lee, J., & Ramkumar, S. (2020). Carotenoid metabolism at the intestinal barrier. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1865(11), 158580-158580. doi:10.1016/j.bbalip.2019.158580
- Wang, C., Berg, C. J., Hsu, C.-C., Merrill, B. A., & Tauber, M. J. (2012). Characterization of carotenoid aggregates by steady-state optical spectroscopy. *The Journal of Physical Chemistry B*, 116(35), 10617-10630. doi:10.1021/jp3069514
- Wang, K., Pozniak, C. J., Ruan, Y., & Fu, B. X. (2023). Unveiling the impact of durum wheat protein quantity and quality on textural properties and microstructure of cooked pasta. *Cereal Chemistry*, 100(2), 484-499. doi:10.1002/cche.10627
- Wang, X., Cui, Y.-j., Qi, J., Zhu, M.-m., Zhang, T.-l., Cheng, M., . . . Wang, G.-c. (2018). Fucoxanthin exerts cytoprotective effects against hydrogen peroxide-induced oxidative damage in L02 cells. *BioMed Research International*, 1085073. doi:10.1155/2018/1085073
- Wellala, C. K. D., Bi, J., Liu, X., Wu, X., Lyu, J., Liu, J., . . . Guo, C. (2022). Effect of high pressure homogenization on water-soluble pectin characteristics and bioaccessibility of carotenoids in mixed juice. *Food Chemistry*, 371, 131073. doi:https://doi.org/10.1016/j.foodchem.2021.131073

- Werner, S., & Böhm, V. (2011). Bioaccessibility of carotenoids and vitamin E from pasta: Evaluation of an in vitro digestion model. *Journal of Agricultural and Food Chemistry*, *59*(4), 1163-1170.
- Wolfe, K. L., Kang, X., He, X., Dong, M., Zhang, Q., & Liu, R. H. (2008). Cellular antioxidant activity of common fruits. *Journal of Agricultural and Food Chemistry*, *56*(18), 8418-8426. doi:10.1021/jf801381y
- Wolfe, K. L., & Liu, R. H. (2007). Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. *Journal of Agricultural and Food Chemistry*, *55*(22), 8896-8907. doi:10.1021/jf0715166
- Wood, J., Batey, I., Hare, R., & Sissons, M. (2001). A comparison of Australian and imported spaghetti. *Food Australia*, *53*(8), 349-352.
- Wright, A. J., Pietrangelo, C., & MacNaughton, A. (2008). Influence of simulated upper intestinal parameters on the efficiency of beta-carotene micellarisation using an in vitro model of digestion. *Food Chemistry*, *107*(3), 1253-1260. doi:https://doi.org/10.1016/j.foodchem.2007.09.063
- Wrigley, C., Batey, I. L., & Miskelly, D. (2017). *Cereal grains : Assessing and managing quality*. Cambridge, UK: Elsevier Science & Technology.
- Xavier, A. A. O., & Mercadante, A. Z. (2019). The bioaccessibility of carotenoids impacts the design of functional foods. *Current Opinion in Food Science*, *26*, 1-8. doi:https://doi.org/10.1016/j.cofs.2019.02.015
- Xiao, Y., Nie, M., Zhao, H., Li, D., Gao, R., Zhou, C., . . . Zhang, Z. (2021). Citrus flavanones enhance the bioaccessibility of β -carotene by improving lipid lipolysis and incorporation

- into mixed micelles. *Journal of Functional Foods*, 87, 104792.
doi:<https://doi.org/10.1016/j.jff.2021.104792>
- Xu, Y., Shrestha, N., Pr at, V., & Beloqui, A. (2021). An overview of in vitro, ex vivo and in vivo models for studying the transport of drugs across intestinal barriers. *Advanced Drug Delivery Reviews*, 175, 113795. doi:<https://doi.org/10.1016/j.addr.2021.05.005>
- Yan, X., Huang, J., Huang, L., Luo, C., Li, Z., Xu, P., . . . Tan, K. (2024). Effects of dietary lipids on bioaccessibility and bioavailability of natural carotenoids. *LWT*, 200, 116171. doi:10.1016/j.lwt.2024.116171
- Yang, C., Fischer, M., Kirby, C., Liu, R., Zhu, H., Zhang, H., . . . Tsao, R. (2018). Bioaccessibility, cellular uptake and transport of luteins and assessment of their antioxidant activities. *Food Chemistry*, 249, 66-76. doi:10.1016/j.foodchem.2017.12.055
- Yang, C., Zhang, H., Liu, R., Zhu, H., Zhang, L., & Tsao, R. (2017). Bioaccessibility, cellular uptake, and transport of astaxanthin isomers and their antioxidative effects in human intestinal epithelial Caco-2 cells. *Journal of Agricultural and Food Chemistry*, 65(47), 10223-10232. doi:10.1021/acs.jafc.7b04254
- Yao, K., McClements, D. J., Yan, C., Xiao, J., Liu, H., Chen, Z., . . . Liu, X. (2021). In vitro and in vivo study of the enhancement of carotenoid bioavailability in vegetables using excipient nanoemulsions: Impact of lipid content. *Food Research International*, 141, 110162-110162. doi:10.1016/j.foodres.2021.110162
- Yonekura, L., & Nagao, A. (2007). Intestinal absorption of dietary carotenoids. *Molecular Nutrition and Food Research*, 51(1), 107-115. doi:<https://doi.org/10.1002/mnfr.200600145>

- Young, A. J., & Lowe, G. M. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics*, *385*(1), 20-27. doi:10.1006/abbi.2000.2149
- Zaripheh, S., & Erdman, J. W., Jr. (2002). Factors that influence the bioavailability of xanthophylls. *The Journal of Nutrition*, *132*(3), 531S-534S. doi:10.1093/jn/132.3.531S
- Zhang, G., Qi, X., He, L., Wang, X., Zhao, Y., Wang, Q., . . . Liu, M. (2024). Non-covalent complexes of lutein/zeaxanthin and whey protein isolate formed at different pH levels: Binding interactions, storage stabilities, and bioaccessibilities. *Current Research in Food Science*, *8*, 100778. doi:https://doi.org/10.1016/j.crfs.2024.100778
- Zhang, S., Ji, J., Zhang, S., Guan, C., & Wang, G. (2020). Effects of three cooking methods on content changes and absorption efficiencies of carotenoids in maize. *Food and Function*, *11*(1), 944-954. doi:10.1039/c9fo02622c
- Zhang, Y., Li, R., Xu, Z., Fan, H., Xu, X., Pan, S., & Liu, F. (2024). Recent advances in the effects of food microstructure and matrix components on the bioaccessibility of carotenoids. *Trends in Food Science and Technology*, *143*, 104301. doi:https://doi.org/10.1016/j.tifs.2023.104301
- Zhang, Y., Wu, H., Wen, H., Fang, H., Hong, Z., Yi, R., & Liu, R. (2015). Simultaneous determination of fucoxanthin and its deacetylated metabolite fucoxanthinol in rat plasma by liquid chromatography-tandem mass spectrometry. *Marine Drugs*, *13*(10), 6521-6536. doi:10.3390/md13106521
- Zhang, Z., Nie, M., Xiao, Y., Zhu, L., Gao, R., Zhou, C., . . . Dai, Z. (2021). Positive effects of ultrasound pretreatment on the bioaccessibility and cellular uptake of bioactive compounds from broccoli: Effect on cell wall, cellular matrix and digesta. *Food Science and Technology*, *149*, 112052. doi:10.1016/j.lwt.2021.112052

- Ziegler, J. U., Schweiggert, R. M., & Carle, R. (2015). A method for the simultaneous extraction and quantitation of lipophilic antioxidants in *Triticum* sp. by HPLC-DAD/FLD-MSn. *Journal of Food Composition and Analysis*, 39, 94-102. doi:<https://doi.org/10.1016/j.jfca.2014.11.011>
- Zou, W., Sissons, M., Gidley, M. J., Gilbert, R. G., & Warren, F. J. (2015). Combined techniques for characterising pasta structure reveals how the gluten network slows enzymic digestion rate. *Food Chemistry*, 188, 559-568. doi:<https://doi.org/10.1016/j.foodchem.2015.05.032>
- Zurak, D., Gunjević, V., Grbeša, D., Svečnjak, Z., Kralik, Z., Košević, M., . . . Kljak, K. (2024). Kernel properties related to carotenoid release during in vitro gastrointestinal digestion in commercial dent maize hybrids. *Food Chemistry*, 435, 137535. doi:<https://doi.org/10.1016/j.foodchem.2023.137535>

APPENDICES

Appendix 1: Durum wheat grains, whole wheat flour, refined semolina and their respective pasta samples

Representation of durum wheat grains



Whole wheat flour



Refined semolina



Whole wheat pasta samples

Refined semolina pasta samples



Whole Wheat Samples



Pasta cooking process

cooked pasta samples

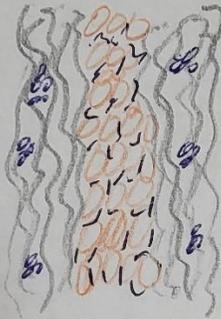


Schematic Representation of cooked pasta samples showing starch and protein modifications

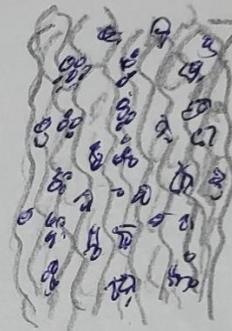
Al-cooked



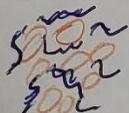
FCI-cooked



OC



 — Intact Starch granules

 Protein matrix surrounding starch granules

 gelatinized starch granules

 Aggregated protein matrix

Appendix 2: Table S3.1. Carotenoid content in durum wheat flour and dry pasta products

Flour type	Wheat variety	Carotenoid content (mg/kg dry weight)					
		all-trans	all-trans	9-cis	13-cis	13'-cis	Total
		Lutein	Zeaxanthin	lutein	lutein	lutein	carotenoid
Raw flour							
Semolina	AAC Spitfire	11.22±0.07 ^a	0.50±0.15 ^{bcd}	1.02±0.08 ^a	1.25±0.08 ^a	0.95±0.03 ^a	14.94±0.27 ^a
	CDC Precision	9.96±0.71 ^{ab}	0.26±0.00 ^d	0.91±0.07 ^a	1.09±0.16 ^{abcd}	0.88±0.08 ^{abc}	13.12±0.40 ^{abc}
	Transcend	8.45±0.46 ^{bc}	0.40±0.08 ^{cd}	0.90±0.04 ^a	1.14±0.03 ^{abc}	0.90±0.04 ^{abc}	11.80±0.63 ^{bcd}
Whole wheat flour	AAC Spitfire	10.03±0.68 ^{ab}	1.19±0.27 ^a	0.91±0.00 ^a	1.12±0.10 ^{abc}	0.88±0.07 ^{abc}	14.13±1.12 ^{ab}
	CDC Precision	8.82±1.14 ^{bc}	0.97±0.18 ^{ab}	0.90±0.03 ^a	1.19±0.02 ^{ab}	0.92±0.00 ^{ab}	12.80±1.36 ^{abc}
	Transcend	8.21±0.40 ^{bcd}	1.04±0.02 ^a	0.93±0.02 ^a	1.20±0.02 ^{ab}	0.94±0.00 ^a	12.33±0.37 ^{bcd}
Dry pasta							
Semolina	AAC Spitfire	7.76±0.13 ^{cd}	0.33±0.02 ^d	1.00±0.02 ^a	0.97±0.01 ^{bcd}	0.83±0.01 ^{abc}	10.88±0.17 ^{cd}
	CDC Precision	8.13±0.14 ^{bcd}	0.23±0.02 ^d	1.04±0.03 ^a	1.00±0.03 ^{bcd}	0.84±0.03 ^{abc}	11.24±0.13 ^{cd}
	Transcend	7.34±0.10 ^{cd}	0.20±0.00 ^d	1.01±0.04 ^a	0.94±0.03 ^{cd}	0.79±0.00 ^{bc}	10.29±0.04 ^d
Whole wheat flour	AAC Spitfire	7.49±0.26 ^{cd}	0.92±0.16 ^{ab}	0.98±0.03 ^a	1.11±0.01 ^{abcd}	0.94±0.03 ^a	11.44±0.49 ^{cd}
	CDC Precision	7.65±0.17 ^{cd}	0.88±0.13 ^{abc}	0.94±0.01 ^a	0.88±0.02 ^d	0.77±0.02 ^c	11.12±0.26 ^{cd}
	Transcend	6.35±0.14 ^d	0.91±0.02 ^{ab}	0.96±0.02 ^a	0.95±0.01 ^{cd}	0.81±0.01 ^{abc}	9.98±0.18 ^d

Values represent mean± SD; n=4. Different letters indicate significant (P<0.05) differences in carotenoid content between semolina and whole wheat flours of the three durum wheat varieties and their dry pasta products within individual carotenoid compound and total carotenoids. LOD and LOQ for lutein are 0.213 µg/ml and 0.642 µg/ml respectively and zeaxanthin (LOD=0.041 µg/ml; LOQ=0.124 µg/ml).

Appendix 3: Table S3.2: ANOVA showing significant main effects and interactions for the evaluation of carotenoids, physical properties, and antioxidant activity of durum wheat pasta for the effects of durum variety (V), milling flour type (F) and cooking duration (C)

Property	V	F	C	VxF	VxC	FxC	VxFxC
Carotenoids							
<i>all-trans lutein</i>	*	NS	NS	NS	*	NS	NS
<i>all-trans zeaxanthin</i>	*	*	*	*	NS	NS	*
<i>9-cis lutein</i>	NS	*	*	NS	NS	NS	NS
<i>13-cis lutein</i>	NS	NS	*	NS	NS	NS	NS
<i>13'-cis lutein</i>	NS	NS	*	NS	NS	NS	NS
Total carotenoids	*	NS	*	NS	*	NS	NS
Apparent retention (%)	*	*	*	*	*	*	*
Cooking quality							
% Water absorption	*	*	*	*	*	NS	*
% Cooking loss	NS	*	*	NS	NS	*	NS
Texture							
Firmness	*	*	*	*	*	*	*
Colour of cooked pasta							
b*	*	*	*	*	*	*	*
Antioxidant							
ABTS	*	*	*	*	*	NS	*

NS: no significant effect (P>0.05); significant at * P<0.05

Appendix 4: Table S3.3. Significant interaction effect between wheat variety and cooking duration on total carotenoids (mg/kg dry weight) in cooked pasta (averaged over milling flour type).

Wheat variety	Cooking duration ^a		
	Al	FCT	OC
AAC Spitfire	8.29±0.21 ^{abc}	8.47±0.14 ^{ab}	8.47±0.14 ^{ab}
CDC Precision	7.17±0.64 ^d	8.25±0.60 ^{abcd}	8.92±0.04 ^a
Transcend	7.28±0.39 ^{cd}	7.70±0.48 ^{bcd}	8.44±0.32 ^{ab}

All values are means ± SD of three determinations. Data with the same lower-case superscripts are not significantly different ($p>0.05$). ^aCooking duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta.

Appendix 5: Table S3.4. Significant interaction between flour type and cooking duration on cooking loss (%) of cooked pasta (averaged over wheat variety).

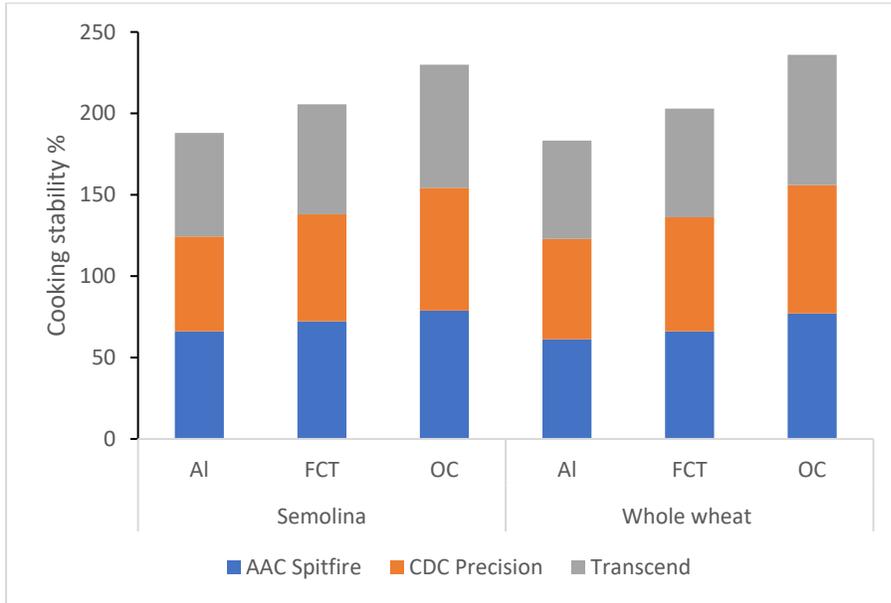
Flour type	Cooking duration ^a		
	Al	FCT	OC
Whole wheat flour	5.60 ^d	7.26 ^b	8.78 ^a
Refined semolina	4.34 ^e	5.57 ^d	6.64 ^c

Values with the same lower-case superscripts are not significantly different ($p > 0.05$). ^aCooking

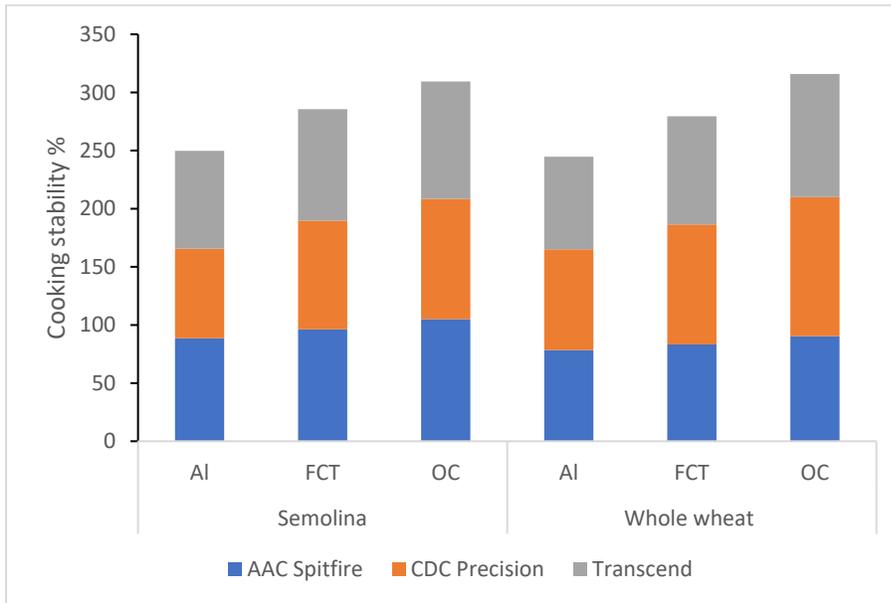
duration: Al, al dente; FCT, fully cooked time; OC, overcooked pasta.

Appendix 6: Figure S3.1. Influence of cooking duration on cooking stability of individual carotenoid species

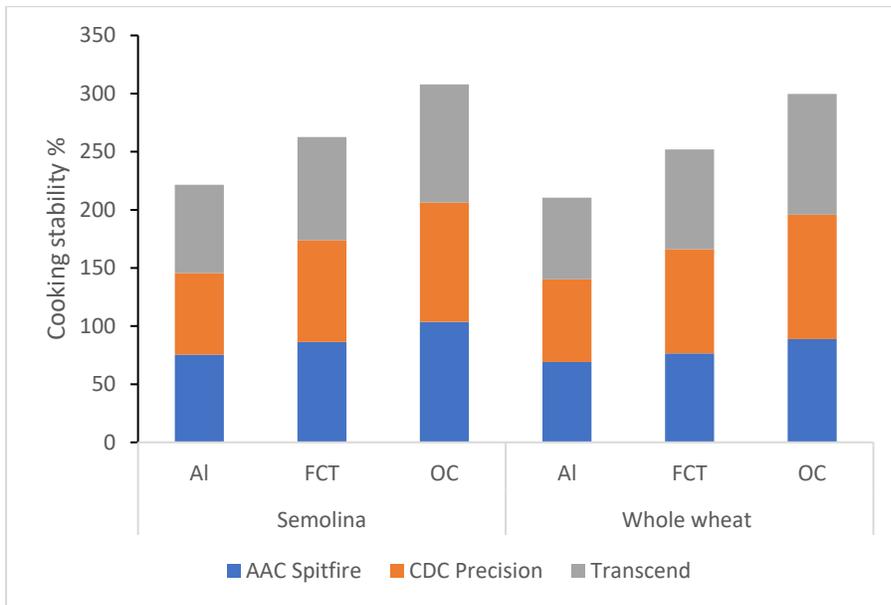
(a)



(b)



(c)



(d)

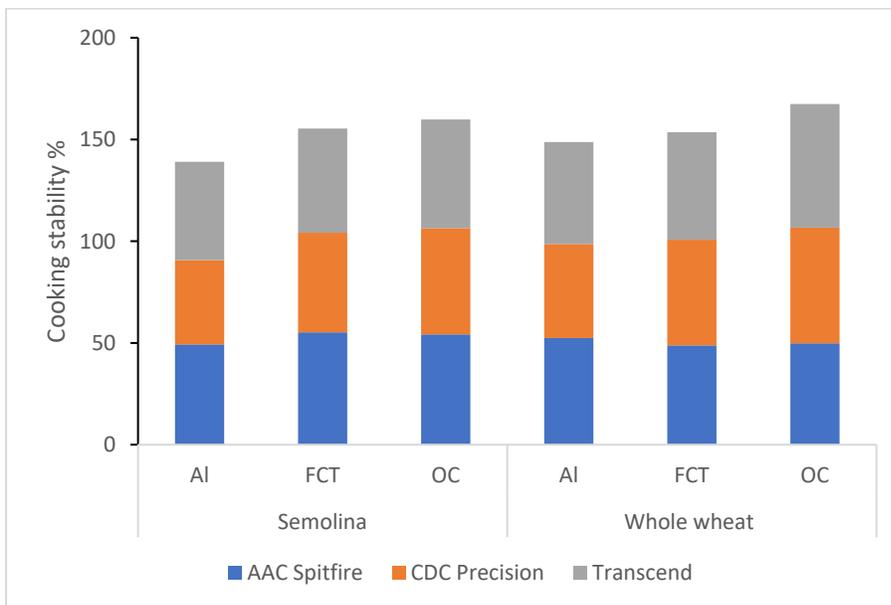


Figure S3.1. Influence of cooking duration on cooking stability of individual carotenoid species and total carotenoids: **A**, 9-cis lutein; **B**, 13-cis lutein; **C**, 13'-cis lutein; **D**, total carotenoids. Cooking duration: AI, al dente; FCT, fully cooked time; OC, overcooked pasta

Appendix 7: Figure S3.2. Representative HPLC separation of carotenoid isomers

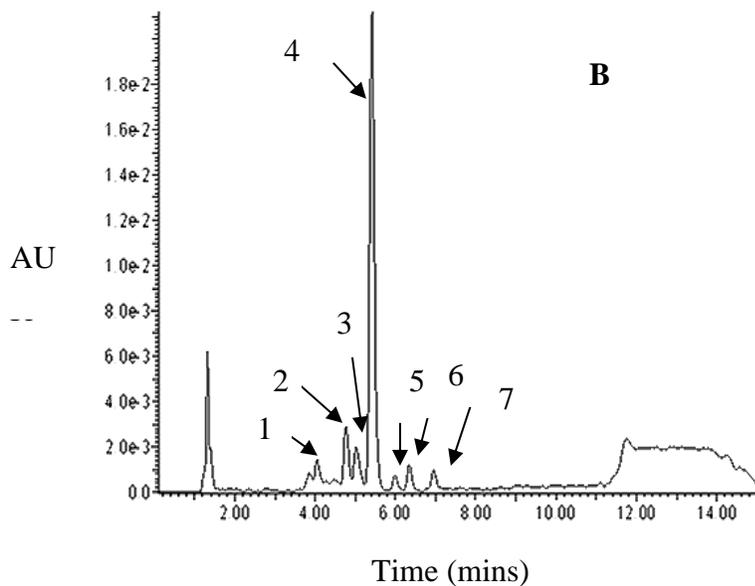
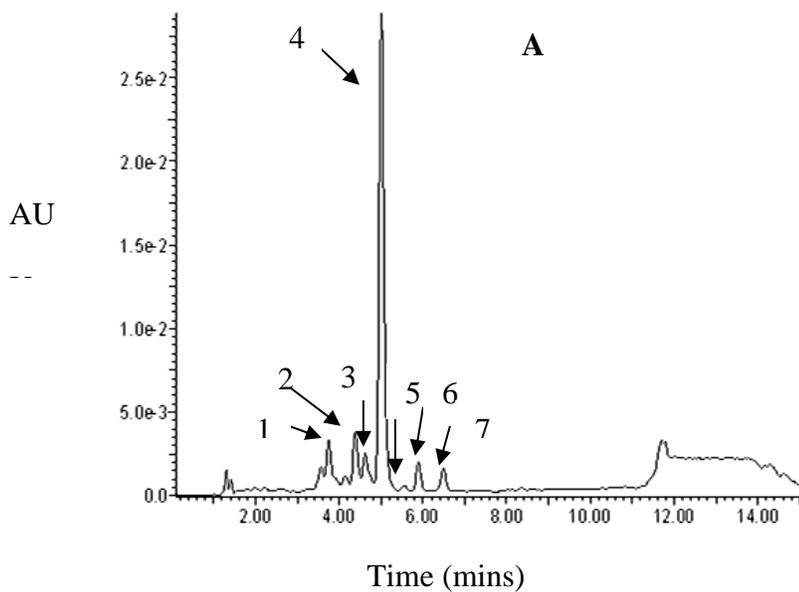


Figure S3.2. Representative HPLC separation of carotenoid isomers from extracts of AAC Spitfire-produced semolina pasta cooked to al dente (A) and AAC Spitfire-produced whole wheat

pasta cooked to al dente (**B**). Carotenoid analysis was completed as described under materials and methods using an S-3 carotenoid column (4.6 x 100 mm) with 3 μ m packing. Peaks: 1, (15-cis)-lutein; 2, (13-cis)-lutein; 3, (13'-cis)-lutein; 4, (all-trans)-lutein; 5, (all-trans)-zeaxanthin; 6, (9-cis)-lutein; 7, (9'-cis)-lutein.

Appendix 8: Table S4.1. Significance of wheat cultivar, cooking duration and their interactions on the concentrations of all- trans carotenoids (mg/100g dry weight) in undigested and digested refined semolina (RS) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)			Filtrate	Residue	Bioaccessibility (%)
				Oral	Oral+ Gastric	Oral + Gastric + Intestinal	Oral Stage	Oral +Gastric Stage	Oral +Gastric+ intestinal stage			
All- trans Lutein	AAC Spitfire	AI	0.60b	0.40c	0.49a	0.88a	66.64bc	83.32a	147.11a	0.98d	0.21c	160.30ab
		FCT	0.85ab	0.48bc	0.52a	0.71a	59.57bc	61.32a	89.97ab	0.96d	0.26c	105.80bc
		OC	0.86ab	0.66abc	0.43a	0.92a	79.61abc	50.22a	107.43ab	0.94d	0.28bc	107.27bc
	CDC Precision	AI	0.74ab	0.66ab	0.52a	0.74a	89.97ab	71.03a	98.62ab	1.55ab	0.27bc	204.96a
		FCT	0.91a	0.68ab	0.49a	0.82a	75.44abc	53.71a	93.19ab	1.60a	0.41a	169.40ab
		OC	0.96a	0.73ab	0.61a	1.07a	74.55abc	61.98a	111.91ab	0.84d	0.45a	86.08c
	Transcend	AI	0.69ab	0.78a	0.40a	0.79a	114.51a	56.40a	119.07ab	1.46abc	0.27c	197.90a
		FCT	0.73ab	0.72ab	0.62a	1.00a	99.35ab	85.28a	137.46ab	1.18bcd	0.40ab	159.76ab
		OC	0.95a	0.47bc	0.50a	0.71a	49.42c	52.66a	75.11b	1.10cd	0.43a	113.17bc
	<i>Pooled SE</i>		<i>0.065</i>	<i>0.056</i>	<i>0.109</i>	<i>0.105</i>	<i>0.084</i>	<i>0.135</i>	<i>0.161</i>	<i>0.085</i>	<i>0.025</i>	<i>13.083</i>
<i>P-value</i>	<i>V</i>	<i>0.156</i>	<i>0.001</i>	<i>0.796</i>	<i>0.859</i>	<i>0.032</i>	<i>0.962</i>	<i>0.541</i>	<i>0.000</i>	<i><.0001</i>	<i>0.013</i>	
	<i>CD</i>	<i>0.000</i>	<i>0.943</i>	<i>0.723</i>	<i>0.530</i>	<i>0.011</i>	<i>0.359</i>	<i>0.187</i>	<i>0.000</i>	<i><.0001</i>	<i><.0001</i>	
	<i>V*CD</i>	<i>0.403</i>	<i>0.000</i>	<i>0.679</i>	<i>0.062</i>	<i>0.001</i>	<i>0.281</i>	<i>0.037</i>	<i>0.001</i>	<i>0.288</i>	<i>0.050</i>	
All trans Zeaxanthin	AAC Spitfire	AI	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		FCT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		OC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	CDC Precision	AI	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		FCT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		OC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Transcend	AI	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		FCT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		OC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different ($p>0.05$). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected ($< LOD$), all-trans lutein ($LOD: 1.04 \mu\text{g/ml}$, $LOQ: 3.14 \mu\text{g/ml}$), all-trans zeaxanthin ($LOD: 0.31 \mu\text{g/ml}$, $LOQ: 0.93 \mu\text{g/ml}$).

Appendix 9: Table S4.2. Significance of wheat cultivar, cooking duration and their interactions on the concentration of cis- carotenoids (mg/100g dry weight) in undigested and digested refined semolina (RS) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)			Bioaccessibility (%)			
				Oral	Oral+ Gastric	Oral + Gastric + Intestinal	Oral Stage	Oral +Gastric Stage	Oral +Gastric+ intestinal stage				
15-cis lutein	AAC Spitfire	AI	0.05a	0.04b	0.04a	0.06a	81.70abc	79.67a	117.13a	0.07ab	ND	129.85ab	
		FCT	0.07a	0.05ab	0.04a	0.05a	69.81bc	58.28a	76.80a	0.06ab	ND	87.65ab	
		OC	0.06a	0.05ab	0.03a	0.05a	86.11abc	49.85a	78.23a	0.06ab	ND	102.37ab	
	CDC Precision	AI	0.05a	0.05ab	0.04a	0.05a	101.83ab	75.25a	91.99a	0.08ab	ND	152.10ab	
		FCT	0.06a	0.04ab	0.03a	0.04a	76.33bc	49.91a	70.27a	0.07ab	ND	116.51ab	
		OC	0.06a	0.05ab	0.03a	0.05a	83.47abc	61.02a	87.15a	0.04b	ND	74.56b	
	Transcend	AI	0.05a	0.06a	0.03a	0.05a	114.22a	62.80a	104.57a	0.11a	ND	195.23a	
		FCT	0.06a	0.05ab	0.04a	0.06a	97.42ab	74.40a	100.11a	0.07ab	ND	122.41ab	
		OC	0.07a	0.04b	0.03a	0.04a	57.16c	50.88a	57.83a	0.05b	ND	76.12b	
	<i>Pooled SE</i>			<i>0.004</i>	<i>0.004</i>	<i>0.006</i>	<i>0.006</i>	<i>0.078</i>	<i>0.097</i>	<i>0.128</i>	<i>0.012</i>		<i>22.121</i>
	<i>P-value</i>			<i>0.350</i>	<i>0.409</i>	<i>0.872</i>	<i>0.268</i>	<i>0.250</i>	<i>0.996</i>	<i>0.767</i>	<i>0.380</i>		<i>0.397</i>
	<i>CD</i>			<i>0.084</i>	<i>0.046</i>	<i>0.468</i>	<i>0.066</i>	<i>0.003</i>	<i>0.076</i>	<i>0.021</i>	<i>0.005</i>		<i>0.002</i>
<i>V*CD</i>			<i>0.259</i>	<i>0.002</i>	<i>0.587</i>	<i>0.258</i>	<i>0.003</i>	<i>0.251</i>	<i>0.158</i>	<i>0.219</i>		<i>0.327</i>	
13-cis lutein	AAC Spitfire	AI	0.06b	0.05b	0.05a	0.08a	83.61ab	80.36a	132.38a	0.09bcd	ND	134.39abc	
		FCT	0.08ab	0.06ab	0.05a	0.07a	71.5b	64.60a	85.28a	0.09bcd	ND	103.24bc	
		OC	0.08ab	0.07ab	0.05a	0.08a	90.76ab	55.17a	99.87a	0.09bcd	ND	112.61abc	
	CDC Precision	AI	0.08ab	0.07ab	0.05a	0.07a	93.38ab	63.06a	86.74a	0.12a	ND	156.90a	
		FCT	0.09a	0.07ab	0.05a	0.08a	82.43ab	51.28a	85.92a	0.13a	ND	130.16abc	

		OC	0.09a	0.08a	0.06a	0.10a	82.90ab	64.31a	103.18a	0.08cd	ND	86.46c
	Transcend	AI	0.07ab	0.08a	0.04a	0.07a	117.76a	53.72a	99.27a	0.11abc	ND	146.19ab
		FCT	0.08ab	0.07ab	0.06a	0.09a	99.92ab	73.53a	116.80a	0.11ab	ND	137.24abc
		OC	0.09ab	0.05b	0.05a	0.06a	56.94b	55.45a	74.45a	0.08d	ND	87.83c
	<i>Pooled SE</i> <i>P-value</i>		<i>0.007</i>	<i>0.005</i>	<i>0.009</i>	<i>0.008</i>	<i>0.097</i>	<i>0.085</i>	<i>0.141</i>	<i>0.005</i>		<i>10.287</i>
		<i>V</i>	<i>0.090</i>	<i>0.011</i>	<i>0.756</i>	<i>0.590</i>	<i>0.490</i>	<i>0.554</i>	<i>0.483</i>	<i>0.000</i>		<i>0.604</i>
		<i>CD</i>	<i>0.003</i>	<i>0.865</i>	<i>0.522</i>	<i>0.297</i>	<i>0.037</i>	<i>0.562</i>	<i>0.480</i>	<i><.0001</i>		<i><.0001</i>
		<i>V*CD</i>	<i>0.806</i>	<i>0.000</i>	<i>0.577</i>	<i>0.051</i>	<i>0.011</i>	<i>0.119</i>	<i>0.070</i>	<i>0.002</i>		<i>0.052</i>
	AAC Spitfire	AI	0.04c	0.03b	0.03a	0.05ab	89.83ab	90.44a	144.55a	0.06bc	ND	164.76a
		FCT	0.05abc	0.04ab	0.04a	0.05b	80.62ab	80.27a	100.28a	0.06c	ND	124.53abc
		OC	0.05ab	0.05a	0.04a	0.06ab	96.58ab	67.82a	116.81a	0.07abc	ND	126.97abc
13'-cis lutein	CDC Precision	AI	0.04bc	0.05ab	0.04a	0.04b	109.63ab	80.16a	101.28a	0.07abc	ND	172.00a
		FCT	0.05ab	0.05a	0.04a	0.05ab	96.37ab	66.99a	102.78a	0.08a	ND	143.14abc
		OC	0.06a	0.05a	0.05a	0.07a	88.79ab	80.29a	125.38a	0.06bc	ND	105.48c
	Transcend	AI	0.04bc	0.05a	0.03a	0.04b	124.32a	62.29a	108.58a	0.07abc	ND	158.50ab
		FCT	0.05abc	0.05a	0.04a	0.06ab	104.54ab	82.71a	128.27a	0.08ab	ND	150.85abc
		OC	0.05ab	0.04ab	0.04a	0.05ab	67.32b	70.29a	93.21a	0.06bc	ND	111.61bc
	<i>Pooled SE</i> <i>P-value</i>		<i>0.003</i>	<i>0.003</i>	<i>0.006</i>	<i>0.005</i>	<i>0.099</i>	<i>0.106</i>	<i>0.149</i>	<i>0.003</i>		<i>10.580</i>
		<i>V</i>	<i>0.052</i>	<i>0.004</i>	<i>0.678</i>	<i>0.500</i>	<i>0.413</i>	<i>0.675</i>	<i>0.605</i>	<i>0.019</i>		<i>0.980</i>
		<i>CD</i>	<i><.0001</i>	<i>0.563</i>	<i>0.247</i>	<i>0.010</i>	<i>0.024</i>	<i>0.841</i>	<i>0.797</i>	<i>0.015</i>		<i><.0001</i>
		<i>V*CD</i>	<i>0.971</i>	<i>0.000</i>	<i>0.643</i>	<i>0.036</i>	<i>0.037</i>	<i>0.329</i>	<i>0.104</i>	<i>0.008</i>		<i>0.228</i>
	AAC Spitfire	AI	0.05b	0.04c	0.04a	0.06a	72.22bcd	86.65a	121.66a	0.06b	ND	118.64abc
		FCT	0.07ab	0.04bc	0.05a	0.05a	62.85cd	68.18a	79.43a	0.06b	ND	87.60c
		OC	0.06ab	0.05abc	0.04a	0.06a	83.37abcd	63.62a	96.34a	0.06b	ND	96.38bc
9-cis lutein	CDC Precision	AI	0.06ab	0.06ab	0.05a	0.05a	94.32abc	74.76a	88.42a	0.09a	ND	146.18a
		FCT	0.07a	0.06ab	0.04a	0.05a	82.28abcd	60.99a	79.56a	0.10a	ND	138.34ab
		OC	0.07a	0.06ab	0.05a	0.07a	78.47abcd	72.74a	97.85a	0.06b	ND	84.71c
		AI	0.06ab	0.06a	0.03a	0.05a	109.10a	58.92a	91.71a	0.08a	ND	136.85ab

9'-cis Lutein	Transcend	FCT	0.06ab	0.06ab	0.05a	0.07a	98.29ab	85.97a	111.35a	0.08a	ND	139.84ab	
		OC	0.07a	0.04bc	0.04a	0.05a	56.82d	56.24a	68.16a	0.06b	ND	85.02c	
		<i>Pooled SE</i>	0.004	0.004	0.008	0.007	0.072	0.127	0.126	0.004		9.392	
		<i>P-value</i>											
		<i>V</i>	0.082	<.0001	0.678	0.736	0.035	0.857	0.555	<.0001		0.018	
		<i>CD</i>	0.002	0.746	0.687	0.668	0.012	0.644	0.412	<.0001		<.0001	
		<i>V*CD</i>	0.225	0.001	0.679	0.127	0.001	0.317	0.051	0.002		0.020	
	AAC Spitfire	AI	0.04c	0.03c	0.04a	0.05a	77.07bcd	91.02a	129.92a	0.05b	ND	124.81bc	
		FCT	0.06ab	0.04bc	0.04a	0.05a	65.60cd	69.96a	83.79ab	0.05b	ND	90.18d	
		OC	0.06ab	0.05abc	0.04a	0.06a	86.99abcd	66.05a	104.15ab	0.06b	ND	94.51cd	
	CDC Precision	AI	0.05bc	0.05ab	0.04a	0.05a	98.53abc	84.59a	96.88ab	0.09a	ND	161.72a	
		FCT	0.06ab	0.05ab	0.04a	0.05a	88.53abcd	68.19a	88.01ab	0.09a	ND	148.17ab	
OC		0.06ab	0.06a	0.05a	0.07a	87.72abcd	76.10a	113.82ab	0.06b	ND	87.52d		
Transcend	AI	0.05bc	0.06a	0.03a	0.05a	117.15a	63.18a	98.63ab	0.08a	ND	149.75ab		
	FCT	0.05bc	0.05ab	0.05a	0.07a	106.78ab	94.78a	125.98ab	0.08a	ND	155.69ab		
	OC	0.07a	0.04bc	0.04a	0.05a	57.37d	61.62a	71.96b	0.06b	ND	86.93d		
	<i>Pooled SE</i>	0.003	0.004	0.008	0.007	0.070	0.149	0.126	0.003		6.915		
	<i>P-value</i>												
	<i>V</i>	0.023	0.000	0.596	0.596	0.010	0.964	0.757	<.0001		<.0001		
	<i>CD</i>	<.0001	0.889	0.557	0.333	0.006	0.596	0.468	<.0001		<.0001		
	<i>V*CD</i>	0.014	0.000	0.839	0.103	0.000	0.385	0.014	<.0001		0.0004		

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different (p>0.05). #Cooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (<LOD).

Appendix 10: Table S4.3. Significance of wheat cultivar, cooking duration and their interactions on the concentration of all- trans carotenoids (mg/100g dry weight) in undigested and digested whole wheat (WWF)

Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)			Filtrate	Residue	Bio-accessibility (%)
				Oral	Oral+ Gastric	Oral + Gastric + Intestinal	Oral Stage	Oral +Gastric Stage	Oral +Gastric+ intestinal stage			
All- trans Lutein	AAC Spitfire	AI	0.97a	1.07a	1.03a	1.00a	109.06ab	100.86a	104.78a	0.97ab	0.24b	99.13b
		FCT	0.84a	0.78a	0.92a	0.92a	94.84ab	108.21a	109.13a	0.77b	0.48ab	96.51b
		OC	0.83a	0.90a	1.41a	1.05a	112.23ab	175.43a	132.44a	0.95ab	0.33ab	116.45ab
	CDC Precision	AI	0.82a	0.90a	1.15a	0.87a	109.99ab	138.8a	107.67a	0.81b	0.26ab	97.52b
		FCT	0.98a	0.67a	1.84a	1.09a	69.78b	198.76a	111.66a	0.87ab	0.41ab	89.17b
		OC	1.09a	1.03a	1.17a	0.96a	95.72ab	118.64a	91.97a	1.24ab	0.56ab	116.71ab
	Transcend	AI	0.78a	1.01a	1.39a	0.99a	131.79a	176.74a	129.21a	1.31a	0.47ab	170.43a
		FCT	0.99a	0.82a	1.29a	1.09a	83.69ab	130.94a	110.45a	1.25ab	0.58ab	126.82ab
		OC	0.95a	1.10a	1.04a	1.12a	119.57ab	120.86a	122.63a	1.12ab	0.58a	119.92ab
	<i>Pooled SE</i>		0.070	0.104	0.232	0.074	0.130	0.321	0.121	0.101	0.063	12.748
	<i>P-value</i>	<i>V</i>	0.363	0.448	0.371	0.256	0.179	0.662	0.231	0.001	0.010	0.002
		<i>CD</i>	0.137	0.010	0.664	0.272	0.009	0.948	0.864	0.255	0.011	0.211
		<i>V*CD</i>	0.052	0.602	0.116	0.296	0.746	0.128	0.274	0.033	0.199	0.070
	AAC Spitfire	AI	0.02a	0.02ab	0.02a	0.03ab	108.4a	89.76a	135.04a	0.02cd	0.03d	114.82c
		FCT	0.02a	0.02bc	0.02a	0.03ab	93.67a	97.74a	137.62a	0.02d	0.05a	113.85c
OC		0.02a	0.02abc	0.03a	0.03ab	101.23a	135.97a	158.35a	0.03bcd	ND	135.35abc	

All trans Zeaxanthin	CDC Precision	Al	0.02a	0.02bc	0.02a	0.02b	101.6a	120.15a	133.22a	0.02d	ND	120.72bc
		FCT	0.02a	0.02c	0.03a	0.03ab	91.98a	181.87a	153.55a	0.02cd	0.04bc	130.29abc
		OC	0.02a	0.02abc	0.02a	0.03ab	96.37a	112.75a	132.06a	0.03abcd	ND	141.79abc
	Transcend	Al	0.02a	0.02ab	0.03a	0.03ab	114.24a	146.58a	155.35a	0.03a	0.04cd	184.42ab
		FCT	0.02a	0.02abc	0.03a	0.03a	114.19a	177.31a	185.42a	0.03ab	0.04bc	197.94a
		OC	0.02a	0.02a	0.03a	0.03a	129.07a	138.26a	175.86a	0.03abc	0.04ab	176.88abc
		<i>Pooled SE</i>	<i>0.001</i>	<i>0.001</i>	<i>0.004</i>	<i>0.002</i>	<i>0.090</i>	<i>0.213</i>	<i>0.136</i>	<i>0.002</i>	<i>0.001</i>	<i>14.593</i>
		<i>P-value</i>										
		<i>V</i>	<i>0.245</i>	<i>0.001</i>	<i>0.093</i>	<i>0.001</i>	<i>0.012</i>	<i>0.047</i>	<i>0.013</i>	<i><.0001</i>	<i>0.029</i>	<i><.0001</i>
		<i>CD</i>	<i>0.145</i>	<i>0.006</i>	<i>0.480</i>	<i>0.124</i>	<i>0.417</i>	<i>0.172</i>	<i>0.260</i>	<i>0.182</i>	<i>0.000</i>	<i>0.632</i>
		<i>V*CD</i>	<i>0.335</i>	<i>0.171</i>	<i>0.255</i>	<i>0.440</i>	<i>0.752</i>	<i>0.183</i>	<i>0.560</i>	<i>0.054</i>	<i>0.001</i>	<i>0.638</i>

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different (p>0.05). #Cooking duration: Al, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (< LOD), all-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

Appendix 11: Table S4.4. Significance of wheat cultivar, cooking duration and their interactions on the concentration of cis- carotenoids (mg/100g dry weight) in undigested and digested whole wheat (WWF) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion Stage			Recovery (%)			Filtrate	Residue	Bioaccessibility (%)
				Oral	Oral+ Gastric	Oral + Gastric + Intestinal	Oral Stage	Oral +Gastric Stage	Oral +Gastric+ intestinal stage			
15-cis lutein	AAC Spitfire	AI	0.06a	0.05a	0.05a	0.04ab	94.46ab	87.85a	77.56a	0.05ab	ND	88.56a
		FCT	0.04a	0.04abc	0.05a	0.04ab	89.88ab	106.86a	86.31a	0.04ab	ND	93.19a
		OC	0.05a	0.04abc	0.06a	0.05a	86.59ab	112.7a	95.56a	0.04ab	ND	88.54a
	CDC Precision	AI	0.04a	0.04abc	0.05a	0.03b	98.38ab	106.27a	81.68a	0.04b	ND	88.90a
		FCT	0.05a	0.03c	0.05a	0.04ab	60.24b	105.98a	75.32a	0.04b	ND	79.33a
		OC	0.05a	0.03bc	0.04a	0.04ab	69.68ab	83.18a	80.52a	0.04ab	ND	92.11a
	Transcend	AI	0.04a	0.05ab	0.05a	0.04ab	111.1ab	118.71a	81.37a	0.05a	ND	118.23a
		FCT	0.04a	0.04abc	0.05a	0.04ab	88.17ab	118.33a	93.56a	0.05ab	ND	118.03a
		OC	0.04a	0.05ab	0.04a	0.05a	124.04a	98.27a	116.06a	0.05ab	ND	124.19a
	<i>Pooled SE</i>		<i>0.004</i>	<i>0.003</i>	<i>0.007</i>	<i>0.003</i>	<i>0.117</i>	<i>0.160</i>	<i>0.101</i>	<i>0.003</i>		<i>12.850</i>
	<i>P-value</i>	<i>V</i>	<i>0.123</i>	<i>0.000</i>	<i>0.530</i>	<i>0.010</i>	<i>0.010</i>	<i>0.589</i>	<i>0.113</i>	<i>0.001</i>		<i>0.006</i>
		<i>CD</i>	<i>0.716</i>	<i>0.000</i>	<i>0.724</i>	<i>0.014</i>	<i>0.086</i>	<i>0.647</i>	<i>0.119</i>	<i>0.357</i>		<i>0.900</i>
		<i>V*CD</i>	<i>0.167</i>	<i>0.654</i>	<i>0.485</i>	<i>0.356</i>	<i>0.274</i>	<i>0.592</i>	<i>0.523</i>	<i>0.127</i>		<i>0.966</i>
AAC Spitfire	AI	0.09a	0.07ab	0.07a	0.08ab	82.74ab	78.25a	93.48a	0.08a	0.05d	95.06a	
	FCT	0.09a	0.07ab	0.08a	0.08ab	74.74ab	92.19a	95.88a	0.08a	0.09a	95.27a	
	OC	0.09a	0.07ab	1.10a	0.10ab	82.15ab	109.79a	112.25a	0.10a	0.06cd	106.78a	
	AI	0.08a	0.06ab	0.07a	0.08b	72.16ab	90.42a	96.73a	0.08a	0.05d	99.26a	

13-cis lutein	CDC Precision	FCT	0.09a	0.05b	0.11a	0.10ab	56.48b	137.87a	117.71a	0.09a	0.07cd	98.09a
		OC	0.11a	0.08ab	0.09a	0.09ab	67.94ab	86.19a	85.80a	0.12a	0.07bc	104.70a
	Transcend	AI	0.08a	0.08ab	0.10a	0.08ab	97.64ab	120.65a	102.19a	0.11a	0.07bc	133.25a
		FCT	0.09a	0.07ab	0.10a	0.10ab	85.92ab	143.49a	119.92a	0.11a	0.08abc	134.11a
		OC	0.09a	0.08a	0.08a	0.10a	113.32a	96.54a	135.18a	0.11a	0.09ab	146.37a
	<i>Pooled SE</i>		<i>0.010</i>	<i>0.006</i>	<i>0.015</i>	<i>0.005</i>	<i>0.112</i>	<i>0.262</i>	<i>0.176</i>	<i>0.008</i>	<i>0.003</i>	<i>18.657</i>
	<i>P-value</i>	<i>V</i>	<i>0.401</i>	<i>0.013</i>	<i>0.755</i>	<i>0.608</i>	<i>0.004</i>	<i>0.469</i>	<i>0.335</i>	<i>0.014</i>	<i>0.001</i>	<i>0.027</i>
		<i>CD</i>	<i>0.194</i>	<i>0.014</i>	<i>0.408</i>	<i>0.001</i>	<i>0.230</i>	<i>0.353</i>	<i>0.555</i>	<i>0.032</i>	<i><.0001</i>	<i>0.748</i>
		<i>V*CD</i>	<i>0.540</i>	<i>0.636</i>	<i>0.392</i>	<i>0.279</i>	<i>0.864</i>	<i>0.649</i>	<i>0.578</i>	<i>0.188</i>	<i>0.001</i>	<i>0.999</i>
	AAC Spitfire	AI	0.05ab	0.03bc	0.04a	0.05cd	65.28abc	70.44a	103.69a	0.05c	0.04de	103.54a
FCT		0.05ab	0.04bc	0.04a	0.06bcd	67.43abc	80.44a	108.40a	0.05bc	0.07ab	105.76a	
OC		0.06ab	0.04ab	0.06a	0.07ab	73.54abc	105.73a	115.51a	0.07abc	0.05cde	114.05a	
13'-cis lutein	CDC Precision	AI	0.05ab	0.03c	0.04a	0.05d	54.02bc	79.06a	103.8a	0.05c	0.04e	104.40a
		FCT	0.06ab	0.03c	0.07a	0.07abc	41.33c	105.07a	107.05a	0.06abc	0.05cd	93.53a
		OC	0.07a	0.04ab	0.06a	0.06abcd	62.38abc	87.14a	94.02a	0.08a	0.05cd	115.89a
	Transcend	AI	0.05b	0.04abc	0.05a	0.05d	84.56ab	108.18a	107.33a	0.07abc	0.05cd	149.97a
		FCT	0.06ab	0.04abc	0.07a	0.07abc	68.18abc	129.02a	118.07a	0.08ab	0.06bc	136.03a
		OC	0.06ab	0.05a	0.05a	0.08a	94.53a	95.42a	147.29a	0.08a	0.08a	159.03a
	<i>Pooled SE</i>		<i>0.005</i>	<i>0.003</i>	<i>0.009</i>	<i>0.003</i>	<i>0.079</i>	<i>0.202</i>	<i>0.149</i>	<i>0.005</i>	<i>0.003</i>	<i>19.399</i>
	<i>P-value</i>	<i>V</i>	<i>0.244</i>	<i>0.001</i>	<i>0.456</i>	<i>0.251</i>	<i>0.000</i>	<i>0.289</i>	<i>0.186</i>	<i>0.001</i>	<i>0.000</i>	<i>0.017</i>
		<i>CD</i>	<i>0.006</i>	<i><.0001</i>	<i>0.061</i>	<i><.0001</i>	<i>0.035</i>	<i>0.528</i>	<i>0.522</i>	<i>0.000</i>	<i><.0001</i>	<i>0.534</i>
		<i>V*CD</i>	<i>0.476</i>	<i>0.514</i>	<i>0.291</i>	<i>0.056</i>	<i>0.701</i>	<i>0.637</i>	<i>0.527</i>	<i>0.222</i>	<i>0.001</i>	<i>0.992</i>
AAC Spitfire	AI	0.06a	0.06a	0.06a	0.06a	102.67a	90.36a	94.13a	0.06ab	0.05b	93.40a	
	FCT	0.06a	0.05a	0.06a	0.06a	91.27a	99.21a	100.02a	0.05b	0.06a	91.22a	
	OC	0.06a	0.05a	0.08a	0.07a	98.50a	144.49a	118.6a	0.06ab	ND	103.86a	
	AI	0.06a	0.05a	0.07a	0.05a	91.26a	116.05a	91.35a	0.05ab	ND	91.40a	

9-cis lutein	CDC Precision	FCT	0.07a	0.04a	0.10a	0.07a	67.29a	160.8a	103.51a	0.06ab	0.06ab	86.97a
		OC	0.07a	0.06a	0.07a	0.06a	85.78a	107.11a	92.22a	0.07ab	ND	107.05a
		AI	0.05a	0.06a	0.08a	0.06a	122.39a	154.08a	116.79a	0.08a	0.05ab	146.88a
	Transcend	FCT	0.06a	0.05a	0.08a	0.07a	88.00a	133.02a	119.48a	0.07ab	0.06ab	128.46a
		OC	0.06a	0.06a	0.06a	0.07a	133.25a	125.37a	141.4a	0.07ab	0.07a	149.53a
	<i>Pooled SE</i>		0.005	0.005	0.012	0.004	0.161	0.242	0.146	0.006	0.003	18.917
	<i>P-value</i>	V	0.101	0.303	0.293	0.224	0.058	0.425	0.047	0.002	0.311	0.007
		CD	0.608	0.028	0.662	0.029	0.139	0.861	0.385	0.284	0.011	0.518
		V*CD	0.504	0.674	0.129	0.257	0.818	0.232	0.788	0.115	0.132	0.992
	AAC Spitfire	AI	0.05a	0.05a	0.05a	0.06a	107.26a	90.607a	112.34a	0.05bcd	ND	98.12a
		FCT	0.05a	0.04a	0.05a	0.05a	93.71a	103.55a	111.44a	0.04d	0.05a	91.93a
		OC	0.05a	0.05a	0.07a	0.06a	101.06a	144.65a	124.18a	0.05abcd	ND	104.46a
	9'-cis Lutein	CDC Precision	AI	0.05a	0.05a	0.06a	0.05a	98.74a	118.52a	101.88a	0.05cd	ND
FCT			0.05a	0.04a	0.10a	0.06a	75.55a	183.00a	116.69a	0.05bcd	0.04b	95.06a
OC			0.06a	0.05a	0.07a	0.06a	91.99a	124.85a	106.42a	0.07ab	ND	121.26a
Transcend		AI	0.05a	0.05a	0.07a	0.06a	115.46a	152.35a	126.77a	0.08a	0.05a	162.61a
		FCT	0.05a	0.04a	0.07a	0.06a	99.31a	150.38a	140.12a	0.07abc	0.05a	154.00a
		OC	0.05a	0.06a	0.06a	0.06a	136.15a	127.7a	143.19a	0.06abcd	0.06a	154.14a
<i>Pooled SE</i>			0.004	0.005	0.011	0.005	0.152	0.267	0.150	0.005	0.001	18.411
<i>P-value</i>		V	0.120	0.541	0.110	0.345	0.092	0.308	0.073	<.0001	0.006	0.001
		CD	0.467	0.011	0.566	0.280	0.226	0.525	0.637	0.143	0.056	0.677
		V*CD	0.776	0.751	0.119	0.526	0.835	0.326	0.946	0.017	-	0.881

All values are means of four determinations (n=4). Pooled SE; Pooled standard error of means assuming equal variances. Data having the same lower-case superscripts within a column and carotenoid are not significantly different (p>0.05). #Cooking duration: AI, *al dente*; FCT, fully cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). ND; Not detected (< LOD).

Appendix 12: Table S4.5. Significance of wheat cultivar, cooking duration, and their interactions on the concentration of total all-trans, total all-cis carotenoids, and total carotenoid content (mg/100 g dry weight) in undigested and digested refined semolina (RS) Pasta

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion stage					
				Oral	Oral+ Gastric	Oral + Gastric + Intestinal	Filtrate	Residue	
Total all-trans	AAC Spitfire	AI	0.60b	0.40c	0.49a	0.88a	0.98d	0.21c	
		FCT	0.85ab	0.48bc	0.52a	0.71a	0.96d	0.26c	
		OC	0.86ab	0.66abc	0.43a	0.92a	0.94d	0.28bc	
	CDC Precision	AI	0.74ab	0.66ab	0.52a	0.74a	1.55ab	0.27bc	
		FCT	0.91a	0.68ab	0.49a	0.82a	1.60a	0.41a	
		OC	0.96a	0.73ab	0.61a	1.07a	0.84d	0.45a	
	Transcend	AI	0.69ab	0.78a	0.40a	0.79a	1.46abc	0.27c	
		FCT	0.73ab	0.72ab	0.62a	1.00a	1.18bcd	0.40ab	
		OC	0.95a	0.47bc	0.50a	0.71a	1.10cd	0.43a	
	<i>Pooled SE</i>		<i>0.065</i>	<i>0.056</i>	<i>0.1094</i>	<i>0.1053</i>	<i>0.08493</i>	<i>0.02549</i>	
	<i>P-value</i>	<i>V</i>	<i>0.156</i>	<i>0.001</i>	<i>0.796</i>	<i>0.859</i>	<i>0.000</i>	<i><.0001</i>	
		<i>CD</i>	<i>0.000</i>	<i>0.943</i>	<i>0.723</i>	<i>0.530</i>	<i>0.000</i>	<i><.0001</i>	
		<i>V*CD</i>	<i>0.403</i>	<i>0.000</i>	<i>0.679</i>	<i>0.062</i>	<i>0.001</i>	<i>0.288</i>	
	Total all-Cis	AAC Spitfire	AI	0.24b	0.19c	0.21a	0.31a	0.33bc	ND
			FCT	0.33ab	0.22bc	0.22a	0.26a	0.33bc	ND
OC			0.32ab	0.27abc	0.19a	0.31a	0.34bc	ND	
CDC Precision		AI	0.29ab	0.28abc	0.21a	0.26a	0.45a	ND	
		FCT	0.34a	0.28abc	0.20a	0.28a	0.47a	ND	
		OC	0.34a	0.29ab	0.25a	0.36a	0.31c	ND	
Transcend		AI	0.28ab	0.32a	0.17a	0.27a	0.46a	ND	
		FCT	0.29ab	0.29ab	0.24a	0.33a	0.42ab	ND	
		OC	0.35a	0.21bc	0.20a	0.25a	0.31c	ND	

			<i>Pooled SE</i>	0.020	0.019	0.037	0.032	0.020		
	<i>P-value</i>	<i>V</i>	0.254	0.004	0.825	0.799	0.001			
		<i>CD</i>	0.001	0.882	0.731	0.584	<.0001			
		<i>V*CD</i>	0.392	0.001	0.672	0.078	0.002			
Total carotenoids (HPLC-PDA)	AAC Spitfire	AI	0.84b	0.59c	0.70a	1.19a	1.31b	0.21c		
		FCT	1.18ab	0.70bc	0.73a	0.97a	1.29b	0.26c		
		OC	1.18ab	0.93abc	0.62a	1.23a	1.29b	0.28bc		
	CDC Precision	AI	1.02ab	0.94abc	0.73a	1.00a	2.00a	0.27bc		
		FCT	1.25a	0.96ab	0.69a	1.10a	2.08a	0.41a		
		OC	1.30a	1.02ab	0.86a	1.42a	1.15b	0.45a		
	Transcend	AI	0.96ab	1.09a	0.56a	1.05a	1.92a	0.27c		
		FCT	1.02ab	1.01ab	0.85a	1.33a	1.60ab	0.40ab		
		OC	1.31a	0.68bc	0.70a	0.96a	1.41b	0.43a		
				<i>Pooled SE</i>	0.084	0.074	0.146	0.137	0.097	0.025
		<i>P-value</i>	<i>V</i>	0.174	0.002	0.812	0.855	<.0001	<.0001	
			<i>CD</i>	0.000	0.957	0.727	0.541	<.0001	<.0001	
		<i>V*CD</i>	0.395	0.000	0.677	0.065	0.001	0.288		
TCC	AAC Spitfire	AI	0.40e	0.38e	0.27b	0.72ab	0.79b	0.33de		
		FCT	0.50b	0.42de	0.33a	0.71ab	0.81b	0.52a		
		OC	0.57a	0.57a	0.25b	0.73ab	0.78b	0.40bc		
	CDC Precision	AI	0.46bc	0.54ab	0.19c	0.65cd	0.90a	0.31e		
		FCT	0.56a	0.51b	0.22c	0.68bc	0.92a	0.44b		
		OC	0.54a	0.41de	0.26b	0.72ab	0.54d	0.42b		
	Transcend	AI	0.42de	0.47c	0.12d	0.53e	0.76bc	0.30e		
		FCT	0.45cd	0.42d	0.20c	0.62d	0.70c	0.35cde		
		OC	0.55a	0.31f	0.28b	0.76a	0.45e	0.37bcd		
			<i>Pooled SE</i>	0.008	0.008	0.006	0.011	0.014	0.013	
	<i>P-value</i>	<i>V</i>	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
		<i>CD</i>	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
		<i>V*CD</i>	<.0001	<.0001	<.0001	<.0001	<.0001	0.000		

Values are means of four determinations (n=4). Pooled SE; Pooled standard error of means

assuming equal variances. Data having the same lower-case superscripts within a column and

variable are not significantly different ($p>0.05$). #Cooking duration: AI, *al dente*; FCT, fully

cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar (V) and cooking duration (CD). TCC; Total carotenoid content. ND; Not detected (<LOD). All-trans lutein (LOD:1.04 $\mu\text{g/ml}$, LOQ: 3.14 $\mu\text{g/ml}$), all-trans zeaxanthin (LOD:0.31 $\mu\text{g/ml}$, LOQ: 0.93 $\mu\text{g/ml}$).

Appendix 13: Table S4.6. Significance of wheat cultivar, cooking duration and their interactions on the concentration of total all-trans, total all-cis carotenoids and total carotenoid content (mg/100 g dry weight) in undigested and digested whole wheat flour (WWF) Pasta.

Carotenoid	Wheat Cultivar	#Cooking duration	Undigested pasta	Digestion stage					
				Oral	Oral+ Gastric	Oral + Gastric + Intestinal	Filtrate	Residue	
Total All-trans	AAC Spitfire	AI	0.99a	1.09a	1.05a	1.03a	0.99ab	0.28b	
		FCT	0.86a	0.80a	0.93a	0.95a	0.79b	0.57a	
		OC	0.85a	0.91a	1.43a	1.08a	0.97ab	0.39ab	
	CDC Precision	AI	0.84a	0.92a	1.17a	0.89a	0.83b	0.33b	
		FCT	1.00a	0.69a	1.87a	1.12a	0.89ab	0.37ab	
		OC	1.11a	1.05a	1.19a	0.99a	1.27ab	0.52ab	
	Transcend	AI	0.80a	1.04a	1.42a	1.02a	1.35a	0.48ab	
		FCT	1.00a	0.84a	1.32a	1.13a	1.28ab	0.59a	
		OC	0.97a	1.12a	1.07a	1.15a	1.15ab	0.61a	
	<i>Pooled SE</i>		<i>0.071</i>	<i>0.105</i>	<i>0.235</i>	<i>0.076</i>	<i>0.1023</i>	<i>0.051</i>	
	<i>P-value</i>	<i>V</i>	<i>0.381</i>	<i>0.430</i>	<i>0.375</i>	<i>0.233</i>	<i>0.001</i>	<i>0.001</i>	
		<i>CD</i>	<i>0.146</i>	<i>0.010</i>	<i>0.662</i>	<i>0.268</i>	<i>0.253</i>	<i>0.002</i>	
		<i>V*CD</i>	<i>0.053</i>	<i>0.598</i>	<i>0.118</i>	<i>0.299</i>	<i>0.033</i>	<i>0.034</i>	
	Total All-Cis	AAC Spitfire	AI	0.31a	0.28ab	0.27a	0.29ab	0.30ab	0.14d
			FCT	0.29a	0.23ab	0.27a	0.29ab	0.27b	0.28ab
OC			0.31a	0.27ab	0.37a	0.34ab	0.32ab	0.11de	
CDC Precision		AI	0.28a	0.23ab	0.29a	0.26b	0.26b	0.09e	
		FCT	0.32a	0.19b	0.42a	0.33ab	0.29ab	0.22c	
		OC	0.36a	0.27ab	0.33a	0.32ab	0.39a	0.13d	
Transcend		AI	0.27a	0.28ab	0.36a	0.28ab	0.38ab	0.23c	
		FCT	0.29a	0.25ab	0.37a	0.34ab	0.38ab	0.25bc	
		OC	0.29a	0.30a	0.30a	0.35a	0.37ab	0.28a	
<i>Pooled SE</i>			<i>0.026</i>	<i>0.021</i>	<i>0.053</i>	<i>0.019</i>	<i>0.025</i>	<i>0.006</i>	
<i>P-value</i>		<i>V</i>	<i>0.258</i>	<i>0.029</i>	<i>0.564</i>	<i>0.376</i>	<i>0.001</i>	<i><.0001</i>	
		<i>CD</i>	<i>0.225</i>	<i>0.009</i>	<i>0.511</i>	<i>0.002</i>	<i>0.061</i>	<i><.0001</i>	
		<i>V*CD</i>	<i>0.474</i>	<i>0.722</i>	<i>0.272</i>	<i>0.271</i>	<i>0.087</i>	<i><.0001</i>	

Total carotenoids (HPLC- PDA)	AAC Spitfire	AI	1.30a	1.37a	1.31a	1.32a	1.29ab	0.35a
		FCT	1.14a	1.03a	1.20a	1.23a	1.06b	0.71a
		OC	1.16a	1.18a	1.80a	1.42a	1.29ab	0.45a
	CDC Precision	AI	1.11a	1.14a	1.46a	1.15a	1.09b	0.37a
		FCT	1.32a	0.88a	2.29a	1.45a	1.18ab	0.48a
		OC	1.48a	1.32a	1.52a	1.31a	1.65ab	0.59a
	Transcend	AI	1.07a	1.32a	1.78a	1.31a	1.73a	0.60a
		FCT	1.30a	1.08a	1.69a	1.46a	1.66ab	0.72a
		OC	1.26a	1.42a	1.37a	1.51a	1.51ab	0.75a
	<i>Pooled SE</i>			0.093	0.126	0.288	0.094	0.127
<i>P-value</i>	<i>V</i>		0.329	0.306	0.416	0.255	0.001	0.020
	<i>CD</i>		0.198	0.010	0.648	0.124	0.213	0.039
	<i>V*CD</i>		0.072	0.618	0.141	0.288	0.040	0.336
TCC	AAC Spitfire	AI	0.78ab	1.04bc	1.15ab	0.65ab	0.83abc	0.49e
		FCT	0.75abc	1.07ab	1.09ab	0.68a	0.76c	1.05a
		OC	0.79a	1.13a	1.09ab	0.65ab	0.92abc	0.62bcd
	CDC Precision	AI	0.64ef	0.96bc	1.02ab	0.64ab	0.74c	0.57cde
		FCT	0.69de	0.96cd	1.16a	0.62ab	0.92abc	0.67bc
		OC	0.73bcd	0.99bcd	0.97b	0.64ab	0.99ab	0.69b
	Transcend	AI	0.59f	0.86e	1.00ab	0.52c	1.02a	0.52de
		FCT	0.66e	0.92de	1.07ab	0.58bc	0.81bc	0.64bc
		OC	0.72cd	0.95d	1.06ab	0.62ab	0.99ab	0.59bcde
	<i>Pooled SE</i>			0.011	0.017	0.035	0.016	0.039
<i>P-value</i>	<i>V</i>		<.0001	<.0001	0.066	<.0001	0.025	<.0001
	<i>CD</i>		<.0001	0.000	0.085	0.044	0.002	<.0001
	<i>V*CD</i>		0.000	0.390	0.027	0.019	0.002	<.0001

Values are means of four determinations (n=4). Pooled SE; Pooled standard error of means

assuming equal variances. Data having the same lower-case superscripts within a column and

variable are not significantly different ($p>0.05$). #Cooking duration: AI, *al dente*; FCT, fully

cooked time; OC, overcooked pasta. Independent variables are represented by the wheat cultivar

(V) and cooking duration (CD). TCC; Total carotenoid content. ND; Not detected (<LOD). All-trans lutein (LOD:1.04 µg/ml, LOQ: 3.14 µg/ml), all-trans zeaxanthin (LOD:0.31 µg/ml, LOQ: 0.93 µg/ml).

Appendix 14: Fig. S4.1. Sample chromatograms of undigested (A) and digested (B) refined semolina pasta and whole wheat flour pasta cooked to al dente.

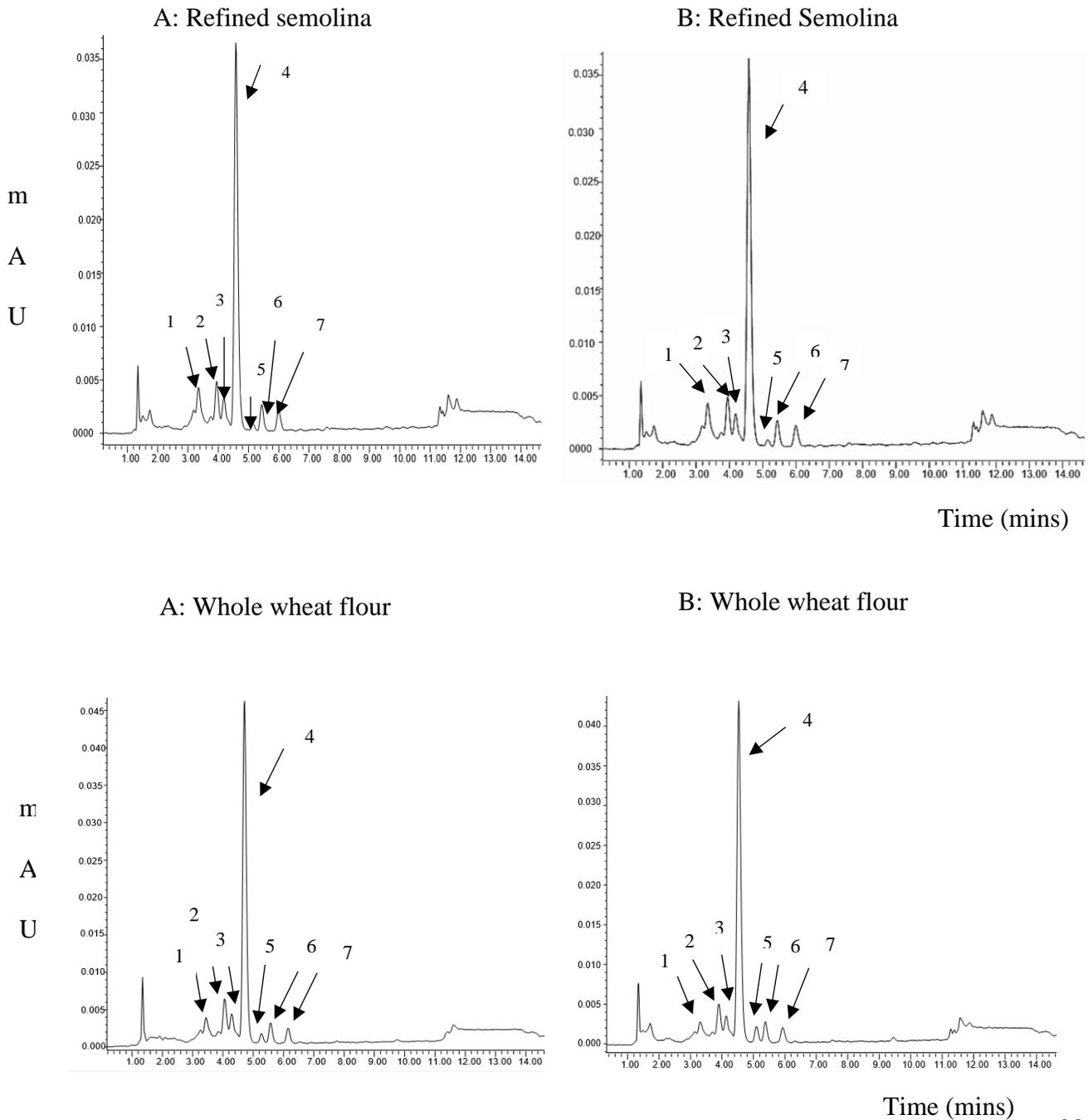


Fig. S4.1. Sample chromatograms obtained by HPLC -PDA at 450 nm of carotenoid extracts from undigested (A) and digested (B) refined semolina pasta and whole wheat flour pasta cooked to al dente of the Transcend wheat cultivar. Peak assignment: 1- 15-cis lutein, 2- 13-cis lutein, 3- 13'-cis lutein, 4- all-trans lutein, 5- all-trans zeaxanthin, 6- 9-cis lutein, 7- 9'-cis lutein.