

**Comparative Study on Nutritional Characteristics and Physicochemical
Properties of Ancient Wheat Species**

By

Sathsara Thakshani Deyalage

A Thesis Submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Food and Human Nutritional Sciences
University of Manitoba
Winnipeg

Copyright © 2024 by Sathsara Thakshani Deyalage

Abstract

Background & objective: Ancient wheat species, such as einkorn, emmer, and spelt, are gaining increased attention due to their environmentally friendly nature, which aligns with sustainable agriculture practices, and the rising consumer demand for nutrient-dense food options. Despite their growing recognition, the full potential of these ancient wheat remains largely unexplored, hindering their future food applications. Therefore, this study aimed to address this gap by comprehensively evaluating the nutritional profiles and physicochemical properties of einkorn, emmer, and spelt in comparison to common hexaploid Canada Western Red Spring (CWRS) wheat.

Methodology: Nutritional characteristics were assessed through analyses of proximate composition, amino acid profile, phenolic acid content, and *in vitro* starch and protein digestibility (IVPD). Physicochemical properties were evaluated with a particular focus on the starch and protein profiles of these species.

Findings: Einkorn demonstrated higher protein content (21.27%) and phenolic acid content (both free and conjugated), along with lower starch levels compared to other wheat species. Spelt had the highest bound phenolic acid content. In terms of starch digestibility, einkorn was characterized by lower glucose release and reduced rapidly digestible starch (RDS) levels, while refined common wheat (CWRS RF) exhibited the highest RDS and glucose release. Among the ancient wheat species, emmer had the highest glucose release and RDS content. IVPD was highest in CWRS RF (86.05%), with einkorn following closely at 84.29%, while spelt and emmer exhibited lower IVPD values. Lysine was the limiting amino acid in

all wheat species. Thermal behavior revealed that spelt had the highest thermal stability with high transition temperatures, while emmer required less energy for gelatinization. Amylose% and amylopectin% showed no significant differences among wheat species. Protein composition analysis indicated significant variations in ancient wheat species, specifically, SDS unextractable glutenin levels were significantly lower in the ancient wheat species compared to common wheat suggesting potential challenges for breadmaking.

Conclusion: This study highlights the nutritional significance of ancient wheat species, particularly einkorn, showcasing its potential with higher levels of protein, phenolic acids, and a low-glycemic food profile, along with substantial *IVPD*. The unique physicochemical characteristics, suggest that processing modifications may be required to optimize the use of ancient wheat in bakery applications.

Key words: Ancient wheat, Health, Nutrition, Physicochemical properties, Sustainability

Acknowledgement

Two years of dedication and hard work have led to the completion of this thesis, a journey that would not have been possible without the opportunity to move to Canada and pursue my MSc at the University of Manitoba. I am deeply grateful to my supervisor, Dr. Maneka Malalgoda, for her invaluable mentorship, unwavering support, and financial contribution throughout this journey. Your encouragement to publish my work and present my findings at various conferences has significantly enhanced my academic profile. Thank you for believing in my abilities, trusting my work, and setting an exemplary standard, particularly in academic writing.

My heartfelt appreciation extends to my thesis committee members: Dr. Sijo Joseph, for offering insightful feedback, reviewing my papers, and providing an opportunity to work at the Agriculture and Agri-Food Lab, which greatly contributed to the success of my experiments. I am also thankful to Dr. Lovemore Malunga for his willingness to be part of my committee and for sharing his technical expertise during my lab work at AAFC. A special mention goes to the late Dr. James House, whose feedback during my proposal and progress meetings was invaluable. Your thoughtful suggestions have left a lasting impression on my work.

I would like to express my gratitude to Alison Ser, Jerry Jin, Yang Qui, Jinghua Mao, and Yuchen Nan for their guidance and assistance with lab equipment at the Ellis Building. My sincere thanks also go to everyone at the Richardson Centre for Food Technology and Research (RCFTR) for providing access to research facilities and instrument training. I would also like to extend my gratitude to all the staff at AAFC, especially Dr. Babak Sobhi, whose

constant support and hands-on training during critical experiments have been instrumental to my success. Special thanks to Lan Shi, Tracy, and Camille for their support. I also appreciate the help from the Graduate Program Support staff; Samantha Berscheid, Emily Gregorchuk, Carola Lange, and Helena Marak, for ensuring a smooth academic journey for all students at the University of Manitoba.

I am also grateful to the University of Manitoba Entrance Scholarship, the U of Manitoba Research Completion Scholarship, and the U of Manitoba Bursary Program for their financial support during these two years.

A special thanks to everyone in the Grain Chemistry and Processing Quality lab for their encouragement and support. My lab group at room 256; Roshema, Anashwar, and Sachini has been there for me every step of the way. Our conversations and small chats always brightened my day, and I will cherish the friendships we have built. You turned the lab into a place of joy, and our teamwork made this journey memorable. I am also thankful to Mackenzie, Reyna, and Indhu for being wonderful lab mates.

I would also like to thank my undergraduate supervisor, Prof. Indira Wickramasinghe, and everyone at the University of Sri Jayewardenepura, as well as my home country, Sri Lanka, for blessing me with the gift of free education, which paved the way for me to pursue further studies in my dream country.

To my beloved husband, Miyuru, words cannot fully express my gratitude. You have been my greatest pillar of strength. Thank you for believing in me and standing by my side through thick and thin. You have selflessly sacrificed your time and career to ensure I could pursue

my dreams in Canada without any obstacles. Your unwavering love, support, and belief in me have uplifted me in ways I could never repay. I am deeply grateful to have you by my side. Above all, I owe everything to my family. My deepest gratitude goes to my parents, whose love and support have been the driving force behind everything I have achieved. Amma, you are the strongest woman I know, and your sacrifices for our family have never gone unnoticed. Dad, your constant encouragement and guidance have always steered me in the right direction. To my sister and brother-in-law, my brother, sister-in-law, and my niece thank you for being my support system throughout this entire journey.

My sister, you are truly the greatest blessing in my life. Our daily video calls have been a source of joy and comfort. Parting from you in two separate countries has been the biggest struggle but talking to you every day has been my capsule of happiness. Despite the challenges you face in your own PhD journey, you have never once hesitated to listen to my long-winded stories, always offering your unwavering support. I am truly grateful to have you by my side. Coming to Canada would have been impossible without the support from you and my brother from another mother, Danuka Aiya.

I also wish to thank Roshema, the first person I turned to when I arrived in Canada. I am truly grateful for all the support you've given me. You've been there to listen to me when I switched into overthinking mode and provided me with much-needed mental support. Your friendship and care have meant the world to me.

Finally, to everyone who has played a role in this journey, I am forever grateful. Your wisdom, kindness, and support have shaped me into who I am today. Though I may not have the space to name you all, my heartfelt thanks extend to each of you.

Dedication

This dissertation is dedicated to my akka, my role model and endless source of inspiration. You have been the guiding force in my life, always there for me, no matter the distance. This is a heartfelt tribute from your nangi to the incredible woman you are. Your belief in me has given me the strength, courage, and confidence to pursue my dreams. I hope I have made you proud.

Table of Content

Abstract.....	i
Acknowledgement.....	iii
Dedication.....	vi
List of Tables.....	xi
List of Figures	xii
List of Abbreviations.....	xiii
Chapter 1 Introduction	1
Chapter 2 Literature Review	5
2.1 Introduction	6
2.2 Role of wheat for global food security	10
2.2.1 Cultivated wheat today: Common wheat	11
2.2.2 Common wheat utilization and sustainability	14
2.3 Chemical composition of ancient wheat species	22
2.3.1 Protein content.....	22
2.3.2 Starch content.....	24
2.3.3 Fiber content	25
2.3.4 Ash content	27
2.3.5 Lipid content	28
2.3.6 Vitamins	30
2.3.7 Phytochemicals.....	31
2.4 Nutritional overview	33
2.5 Starch chemistry and nutritional quality.....	36
2.5.1 Granular structure and distribution	36

2.5.2 Starch digestibility	38
2.6 Wheat protein chemistry and nutritional quality	42
2.6.1 Protein composition and functionality	42
2.6.2 Nutritional value of wheat protein	47
2.6.3 Digestibility of wheat protein	49
2.7 Conclusion	53
2.8 References	54
Chapter 3 Determining the Nutritional Potential of Ancient Wheat Species: A closer Look at Starch and Protein Digestibility, and Phenolic Acid Content	86
Abstract	86
3.1 Introduction	87
3.2 Materials and methods	89
3.2.1 Proximate composition	89
3.2.2 Determination of the amino acid profile	90
3.2.3 Arabinoxylan (AX) quantification	91
3.2.4 Phenolic acid profile	92
3.2.5 Assessing nutritional characteristics of ancient wheat species	94
3.2.6 Statistical analysis	98
3.3 Results and discussion	98
3.3.1 Proximate composition	98
3.3.2 Amino acid (AA) composition	100
3.3.3 Arabinoxylan quantification	103
3.3.4 Phenolic acids profile	105
3.3.5 <i>In vitro</i> starch digestibility	109

3.3.6 <i>In vitro</i> glucose determination	111
3.3.7 <i>In vitro</i> protein digestibility.....	112
3.4 Conclusion	116
3.5 References	117
Chapter 4 Comparative Evaluation of Starch and Protein Characteristics in Ancient Wheat species	126
4.1 Introduction	127
4.2 Materials and methods	129
4.2.1 Scanning electron microscopy (SEM) analysis of kernel morphology	129
4.2.2 Starch extraction.....	130
4.2.3 Scanning electron microscopy for starch granule visualization	130
4.2.4 Granule size distribution	131
4.2.5 Characterization of amylose and amylopectin.....	131
4.2.6 Thermal properties of starch gelatinization	132
4.2.7 Swelling power of starch	133
4.2.8 Protein composition analysis.....	133
4.3 Results and discussion.....	135
4.3.1 Scanning electron microscopy analysis of kernel morphology	135
4.3.2 Scanning electron microscopy for starch granule visualization	142
4.3.3 Granule size distribution	146
4.3.4 Characterization of amylose and amylopectin.....	148
4.3.5 Thermal properties of starch gelatinization	150
4.3.6 Swelling power of starch	154
4.3.7 Protein composition analysis.....	155

4.4 Conclusion	159
4.5 References	160
Chapter 5 Conclusion and Future Direction	166
5.1 Conclusion	166
5.2 Future work	170
Author Contribution	172

List of Tables

Table 2.1 Studies conducted on ancient wheat flour-based products	19
Table 2.2 Proximate composition of einkorn, emmer, spelt and common wheat.....	34
Table 3.1 Proximate composition of flour samples from different wheat species (% as-is basis).....	100
Table 3.2 Amino acid composition of flour samples from different wheat species (mg/g crude protein)	101
Table 3.3 Amino acid score (AAS) of flour samples from different wheat species	102
Table 3.4 Content of free, conjugated, and bound phenolic acids in flour samples from different wheat species (mg/kg).....	107
Table 3.5 <i>In vitro</i> starch digestibility and protein digestibility (IVPD) of the bread samples of einkorn, emmer, spelt, and common wheat species.....	115
Table 4.1 Granule size distribution of starch extracted from different wheat species.	147
Table 4.2 Amylose and amylose/ amylopectin (% as-is basis) of starch extracted from different wheat species.....	150
Table 4.3 Thermal behaviour of starch extracted from different wheat species.	153
Table 4.4 Swelling power values amylose and amylose/ amylopectin (% as-is basis) of starch extracted from different wheat species.....	155
Table 4.5 Protein composition of protein extracted from different wheat species as analyzed by SE-HPLC.....	158

List of Figures

Figure 2.1 Origin and domestication of <i>Triticum</i> spp., einkorn, emmer, spelt, durum, and common wheat (Adapted from Kulathunga et al., 2020)	13
Figure 2.2 Relationship between starch structure, nutrition and functional properties....	42
Figure 2.3 Wheat protein functionality and nutritional quality.....	53
Figure 3.1 Arabinoxylan (AX) content of the flour samples from different wheat species (% as-is basis).	104
Figure 3.2 <i>In vitro</i> glucose determination curve of the bread samples of einkorn, emmer, spelt, and common wheat species.....	112
Figure 4.1 The microstructure of the transverse section of wheat. A) Einkorn, B) Emmer, C) Spelt, D) Common wheat.	137
Figure 4.2 The microstructure of Aleurone layers. A) Einkorn, B) Emmer, C) Spelt, D) Common wheat.....	140
Figure 4.3 The microstructure of the endosperm of wheat kernels. A) Einkorn, B) Emmer, C) Spelt, D) Common wheat.....	141
Figure 4.4 Scanning electron microscope images of starch extracted from different wheat species: A) Einkorn, B) Emmer, C) Spelt, D) Common wheat :CWRS Whole wheat, and E) Common wheat :CWRS Refined flour.....	145
Figure 4.5 Granule size distribution of starch extracted from different wheat species	148

List of Abbreviations

DF - Dietary Fiber

SCFs - Short-Chain Fatty Acids

SFA - Saturated Fatty Acids

PA - Palmitic Acid

PUFA - Polyunsaturated Fatty Acids

ALA - Alpha-Linolenic Acid

LA - Linoleic Acid

OA - Oleic Acid

MUFA - Monounsaturated Fatty Acids

SDI - Starch Digestion Index

HRS - Hard Red Spring Wheat

HRW- Hard Red Winter

GLI - Gliadin

GLU - Glutenin

LMW-GS - Low Molecular Weight
Glutenin Subunits

HMW-GS - High Molecular Weight
Glutenin Subunits

PER - Protein Efficiency Ratio

PDCAAS - Protein Digestibility-Corrected
Amino Acid Score

AACCI - American Association of Cereal
Chemists International

AOAC - Association of Official Analytical
Chemists

ISO - International Organization for
Standardization

ANOVA - Analysis of Variance

CWRS WW - Canada Western Red Spring
Whole Wheat

CWRS RF - Canada Western Red Spring
Refined Flour

SDF - Soluble Dietary Fiber

IDF - Insoluble Dietary Fiber

AMG - Amyloglucosidase

RAG - Rapidly Available Glucose

RDS - Rapidly Digestible Starch

SDS - Slowly Digestible Starch

TS - Total Starch

RS - Resistant Starch

IVPD - *In vitro* Protein Digestibility

AAS - Amino Acid Score

UHPLC - **Ultra**-High Performance Liquid Chromatography

AX - Arabinoxylan

PAA/AMG - Pancreatic α -Amylase and Amyloglucosidase

GOPOD - Glucose Oxidase-Peroxidase

SAA - Sulfur-Containing Amino Acids

AAA - Aromatic Amino Acids

IAA - Indispensable Amino Acids

DAA - Dispensable Amino Acids

SE-HPLC - Size-Exclusion High-Performance Liquid Chromatography

DSC - Differential Scanning Calorimeter

SDS EP - Sodium dodecyl sulfate Extractable Protein

SDS UP - Sodium dodecyl sulfate Unextractable Protein

Chapter 1 Introduction

Species such as einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and spelt (*Triticum spelta*), are among the earliest cultivated wheats and have been integral to human diets for centuries (Özbek & Baloch, 2022). These wheat species are collectively known as ancient wheat species. The thick, tough glumes of these hulled wheats provide excellent protection to kernels both in the field and during storage; however, they require an additional processing step, known as dehulling, which is usually performed prior to milling (Nesbitt & Samuel, 1996). The development of hexaploid common wheat (*Triticum aestivum*), marked by the loss of strong glumes/hull, was a pivotal trait in the wheat domestication process (Arzani, 2019; de Sousa et al., 2021). The emergence of high-yielding common wheat, which offered greater processing efficiency, led to the gradual decline in the cultivation of ancient wheat species, resulting in a significant reduction in their use despite their historical importance (Arzani & Ashraf, 2017).

However, the growing awareness of climate change has led to renewed interest in ancient wheat species, which exhibit greater resilience to drought and extreme weather conditions compared to common wheat (Zamaratskaia et al., 2021). These species are valued for their ability to thrive in challenging environments, their suitability for organic farming, and their resistance to pathogens with minimal need for pesticides and fertilizers (Guzmán & Alvarez, 2021; Moudrý et al., 2011). This increasing interest is further fueled by the desire to preserve genetic diversity in agriculture (Lacko-bartošová et al., 2021).

Globally, wheat is a key staple in the diets of a large segment of the population, significantly influencing the nutritional quality of meals and, consequently, the health of individuals

worldwide (Arendt & Zannini, 2013). Currently, the bakery sector is dominated by refined flour-based products, which lack the nutritional characteristics of wholegrains (Arzani & Ashraf, 2017). In contrast, ancient wheat species are commonly consumed as whole grains, ensuring the retention of vital nutritional components, as whole grains consist of the intact, ground, flaked, or cracked caryopsis, which includes the starchy endosperm, germ, and bran (Bordoni et al., 2017; Kulathunga et al., 2021). These whole grains are also associated with numerous health benefits, including a reduced risk of cardiovascular disease, type 2 diabetes, and certain types of cancers (Kulathunga et al., 2021; Shewry, 2009; Wang et al., 2020). Previous literature has reported that ancient wheat species retain higher levels of health-related compounds, including minerals, phytochemicals, and greater nutrient density when compared to conventionally produced cereals (Hammed & Simsek, 2014; Johansson et al., 2021; Roumia et al., 2023). Therefore, in light of the recent demand for nutrient-dense food and interest in sustainable agriculture, ancient wheat species present a promising opportunity to supplement the global wheat industry (Dinu et al., 2018; Kulathunga et al., 2020; Longin & Würschum, 2016). However, despite their potential, there is a limited number of studies comprehensively exploring their nutritional characteristics. This gap in knowledge highlights the need for further research to fully understand their potential and support their expansion in the marketplace. Therefore, the first chapter of this study investigates the detailed nutritional profile of ancient wheat species, hypothesizing that significant differences exist between ancient wheat species and common wheat. The study focuses on proximate composition, arabinoxylan content, amino acid and phenolic acid

profile as well as the digestibility characteristics of these grains, as these parameters are crucial for understanding their functional and health-related properties.

While the first chapter focused on the nutritional profile of ancient wheat species, the second chapter continues on assessing the physicochemical properties of these grains, with a particular emphasis on their starch and protein profiles. The widespread use of common wheat in the food industry can be attributed to its unique dough rheological properties, which are essential for producing a variety of bakery products, particularly leavened bread. The viscoelastic nature of wheat dough, primarily influenced by gluten proteins, which enables gas retention during fermentation, resulting in dough expansion and proper baking (Schofield, 1994; Shewry et al., 2002). In addition to gluten, the starch properties of wheat also play a significant role in its functional characteristics, influencing parameters such as gelatinization and swelling power (Maningat et al., 2009). Therefore, the hypothesis guiding this investigation is that significant differences exist between ancient and common wheat species in terms of starch characteristics and protein composition, which may have important implications for their functionality in diverse food applications. To this end, the overarching objective of the study was to characterize ancient grains for the nutritional potential and physicochemical properties. The specific objectives of outlined below.

Specific objectives

Chapter 01

To explore the nutritional potential and health implications of ancient wheat: including einkorn, emmer, and spelt by investigating the chemical composition and digestibility characteristics of einkorn, emmer and spelt, in comparison to Canada Red Spring Wheat (CWRS).

Chapter 02

To investigate the starch and protein characteristics of ancient wheat species, including einkorn, emmer, and spelt, and assess their functionality for potential use in food applications in comparison to CWRS wheat.

Chapter 2 Literature Review

Nutritional Characteristics and Physicochemical Properties of Ancient Wheat Species for Food Applications

A version of this chapter (section 2.1-2.8) has been published in Food Bioscience

Deyalage, S. T., House, J. D., Thandapilly, S. J., & Malalgoda, M. (2024). Nutritional

Characteristics and Physicochemical Properties of Ancient Wheat Species for Food

Applications. Food Bioscience, 105397. <https://doi.org/10.1016/j.fbio.2024.105397>

Creative Commons Attribution 4.0 International License: [Link](#)

Abstract

Wheat is fundamental to human civilization and has played a prominent role in feeding the world and enhancing food security globally. As a staple food commodity, wheat contributes a significant portion of the human daily caloric intake in many parts of the world. However, given the need for diversifying the global food system, there is an increasing interest in identifying wheat species which are healthy and can be grown sustainably. The earliest cultivated forms of wheat (einkorn, emmer, and spelt) have been reintroduced recently for their potential for producing nutritionally rich food products with exceptional health benefits. Several studies revealed notable differences in the nutritional composition of ancient wheat compared to common wheat particularly in terms of protein and starch content, dietary fiber, minerals, and vitamins. Additionally, ancient wheat exhibited higher levels of bioactive compounds, such as phenolics and carotenoids, which are associated with potential health- promoting effects. Furthermore, investigations into the structural and physicochemical properties of ancient wheat demonstrated distinct characteristics,

predominantly the variations in gluten protein composition, potentially leading to differences in dough rheology and breadmaking qualities. This review consolidates current knowledge on the nutritional characteristics and structural and physicochemical properties of ancient wheat species, providing valuable insights into their potential significance. Exploring ancient wheat facilitates the development of novel food products which could ultimately promote a healthy and sustainable food system.

Keywords: Einkorn, Emmer, Spelt, Sustainability, Starch, Protein, Nutrition

2.1 Introduction ¹

Wheat is fundamental to human civilization and has played a prominent role in feeding the world and enhancing food security globally (Shiferaw et al., 2013). It is one of the oldest domesticated crops, originating in the pre-pottery Neolithic East Fertile Crescent around 10,000 years ago (Harlan & Zohary, 1966). The domestication of wheat signifies the transition from a lifestyle centered around hunting and gathering to an agrarian economy,

¹ DF: Dietary fiber; IDF: Insoluble dietary fiber; SDF: Soluble dietary fiber; SCFs: Short-chain fatty acids; AX: Arabinoxylan; SFA: Saturated fatty acids; PA: Palmitic acid; PUFA: Polyunsaturated fatty acids; ALA: Alpha-linolenic; LA: Linoleic acids; OA: Oleic acid; MUFA: Monounsaturated fatty acids; RDS - Rapidly Digestible Starch; SDS - Slowly Digestible Starch; RS - Resistant Starch; DSI: Starch digestion index; RAG - Rapidly Available Glucose; HRS: Hard Red Spring wheat; GLI: Gliadin; GLU: glutenin; LMW-GS : Low molecular weight glutenin subunits; HMW-GS: High molecular weight glutenin subunits; PD: Protein digestibility; PER: Protein Efficiency Ratio; PDCAAS: Protein Digestibility-Corrected Amino Acid Score

marking the dawn of human civilization (Eckardt, 2010). Human nutrition is closely related to grain consumption and about 35% of humans' calorie intake comes from main cereal crops such as rice, wheat, and corn (Soto-Gómez & Pérez-Rodríguez, 2021). In fact, wheat is one of the world's three major cereals, accounting for a considerable portion of the human dietary structure (Li et al., 2022). Wheat is also considered an important source of protein as well as dietary fiber, B vitamins, minerals, and various other phytochemicals in the human diet (Shewry & Hey, 2015b). The success of wheat can be attributed to its high yields and adaptability, but these factors alone do not fully explain its prevailing dominance in the temperate world. A crucial aspect contributing to wheat's superiority over other temperate crops lies in the unique qualities of the dough produced from wheat flour. This characteristic enables the processing of wheat into a broad range of food products, encompassing bread, baked goods, pasta, noodles, and other processed foods. These properties are determined by the structural arrangements and interactions of the storage proteins, which collectively form the 'gluten' protein network (Shewry, 2009; Uthayakumaran & Wrigley, 2010, pp. 59-111). The earliest cultivated wheat cultivars were landraces identified by farmers and domesticated from wild populations, primarily due to superior yield and other favorable characteristics; this marks a non-scientific form of plant breeding (Shewry, 2009). These ancient wheat species are characterized as hulled wheat enclosed by a tough glume, which requires removal from the grain before the milling process (Abdel-Aal & Hucl, 2002). These thick, tough glumes offer superior protection to the grains in both field conditions and during storage (Nesbitt & Samuel, 1996). However, due to the retention of its glumes after harvesting, ancient wheat species are prone to chaff contamination, which prevents their

immediate use in food processing. To prepare hulled wheat for milling, its spikes must undergo processing, such as dehulling, to remove the attached hulls. This extra step is a disadvantage compared to common wheat, where grains can be threshed directly (Roumia et al., 2023). The gradual domestication of wheat species resulted in changes to the structure of the rachis and glumes. The fragility of the glumes in common wheat species offers a technological benefit by making it easier to grind the grains into flour (de Sousa et al., 2021). The dehulling process typically utilizes traditional equipment, including roll hullers, horizontal dehullers and impact dehullers, to separate the hulls from the kernels effectively. Since no dehulling system achieves complete efficiency, additional equipment is often necessary. Sorting dehulled products after each stage is important to separate cleaned grains, and this can be accomplished using sieves, paddy machines, or triers, leveraging the size differences between the kernels and grains (Sots et al., 2024). The efficiency of the process is influenced by the type of equipment used and the manner in which the glume is attached to the caryopsis (Belcar et al., 2020).

The emergence of hexaploid common wheat (*Triticum aestivum*), was initiated 8500-9000 years ago from the hybridization of cultivated emmer *Triticum turgidum* with unrelated wild grass *Aegilops tauschii*, the combination entails merging the genome with the AABB tetraploid, resulting in the formation of AABBDD hexaploid wheat (**Figure 2.1**) (Khan & Shewry, 2009; Levy & Feldman, 2022).

In the 20th century, the cultivation of "ancient" wheat, including einkorn (*Triticum monococcum* L., diploid), emmer (*Triticum dicoccum* L., tetraploid), and also spelt (*Triticum aestivum* ssp. *spelta*, hexaploid), remained considerably low compared to the cultivation of

common wheat (*Triticum aestivum* L., hexaploid) and durum wheat (*Triticum durum* L., tetraploid) (Gesslits et al., 2019). Wheat cultivars that have been released over the last decades have been recognized for their higher yields and for fulfilling the expected requirements in the baking industry making such cultivars feasible. However, several ancient wheat species have been reintroduced in recent decades and these underutilized species are rapidly becoming significant contributors to the development of a more diverse global agricultural food system, and appear to hold enormous potential for future livelihoods (Cheng, 2018; Rodríguez-Quijano et al., 2019). The resurgence of ancient wheat species is fueled by their multifaceted appeal to farmers, bakers, and consumers. Farmers are inclined towards these species for crop diversification, offering advantages such as limited fertilizer requirements, adaptability to diverse conditions, and resilience to stresses. Bakers find them valuable for creating specialty products, enhancing both organoleptic and nutritional qualities to compete effectively (Belcar et al., 2021; Kohajdová & Karovičová, 2008). Simultaneously, consumers increasingly favor products derived from ancient wheat species due to their heightened nutritional value, unique taste, flavor, and associated health benefits (Dinu et al., 2018; Nakov et al., 2018). Notably, certain studies have identified high levels of bioactive or functional compounds in ancient wheat species indicating an increased potential for health benefits and positive physiological effects (Dhanavath & Prasada Rao, 2017; Zrcková et al., 2019).

With growing consumer awareness about nutrient-dense grain-based food, the consumption of ancient wheat-based products has increased, therefore there has been a revived interest in these wheat species in the last 20 years (Geisslitz et al., 2018). Currently, ancient wheat

species have reflected the potential in certain markets in the form of organic or healthy foods and multi-grain food products (Abdel-Aal & Hucl, 2002). The renewed focus on these ancient wheat species is also driven by the rising demand for traditional food products, the need for species adaptable to marginal areas, and the essential goal of conserving genetic diversity (Lacko-Bartošová & Čurná, 2015). The foregoing information ensures these underexplored species can serve to diversify food sources and boost nutritional enrichment, while simultaneously fostering sustainable and resilient agricultural systems (Cheng, 2018). However, a notable gap persists, particularly in a more comprehensive analysis of the nutritional potential of emmer, einkorn, and spelt, as previous reviews have predominantly focused on their proximate composition, offering limited insights into various nutritional characteristics. In response, this comprehensive review aims to bridge this gap by conducting a more thorough analysis of the available literature, encompassing the proximate composition and various other nutritional parameters of emmer, einkorn, and spelt. Additionally, this review expands its scope on the structural and physicochemical attributes of ancient wheat species, which are critical factors influencing flour performance in various food applications. By exploring both nutritional and structural aspects, this review aims to be a pivotal resource, offering valuable insights for dietary and industrial applications of ancient wheat.

2.2 Role of wheat for global food security

Cereal grains play a fundamental role in the global diet, serving as the primary source of dietary energy and carbohydrates (Poutanen et al., 2022). Carbohydrates are generally regarded as the principal component of cereals, supplying the dietary energy that is essential

for relieving hunger and facilitating human function (Shewry & Hey, 2015b). In addition, the composition and structure of these dietary carbohydrates have significant effects on the gut microbiota (Poole et al., 2021a).

Wheat alone plays a critical role in safeguarding global food and nutrition security by supplying around 18% of daily caloric needs in developing countries, in contrast to the global average of 19%, and 21% in high-income countries (Jiang et al., 2022; Shiferaw et al., 2013). Furthermore, as a substantial protein source in the human diet, wheat contributes 20% to the overall protein intake of the global population, and will continue to be center stage in universal efforts to feed the growing population (Arzani & Ashraf, 2017; Poole et al., 2021b).

2.2.1 Cultivated wheat today: Common wheat

The genus *Triticum* consists of a diverse array of species; however, only two species are cultivated on a commercial scale; *T. aestivum* –genetically hexaploid with genomes A, B, and D, known as common wheat or bread wheat, and *T. durum*-genetically tetraploid, with genomes A and B, also known as durum wheat (Iqbal et al., 2022; Uthayakumaran & Wrigley, 2010). Common wheat is also classified as either red or white wheat (depending on the level of red pigmentation present in the seed coat), hard and soft wheat (depending on protein level and the seed's resistance to crushing), and winter and spring (according to their growth habit). In regions with cold climates, spring wheat is planted during the spring, undergoes growth throughout the summer, and is harvested in the fall before the onset of frost. Conversely, in moderate climates, winter wheat is sown in the fall, initiating growth, and then enters a dormant phase over winter. Substantial plant growth for winter wheat takes place the following spring, with harvest occurring in the summer (Hui et al., 2008). That means

for winter wheat, the plant requires exposure to low temperatures before it can initiate reproductive development. This process is known as vernalization. Additionally, high-density crops such as winter wheat contribute significantly to reducing erosion risks, thereby preserving the arable layer over extended periods, potentially spanning thousands of years, despite periodic tilling activities along the slope. Additionally, winter wheat offers benefits in reducing soil erosion and mitigating spring runoff, further enhancing its ecological value in sustainable agriculture practices (Basic et al., 2004).

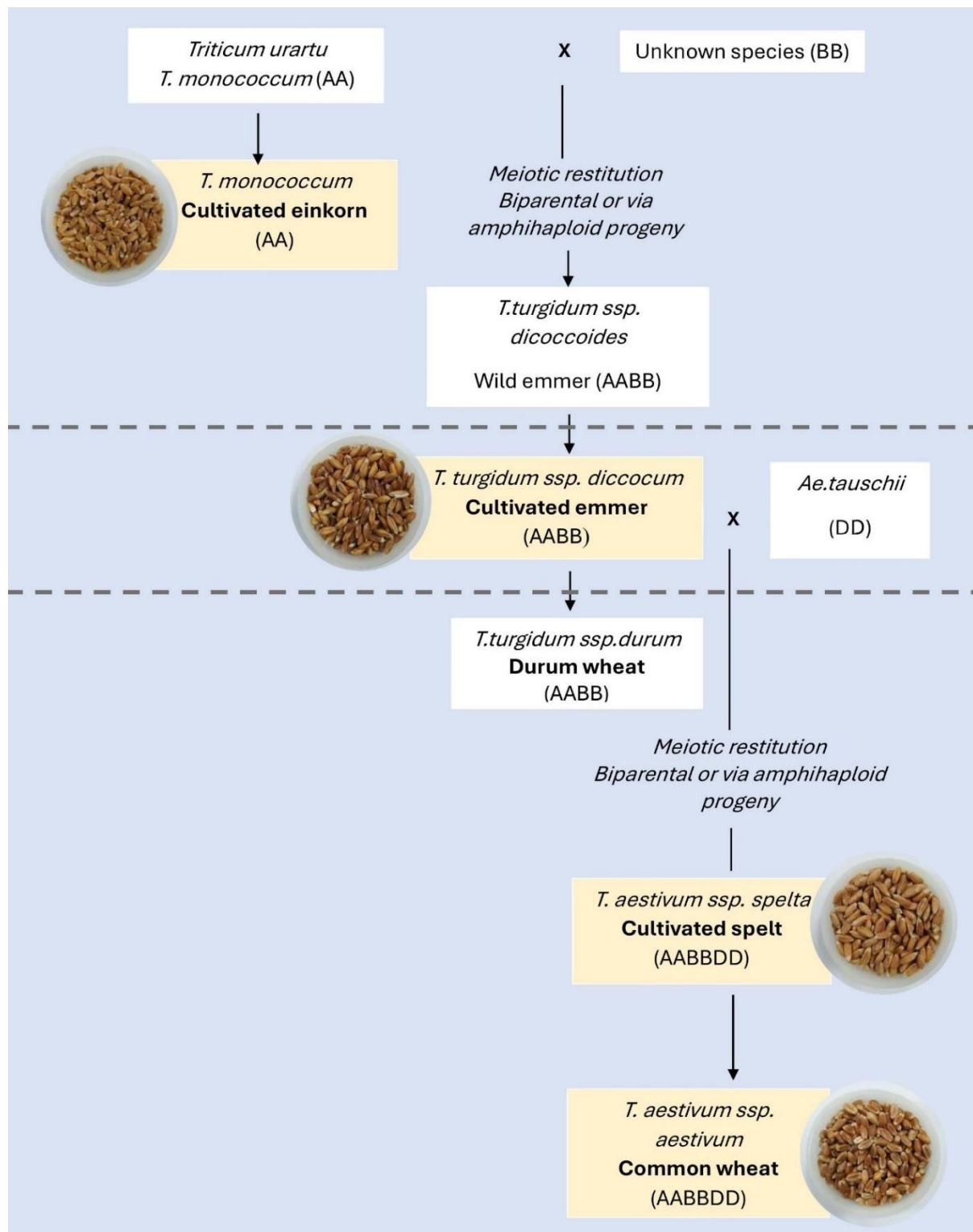


Figure 2.1 Origin and domestication of *Triticum* spp., einkorn, emmer, spelt, durum, and common wheat (Adapted from Kulathunga et al., 2020)

In contrast to winter wheat, spring wheat does not necessarily require vernalization, but some cultivars can still respond to cold by flowering early. This suggests that the vernalization response is a key factor in regulating the reproductive development of wheat and plays an important role in the adaptation of wheat to different environments (Iqbal et al., 2011; Shindo & Sasakuma, 2002). Red wheat species demonstrate increased dormancy compared to white wheat species, making them preferable in climates that are conducive to pre-harvest sprouting (Arendt & Zannini, 2013; Khan & Shewry, 2009). In addition to vernalization, photoperiod sensitivity is another crucial factor influencing reproductive development and adaptation of wheat to various environments. Photoperiod genes regulate the plant's response to day length, controlling flowering time once the vernalization requirement is met (Amo et al., 2022). Photoperiod sensitivity ensures that wheat flowers only after reaching a critical day length, but photoperiod insensitivity has been selected by breeders in some cultivars to enhance yield under different climatic conditions (Langer et al., 2014). This adaptation, controlled by the interaction of vernalization and photoperiod genes, affects spike development, organ formation, and yield components. In regions with cold winters, vernalized wheat flowers rapidly as day length increases in spring, optimizing yield potential (Hyles et al., 2020; Steinfert et al., 2017).

2.2.2 Common wheat utilization and sustainability

The projected total utilization of wheat is estimated to reach close to 780 million tons during the 2023/24 period, and the demand for wheat on a global scale is anticipated to escalate alongside population growth (Food and Agriculture Organization [FAO], 2023). According to recent projections by the United Nations, the global population may reach 8.5 billion in

2030, 9.7 billion in 2050, and 10.4 billion in 2100 (United Nations, 2022). As the world population is expected to grow, the global demand for wheat is predicted to rise by 60%. In order to address this requirement, there is a necessity for an annual increase in wheat production from its current level of less than 1% to a minimum of 1.6% (Bezabeh et al., 2022).

Currently, hexaploid common wheat accounts for over 95% of global wheat production, with tetraploid durum wheat accounting for the majority of the remaining 5% (Shewry, 2009).

Other species within the *Triticum* genus such as einkorn, emmer, and spelt (*T. monococcum*, *T. dicoccum*, *T. spelta*) which had played a significant role in global food production for thousands of years, are now grown and utilized to a much lesser extent (Longin & Würschum, 2016). Common wheat, which acts as a dominant staple crop, has been developed mainly for intensive agriculture, with management practices geared towards maximizing yields through the use of high levels of inputs (Mijatović et al., 2013). However, climate change and global warming can have a severe impact on wheat production and can negatively affect both crop yield and quality. By using these ancient wheat species, which have been naturally adapted to specific environmental conditions over time, there is a greater potential to achieve greater resilience and adaptability in wheat production. Emmer and einkorn, known for their enhanced disease resistance, particularly in extreme conditions, emerge as valuable genetic resources. As climates undergo variations, including prolonged periods of extreme temperatures and intense rainfall, the adaptability of these ancient wheats gains significance. Thriving in marginal areas with poor soils and adverse climatic conditions, ancient wheat offer a promising source for desirable traits in modern plant breeding. Their

special attributes, such as disease tolerance and improved nitrogen use efficiency, play a pivotal role in fostering sustainability in wheat cultivation (Bencze et al., 2020; Guzmán & Alvarez, 2021; Migliorini et al., 2016)

2.3.3 Resurgence of ancient wheat species

The cultivation of ancient wheat experienced a significant decline in the 1960s, primarily attributed to their lower yields (reduced productivity) and the challenging processing caused by the presence of hulls which require an additional step in flour production (Gesslits et al., 2019). In terms of yield, ancient grains generally produce significantly lower yields compared to hexaploid common wheat, with spelt at 37%, emmer at 55%, and einkorn at 62% (Geisslitz et al., 2018). Furthermore, they encounter challenges related to functionality, which is linked to differences in their physicochemical properties compared to common wheat, primarily due to the inferior gluten quality inherent to these ancient species (Belcar et al., 2020). However, there has been a growing interest in cultivating these ancient wheat species due to their unique characteristics. One of the main factors is the increased resistance of ancient wheat species to pathogens and adverse conditions, making them particularly suitable for organic farming (Rodríguez-Quijano et al., 2019). Underutilized grains are also in demand because they are perceived as healthy, owing to their common use as whole grains which contain higher contents of fiber and micronutrients (Costanzo et al., 2019).

The potential expansion of marketing opportunities due to increasing consumer demands grant these ancient wheat species a better chance of survival as well as a means to preserve their genetic heritage (Piergiovanni, 2013). According to previous literature, these ancient wheat species play a crucial role in ensuring food security, not only as a source of readily

available genetic material for breeders but also due to their ability to thrive in marginal environments and their lower sensitivity to environmental changes (Migliorini et al., 2016; Yang et al., 2022; Zaharieva et al., 2010).

Nowadays, various food items such as bread, pasta, snacks, crackers, cookies, breakfast cereals, as well as beverages can be prepared from ancient wheat species. The choice of which wheat species to use for each product is determined by its suitability for the specific culinary application (Kulathunga et al., 2020). However, the scientific literature on this subject remains relatively limited. A summary of findings on ancient wheat-based products is presented in **Table 2.1**. According to Brandolini and Hidalgo (2011) einkorn bread displays a lighter color than those of common and durum wheat breads, indicating lower heat damage during baking due to its reduced α - and β -amylase activity, which limits starch degradation. A comparative analysis of ancient wheats revealed that breads made from spelt and einkorn are of good quality; however, emmer wheat failed to meet essential quality parameters for organoleptic evaluation (Belcar et al., 2021). Although bread with einkorn alone typically has the lowest loaf volume, replacing 30% - 50% of einkorn flour with common wheat flour significantly enhances volume, structure, and crumb elasticity, resulting in a high organoleptic score (Piasecka-Jóźwiak, 2015). In another study, baked goods formulated with 100% spelt flour exhibited lower overall and specific volumes, characterized by an open grain structure and coarse texture. Among these formulations, those containing 15% spelt flour were determined to be the most acceptable in terms of sensory quality (Kohojdova & Karovicova, 2007). Furthermore, the incorporation of increased ratios of germinated ancient wheat flour (emmer and einkorn) significantly

enhanced both the functional components and nutritional value of the bread. Additionally, the use of low ratios of this flour contributed positively to the technological quality of the final product (Tekmile Cankurtaran-Kömürcü & Bilgiçli, 2023).

In recent years, there has been a growing trend toward incorporating ancient wheat species into sourdough applications due to their nutritional benefits and distinct sensory qualities (Şerban et al., 2021). The comparison between common and ancient wheat revealed that sourdough bread made from ancient wheat was perceived as less sour in taste, aroma, and flavor, resulting in more sensory appeal than that made from common wheat (Škrobot et al., 2022).

Despite their increasing demand, the application of ancient wheat in bread production presents several technological challenges. Specifically, ancient wheat flours tend to produce doughs that are low in strength, highly tenacious, and exhibit limited extensibility (Cappelli et al., 2018). The baking quality of wheat flours is principally determined by gluten quality and quantity. Common wheat is recognized as the ideal grain for bread-making due to its ability to produce a viscoelastic dough when mixed with water, owing to its high-quality gluten, which provides essential elasticity and strength. In contrast, ancient wheat species yield softer doughs with lower elasticity and greater extensibility, primarily due to their inferior gluten quality. This limitation makes them less effective for traditional baking compared to common wheat, significantly affecting the texture and sensory qualities of the final products (Geisslitz et al., 2019; Wrigley et al., 2006). Furthermore, ancient wheat species are generally consumed in their whole grain form, which limits the extent of refining. However, the presence of wheat bran into bread formulations can negatively affect the final

product due to fiber components disrupting the gluten matrix, diluting gluten content, and competing with water for binding, which compromises the viscoelastic properties of the dough (Boita et al., 2016). Given these considerations, further research is vital to investigate processing techniques and develop new formulations to produce desirable products from ancient wheat species (Valsalan et al., 2023).

Table 2.1 Studies conducted on ancient wheat flour-based products

Flour type	Food	Characteristics/findings	References
Einkorn	Specialty	Deep yellow crumb colour	Brandolini and Hidalgo, 2011
	bread	High bread making qualities for some genotypes	
Einkorn	Bread	Bread volume was mainly dependent on the content of glutenins Einkorn lines characterized by elevated levels of glutenin and reduced GLI/GLU ratios exhibit favorable characteristics for the production of bread.	Wieser and Peter (2009)
Einkorn	Bread	Poor loaf volumes The application of individual enzymes and emulsifiers can significantly improve the crumb firmness of whole-grain einkorn breads	Grausgruber et al. (2008)
Einkorn	Bread with einkorn +	Improved bread volume, structure, and crumb elasticity	Piasecka-Józwiak et al. (2015)

	common wheat flour		
Einkorn	Bread	lowest bread volumes	Abdel-Aal et al. (1997)
Einkorn	Water biscuits	Preserved nutritional quality compared to common wheat	Hidalgo and Brandolini, (2011)
Einkorn	Cookies	Thinner, larger, slightly darker, and higher breaking resistance, higher carotenoid content and less heat damage than the cookies made of common wheat	Hidalgo et al. (2019)
Einkorn	Pasta	Similar technological characteristics and better nutritional value than durum wheat pasta. lower amount of solid losses into cooking water lutein was present in good quantities in einkorn pastas even after storage or cooking	Brandolini et al. (2018)
Emmer	Breads	The use of ancient wheat flour (einkorn and emmer) showed lower bread volume compared to common wheat flour	Cankurtaran-Kömürcü and Bilgiçli (2023)

		Increasing ratios of germinated ancient wheat flour increased the functional component and nutritional value of the bread	
	Breads	Inferior baking quality due to lower gluten index (23%–39%)	Konvalina et al. (2013)
Emmer	Pasta	Favorable organoleptic quality	Fares et al. (2008)
Emmer	Puff pastry	Bright crust colour Regular form Large volume	Zaparenko et al. (2020)
Spelt	Breads	Greater crumb elasticity in comparison to the breads made with common wheat significantly lower crumb cell homogeneity	Callejo et al. (2015)
Spelt + Common wheat flour blends	Breads	Lower volume, specific volume and a rather open grain and coarse texture 15% of spelt flour was the most acceptable	Kohojdova and Karovicova (2007)
Spelt	Bread	Bread from spelt flour exhibits lower specific volume, darker crumb and crust color and increased crust and crumb hardness	Frakolaki et al. (2018)

Green spelt	Bread	Green spelt is an excellent additive to improve the dietary value of bakery products	Kraska et al. (2020)
Spelt	Pasta	The combination of high protein >13.5% and high temperature drying resulted in pastas with satisfactory cooking quality	Marconi et al. (2002)
Spelt	crackers	Acceptable quality with lower snap back, and lower hardness Low oven spring (negatively reflected to cracker texture)	Filipčev et al. (2013)
Spelt	crackers	lower tendency toward shape deformation Less hard textures.	Filipčev et al. (2012)

2.3 Chemical composition of ancient wheat species

2.3.1 Protein content

Ancient wheat species are reported to have consistently higher protein content compared to common wheat cultivated under the same growing conditions (Bencze et al., 2020). In the recent comprehensive review published by Arzani and Ashraf (2017), variations in protein content among these wheat species are highlighted. The reviewed literature revealed that einkorn displayed a crude protein range (dry matter basis :dm) (% nitrogen x 5.7) of 13.8%-22.8%, with emmer falling within 13.5% and 23.92%, spelt exhibiting a content of 12.8%-

18.7%, and common wheat encompassing levels ranging from 8.5% to 16.8% (**Table 2.2**) (Akar et al., 2019; Brandolini et al., 2008). According to Biel et al. (2021), einkorn exhibited the highest protein content (dm) at 18.1%, while emmer had a protein content of 15.4%. Spelt followed with a protein content of 12.8%, and common wheat had the lowest protein content at 11.0%. In accordance with these results, Akar et al. (2019) also reported the highest protein content (dm) for einkorn (15.76%-24.75%) followed by emmer (13.51%-23.92%) wheat. The lower protein content of common wheat can be attributed to its larger size and heavier grains that yield an increased proportion of starchy endosperm and consequently a lower protein content (Arzani & Ashraf, 2017). Therefore, this decline in protein content is could be due to the breeding of high-yielding cultivars, which leads to a dilution effect on grain protein content (Shewry et al., 2016). In order to maintain bread quality despite the decrease in total protein, newer cultivars have been selected for an increased amount of functional protein, particularly polymeric high molecular weight (MW) glutenin proteins (Ohm et al., 2010).

However, Kulathunga et al. (2021) compared the protein content of hulled wheat cultivars with Hard Red Spring (HRS) wheat and reported lower levels for hulled wheat compared to HRS wheat. The protein content in the wheat kernel is significantly influenced by the cultivar/genotype, environmental conditions, fertilizer use, and other agricultural practices (Biel et al., 2021; Tran et al., 2020; Čurná & Lacko-Bartošová, 2017; Løje et al., 2003). However, as per the findings of Shewry et al. (2013), the influence of nitrogen fertilization on grain protein content was more pronounced than that of the genotype. The gluten protein composition of ancient and common wheat in terms of gliadin to glutenin ratio (GLI/GLU)

were determined by Geisslitz et al. (2019) as this is essential for breadmaking quality, especially for good loaf volume. The GLI/GLU ratios of common wheat were shown to be lower compared to ancient wheat (Geisslitz et al., 2019). Achieving optimal dough characteristics relies on a proper balance between gliadin which contributes to dough viscosity and glutenin which contributes to dough strength and elasticity (Uthayakumaran & Wrigley, 2010). Therefore, from a processing standpoint, ancient wheat species require process modifications or ingredients to enhance the quality of products developed from these flours.

2.3.2 Starch content

In wheat, starch is the principal carbohydrate stored in the wheat endosperm and acts as a multifunctional ingredient for the food and non-food industries. During grain development, starch is accumulated within the endosperm in the form of distinct semicrystalline aggregates referred to as starch granules (Rosicka-kaczmarek, 2018). While starch primarily consists of carbohydrates, there are also minor constituents such as proteins, ash, lipids, and dietary fiber present both on the surface and within the granules. Starch granules are comprised of two glucose polymers classified as amylose and amylopectin (Kim & Kim, 2021). Amylose is a linear molecule of α -(1,4) linked α -D-glucopyranosyl units, with certain molecules being slightly branched by α -(1,6) linkages. Amylopectin is highly branched and it is formed mainly by α -(1,4) linkages but with 5–6% of (1,6) bonds at the branch points (Buléon et al., 1998). The ratio of these glucose polymers differs according to the botanical origin of the starch (Tester et al., 2004). According to previous literature, the total starch content (dm) in ancient wheat species range as follows: einkorn (60.6%-71.4%) (Abdel-Aal

et al., 1995; Brandolini et al., 2008; Kulathunga et al., 2021), emmer (63.67%-70.7%) (Brandolini et al., 2008; Kulathunga et al., 2021; Lacko - Bartošová & Čurná, 2015), spelt (60.9%-65.8%) and the value for common wheat exhibited a range from 60.5% to 72.9% (Abdel-Aal et al., 1995; Brandolini et al., 2008; Kulathunga et al., 2021). The variations in starch content could be attributed to the association between genotype and environmental conditions (Massaux et al., 2008). The amylose content of einkorn on a dry basis ranged from 23.2% to 28.6%, spelt 28.6%-28.9% while common wheat was reported to have a range of 23.2%-27.6% (Brandolini et al., 2008). According to Şerban et al. (2021) starch in spelt may exhibit 2.1% up to 12% more amylose compared to hard red winter (HRW) wheat.

2.3.3 Fiber content

Dietary fiber (DF) is defined as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. DF includes polysaccharides, oligosaccharides, lignin, and associated plant substances. It promotes beneficial physiological effects including laxation and/ or blood cholesterol attenuation and/or blood glucose attenuation” (The American Association of Cereal Chemists [AACC], 2001). The fermentation of DF leads to the generation of short-chain fatty acids which are critical for maintaining intestinal and immune homeostasis in the human body (Markowiak-kope & Slizewska, 2020). Dietary fiber is categorized as insoluble dietary fiber (IDF) or soluble dietary fiber (SDF) based on its solubility in an aqueous solution following the breakdown by starch and protein degrading enzymes (McCleary et al., 2012). According to Løje et al. (2003), the dietary fiber content (dm) was observed to be the highest in common wheat at 12.3%, while spelt had a content

of 10.3%, einkorn had 8.7%, and emmer had 7.9%. Furthermore, the HEALTHGRAIN study (which was focused on developing novel and healthy food products based on wholegrains of wheat and other cereals) also reported the highest amount of dietary fiber (dm) for common wheat genotypes (11.5-18.3 g/100 g) compared to ancient wheat species; spelt, ranging from 10.7 to 13.9 g/100 g, einkorn, ranging from 9.3 to 12.8 g/100 g, and emmer, ranging from 7.2 to 12.0 g/100g). In contrast, Biel et al. (2021) observed the highest values in einkorn and emmer whereas the lowest value was identified in common wheat. These contradictory results can be explained by the differences in genotype, location, and agricultural practices. Moreover, the fiber content can vary depending on the method of analysis (Kulathunga & Simsek, 2022). The latter authors reported significantly lower levels of insoluble dietary fiber as well as total dietary fiber and higher levels of low molecular soluble dietary fiber (LW-SDF) in ancient wheat species than in common wheat. Due to the higher concentrations of LW-SDF (raffinose, stachyose, fructo-, and galacto-oligosaccharides) in einkorn, it could be explored as a potential candidate prebiotic source in novel food applications since SDF can be easily fermented by gut microbiota, resulting in the production of short-chain fatty acids (SCFAs) (Kulathunga & Simsek, 2022; Løje et al., 2003). Arabinoxylan (AX) constitutes a significant portion of the dietary fiber present in cereal grains., and it consists of a linear β (1-4)-linked xylan backbone with α -1-arabinofuranose units connected laterally via (1-3) and/or (1-2) linkage. The majority of xyloses are mono substituted; however, the level of substitution is regulated by wheat type and the maturity of the wheat grain. According to previous findings, AX has been identified as a prebiotic and its utilization has proven highly

beneficial in promoting both the variety and function of gut microbiota (Lu et al., 2000; Schupfer et al., 2021).

Due to its comparatively high level of arabinose substitution along the xylan backbone, arabinoxylan generated from wheat is recognized to be highly fermentable (Chudan et al., 2023). Mainly AX structures are classified into branched and linear molecules, and branched AXs structures have been recognized for their greater efficacy in promoting the proliferation of gut microbiota and boosting the production of short-chain fatty acids (SCFAs) according to *in vitro* studies available (Schupfer et al., 2021). Gebruers et al. (2008) observed a higher variation of AX content (dm) in common wheat flour and the value ranged from 0.30% to 1.40%. In einkorn the variation was from 1.45% to 2.35% and for spelt; from 1.60% to 2.15%. Emmer exhibited values with the lowest variation from 1.40% to 1.95%. Apart from AX, β -glucan is also present in wheat grain as one of the major cell wall polysaccharides. The monomer units of β -D-glucose are linked by glycosidic bonds at β (1 $\rightarrow$ 3), (1 $\rightarrow$ 4), and/or (1 $\rightarrow$ 6), occurring in either a branched or an unbranched configuration (Ahmad & Kaleem, 2018). According to previous literature, total β -glucan content (dm) in emmer ranged from 0.3% to 0.4%, from 0.23% to 0.90% in spelt, and from 0.25% to 0.48% in einkorn (Gebruers et al., 2008; Grausgruber et al., 2004).

2.3.4 Ash content

Cereals are a source of several minerals, and these minerals are important for nutritional and technological reasons (Biel et al., 2021). The content of ash and the color of the flour are indirectly related to the processing methods employed and product quality. The mineral concentration gradually increases from the inner part of the wheat kernel towards the outer

seed coat, reaching its highest point in the aleurone and bran layers (Khan & Shewry, 2009). As reported by Biel et al. (2021), einkorn demonstrated the highest level of crude ash content (dm), measuring 2.65%. Subsequently, emmer displayed a content of 2.16%, spelt contained 1.86%, and common wheat possessed the lowest content at 1.52%. The lower levels of minerals in common wheat can be an outcome of mineral dilution caused by higher grain yields, which are aimed at maximizing milling output. Hence, this parameter may serve as an indication of the primitive nature of ancient wheat (Rasmusson et al., 1971). The ash content of flour can vary across different years, regions, and classes, even when the level of flour refinement remains constant (Khan & Shewry, 2009). Einkorn, emmer, and spelt have demonstrated significantly higher amounts of zinc (34%-54%), iron (31%-33%) and Copper (3%-28%) compared to common wheat (Suchowilska et al., 2012). Therefore, the consumption of these ancient wheat species with other plant-based foods, has the potential to provide a substantial contribution to the intake of minerals (Zamaratskaia et al., 2021). According to Biel et al. (2021), einkorn exhibited a higher content of potassium, magnesium, and calcium, and the lowest content of magnesium and potassium was observed in common wheat and the reported values were 40% and 27% lower compared to einkorn. Emmer is characterized by higher efficiency of zinc absorption and it could be a valuable parental genotype for breeding new cultivars (Genc & McDonald, 2008).

2.3.5 Lipid content

Cereal lipids are a complex family of constituents, that exist as free and bound to various other components in the grain, such as proteins and starch (Ruibal-Mendieta et al., 2002). They are more abundant in the germ followed by the aleurone layer and then the endosperm

(Khan & Shewry, 2009). According to Kulathunga et al. (2021), ancient wheat species are reported to have a slightly higher content of crude fat than common wheat. The reported values are; 2.1%–2.5% in einkorn, 2.0%–2.3% in emmer, 1.0%-1.9% in spelt and 0.6%–1.4% (dm) in common wheat. Piergiovanni et al. (1996) observed lipid values (dm) in fifty emmer accessions ranging from 1.4% to 2.8%, with a mean of 2.0%. For thirty-seven spelt accessions, the observed lipid values varied from 1.7% to 2.5%, with a mean of 2.1%. Ancient wheats were low in saturated fatty acids (SFA) such as palmitic acid (PA) and polyunsaturated fatty acids (PUFA), including alpha-linolenic (ALA) and linoleic acids (LA). Additionally, they were higher in monounsaturated fatty acids (MUFA), such as oleic acid (OA), compared to common wheat (Hidalgo et al., 2009; Kulathunga et al., 2021). In terms of the fatty acid profile, LA, PA, and OA are by far the predominant fatty acids in einkorn, and LA, followed by OA, PA and ALA are highest in emmer (Hidalgo & Brandolini, 2014; Čurná & Lacko-Bartošová, 2017). The major fatty acids observed in spelt were LA, PA, OA and ALA (Grela, 1996). From a nutritional perspective, a high MUFA content in foods, coupled with a low SFA content, contributes to the prevention of cardiovascular disease since MUFA can influence lipid and cholesterol synthesis, reducing the risk of atherosclerosis and thrombosis (Hidalgo et al., 2009). As with other grain macromolecules, it should be emphasized that lipid content and composition can be influenced by wheat class and cultivar (genetic variation), environmental factors during growth, and the effects of the genetic x environment interaction (Chung et al., 2009)

2.3.6 Vitamins

Ancient wheat species and common hulless wheat species have a large variability in terms of B complex vitamins and tocopherols (Karakas et al., 2021). Einkorn is reported to have a higher content of Vitamin B2, B6, and Vitamin A compared to spelt and common wheat (Abdel-Aal et al., 1995; Hammed & Simsek, 2014). Gabrovská et al. (2002) observed higher levels of vitamin B5 (pantothenic acid) (1.14 mg/100 g) in emmer compared to einkorn (0.47 mg/100 g) and spelt (0.60 mg/100 g) on a dry matter basis. In a study conducted by Ranhotra et al. (1995), three B vitamins, namely B1 (thiamine), B2 (riboflavin), and B3 (niacin), were studied. The results revealed that spelt exhibited a higher concentration of niacin (5%) compared to HRW wheat. Folate has been studied using ancient and common wheat species in the HEALTHGRAIN project and higher quantities were reported in durum (0.74 µg/g dm) and emmer (0.69 µg/g dm). Folate has the ability to prevent neural tube defects in the fetus and there is a link between inadequate or suboptimal folate intake and the occurrence of various health problems (Piironen et al., 2008). Tocopherols, members of the vitamin E family of bioactive compounds, are lipid-soluble antioxidants, classified into two main classes namely tocopherols and tocotrienols (Giambanelli et al., 2013). According to Lampi, Nurmi, Ollilainen, and Piironen (2008), ancient wheat species contain lower contents of tocopherol in comparison to both winter wheat and spring wheat. However, einkorn exhibited higher tocotrienol levels than other wheat species like emmer, spelt, and durum wheat. Einkorn showcased the highest proportion of tocotrienol, constituting 79.2% of the total tocopherols, while spring wheat genotypes displayed 59.0%. Average values for other wheat types ranged between 59.7% and 69.1%. Shewry and Hey (2015a) reported the highest Vitamin E content

for einkorn, ranging from 19.6 to 109.89 µg/g. In comparison, spelt exhibited values ranging from 8.9 to 69.18 µg/g, then emmer from 19.7 to 69.85 µg/g and common wheat from 23.3 to 79.7 µg/g. Grela (1996) observed that the vitamin E concentration in the spelt grain is 143% higher than in common wheat.

2.3.7 Phytochemicals

Wheat stands as a plentiful reservoir of health-promoting compounds, particularly in relation to phenols that are recognized for their remarkable diversity and prevalence among phytochemicals. These phytochemicals have gained significant attention lately due to their potential to act as antioxidants and protect against various diseases, such as coronary heart disease, cancer and stroke (Quiñones et al., 2013). According to recent literature, ancient wheat has been recognized as a potential source of various health-promoting compounds such as alkylresorcinols, phenolic acids, and carotenoids (Zencirci et al., 2022).

2.4.7.1 Alkylresorcinol

Alkylresorcinol is one of the major phenolic compounds present in whole wheat products and it is more abundant in the outer layers of the grain (Landberg et al., 2008). Alkylresorcinols exhibit a myriad of biological properties and are being investigated for their potential effects on human health, including cancer prevention, cholesterol reduction, immune regulation, and antimicrobial and anticancer properties (Zabolotneva et al., 2022). According to the HEALTHGRAIN study, ancient wheats with small seeds (einkorn, emmer, and spelt) were reported to have a higher alkylresorcinol content compared to large-seeded common wheats (durum and bread wheat) (Andersson et al., 2008; Shewry & Hey, 2015a). As per the findings of Ziegler et al. (2015), significant variation in alkylresorcinol content

was observed among genotypes within each wheat species analyzed. The mean alkylresorcinol contents for the respective species were as follows: 737 $\mu\text{g/g dm}$ in einkorn, 697 $\mu\text{g/g dm}$ in emmer, 743 $\mu\text{g/g dm}$ in spelt, and 761 $\mu\text{g/g dm}$ in common wheat samples.

2.4.7.2 Phenolic acids

Ferulic acid is the predominant phenolic acid in ancient wheat species and it has several health benefits such as antioxidant and anti-inflammatory effects (Srinivasan et al., 2007). It is more concentrated in wheat bran (Kaplan Evlice et al., 2022). In terms of ferulic acid content, spelt and common wheat exhibited similar levels, with approximately 400 $\mu\text{g/g dm}$. Emmer displayed a higher ferulic acid content (476 $\mu\text{g/g dm}$), while einkorn had a lower content (298 $\mu\text{g/g dm}$) (Li et al., 2008). Furthermore, Serpen et al. (2008) discovered a nearly 2.1-fold increase in ferulic acid content in emmer compared to the einkorn samples. In addition to ferulic acid, syringic acid, p-coumaric acid, and vanillic acid were also identified in these ancient wheat species (Kaplan Evlice et al., 2022).

2.4.7.3 Carotenoids

The predominant carotenoid identified in ancient wheat species is lutein, which has been found in elevated levels in einkorn, and emmer in comparison to common wheat (Lafiandra et al., 2012). As reported by Serpen et al. (2008), einkorn demonstrated, on average, nearly a two-fold higher lutein content than emmer, implying a high antioxidant activity. Due to this reason, there is a possibility for einkorn-based products to elevate the daily uptake of lutein because this compound has demonstrated potential advantages in addressing a range of health issues, encompassing neurological disorders, eye conditions, and skin irritation (Grausgruber et al., 2008; Zabolotneva et al., 2022; Ziegler et al., 2015).

2.4 Nutritional overview

Ancient wheat species have gained renewed attention for their higher contents of protein, fiber, and various vitamins and minerals as well as phytochemicals such as phytosterols, phenolic compounds like ferulic acid and lignans, flavonoids, and carotenoids (Dinu et al., 2018; Shewry, 2018; Valsalan et al., 2023). The nutritional profile is influenced by genetic and environmental factors. Therefore, ancient wheat species need to be explored thoroughly to optimize the sustainable supply of protein, minerals, fiber, as well as phytochemicals. A limited number of studies have been conducted to identify the impact of ancient wheat on health. Thorup et al. (2014) carried out an *in-vivo* study to compare the effects of einkorn, emmer, and spelt diets on glycemic control, plasma lipid profile, and acute glycemic responses. In this study, the authors noted that the development and progression of type 2 diabetes mellitus seemed to be less evident for individuals following ancient wheat-based diets. Baldi et al. (2022) investigated the impact of ancient wheat pasta on gut microbiota composition and bacteria-derived metabolites. According to the findings, significant changes were reported in the composition of gut microbiota at the genus level when the subjects were given a replacement diet with either ancient or common wheat pasta for a short period. However, the consumption of ancient wheat pasta had a greater beneficial impact on anti-inflammatory SCFAs compared to common wheat pasta. Moreover, the consumption of bread made from ancient wheat appeared to delay the onset of hunger compared to breads made of white flour of common wheat, most likely because ancient grains are usually used as wholegrain products (Patijn et al., 2018).

Table 2.2 Proximate composition of einkorn, emmer, spelt and common wheat

Component		Einkorn	Emmer	Spelt	Common wheat	References
Protein (N x 5.7)	dm	13.8 ±3.2	13.5 ±0.9	15.4±0.8	8.5±0.4	Løje et al. (2003)
Protein (N x 5.7)	dm	18.1	-	16.3-17.5	16.8	Abdel-Aal et al. (1995)
Protein (N x 5.7)	dm	18.2 ±1.48	-	-	-	
Protein (N x5.33)	-	15.76-24.75	13.51-23.92	-	-	Akar et al. (2019)
Protein (N x 5.7)	dm	18.1± 0.41	15.4± 0.39	12.8± 0.31	11.0 ± 0.26	Biel et al. (2021)
Protein (N x 5.7)	dm	-	16.7 0.16	17.1 0.16	-	Piergiovanni et al. (1996)
Protein (N x 5.7)	dm	-	-	14.0	11.1 (refined)	Skrabanja et al. (2001)
Protein (N x 5.7)	dm	15.5-22.8	14.0-16.0	17.1-18.7	13.7-15.7	Brandolini et al. (2008)
Protein *NIR technique		22.40 ± 0.40	20.80 ± 0.35	17.30 ± 0.38	13.50 ± 0.17	Belcar et al. (2020)
Protein *N is not mentioned	dm	14.6 ± 0.8	14.5± 0.7	15.2±0.6	17.3 ±0.8	Kulathunga et al.(2021)
Starch	dm	61.8	-	60.9-65.8	62.4	Abdel-Aal et al. (1995)

	dm	62.2 ± 0.6	65.9 ± 1.2 a	61.6 ± 0.4	62.3 ± 1.7	Kulathunga et al.(2021)
	dm	60.6 -71.4	70.2 - 70.7	62.1 - 65.5	65.3 - 72.9	Brandolini et al. (2008)
Ash	dm	2.21	2.10	2.04	1.90	Gebruers et al.(2008)
	dm	2.65 ± 0.08	2.16 ± 0.07	1.86 ± 0.07	1.52 ± 0.06	Biel et al.(2021)
	dm	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	Kulathunga et al.(2021)
	dm	2.4 ± 0.2	2.3 ± 0.2	2.1	1.9 ± 0.1	Løje et al. (2003)
	dm	11.0	9.8	12.0	14.7	Gebruers et al. (2008)
Fiber	dm	8.7 ± 0.7	7.9	10.3	12.3± 0.7	Løje et al. (2003)
	dm	8.7	-	9.8-10.3	12.5	Abdel-Aal et al. (1995)
	dm	1.32	1.71	2.56	3.05	Grausgruber et al. (2004)
	dm	2.48	-	2.21-2.48	2.14	Abdel-Aal et al. (1995)
Lipid	dm	2.44	2.01	2.19	1.83	Grausgruber et al. (2004)
	dm	2.1–2.5	2.0–2.3	1.0-1.9	0.6–1.4	Kulathunga et al. (2021)

2.5 Starch chemistry and nutritional quality

2.5.1 Granular structure and distribution

Wheat starch granules were originally classified into A and B types based on their size and shape (Li et al., 2016). The relative distribution of these A- and B-type starch granules may differ across various wheat species (Shang et al., 2023). A-type starch granules, characterized by their discoid or lenticular shape, have a diameter that falls within the range of 10-35 μm . These particular granules constitute approximately 3% of the total number of starch granules found in the endosperm. B-type starch granules, usually spherical and irregular, with a diameter ranging from 1 to 10 μm , account for more than 90% of the total number of starch granules (Moiraghi et al., 2019). However, C-type granules, characterized by a diameter of $\leq 3 \mu\text{m}$, were further distinguished from B-granules through enhanced separation accuracy (Karlsson et al., 1983).

Due to the dissimilarities in size, shape, and structural characteristics, functional properties and physicochemical (swelling power, water-binding capacity, and solubility, retrogradation and pasting) properties of starch greatly vary (**Figure 2.2**) (Lindeboom et al., 2004). Granules of smaller sizes (i.e., B- and C-types) possess relatively greater surface areas compared to larger granules (i.e., A-type), thereby facilitating stronger associations with protein, lipid, and other substances. Starches containing a considerable proportion of large granules exhibit high temperatures for gelatinization and pasting while exhibiting lower values for water solubility and swelling power (Zi et al., 2019). Furthermore, wheat processing quality is also significantly impacted by small starch granules. Primarily, the increased surface-to-volume ratio of the B granules is linked to higher water absorption

rates compared to A granules, and this has implications for both dough mixing and the baking characteristics of the final products (Stoddard, 1999). The results of Stoddard (1999) indicate that bread made with flour showing a higher proportion of B-type granules has a lower volume than conventional bread, while bread made flour containing an increased proportion of A granules has a lower water absorption without affecting the final volume. According to previous literature, it has been observed that the porosity of dough can be influenced by the granule size of starch. Especially, larger granule sizes are associated with increased porosity, whereas smaller granules tend to foster greater granule-granule interactions and irregular shapes of starch granules, leading to the development of a more compact and uniform protein network (Zhou et al., 2021). Therefore, small B-type starch granules could facilitate the development of a continuous gluten network (Shang et al., 2023). Stoddard (1999) observed a higher frequency of B-type granules in einkorn wheat (38%–40%) compared to durum wheat (22%–32%) but not to common wheat (34%–42%). Additionally, einkorn demonstrated the smallest A-type granules with a diameter of 13.2 μm . According to Kulathunga et al. (2021), starch granules observed in emmer wheat were compact and densely packed and the granules were firmly cemented by a protein matrix. But in einkorn wheat, small and large granules were entirely embedded in the protein matrix, while spelt starch granules were observed to be notably less firmly embedded in the interior protein matrix than emmer and HRS wheat. The diverse range of starch granule size, their composition and characteristics observed in the previous literature could be attributed to variations in wheat genotypes, the methods employed for starch isolation, granule separation techniques, or the procedure used for granule size measurement, as well as the

designated cutoff point used to distinguish different starch granules (Moiraghi et al., 2019). Additionally, these observations could have implications for the functionality of ancient grains in food systems adding to the deficiencies caused by the absence of high molecular weight glutenins.

2.5.2 Starch digestibility

The nutritional quality of starch is linked to parameters such as the rate of starch digestion and glucose absorption (Zhang & Hamaker, 2017). Starch and its derivatives are digested in successive stages inside the human body. Salivary α -amylase cleaves starch polymeric chains into shorter oligosaccharides in the mouth, while human pancreatic α -amylase further hydrolyzes partially digested material in the gut. Maltose and branched dextrins are converted to glucose by enzymes (maltoseglucoamylase and sucraseisomaltase) before entering the blood circulation (Lehmann & Robin, 2007). Following the digestibility in the upper digestive tract and rate and degree of digestive enzyme hydrolysis, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). In *in vitro* digestion, RDS is digested during the first 20 minutes causing a rapid surge in glucose release following ingestion. SDS is digested *in vitro* between 20 and 120 minutes, which can cause a slower release of glucose in the human body. RS is the residual portion and remains unhydrolyzed after 120 minutes, yet it has the capacity to undergo fermentation by human colonic microbiota (Englyst et al., 1992; Englyst & Hudson, 1996; Li et al., 2019).

Furthermore, starch accounts for approximately 60%-70% of the 'available' or 'glycemic' carbohydrates. The fraction digested by α -amylase in the upper gastrointestinal tract and

absorbed into the portal blood is referred to as available carbohydrates (mainly as glucose) (Roder et al., 2005). Since ancient wheat species contain high levels of carbohydrates, determining the glycemic response is an important criterion when considering its nutritional and physiological benefits.

The relative rate of starch digestion can be measured by the starch digestion index (SDI) (the proportion of rapidly digested starch to the total starch content) and the rapid release of glucose can be quantitatively determined as rapidly available glucose (RAG). The RAG fraction encompasses the combined quantity of free glucose, glucose originating from sucrose, and glucose liberated from rapidly digestible starch or starch subjected to digestion for 20-min threshold (Abdel-Aal & Rabalski, 2008).

The digestibility of starches from various wheat types is related to their morphological, physicochemical, and structural characteristics (Shevkani et al., 2016). Starch which contains high amylose content demonstrates reduced digestibility because of its compact linear structure (Martens et al., 2018). Additionally, a lower rate of starch digestibility may be also attributed to the formation of complexes between amylose and fatty acids, which commonly takes place on the surface of the starch granule. Furthermore, starch digestibility is also influenced by amylopectin chain length distribution, as reported by Martens et al. (2018). Świeca et al. (2014) determined the starch digestibility of six spelt cultivars and reported a range from 33.3% to 45.9%. The highest starch digestibility was observed for Ostro, Schwabenspelz, and Spelt I.N.Z., while the lowest digestibility was observed for Ceralio. Based on these findings, it could be speculated that one of the dominant factors affecting starch digestibility could be the RS content because a significantly higher RS content

was reported for the Ceralio cultivar in this study. Abdel-Aal and Rabalski (2008) reported an approximately eight to ten times higher RS content in spelt compared to common wheat. The consumption of diets rich in RS or amylose is associated with reduced postprandial glycemic and insulinemic responses among human subjects (Abdel-Aal & Rabalski, 2008). Kulathunga and Simsek (2023) reported a higher SDS fraction in ancient wheat compared to HRS wheat. The average value of SDS starch fractions in bread samples exhibited a descending order of einkorn, spelt, emmer, and HRS. Bonafaccia et al. (2000) compared the starch digestibility of spelt bread to common wheat breads and spelt breads exhibited elevated values of RDS and SDI and this is possibly due to greater water absorption capacity. Comparable results have been previously documented by Skrabanja et al. (2001), the SDI of spelt wheat bread was reported to be higher (SDI = 80) than common wheat bread (SDI = 68) and this may be attributed to the extensive protein matrix within the spelt endosperm, potentially leading to increased mechanical damage to starch grains during the milling process. During milling, the strong adhesion between starch and protein matrix may cause starch granules to shatter, resulting in damaged starch which can be highly accessible to enzymes. This effect could be further amplified in spelt wheat due to its higher protein content, potentially causing proteins to bind tightly within the matrix of the starch granules. As a result, milling spelt wheat is likely to induce greater breakage of these granules and consequent elevation of SDI values (Bonafaccia et al., 2000).

In contrast, Abdel-Aal & Rabalski. (2008) observed lower values for SDI and rapidly available glucose for spelt whole grain flour and doughs, and this could be attributed to the higher levels of resistant starch and a lower rate and extent of starch digestion compared to

common wheat products. Nakov et al. (2018) studied the effect of einkorn whole meal flour addition on *in vitro* starch digestion of cookies and reported that the rate of starch digestion between einkorn (100%) cookies and common wheat (100%) was comparable for up to 120 min but decreased after 180 min. Furthermore, in this study, einkorn-enriched cookies exhibited higher levels of ash, polyphenols, carotenoids, antioxidants, and β -glucans compared to cookies made with refined wheat flour. Therefore, the results suggest that einkorn-enriched cookies possess improved physicochemical and nutritional properties compared to regular common wheat cookies, thereby offering consumers a new cereal-based product with potential health benefits. It is worthwhile noting that comparing the outcomes of various studies directly is challenging due to the differences in the starting material (growth conditions, cultivar, and flour preparations) and analytical methods.

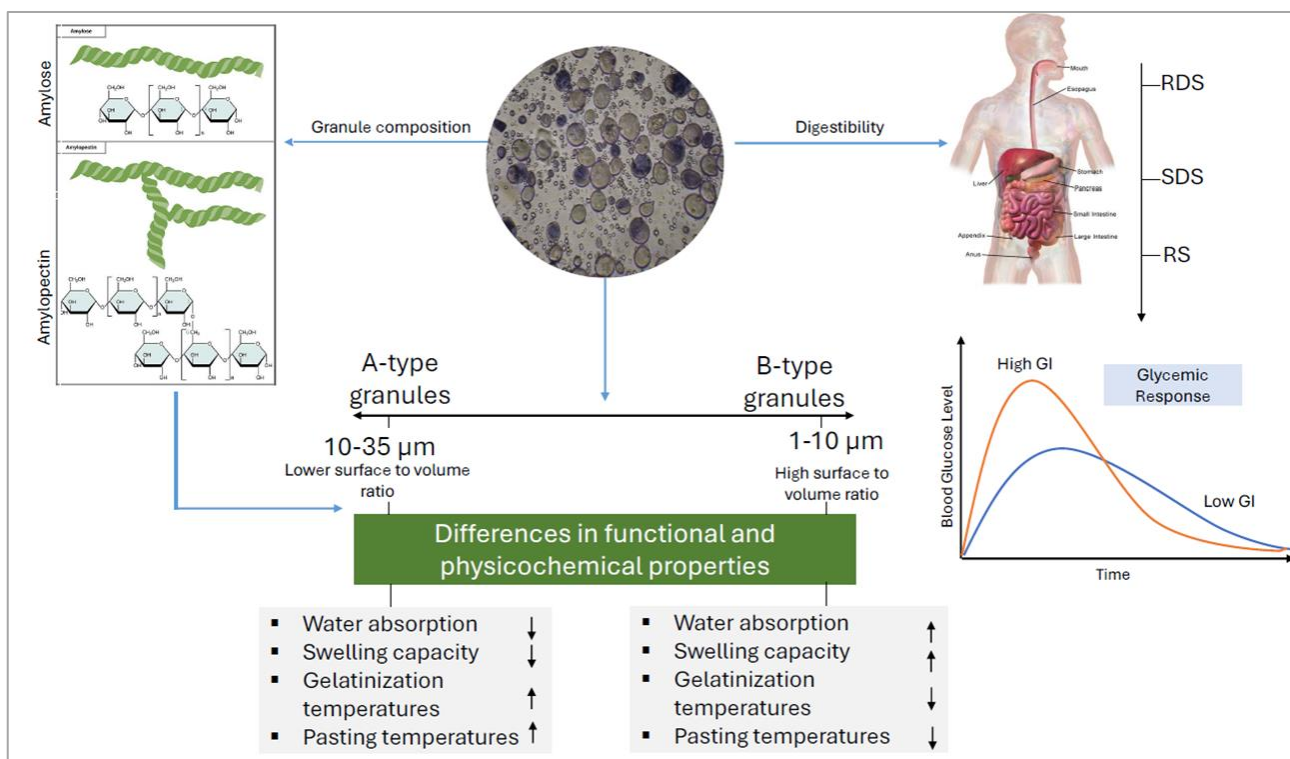


Figure 2.2 Relationship between starch structure, nutrition and functional properties

2.6 Wheat protein chemistry and nutritional quality

2.6.1 Protein composition and functionality

Wheat proteins can be classified systematically based on their extraction and solubility in different mediums; albumins can be dissolved in water, globulins in weak aqueous salt solutions, gliadins in mixtures of alcohol and water, and glutenins in diluted acids or alkalis (Uthayakumaran & Wrigley, 2010). Flour proteins interact in the presence of water resulting in a unique viscoelastic network termed gluten which is needed for breadmaking (Shewry et al., 2002). This gluten protein fraction consists of the monomeric gliadins and polymeric glutenins; gliadin protein fraction consists of mainly single polypeptide chains (monomers) linked by hydrogen bonds and hydrophobic interactions, whereas glutenins consist of

polymers stabilized by inter-chain disulfide bonds (Shewry et al., 1986). Monomeric gliadin proteins are categorized into α/β , γ - and ω -gliadins, depending on the order of mobility on electrophoresis under low pH conditions (Ma et al., 2019). The ω -gliadins are characterized by having substantial amounts of glutamine, proline, and phenylalanine residues which constitute approximately 80% of the total residues but, there are minimal or no sulfur-containing amino acids such as cysteine and methionine. In contrast, α/β -and γ -gliadins exhibit small proportions of proline, glutamine, and phenylalanine but contain about 2%-3% cysteine and methionine; all cysteine residues could be involved in the formation of intramolecular disulfide bonds (Shewry et al., 1986). Polymeric glutenin proteins, exhibiting molecular masses within the range of approximately 300 kDa to one million kDa, are further divided into two subunit groups: low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). LMW-GS subunits are systematically grouped into B, C, and D categories based on their biochemical characteristics (Kaplan Evlice et al., 2022; Wieser et al., 1998).

The HMW glutenin subunits are distinct from the gliadins due to their high level of glycine and a relatively lower percentage of proline (Shewry et al., 1986). A comparison of amino acid sequences demonstrated that the LMW-GS have a close relationship with α and γ -gliadins (Veraverbeke & Delcour, 2002). The formation of glutenin polymers occurs through inter-chain disulfide bonds, while gliadin monomers interact with glutenin polymers by means of non-covalent forces, particularly hydrogen bonds (Schofield, 1994, pp. 73-106). The high molecular weight polymeric structure of glutenin can account for its partial insolubility and its impact on food-product quality. Hence, classifying these proteins into

monomeric and polymeric forms provides useful insight into their functional characteristics (Payne & Lawrence, 1983). However, the quantity and distribution of polymeric protein in the wheat is influenced by both genetic and environmental factors, this will also serve as the foundation for the functionality of protein polymers during dough development (Kuktaite et al., 2004).

When kneading/mixing flour with water, gluten proteins enable the formation of a cohesive visco-elastic dough that has resistance to extension (**Figure 2.3**). This dough is capable of holding gas produced during fermentation within evenly distributed discrete cells in its structure. This network formation attains uniform and evenly distributed gas cells in a loaf crumb. In contrast, sub-optimally developed gluten permits gas cells to expand excessively during fermentation, causing a disintegration of cell walls and resulting in the cells coalescing together, leading to an open texture with a coarse wall structure (Ma et al., 2019; Schofield, 1994). Glutenin quality depends on glutenin structure, molecular size distribution, and subunit composition. A significant percentage of HMW-GS enhances dough stability against overmixing and strengthens dough (Joye et al., 2009). Hence, it is widely accepted that the cohesiveness and elasticity of dough are attributed to glutenin proteins that form polymeric protein networks, whilst gliadins act as plasticizers of the glutenin network and provide viscosity and extensibility (Lafiandra & Shewry, 2022; Veraverbeke & Delcour, 2002). An optimum balance between viscosity and elasticity is essential for the production of high-quality breads. Low bread loaf volume results from insufficiently elastic gluten proteins, while an increased level of elasticity than expected causes decreased expansion of gas cells resulting in lower loaf volume (Veraverbeke et al., 2002). Therefore, the key

parameter associated with baking quality is the balance between gliadin and glutenin proteins in wheat flour. An optimum ratio of glutenin to gliadin protein is directly linked to maintaining the balance of dough strength and extensibility. The hydrogen bonds involved in the interaction between gliadin and glutenin molecules of various flours exhibit differences in terms of their quantity. The rate of interaction is governed by the specific surface area of glutenin proteins, and a larger molecule size corresponds to a reduced specific surface area. Thus, more time is required for complete mixing to achieve full dough development. In addition, it is crucial to consider the composition of both gliadin and glutenin proteins because the balance of HMW-to-LMW glutenin subunit ratio within the polymeric fraction plays a crucial role in influencing the strength and extensibility of the dough (Sapirstein & Fu, 2000; Wrigley et al., 2006).

Therefore, understanding the specific roles of storage protein fractions on bread texture is critical for technological applications (Kaplan Evlice et al., 2022). Furthermore, the structure as well as functionality of gluten have been linked with cysteine, as the majority of cysteines form either intra-chain disulfide bonds within a protein or inter-chain disulfide bonds between proteins. These disulfide bonds can undergo mobilization during the process of dough formation and development through disulfide-interchange reactions (Zaidel et al., 2008).

In some literature, wheat gluten is classified into three main groups: sulphur-rich (with a molecular weight around 50 kDa, including α -, β -, and γ -gliadins, as well as B- and C-LMW glutenins), sulfur-poor proteins (with a molecular weight around 50 kDa, comprising ω -gliadins and D-LMW glutenins), and high molecular weight proteins (with a molecular

weight around 100 kDa, known as HMW glutenins) on the basis of structural and genetic relationships among different polypeptides in gliadin and glutenin protein fractions (Žilic, 2013). The proportion of S-poor and S-rich subunits can fluctuate according to variations in the sulphur availability in the soil thereby altering the overall protein composition (Ma et al., 2019).

Wieser (2000) analyzed quantitative and qualitative compositions of gluten proteins in ancient wheat species, common wheat, and durum wheat. The quantities of total glutenins and HMW-GS in common wheat were highest, while the ratio of gliadins to glutenins was lowest. The process of breeding and selecting common wheat for enhanced breadmaking quality may have led to a reduction in the GLI/GLU ratio, as a higher ratio has adverse effects on bread volume (Barak et al., 2013). The protein composition analysis of emmer revealed that the levels of total glutenins and HMW-GS and LMW-GS subunits were very low, while the ratio of gliadins to glutenins was substantially higher compared to other types of wheat (Wieser, 2000). In comparison to common wheat, einkorn flours exhibit comparable or elevated levels of total gluten proteins with a significant imbalance between gliadins and glutenins. Additionally, it is accompanied by notably low quantities of HMW-GS (Wieser et al., 2009). Corbellini et al. (1999) characterized twenty-four einkorn lines for the production of bread and cookies, considering the composition of protein subunits. According to the results of the study, the composition of HMW-GS and α -, β - and γ -gliadins was similar in all the lines. However, LMW-GS exhibited a significant correlation with baking quality. Wieser et al. (2009) observed that einkorn flours with a high level of glutenins, total gluten, or low ratios of gliadins to glutenins exhibited favorable baking qualities. In consequence, einkorn

lines that did not possess these traits displayed lower bread volumes and were not suited to be used in breadmaking applications.

Takač et al. (2021) observed higher GLU/GLI and HMW/LMW in the bread wheat cultivars than in spelt wheat cultivars. Gliadins in gluten matrix form only non-covalent interactions with other proteins, making them less effective in affecting both viscoelastic properties and breadmaking quality in the dough. A relatively higher proportion of monomeric gliadins in spelt, combined with lower levels of HMW-GS/LMW-GS, leads to less favorable viscoelastic properties of the gluten matrix. In comparison to bread wheat, spelt doughs have less stability and elasticity, and weak gluten structure. Geisslitz et al. (2019) analyzed the gluten protein composition of ancient wheat, common, and durum wheat species. The content of glutenins showed a significant decrease from common wheat (mean 16.6 mg/g) and spelt (mean 19.0 mg/g) to einkorn (mean 10.3 mg/g). Meanwhile, durum wheat had a mean of 16.0 mg/g, and emmer displayed a mean of 12.8 mg/g, signifying intermediate levels in terms of glutenin content compared to the aforementioned wheat species. Due to these differences, significant variations were observed in the GLI/GLU ratios reporting the lowest ratio in common wheat (2.5) and the highest ratio in einkorn (6.5), and spelt (3.3), durum wheat (4.0) and emmer (4.9).

2.6.2 Nutritional value of wheat protein

The nutritional value and the quality of dietary proteins are influenced by both the amino acid composition and the digestibility of grain proteins. The digestibility of grain proteins is particularly dependent on processing, anti-nutritional factors, and protein structure and composition (Lavoignat et al., 2022). The amino acid composition of proteins, particularly

essential amino acids, can significantly affect their nutritional value (Schaafsma, 2000). The proportion of amino acids can vary based on the wheat cultivar, growing location, fertilization, and stress conditions (Abid et al., 2018). The Food and Agriculture Organization advises treating amino acids as individual nutritional components of the diet, emphasizing not just the amount of protein consumed but also its amino acid composition (FAO, 2013). Among the twenty naturally occurring amino acids, nine are deemed essential due to their inability to be synthesized within the human body from substances typically available to the cells at a rate corresponding with the demands for normal growth. It has been known that wheat protein is low in certain essential amino acids, particularly lysine, and threonine, the second most deficient amino acid. However, wheat protein has a higher content of glutamine and proline which are considered functional amino acids in dough formation (Abdel-Aal & Hucl, 2002). Furthermore, the lower level of essential amino acids in white flour can be attributed to the high amount of lysine-poor prolamin storage proteins/gluten proteins found in the starchy endosperm (Shewry & Hey, 2015b). Additionally, glutamine and glutamic acid serve critical roles in a variety of biological processes, including intracellular protein turnover, gene expression regulation, oxidative defense, and nutrition metabolism (Wu, 2010). Akar et al. (2019) reported the highest protein and essential amino acid levels in einkorn compared to emmer and durum wheat lines. Additionally, einkorn had higher non-essential amino acids except for arginine and proline when compared to other analyzed wheat species. Therefore, einkorn has the potential to be used in pasta production in conjunction with durum wheat to enhance the overall nutritional value of the final product. Dvořáček et al. (2002) also determined the contents of lysine, threonine, and sulfur-

containing amino acids in spelt, emmer, and common wheat. A slightly higher content of lysine was observed in spelt compared to common wheat. According to previous literature, the genotype contributed more to the total diversity of essential and non-essential amino acids than agricultural practices (Sulek et al., 2023).

2.6.3 Digestibility of wheat protein

Proteins are usually folded in a certain conformation in their native state, which is determined by the unique amino acid composition. Protein digestibility is hampered by this tightly folded shape. Furthermore, proteins frequently occur in supramolecular structures like protein bodies and/or are physically embedded in cellular structures. These supramolecular structures further restrict hydrolytic enzymes from accessing their substrates (Bhattarai et al., 2017; Pernollet, 1978). Therefore, for dietary proteins to be useful to the human body after ingestion, they must be hydrolyzed into their fundamental components/basic building blocks, such as small peptides and amino acids. It has been well-documented that the human digestive system has a variety of peptidases and enzymes that can hydrolyze proteins. Alkaline amino acids, such as arginine, lysine, and histidine, are typically more abundant in proteins with a storage function because they are more nitrogen-rich (Joye, 2019). After enzymatic hydrolysis, the enterocytes in the small intestine should promptly and effectively absorb the small peptides and amino acids (Lundquist & Artursson, 2016). Due to the presence of proline-rich domains it has been reported that, gluten proteins can be resistant to enzymatic breakdown. Understanding changes in protein digestibility and identifying particular gluten protein fractions linked to high or low protein digestibility in diverse wheat cultivars is essential for gaining insights into potential changes that have

occurred in protein digestibility over time (Gulati et al., 2020). The digestibility of gluten proteins is greatly impacted by their structural form, the isolated protein fraction, when purified, exhibits a faster digestion rate compared to the protein present in flour. The presence of gluten-starch complexes in flour may hinder access to digestive enzymes, thus slowing down the digestion of flour proteins (Smith et al., 2015).

Abdel-Aal and Hucl (2002) investigated the protein digestibility of whole meals of einkorn and spelt and their products following a multienzyme technique using trypsin, chymotrypsin, and peptidase and determined that the protein digestibility (PD%) for whole meals of einkorn and spelt are comparable to HRS wheat. Ranhotra et al. (1995) assessed the protein quality using Protein Efficiency Ratio (PER) and reported protein digestibility values of 80.1% for spelt, 78.9% for common wheat, and 91.6% for casein. Based on these findings, they suggested that spelt could be more easily digested than common wheat even though minor differences were observed in the study. Despite spelt having a significantly lower lysine content compared to common wheat, overall growth response and PER values showed no significant differences. Therefore, the authors proposed that, practically, lysine and other amino acids from spelt might be more readily digestible than HRW wheat. In the study of Ma and Baik (2021), a thorough analysis was undertaken for diverse wheat types, including common wheat of five different US classes and three ancient wheat classes (spelt, emmer, and einkorn) to investigate *in vitro* protein digestibility of cooked flours and protein-specific fractions employing a trypsin, chymotrypsin, and protease three-enzyme system for digestion. According to this study, *in vitro* protein digestibility of three einkorn wheat flours was substantially higher compared to HRS wheat flours. The inconsistencies observed in the

digestibility of flours across different studies may be attributed to the variations in protein compositions of the wheat cultivars used.

Furthermore, avoiding the potential immunogenic effects of undigested peptides requires high protein digestibility in final products (Smith et al., 2015). The D genome of common wheat appears to have more immunogenic epitopes than the A and B genomes. As a result, diploid and tetraploid wheats (*T. monococcum* and *T. durum*) are expected to have fewer immunogenic epitopes that trigger celiac disease in comparison to common wheat (Herpen et al., 2006). Furthermore, Ciclitira (2013) investigated the effect of *in vitro* gastro-intestinal digestion on the antigenic properties of gliadin from einkorn (compared to common wheat) and reported that gliadin proteins of einkorn are adequately distinct from those of common wheat, thereby identifying a low-level of antigenicity following *in vitro* simulation of human digestion. According to previous literature, spelt, emmer, and einkorn possess proteins that are more readily digestible and elicit fewer adverse responses than those present in common wheat species (Shewry, 2018). Although these findings suggest that these proteins in ancient grains are more digestible than common wheat, they still contain immunogenic epitopes that may trigger symptoms of celiac disease (Malalgoda et al., 2019). As a result, these grains are currently considered unsafe for individuals with celiac disease. This highlights the need for further investigation and confirmation through future studies to determine their potential role in influencing the incidence of celiac disease (Malalgoda & Simsek, 2017). Additionally, the identification and development of wheat cultivars with improved protein digestibility would benefit from the determination of the protein digestibility of common and ancient

wheat species as well as the identification of the grain and protein properties impacting protein digestibility (Ma & Baik, 2021).

2.7.3.1 Protein complementation with wheat proteins

Protein complementation is a crucial strategy for enhancing the nutritional quality of plant-based diets, particularly by combining different protein sources throughout the day to ensure a balanced intake of essential amino acids (Dimina et al., 2022; Hertzler et al., 2020). Plant proteins are often perceived as having lower nutritional quality than animal proteins, largely because of their lower essential amino acid content. For instance, cereals such as wheat, as discussed earlier, are notably deficient in lysine, an amino acid abundant in animal proteins and crucial for meeting amino acid requirements (Dimina et al., 2022). However, pulses, which are high in lysine but low in sulfur-containing amino acids such as methionine, cysteine, and tryptophan, can complement cereals that have an opposing amino acid profile (Rajpurohit & Li, 2023). Therefore, consuming these complementary foods can help meet the dietary requirements for essential amino acids (Roy et al., 2010; Sá & House, 2024). Research has shown that combining cereals and legumes significantly enhances the Protein Digestibility-Corrected Amino Acid Score (PDCAAS), with wheat flour and pea flour mixtures achieving a PDCAAS value of 0.82 (Rajpurohit & Li, 2023). Additionally, Coda et al. (2017) demonstrated that the concentration of essential amino acids in bread increased when faba bean flour was added to wheat flour, particularly after fermentation. This indicates that legume-cereal mixtures can effectively improve nutritional quality of these proteins (Hertzler et al., 2020). With the strong consumer demand for plant protein-based products,

projected to rise significantly in the next decade, there is a need to continue researching optimal blends of cereals and pulses.

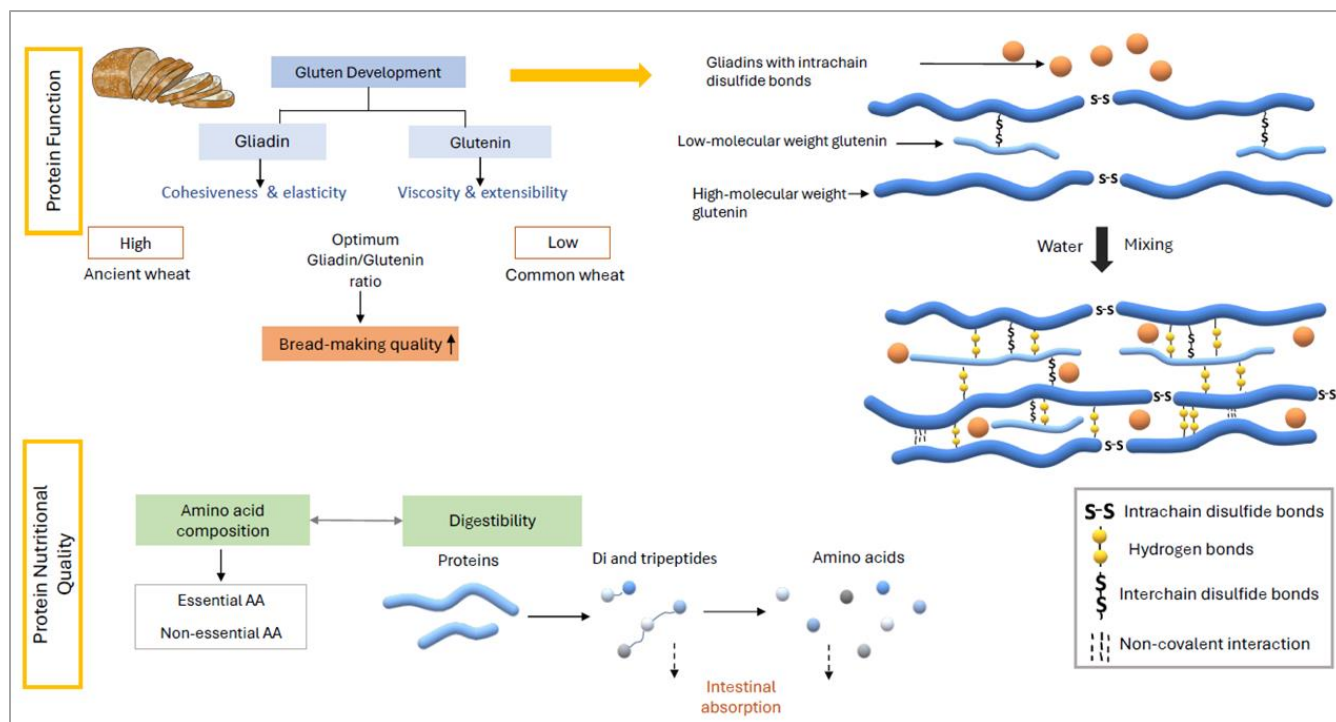


Figure 2.3 Wheat protein functionality and nutritional quality

2.7 Conclusion

By incorporating ancient wheat into daily diets, individuals can access a rich source of macromolecules and micronutrients, potentially leading to various health benefits. The elevated RS content in ancient wheat species plays a significant role in starch digestibility, contributing to the positive impact on lowering postprandial glycemic and insulin responses. The significance of ancient wheat has been overlooked and it is undervalued commercially. A thorough investigation of the principal molecules present in these ancient wheat species, such as starch and proteins, that influence different functional characteristics and end

product quality is still underexplored in the academic literature. Notably, the protein composition in these ancient wheat species exhibits distinctive features, including low total glutenins and higher gliadin-to-glutenin ratios compared to common wheat. These unique traits provide perspectives that hold potential for optimizing the performance of ancient wheat in different applications. Therefore, detailed studies on their unique structural and physicochemical characteristics are critical as it provides valuable insights for integrating these underutilized species into the commercial wheat industry, facilitating the formulation of innovative food products and promoting food diversification. Furthermore, additional research on ancient wheat processing and product development is required to obtain end products with favorable product characteristics while maximizing the market potential of these unique grains.

2.8 References

AACC (American Association of Cereal Chemists), 2001. The definition of dietary fiber.

Cereal Foods World, 46, 112-126.

Abang Zaidel, D. N., Chin, N. L., Abdul Rahman, R., & Karim, R. (2008). Rheological characterisation of gluten from extensibility measurement. *Journal of Food Engineering*, 86(4), 549–556. <https://doi.org/10.1016/j.jfoodeng.2007.11.005>

Abdel-Aal, E. S.M., & Hucl, P. (2002). Amino acid composition and *in vitro* protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis*, 15(6), 737–747. <https://doi.org/10.1006/jfca.2002.1094>

Abdel-Aal, E. S.M., Hucl, P., & Sosulski, F. W. (1995). Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chemistry*, 72(6), 621–624.

- Abdel-Aal, E. S.M., Hucl, P., Sosulski, F. W., & Bhirud, P. R. (1997). Kernel, milling and baking properties of spring-type spelt and einkorn wheats. *Journal of Cereal Science*, 26(3), 363–370. <https://doi.org/10.1006/jcrs.1997.0139>
- Abdel-Aal, El Sayed M., & Rabalski, I. (2008). Effect of baking on nutritional properties of starch in organic spelt whole grain products. *Food Chemistry*, 111(1), 150–156. <https://doi.org/10.1016/j.foodchem.2008.03.050>
- Abid, M., Ali, S., Qi, L. K., Zahoor, R., Tian, Z., Jiang, D., Snider, J. L., & Dai, T. (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Scientific Reports*, 8(1), 1–15. <https://doi.org/10.1038/s41598-018-21441-7>
- Ahmad, A., & Kaleem, M. (2018). β -Glucan as a Food Ingredient. In *Biopolymers for Food Design*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-811449-0.00011-6>
- Akar, T., Cengiz, M. F., & Tekin, M. (2019). A comparative study of protein and free amino acid contents in some important ancient wheat lines. *Quality Assurance and Safety of Crops and Foods*, 11(2), 191–200. <https://doi.org/10.3920/QAS2018.1382>
- Amo, A., Serikbay, D., Song, L., Chen, L., & Hu, Y.-G. (2022). Vernalization and photoperiod alleles greatly affected phenological and agronomic traits in bread wheat under autumn and spring sowing conditions. *Crop and Environment*, 1(4), 241–250. <https://doi.org/10.1016/j.crope.2022.11.002>
- Andersson, A. A. M., Kamal-Eldin, A., Fraś, A., Boros, D., & Åman, P. (2008). Alkylresorcinols in wheat varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9722–9725. <https://doi.org/10.1021/jf8011344>

- Arendt, E. K., & Zannini, E. (2013). Wheat and other Triticum grains. In *Cereal Grains for the Food and Beverage Industries*. <https://doi.org/10.1533/9780857098924.1>
- Arzani, A., & Ashraf, M. (2017). Cultivated Ancient Wheats (Triticum spp.): A Potential Source of Health-Beneficial Food Products. *Comprehensive Reviews in Food Science and Food Safety*, 16(3), 477–488. <https://doi.org/10.1111/1541-4337.12262>
- Arzani, A. (2019). Emmer (Triticum turgidum ssp. dicoccum) flour and bread. In *Flour and Breads and Their Fortification in Health and Disease Prevention* (2nd ed.). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-814639-2.00007-1>
- Baldi, S., Dinu, M., Pagliai, G., Colombini, B., Di Gloria, L., Curini, L., Pallecchi, M., Ramazzotti, M., Bartolucci, G., Benedettelli, S., Amedei, A., & Sofi, F. (2022). Effect of ancient wheat pasta on gut microbiota composition and bacteria-derived metabolites: A randomized controlled trial. *Frontiers in Nutrition*, 9(August), 1–9. <https://doi.org/10.3389/fnut.2022.971666>
- Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. *Lwt*, 51(1), 211–217. <https://doi.org/10.1016/j.lwt.2012.09.011>
- Basic, F., Kisic, I., Mesic, M., Nestroy, O., & Butorac, A. (2004). Tillage and crop management effects on soil erosion in central Croatia. *Soil and Tillage Research*, 78(2), 197–206. <https://doi.org/10.1016/j.still.2004.02.007>
- Belcar, J., Sobczyk, A., Sekutowski, T. R., Stankowski, S., & Gorzelany, J. (2021). Evaluation of Flours from Ancient Varieties of Wheat (Einkorn, Emmer, Spelt) used in Production of Bread. *Acta Universitatis Cibiniensis. Series E: Food Technology*, 25(1), 53–66.

<https://doi.org/10.2478/aucft-2021-0005>

- Belcar, J., Sobczyk, A., Sobolewska, M., Stankowski, S., & Gorzelany, J. (2020). Characteristics of Technological Properties of Grain and Flour from Ancient Varieties of Wheat (Einkorn, Emmer and Spelt). *Acta Universitatis Cibiniensis. Series E: Food Technology*, 24(2), 269–278. <https://doi.org/10.2478/aucft-2020-0024>
- Bencze, S., Makádi, M., Aranyos, T. J., Földi, M., Hertelendy, P., Mikó, P., Bosi, S., Negri, L., & Drexler, D. (2020). Re-introduction of ancient wheat cultivars into organic agriculture-Emmer and Einkorn cultivation experiences under marginal conditions. *Sustainability (Switzerland)*, 12(4). <https://doi.org/10.3390/su12041584>
- Bezabeh, M. W., Hailemariam, M. H., Sogn, T. A., & Eich-Greatorex, S. (2022). Wheat (*Triticum aestivum*) production and grain quality resulting from compost application and rotation with faba bean. *Journal of Agriculture and Food Research*, 10(September 2021), 100425. <https://doi.org/10.1016/j.jafr.2022.100425>
- Bhattarai, R. R., Dhital, S., Wu, P., Chen, X. D., & Gidley, M. J. (2017). Digestion of isolated legume cells in a stomach-duodenum model: Three mechanisms limit starch and protein hydrolysis. *Food and Function*, 8(7), 2573–2582. <https://doi.org/10.1039/c7fo00086c>
- Biel, W., Jaroszewska, A., Stankowski, S., Sobolewska, M., & Kępińska-Pacelik, J. (2021). Comparison of yield, chemical composition and farinograph properties of common and ancient wheat grains. *European Food Research and Technology*, 247(6), 1525–1538. <https://doi.org/10.1007/s00217-021-03729-7>
- Boita, E. R. F., Oro, T., Bressiani, J., Santetti, G. S., Bertolin, T. E., & Gutkoski, L. C. (2016).

- Rheological properties of wheat flour dough and pan bread with wheat bran. *Journal of Cereal Science*, 71, 177–182. <https://doi.org/10.1016/j.jcs.2016.08.015>
- Bonafaccia, G., Galli, V., Francisci, R., Mair, V., Skrabanja, V., & Kreft, I. (2000). Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68(4), 437–441. [https://doi.org/10.1016/S0308-8146\(99\)00215-0](https://doi.org/10.1016/S0308-8146(99)00215-0)
- Bordoni, A., Danesi, F., Di Nunzio, M., Taccari, A., & Valli, V. (2017). Ancient wheat and health: a legend or the reality? A review on KAMUT khorasan wheat. *International Journal of Food Sciences and Nutrition*, 68(3), 278–286. <https://doi.org/10.1080/09637486.2016.1247434>
- Brandolini, A., & Hidalgo, A. (2011). Einkorn (*Triticum monococcum*) Flour and Bread. In *Flour and Breads and their Fortification in Health and Disease Prevention* (pp. 79–88). Elsevier. <https://doi.org/10.1016/B978-0-12-380886-8.10008-X>
- Brandolini, A., Hidalgo, A., & Moscaritolo, S. (2008). Chemical composition and pasting properties of einkorn (*Triticum monococcum* L. subsp. *monococcum*) whole meal flour. *Journal of Cereal Science*, 47(3), 599–609. <https://doi.org/10.1016/j.jcs.2007.07.005>
- Brandolini, A., Lucisano, M., Mariotti, M., & Hidalgo, A. (2018). A study on the quality of einkorn (*Triticum monococcum* L . ssp . *monococcum*) pasta. *Journal of Cereal Science*, 82(May), 57–64. <https://doi.org/10.1016/j.jcs.2018.05.010>
- Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules*, 23(2), 85–112.

[https://doi.org/10.1016/S0141-8130\(98\)00040-3](https://doi.org/10.1016/S0141-8130(98)00040-3)

- Callejo, M. J., Vargas-Kostiuk, M., & Rodríguez-Quijano, M. (2015). Selection, training and validation process of a sensory panel for bread analysis: Influence of cultivar on the quality of breads made from common wheat and spelt wheat. *Journal of Cereal Science*, 61, 55–62. <https://doi.org/10.1016/j.jcs.2014.09.008>
- Cankurtaran-Kömürcü, T., & Bilgiçli, N. (2023). Utilization of germinated ancient wheat (Emmer and Einkorn) flours to improve functional and nutritional properties of bread. *Innovative Food Science & Emerging Technologies*, 84, 103292. <https://doi.org/10.1016/j.ifset.2023.103292>
- Cappelli, A., Cini, E., Guerrini, L., Masella, P., Angeloni, G., & Parenti, A. (2018). Predictive models of the rheological properties and optimal water content in doughs: An application to ancient grain flours with different degrees of refining. *Journal of Cereal Science*, 83, 229–235. <https://doi.org/10.1016/j.jcs.2018.09.006>
- Cheng, A. (2018). Review: Shaping a sustainable food future by rediscovering long-forgotten ancient grains. *Plant Science*, 269(January), 136–142. <https://doi.org/10.1016/j.plantsci.2018.01.018>
- Chudan, S., Ishibashi, R., Nishikawa, M., Tabuchi, Y., Nagai, Y., Ikushiro, S., & Furusawa, Y. (2023). Effect of Wheat-Derived arabinoxylan on the gut microbiota composition and colonic regulatory T cells. *Molecules*, 28(7), 3079. <https://doi.org/10.3390/molecules28073079>
- Coda, R., Varis, J., Verni, M., Rizzello, C. G., & Katina, K. (2017). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *Lwt*, 82, 296–302.

<https://doi.org/10.1016/j.lwt.2017.04.062>

Corbellini, M., Empilli, S., Vaccino, P., Brandolini, A., Borghi, B., Heun, M., & Salamini, F. (1999). Einkorn characterization for bread and cookie production in relation to protein subunit composition. *Cereal Chemistry*, 76(5), 727–733.

<https://doi.org/10.1094/CCHEM.1999.76.5.727>

Costanzo, A., Amos, D. C., Dinelli, G., Sferrazza, R. E., Accorsi, G., Negri, L., & Bosi, S. (2019). Performance and nutritional properties of einkorn, emmer and rivet wheat in response to different rotational position and soil tillage. *Sustainability*, 11(22), 6304.

<https://doi.org/10.3390/su11226304G>

Čurná, V., & Lacko-Bartošová, M. (2017). Chemical composition and nutritional value of emmer wheat (*Triticum dicoccon schrank*): A review. *Journal of Central European Agriculture*, 18(1), 117–134. <https://doi.org/10.5513/JCEA01/18.1.1871>

de Sousa, T., Ribeiro, M., Sabença, C., & Igrejas, G. (2021). The 10,000-Year Success Story of Wheat! *Foods*, 10(9), 2124. <https://doi.org/10.3390/foods10092124>

Deyalage, S. T., House, J. D., Thandapilly, S. J., & Malalgoda, M. (2024). Nutritional Characteristics and Physicochemical Properties of Ancient Wheat Species for Food Applications. *Food Bioscience*, 105397. <https://doi.org/10.1016/j.fbio.2024.105397>

Creative Commons Attribution 4.0 International License

Dhanavath, S., & Prasada Rao, U. J. S. (2017). Nutritional and Nutraceutical Properties of *Triticum dicoccum* Wheat and Its Health Benefits: An Overview. *Journal of Food Science*, 82(10), 2243–2250. <https://doi.org/10.1111/1750-3841.13844>

Dimina, L., Rémond, D., Huneau, J. F., & Mariotti, F. (2022). Combining Plant Proteins to

- Achieve Amino Acid Profiles Adapted to Various Nutritional Objectives—An Exploratory Analysis Using Linear Programming. *Frontiers in Nutrition*, 8(February), 1–11. <https://doi.org/10.3389/fnut.2021.809685>
- Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., & Sofi, F. (2018). Ancient wheat species and human health: Biochemical and clinical implications. *Journal of Nutritional Biochemistry*, 52, 1–9. <https://doi.org/10.1016/j.jnutbio.2017.09.001>
- Dvořáček, V., Čurn, V., & Moudrý, J. (2002). Evaluation of amino acid content and composition in spelt wheat varieties. *Cereal Research Communications*, 30(1–2), 187–193. <https://doi.org/10.1007/bf03543407>
- Eckardt, N. A. (2010). Evolution of domesticated bread wheat. *Plant Cell*, 22(4), 993. <https://doi.org/10.1105/tpc.110.220410>
- Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European journal of clinical nutrition*, 46, S33-50. PMID: 1330528
- Englyst, H. N., & Hudson, G. J. (1996). The classification and measurement of dietary carbohydrates. *Food Chemistry*, 57(1), 15–21. [https://doi.org/10.1016/0308-8146\(96\)00056-8](https://doi.org/10.1016/0308-8146(96)00056-8)
- FAO. (2023). FAO Cereal Supply and Demand Brief - September 2022. *FAO Cereal Supply and Demand Brief*, 23. <https://www.fao.org/worldfoodsituation/csdb/en/>
- FAO, F. A. (2013). Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. In *FAO food and nutrition paper* (Vol. 92).
- 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. <https://doi.org/10.1002/jsfa.3350>
- Filipčev, B., Šimurina, O., Bodroža-Solarov, M., & Brkljača, J. (2013). Dough rheological properties in relation to cracker-making performance of organically grown spelt cultivars. *International Journal of Food Science and Technology* 2013, 48(11), 2356–2362. <https://doi.org/10.1111/ijfs.12225>
- Filipčev, B. V., Bodroža-Solarov, M. I., & Brkljača, J. (2012). Characterization of organically grown spelt cultivars for cracker-making applications. *Food and Feed Research*, 39(2), 61–68.
- Frakolaki, G., Giannou, V., Topakas, E., & Tzia, C. (2018). Chemical characterization and breadmaking potential of spelt versus wheat flour. *Journal of Cereal Science*, 79, 50–56. <https://doi.org/10.1016/j.jcs.2017.08.023>
- Gabrovská, D., Fiedlerová, V., Holasová, M., Mašková, E., Smrčinov, H., Rysová, J., Winterová, R., Michalová, A., & Hutař, M. (2002). The nutritional evaluation of underutilized cereals and buckwheat. *Food and Nutrition Bulletin*, 23(3 SUPP), 246–249. <https://doi.org/10.1177/15648265020233s148>
- Gebruers, K., Dornez, E., Boros, D., Dynkowska, W., Bedő, Z., Rakszegi, M., Delcour, J. A., & Courtin, C. M. (2008). Variation in the content of dietary fiber and components thereof in wheats in the HEALTHGRAIN Diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9740–9749. <https://doi.org/10.1021/jf800975w>
- Geisslitz, N., Longin, N., Scherf, N., & Koehler, N. (2019). Comparative study on gluten

- protein composition of ancient (Einkorn, emmer and spelt) and modern wheat species (Durum and common wheat). *Foods*, 8(9), 409.
<https://doi.org/10.3390/foods8090409>
- Geisslitz, S., Wieser, H., Scherf, K. A., & Koehler, P. (2018). Gluten protein composition and aggregation properties as predictors for bread volume of common wheat, spelt, durum wheat, emmer and einkorn. *Journal of Cereal Science*, 83(August), 204–212.
<https://doi.org/10.1016/j.jcs.2018.08.012>
- Genc, Y., & McDonald, G. K. (2008). Domesticated emmer wheat [*T. turgidum* L. subsp. *dicoccon* (Schränk) Thell.] as a source for improvement of zinc efficiency in durum wheat. *Plant and Soil*, 310(1–2), 67–75. <https://doi.org/10.1007/s11104-008-9630-4>
- Giambanelli, E., Ferioli, F., Koçaoglu, B., Jorjadze, M., Alexieva, I., Darbinyan, N., & D'Antuono, L. F. (2013). A comparative study of bioactive compounds in primitive wheat populations from Italy, Turkey, Georgia, Bulgaria and Armenia. *Journal of the Science of Food and Agriculture*, 93(14), 3490–3501.
<https://doi.org/10.1002/jsfa.6326>
- Grausgruber, H., Miesenberger, S., Schoenlechner, R., & Vollmann, J. (2008). Influence of dough improvers on whole-grain bread quality of einkorn wheat. *Acta Alimentaria*, 37(3), 379–390. <https://doi.org/10.1556/AAlim.2008.0009>
- Grausgruber, Heinrich, Scheiblaue, J., Schönlechner, R., Ruckebauer, P., & Berghofer, E. (2004). Variability in chemical composition and biologically active constituents of cereals. *Genetic Variation for Plant Breeding*, 26(May 2014), 23–26.

- Grela, E. R. (1996). Nutrient Composition and Content of Antinutritional Factors in Spelt (*Triticum spelta*L) Cultivars. *Journal of the Science of Food and Agriculture*, 71(3), 399-404.
- Gulati, P., Brahma, S., Graybosch, R. A., Chen, Y., & Rose, D. J. (2020). In vitro digestibility of proteins from historical and modern wheat cultivars. *Journal of the Science of Food and Agriculture*, 100(6), 2579–2584. <https://doi.org/10.1002/jsfa.10283>
- Guzmán, C., & Alvarez, J. B. (2021). Ancient wheats role in sustainable wheat cultivation. In *Trends in Wheat and Bread Making*. <https://doi.org/10.1016/B978-0-12-821048-2.00002-7>
- Hammed, A. M., & Simsek, S. (2014). REVIEW: Hulled wheats: A Review of Nutritional properties and Processing methods. *Cereal Chemistry*, 91(2), 97–104. <https://doi.org/10.1094/cchem-09-13-0179-rw>
- Harlan, J. R., & Zohary, D. (1966). Distribution of wild wheats and barley: the present distribution of wild forms may provide clues to the regions of early cereal domestication. *Science*, 153(3740), 1074-1080.
- Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M., & Allgeier, C. (2020). Plant proteins: Assessing their nutritional quality and effects on health and physical function. *Nutrients*, 12(12), 1–27. <https://doi.org/10.3390/nu12123704>
- Hidalgo, A., & Brandolini, A. (2011). Heat damage of water biscuits from einkorn , durum and bread wheat flours. *Food Chemistry*, 128(2), 471–478. <https://doi.org/10.1016/j.foodchem.2011.03.056>
- Hidalgo, A., & Brandolini, A. (2013). Nutritional properties of einkorn wheat (*Triticum*

- monococcum L.). *Journal of the Science of Food and Agriculture*, 94(4), 601–612.
<https://doi.org/10.1002/jsfa.6382>
- Hidalgo, A., Brandolini, A., & Ratti, S. (2009). Influence of genetic and environmental factors on selected nutritional traits of *Triticum monococcu*. *Journal of Agricultural and Food Chemistry*, 57(14), 6342–6348. <https://doi.org/10.1021/jf901180q>
- Hidalgo, A., Lucisano, M., Mariotti, M., & Brandolini, A. (2019). Physico-chemical and nutritional characteristics of einkorn flour cookies. In *Journal of Food Processing and Preservation* (Vol. 43, Issue 9). <https://doi.org/10.1111/jfpp.14079>
- Hui, Y. H., Corke, H., De Leyn, I., Nip, W. K., & Cross, N. A. (Eds.). (2008). *Bakery products: science and technology*. John Wiley & Sons.
- Hyles, J., Bloomfield, M. T., Hunt, J. R., Trethowan, R. M., & Trevaskis, B. (2020). Phenology and related traits for wheat adaptation. *Heredity*, 125(6), 417–430.
<https://doi.org/10.1038/s41437-020-0320-1>
- Iqbal, M., Fayyaz, M., Shahzad, A., Ahmed, I., Ali, G. M., Masood, S., & Spaner, D. (2011). Effect of vernalization on grain fill duration and grain weight in spring wheat. *Pakistan Journal of Botany*, 43(1), 689–694.
- Iqbal, M. J., Shams, N., & Fatima, K. (2022). Nutritional quality of wheat. In *IntechOpen eBooks*. <https://doi.org/10.5772/intechopen.104659>
- Jiang, T., Wang, B., Xu, X., Cao, Y., Liu, D. L., He, L., Jin, N., Ma, H., Chen, S., Zhao, K., Feng, H., Yu, Q., He, Y., & He, J. (2022). Identifying sources of uncertainty in wheat production projections with consideration of crop climatic suitability under future climate.

Agricultural and Forest Meteorology, 319(April), 108933.

<https://doi.org/10.1016/j.agrformet.2022.108933>

Johansson, E., Prieto-Linde, M. L., & Larsson, H. (2021). Locally adapted and organically grown landrace and ancient spring cereals—a unique source of minerals in the human diet. *Foods*, 10(2), 1–17. <https://doi.org/10.3390/foods10020393>

Joye, I. (2019). Protein digestibility of cereal products. *Foods*, 8(6), 199. <https://doi.org/10.3390/foods8060199>

Joye, I. J., Lagrain, B., & Delcour, J. A. (2009). Endogenous redox agents and enzymes that affect protein network formation during breadmaking - A review. *Journal of Cereal Science*, 50(1), 1–10. <https://doi.org/10.1016/j.jcs.2009.04.002>

Karakas, F. P., Keskin, C. N., Agil, F., & Zencirci, N. (2021). Profiles of vitamin B and E in wheat grass and grain of einkorn (*Triticum monococcum* spp. *monococcum*), emmer (*Triticum dicoccum* ssp. *dicoccum* Schrank.), durum (*Triticum durum* Desf.), and bread wheat (*Triticum aestivum* L.) cultivars by LC-ESI-MS/MS analysis. *Journal of Cereal Science*, 98, 103177. <https://doi.org/10.1016/j.jcs.2021.103177>

Karlsson, R., Olered, R., & Eliasson, A. (1983). Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye. *Starch - Stärke*, 35(10), 335–340. <https://doi.org/10.1002/star.19830351002>

Khan, K., & Shewry, P. R. (2009). *Wheat: Chemistry and Technology*. American Association of Cereal Chemists.

Kim, K. H., & Kim, J. Y. (2021). Understanding wheat starch metabolism in properties,

- environmental stress condition, and molecular approaches for value-added utilization. *Plants*, 10(11). <https://doi.org/10.3390/plants10112282>
- Kohajdová, Z., & Karovicova, J. (2008). Nutritional value and baking application of spelt wheat. *Acta Scientiarum Polonorum Technologia Alimentaria*, 7(3), 5-14.
- Kohajdová, Z., & Karovicova, J. (2007). Effect of incorporation of spelt flour on the dough properties and wheat bread quality. *Żywność Nauka Technologia Jakość*, 14(4).
- Konvalina, P., Bradová, J., Capouchová, I., Stehno, Z., & Moudrý, J. S. (2013). Baking quality and high molecular weight glutenin subunit composition of Emmer wheat, old and new varieties of bread wheat. *Romanian Agricultural Research*, 30 , 83–89
- Kraska, P., Andruszczak, S., Gawlik-Dziki, U., Dziki, D., & Kwiecińska-Poppe, E. (2020). Wholemeal spelt bread enriched with green spelt as a source of valuable nutrients. *Processes*, 8(4), 389.
- Kuktaite, R., Larsson, H., & Johansson, E. (2004). Variation in protein composition of wheat flour and its relationship to dough mixing behaviour. *Journal of Cereal Science*, 40(1), 31–39. <https://doi.org/10.1016/j.jcs.2004.04.007>
- Kulathunga, J., Reuhs, B. L., & Simsek, S. (2020). A review: Novel trends in hulled wheat processing for value addition. *Trends in Food Science and Technology*, 106(September), 232–241. <https://doi.org/10.1016/j.tifs.2020.09.033>
- Kulathunga, J., Reuhs, B. L., Zwinger, S., & Simsek, S. (2021). Comparative study on kernel quality and chemical composition of ancient and modern wheat species: Einkorn, emmer, spelt and hard red spring wheat. *Foods*, 10(4). <https://doi.org/10.3390/foods10040761>

- Kulathunga, J., & Simsek, S. (2022). Dietary fiber variation in ancient and modern wheat species: Einkorn, emmer, spelt and hard red spring wheat. *Journal of Cereal Science*, 104(October 2021), 103420. <https://doi.org/10.1016/j.jcs.2022.103420>
- Kulathunga, J., & Simsek, S. (2023). Pasting properties, baking quality, and starch digestibility of einkorn, emmer, spelt, and hard red spring wheat. *Cereal Chemistry*, October 2022. <https://doi.org/10.1002/cche.10644>
- Lacko - Bartošová, M., & Čurná, V. (2015). Nutritional Characteristics of Emmer Wheat Varieties. *Journal of Microbiology, Biotechnology and Food Sciences*, 4(special issue 3 (Food Sciences)), 95–98. <https://doi.org/10.15414/jmbfs.2015.4.special3.95-98>
- Lafiandra, D., Masci, S., Sissons, M., Dornez, E., Delcour, J. A., Courtin, C. M., & Caboni, M. F. (2012). Kernel components of technological value. In *Elsevier eBooks* (pp. 85–124). <https://doi.org/10.1016/b978-1-891127-65-6.50011-8>
- Lacko-Bartošová, M., Lacko-Bartošová, L., Konvalina, P., Matejková, E., & Bieliková, D. (2021). Rheological dough properties of organic spelt and emmer wheat for assessment of bread making quality. *Zemdirbyste-Agriculture*, 108(3), 279–286. <https://doi.org/10.13080/z-a.2021.108.036>
- Lafiandra, D., & Shewry, P. R. (2022). Wheat Glutenin polymers 2. The role of wheat glutenin subunits in polymer formation and dough quality. *Journal of Cereal Science*, 106(July). <https://doi.org/10.1016/j.jcs.2022.103487>
- Lampi, A. M., Nurmi, T., Ollilainen, V., & Piironen, V. (2008). Tocopherols and tocotrienols in wheat genotypes in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9716–9721.

- Landberg, R., Kamal-Eldin, A., Salmenkallio-Marttila, M., Rouau, X., & Åman, P. (2008). Localization of alkylresorcinols in wheat, rye and barley kernels. *Journal of Cereal Science*, 48(2), 401–406. <https://doi.org/10.1016/j.jcs.2007.09.013>
- Langer, S. M., Longin, C. F. H., & Würschum, T. (2014). Flowering time control in European winter wheat. *Frontiers in plant science*, 5, 537.
- Lavoignat, M., Denis, S., Faye, A., Halupka, L., Perrochon, S., Rhazi, L., Giraudeau, P., Déjean, S., Branlard, G., Bancel, E., & Ravel, C. (2022). Differences in bread protein digestibility traced to wheat cultivar traits. *Journal of Cereal Science*, 107, 103533. <https://doi.org/10.1016/j.jcs.2022.103533>
- Lehmann, U., & Robin, F. (2007). Slowly digestible starch – its structure and health implications: a review. *Trends in Food Science & Technology*, 18(7), 346–355. <https://doi.org/10.1016/j.tifs.2007.02.009>
- Levy, A. A., & Feldman, M. (2022). Evolution and origin of bread wheat. *The Plant Cell*, 34(7), 2549–2567. <https://doi.org/10.1093/plcell/koac130>
- Li, Lei, Chen, S., Deng, M., & Gao, Z. (2022). Optical techniques in non-destructive detection of wheat quality: A review. *Grain and Oil Science and Technology*, 5(1), 44–57. <https://doi.org/10.1016/j.gaost.2021.12.001>
- Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the HEALTHGRAIN Diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9732–9739. <https://doi.org/10.1021/jf801069s>
- Li, M., Xie, Y., Chen, H., & Zhang, B. (2019). Effects of heat-moisture treatment after citric acid esterification on structural properties and digestibility of wheat starch, A- and

- B-type starch granules. *Food Chemistry*, 272, 523–529. <https://doi.org/10.1016/j.foodchem.2018.08.079>
- Li, W., Yan, S., Shi, X., Zhang, C., Shao, Q., Xu, F., & Wang, J. (2016). Starch granule size distribution from twelve wheat cultivars in east China's Huaibei region. *Canadian Journal of Plant Science*, 96(2), 176–182. <https://doi.org/10.1139/cjps-2015-0048>
- Lindeboom, N., Chang, P. R., & Tyler, R. T. (2004). Analytical, Biochemical and Physicochemical Aspects of Starch Granule Size, with Emphasis on Small Granule Starches: A Review. *Starch - Stärke*, 56(3–4), 89–99. <https://doi.org/10.1002/star.200300218>
- Løje, H., Møller, B., Laustsen, A. M., & Hansen, Å. (2003). Chemical composition, functional properties and sensory profiling of einkorn (*Triticum monococcum* L.). *Journal of Cereal Science*, 37(2), 231–240. <https://doi.org/10.1006/jcrs.2002.0498>
- Longin, C. F. H., & Würschum, T. (2016). Back to the Future – Tapping into Ancient Grains for Food Diversity. *Trends in Plant Science*, 21(9), 731–737. <https://doi.org/10.1016/j.tplants.2016.05.005>
- Lu, Z. X., Walker, K. Z., Muir, J. G., Mascara, T., & O'Dea, K. (2000). Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *The American journal of clinical nutrition*, 71(5), 1123–1128.
- Lundquist, P., & Artursson, P. (2016). Oral absorption of peptides and nanoparticles across the human intestine: Opportunities, limitations and studies in human tissues. *Advanced Drug Delivery Reviews*, 106, 256–276.

<https://doi.org/10.1016/j.addr.2016.07.007>

- Ma, F., & Baik, B. K. (2021). Influences of grain and protein characteristics on in vitro protein digestibility of modern and ancient wheat species. *Journal of the Science of Food and Agriculture*, 101(11), 4578–4584. <https://doi.org/10.1002/jsfa.11100>
- Ma, W., Yu, Z., She, M., Zhao, Y., & Islam, S. (2019). Wheat gluten protein and its impacts on wheat processing quality. *Frontiers of Agricultural Science and Engineering*, 6(3), 279–287. <https://doi.org/10.15302/J-FASE-2019267>
- Maningat, C. C., Seib, P. A., Bassi, S. D., Woo, K. S., & Lasater, G. D. (2009). Wheat Starch. In *Starch* (Third Edit, pp. 441–510). Elsevier. <https://doi.org/10.1016/B978-0-12-746275-2.00010-0>
- Malalgoda, M., Ohm, J. B., & Simsek, S. (2019). Celiac antigenicity of ancient wheat species. *Foods*, 8(12), 1–6. <https://doi.org/10.3390/foods8120675>
- Malalgoda, M., & Simsek, S. (2017). Celiac disease and cereal proteins. *Food Hydrocolloids*, 68, 108–113. <https://doi.org/10.1016/j.foodhyd.2016.09.024>
- Marconi, E., Carcea, M., Schiavone, M., & Cubadda, R. (2002). Spelt (*Triticum spelta* L.) pasta quality: Combined effect of flour properties and drying conditions. *Cereal chemistry*, 79(5), 634-639.
- Markowiak-Kopeć, P., & Śliżewska, K. (2020). The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients*, 12(4), 1107.
- Martens, B. M. J., Gerrits, W. J. J., Bruininx, E. M. A. M., & Schols, H. A. (2018). Amylopectin structure and crystallinity explains variation in digestion kinetics of starches across botanic sources in an in vitro pig model. *Journal of Animal Science and Biotechnology*,

- 9(1), 1–13. <https://doi.org/10.1186/s40104-018-0303-8>
- Massaux, C., Sindic, M., Lenartz, J., Sinnaeve, G., Bodson, B., Falisse, A., Dardenne, P., & Deroanne, C. (2008). Variations in physicochemical and functional properties of starches extracted from European soft wheat (*Triticum aestivum* L.): The importance to preserve the varietal identity. *Carbohydrate Polymers*, 71(1), 32–41. <https://doi.org/10.1016/j.carbpol.2007.05.012>
- McCleary, B. V., DeVries, J. W., Rader, J. I., Cohen, G., Prosky, L., Mugford, D. C., Champ, M., & Okuma, K. (2012). Determination of insoluble, soluble, and total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: Collaborative study. *Journal of AOAC International*, 95(3), 824–844. https://doi.org/10.5740/jaoacint.CS2011_25
- Migliorini, P., Spagnolo, S., Torri, L., Arnoulet, M., Lazzerini, G., & Ceccarelli, S. (2016). Agronomic and quality characteristics of old, modern and mixture wheat varieties and landraces for organic bread chain in diverse environments of northern Italy. *European Journal of Agronomy*, 79, 131–141. <https://doi.org/10.1016/j.eja.2016.05.011>
- Mijatović, D., Van Oudenhoven, F., Eyzaguirre, P., & Hodgkin, T. (2013). The role of agricultural biodiversity in strengthening resilience to climate change: Towards an analytical framework. *International Journal of Agricultural Sustainability*, 11(2), 95–107. <https://doi.org/10.1080/14735903.2012.691221>
- Moudrý, J., Konvalina, P., Stehno, Z., Capouchová, I., & Moudrý, J. (2011). Ancient wheat species can extend biodiversity of cultivated crops. *Scientific Research and Essays*,

6(20), 4273–4280. <https://doi.org/10.5897/sre11.928>

- Moiraghi, M., Sciarini, L. S., Paesani, C., León, A. E., & Pérez, G. T. (2019). Flour and starch characteristics of soft wheat cultivars and their effect on cookie quality. *Journal of Food Science and Technology*, 56(10), 4474–4481. <https://doi.org/10.1007/s13197-019-03954-9>
- Nakov, G., Brandolini, A., Ivanova, N., Dimov, I., & Stamatovska, V. (2018). The effect of einkorn (*Triticum monococcum* L.) whole meal flour addition on physico-chemical characteristics, biological active compounds and in vitro starch digestion of cookies. *Journal of Cereal Science*, 83(August), 116–122. <https://doi.org/10.1016/j.jcs.2018.08.004>.
- Nesbitt, M., & Samuel, D. (1996). From staple crop to extinction? The archaeology and history of the hulled wheats.
- Ohm, J. B., Hareland, G., Simsek, S., Seabourn, B., Maghirang, E., & Dowell, F. (2010). Molecular weight distribution of proteins in hard red spring wheat: Relationship to quality parameters and intrasample uniformity. *Cereal Chemistry*, 87(6), 553–560. <https://doi.org/10.1094/CCHEM-06-10-0088>
- Patiñ, O., Voshol, P., Battjes-Fries, M., & Nuijten, E. (2018). Health effects of ancient wheat species compared to modern wheat varieties. Louis Bolk Institute. Retrieved from www.louisbolk.org/publications
- Payne, P.I., & Lawrence, G.J. (1983). Detection by gel electrophoresis of oligomers formed by the association of High-Molecular-Weight glutenin protein subunits of wheat endosperm. *Journal of Experimental Botany*, 34(3), 254–

267. <https://doi.org/10.1093/jxb/34.3.254>

Pernollet, J. C. (1978). Protein bodies of seeds: Ultrastructure, biochemistry, biosynthesis and degradation. *Phytochemistry*, 17(9), 1473–1480.

[https://doi.org/10.1016/S0031-9422\(00\)94623-5](https://doi.org/10.1016/S0031-9422(00)94623-5)

Piasecka-Jóźwiak, K. . S. E. . R. J. . C. B. (2015). Characteristic of organic flour produced from einkorn wheat and rheological properties of einkorn dough in terms of bread obtaining. *Journal of Research and Applications in Agricultural Engineering*, 60(4), 61–66.

Piergiovanni, A R, Laghetti, G., & Perrino, P. (1996). Characteristics of meal from hulled wheats (*Triticum dicoccon schrank* and *T. spelta* L.): An evaluation of selected accessions. *Cereal Chemistry*, 73(6), 732–735.

Piergiovanni, Angela R. (2013). Evaluation of genetic variation and grain quality of old bread wheat varieties introduced in north-western Italian environments. *Genetic Resources and Crop Evolution*, 60(1), 325–333. <https://doi.org/10.1007/s10722-012-9838-2>

Poole, N., Donovan, J., & Erenstein, O. (2021a). Continuing cereals research for sustainable health and well-being. *International Journal of Agricultural Sustainability*. <https://doi.org/10.1080/14735903.2021.1975437>

Poole, N., Donovan, J., & Erenstein, O. (2021b). Viewpoint: Agri-nutrition research: Revisiting the contribution of maize and wheat to human nutrition and health. *Food Policy*, 100, 101976. <https://doi.org/10.1016/j.foodpol.2020.101976>

Poutanen, K. S., Kårlund, A. O., Gómez-Gallego, C., Johansson, D. P., Scheers, N. M.,

- Marklinder, I. M., Eriksen, A. K., Silventoinen, P. C., Nordlund, E., Sozer, N., Hanhineva, K. J., Kolehmainen, M., & Landberg, R. (2022). Grains - a major source of sustainable protein for health. *Nutrition Reviews*, *80*(6), 1648–1663.
<https://doi.org/10.1093/nutrit/nuab084>
- Quiñones, M., Miguel, M., & Aleixandre, A. (2013). Beneficial effects of polyphenols on cardiovascular disease. *Pharmacological Research*, *68*(1), 125–131.
<https://doi.org/10.1016/j.phrs.2012.10.018>
- Rajpurohit, B., & Li, Y. (2023). Overview on pulse proteins for future foods: ingredient development and novel applications. *Journal of Future Foods*, *3*(4), 340–356.
<https://doi.org/10.1016/j.jfutfo.2023.03.005>
- Ranhotra, G., Gelroth, J., Glaser, B., & Lorenz, K. (1995). Baking and nutritional qualities of a spelt wheat sample. *LWT*, *28*(1), 118–122. [https://doi.org/10.1016/s0023-6438\(95\)80022-0](https://doi.org/10.1016/s0023-6438(95)80022-0)
- Rasmusson, D. C., Hester, A. J., Fick, G. N., & Byrne, I. (1971). Breeding for mineral content in wheat and barley1. *Crop Science*, *11*(5), 623–626. <https://doi.org/10.2135/cropsci1971.0011183x001100050005x>
- Roder, N., Ellis, P. R., & Butterworth, P. J. (2005). Starch molecular and nutritional properties: a review. *Advances in Molecular Medicine*, *1*(12), 5–14.
- Rodríguez-Quijano, M., Vargas-Kostiuk, M., Ribeiro, M., & Callejo, M. J. (2019). Triticum aestivum ssp. vulgare and ssp. spelta cultivars. 1. Functional evaluation. *European Food Research and Technology*, *245*(8), 1561–1570.
<https://doi.org/10.1007/s00217-019-03263-7>

- Rosicka-Kaczmarek, J., Kwaśniewska-Karolak, I., Nebesny, E., & Komisarczyk, A. (2018). The functionality of wheat starch. In *Elsevier eBooks* (pp. 325–352). <https://doi.org/10.1016/b978-0-08-100868-3.00008-1>
- Roumia, H., Kókai, Z., Mihály-Langó, B., Csobod, É. C., & Benedek, C. (2023). Ancient Wheats—A Nutritional and Sensory Analysis Review. *Foods*, 12(12), 2411. <https://doi.org/10.3390/foods12122411>
- Roy, F., Boye, J. I., & Simpson, B. K. (2010). Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International*, 43(2), 432–442. <https://doi.org/10.1016/j.foodres.2009.09.002>
- Ruibal-Mendieta, N. L., Delacroix, D. L., & Meurens, M. (2002). A comparative analysis of free, bound and total lipid content on spelt and winter wheat wholemeal. *Journal of Cereal Science*, 35(3), 337–342. <https://doi.org/10.1006/jcrs.2001.0434>
- Sá, A. G. A., & House, J. D. (2024). Protein quality of cereals: Digestibility determination and processing impacts. *Journal of Cereal Science*, 117(November 2023), 103892. <https://doi.org/10.1016/j.jcs.2024.103892>
- Sapirstein, H. D., & Fu, B. X. (2000). Evidence for varying interaction of gliadin and glutenin proteins as an explanation for differences in dough strength of different wheats. *SPECIAL PUBLICATION-ROYAL SOCIETY OF CHEMISTRY*, 261, 425-429.
- Schaafsma, G. (2000). The Protein Digestibility–Corrected Amino Acid Score. *Journal of Nutrition*, 130(7), 1865S-1867S. <https://doi.org/10.1093/jn/130.7.1865s>
- Schofield, J. D. (1994). Wheat proteins: structure and functionality in milling and breadmaking. In *Springer eBooks* (pp. 73–106). <https://doi.org/10.1007/978-1->

- Schupfer, E., Pak, S. C., Wang, S., Micalos, P. S., Jeffries, T., Ooi, S. L., Golombick, T., Harris, G., & El-Omar, E. (2021). The effects and benefits of arabinoxylans on human gut microbiota – A narrative review. *Food Bioscience*, 43, 101267. <https://doi.org/10.1016/j.fbio.2021.101267>
- Şerban, L. R., Păucean, A., Man, S. M., Chiş, M. S., & Mureşan, V. (2021). Ancient wheat species: Biochemical profile and impact on sourdough bread characteristics—a review. *Processes*, 9(11). <https://doi.org/10.3390/pr9112008>
- Serpen, A., Gökmen, V., Karagöz, A., & Köksel, H. (2008). Phytochemical quantification and total antioxidant capacities of Emmer (*Triticum dicoccon* Schrank) and Einkorn (*Triticum monococcum* L.) wheat landraces. *Journal of Agricultural and Food Chemistry*, 56(16), 7285–7292. <https://doi.org/10.1021/jf8010855>
- Shang, J., Zhao, B., Liu, C., Li, L., Hong, J., Liu, M., Zhang, X., Lei, Y., & Zheng, X. (2023). Impact of wheat starch granule size on viscoelastic behaviors of noodle dough sheet and the underlying mechanism. *Food Hydrocolloids*, 134, 108111. <https://doi.org/10.1016/j.foodhyd.2022.108111>
- Shevkani, K., Singh, N., Bajaj, R., & Kaur, A. (2016). Wheat starch production, structure, functionality and applications—a review. *International Journal of Food Science & Technology*, 52(1), 38–58. <https://doi.org/10.1111/ijfs.13266>
- Shewry, P., Pellny, T. K., & Lovegrove, A. (2016). Is modern wheat bad for health? *Nature Plants*, 2(7), 1–3. <https://doi.org/10.1038/NPLANTS.2016.97>
- Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, 60(6), 1537–1553.

<https://doi.org/10.1093/jxb/erp058>

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security*, 4(3), 178–202. <https://doi.org/10.1002/fes3.64>

Shewry, Peter R. (2018). Do ancient types of wheat have health benefits compared with modern bread wheat? *Journal of Cereal Science*, 79, 469–476.
<https://doi.org/10.1016/j.jcs.2017.11.010>

Shewry, P. R., Halford, N. G., Belton, P. S., & Tatham, A. S. (2002). The structure and properties of gluten: an elastic protein from wheat grain. *Philosophical Transactions of the Royal Society B Biological Sciences*, 357(1418), 133–142. <https://doi.org/10.1098/rstb.2001.1024>

Shewry, Peter R., Hawkesford, M. J., Piironen, V., Lampi, A. M., Gebruers, K., Boros, D., Andersson, A. A. M., Åman, P., Rakszegi, M., Bedo, Z., & Ward, J. L. (2013). Natural variation in grain composition of wheat and related cereals. *Journal of Agricultural and Food Chemistry*, 61(35), 8295–8303. <https://doi.org/10.1021/jf3054092>

Shewry, Peter R., & Hey, S. (2015a). Do “ancient” wheat species differ from modern bread wheat in their contents of bioactive components? *Journal of Cereal Science*, 65, 236–243. <https://doi.org/10.1016/j.jcs.2015.07.014>

Shewry, Peter R., & Hey, S. J. (2015b). The contribution of wheat to human diet and health. *Food and Energy Security*, 4(3), 178–202. <https://doi.org/10.1002/FES3.64>

Shewry, Peter R., Tatham, A. S., Forde, J., Kreis, M., & Mifflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science*, 4(2), 97–106. [https://doi.org/10.1016/S0733-5210\(86\)80012-1](https://doi.org/10.1016/S0733-5210(86)80012-1)

- Shiferaw, B., Smale, M., Braun, H. J., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. *Food Security*, 5(3), 291–317.
<https://doi.org/10.1007/s12571-013-0263-y>
- Shindo, C., & Sasakuma, T. (2002). Genes responding to vernalization in hexaploid wheat. *Theoretical and Applied Genetics*, 104(6–7), 1003–1010.
<https://doi.org/10.1007/s00122-002-0886-4>
- Skrabanja, V., Kovac, B., Golob, T., Liljeberg Elmståhl, H. G. M., Björck, I. M. E., & Kreft, I. (2001). Effect of spelt wheat flour and kernel on bread composition and nutritional characteristics. *Journal of Agricultural and Food Chemistry*, 49(1), 497–500.
<https://doi.org/10.1021/jf000819w>
- Škrobot, D., Dapčević-Hadnađev, T., Tomić, J., Maravić, N., Popović, N., Jovanov, P., & Hadnađev, M. (2022). Techno-Functional Performance of Emmer, Spelt and Khorasan in Spontaneously Fermented Sourdough Bread. *Foods*, 11(23), 3927.
<https://doi.org/10.3390/foods11233927>
- Smith, F., Pan, X., Bellido, V., Toole, G. A., Gates, F. K., Wickham, M. S. J., Shewry, P. R., Bakalis, S., Padfield, P., & Mills, E. N. C. (2015). Digestibility of gluten proteins is reduced by baking and enhanced by starch digestion. *Molecular Nutrition & Food Research*, 59(10), 2034–2043. <https://doi.org/10.1002/mnfr.201500262>
- Sots, S., Kustov, I., & Donii, O. (2024). Authentic wheat grain is the raw material for modern cereal products. *Grain Products and Mixed Fodder's*, 23(4), 7–12.
<https://doi.org/10.15673/gpmf.v23i4.2741>

- Srinivasan, M., Sudheer, A. R., & Menon, V. P. (2007). Ferulic acid: Therapeutic potential through its antioxidant property. *Journal of Clinical Biochemistry and Nutrition*, 40(2), 92–100. <https://doi.org/10.3164/jcbn.40.92>
- Steinfort, U., Trevaskis, B., Fukai, S., Bell, K. L., & Dreccer, M. F. (2017). Vernalisation and photoperiod sensitivity in wheat: Impact on canopy development and yield components. *Field Crops Research*, 201, 108–121. <https://doi.org/10.1016/j.fcr.2016.10.012>
- Stoddard, F. L. (1999). Survey of Starch Particle-Size distribution in wheat and related species. *Cereal Chemistry*, 76(1), 145–149. <https://doi.org/10.1094/cchem.1999.76.1.145>
- Suchowilska, E., Wiwart, M., Kandler, W., & Krska, R. (2012). A comparison of macro- and microelement concentrations in the whole grain of four Triticum species. *Plant, Soil and Environment*, 58(3), 141–147. <https://doi.org/10.17221/688/2011-pse>
- Sułek, A., Cacak-Pietrzak, G., Różewicz, M., Nieróbca, A., Grabiński, J., Studnicki, M., Sujka, K., & Dziki, D. (2023). Effect of Production Technology Intensity on the Grain Yield, Protein Content and Amino Acid Profile in Common and Durum Wheat Grain. *Plants*, 12(2). <https://doi.org/10.3390/plants12020364>
- Šuligoj, T., Gregorini, A., Colomba, M., Ellis, H. J., & Ciclitira, P. J. (2013). Evaluation of the safety of ancient strains of wheat in coeliac disease reveals heterogeneous small intestinal T cell responses suggestive of coeliac toxicity. *Clinical Nutrition*, 32(6), 1043–1049. <https://doi.org/10.1016/j.clnu.2013.02.003>
- Świeca, M., Dziki, D., Gawlik-Dziki, U., Różyło, R., Andruszczak, S., Kraska, P., Kowalczyk, D.,

- Pałys, E., & Baraniak, B. (2014). Grinding and nutritional properties of six spelt (*Triticum aestivum* ssp. *spelta* L.) cultivars. *Cereal Chemistry*, 91(3), 247–254.
<https://doi.org/10.1094/CCHEM-02-13-0019-R>
- Takač, V., Tóth, V., Rakszegi, M., Mikić, S., Mirosavljević, M., & Kondić-špika, A. (2021). Differences in processing quality traits, protein content and composition between spelt and bread wheat genotypes grown under conventional and organic production. *Foods*, 10(1). <https://doi.org/10.3390/foods10010156>
- Tester, R. F., Karkalas, J., & Qi, X. (2004). Starch—composition, fine structure and architecture. *Journal of Cereal Science*, 39(2), 151–165. <https://doi.org/10.1016/j.jcs.2003.12.001>
- Thorup, A. C., Gregersen, S., & Jeppesen, P. B. (2014). Ancient wheat diet delays diabetes development in a type 2 diabetes animal model. *The Review of Diabetic Studies*, 11(3–4), 245–257. <https://doi.org/10.1900/rds.2014.11.245>
- Tran, K. D., Konvalina, P., Capouchova, I., Janovska, D., Lacko-Bartosova, M., Kopecky, M., & Tran, P. X. T. (2020). Comparative study on protein quality and rheological behavior of different wheat species. *Agronomy*, 10(11).
<https://doi.org/10.3390/agronomy10111763>
- United Nations. (2022). *World Population Prospects 2022*. United Nations Publication.
[https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp2022_summary_of_results.pdf](https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pdf/files/wpp2022_summary_of_results.pdf)
- Uthayakumaran, S., & Wrigley, C. (2010). Wheat: characteristics and quality requirements. In *Elsevier eBooks* (pp. 59–111). <https://doi.org/10.1533/9781845699529.2.59>

- Valsalan, A., Koksel, F., Rosell, C. M., & Malalgoda, M. (2023). Ingredient Technologies and Process Modifications for Increasing the Use of Ancient Grains in Bakery Applications. *Food Reviews International*, 00(00), 1–20.
<https://doi.org/10.1080/87559129.2023.2279613>
- Van Herpen, T. W. J. M., Goryunova, S. V., Van Der Schoot, J., Mitreva, M., Salentijn, E., Vorst, O., Schenk, M. F., Van Veelen, P. A., Koning, F., Van Soest, L. J. M., Vosman, B., Bosch, D., Hamer, R. J., Gilissen, L. J. W. J., & Smulders, M. J. M. (2006). Alpha-gliadin genes from the A, B, and D genomes of wheat contain different sets of celiac disease epitopes. *BMC Genomics*, 7(1). <https://doi.org/10.1186/1471-2164-7-1>
- Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food Science and Nutrition*, 42(3), 179–208. <https://doi.org/10.1080/10408690290825510>
- Piironen, V., Edelmann, M., Kariluoto, S., & Bedo, Z. (2008). Folate in wheat genotypes in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9726-9731.
- Wang, J., Chatzidimitriou, E., Wood, L., Hasanalieva, G., Markellou, E., Iversen, P. O., Seal, C., Baranski, M., Vigar, V., Ernst, L., Willson, A., Thapa, M., Barkla, B. J., Leifert, C., & Rempelos, L. (2020). Effect of wheat species (*Triticum aestivum* vs *T. spelta*), farming system (organic vs conventional) and flour type (wholegrain vs white) on composition of wheat flour – Results of a retail survey in the UK and Germany – 2. Antioxidant activity, and phenoli. *Food Chemistry: X*, 6(May), 100091.

<https://doi.org/10.1016/j.fochx.2020.100091>

Wieser, H. (2000). Comparative investigations of gluten proteins from different wheat species. *European Food Research and Technology*, 211(4), 262–268.

<https://doi.org/10.1007/s002170000165>

Wieser, H., Antes, S., & Seilmeier, W. (1998). Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. *Cereal Chemistry*, 75(5), 644–650. <https://doi.org/10.1094/CCHEM.1998.75.5.644>

Wieser, H., Mueller, K., & Koehler, P. (2009). Studies on the protein composition and baking quality of einkorn lines. *European Food Research and Technology*, 229(3), 523–532. <https://doi.org/10.1007/s00217-009-1081-5>

Wrigley, C., Békés, F., & Bushuk, W. (2006). Chapter 1 Gluten: A balance of gliadin and glutenin. In *AACC International, Inc. eBooks* (pp. 3–32). <https://doi.org/10.1094/9781891127519.002>

Wu, G. (2010). Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, 1(1), 31–37. <https://doi.org/10.3945/an.110.1008>

Yang, F., Zhang, J., Liu, Q., Liu, H., Zhou, Y., Yang, W., & Ma, W. (2022). Improvement and Re-Evolution of Tetraploid Wheat for Global Environmental Challenge and Diversity Consumption Demand. *International Journal of Molecular Sciences*, 23(4). <https://doi.org/10.3390/ijms23042206>

Zabolotneva, A. A., Shatova, O. P., Sadova, A. A., Shestopalov, A. V., & Roumiantsev, S. A. (2022). An overview of alkylresorcinols biological properties and effects. *Journal of Nutrition and Metabolism*, 2022, 1–12. <https://doi.org/10.1155/2022/4667607>

- Zaharieva, M., Ayana, N. G., Hakimi, A. Al, Misra, S. C., & Monneveux, P. (2010). Cultivated emmer wheat (*Triticum dicoccon* Schrank), an old crop with promising future: A review. *Genetic Resources and Crop Evolution*, 57(6), 937–962.
<https://doi.org/10.1007/s10722-010-9572-6>
- Zamaratskaia, G., Gerhardt, K., & Wendin, K. (2021). Biochemical characteristics and potential applications of ancient cereals - An underexploited opportunity for sustainable production and consumption. *Trends in Food Science and Technology*, 107(November 2020), 114–123. <https://doi.org/10.1016/j.tifs.2020.12.006>
- Zaparenko, A., Didenko, S., Holyk, O., & Goloventsov, Y. (2020). Investigation of the technological properties of emmer flour. *Food Science and Technology*, 14(2).
<https://doi.org/10.15673/fst.v14i2.1717>.
- Zencirci, N., Ulukan, H., Baloch, F. S., Mansoor, S., & Rasheed, A. (Eds.). (2022). *Ancient Wheats*. Springer.
- Zhang, G., & Hamaker, B. R. (2017). The nutritional property of endosperm starch and its contribution to the health benefits of whole grain foods. *Critical Reviews in Food Science and Nutrition*, 57(18), 3807–3817.
<https://doi.org/10.1080/10408398.2015.1130685>
- Zhou, T., Zhang, L., Liu, Q., Liu, W., & Hu, H. (2021). Rheological behaviors and physicochemical changes of doughs reconstituted from potato starch with different sizes and gluten. *Food Research International*, 145(February), 110397.
<https://doi.org/10.1016/j.foodres.2021.110397>

- Zi, Y., Shen, H., Dai, S., Ma, X., Ju, W., Wang, C., Guo, J., Liu, A., Cheng, D., Li, H., Liu, J., Zhao, Z., Zhao, S., & Song, J. (2019). Comparison of starch physicochemical properties of wheat cultivars differing in bread- and noodle-making quality. *Food Hydrocolloids*, 93, 78–86. <https://doi.org/10.1016/j.foodhyd.2019.02.014>
- Ziegler, J. U., Steingass, C. B., Longin, C. F. H., Würschum, T., Carle, R., & Schweiggert, R. M. (2015). Alkylresorcinol composition allows the differentiation of *Triticum* spp. having different degrees of ploidy. *Journal of Cereal Science*, 65, 244–251. <https://doi.org/10.1016/j.jcs.2015.07.013>
- Žilić, S. (2013). Wheat gluten: Composition and health effects. *Gluten*, 71-86.
- Zrcková, M., Capouchová, I., Paznocht, L., Eliášová, M., Dvořák, P., Konvalina, P., Janovská, D., Orsák, M., & Bečková, L. (2019). Variation of the total content of polyphenols and phenolic acids in einkorn, emmer, spelt and common wheat grain as a function of genotype, wheat species and crop year. *Plant, Soil and Environment*, 65(5), 260–266. <https://doi.org/10.17221/134/2019-PSE>

Chapter 3 Determining the Nutritional Potential of Ancient Wheat Species: A closer Look at Starch and Protein Digestibility, and Phenolic Acid Content

Abstract

The recent trend towards healthy food choices and sustainable agriculture has revived interest in underutilized wheat species such as emmer, einkorn, and spelt. Given these developments, the objective of the study was to compare the differences in chemical composition and digestibility characteristics among ancient wheat species. Einkorn demonstrated higher protein (21.27%) and phenolic acids (free and conjugated) along with lower starch levels than other samples. Notably, its starch digestibility was marked by lower glucose release and reduced rapidly digestible starch (RDS) levels, while refined flour from common wheat exhibited the highest RDS with glucose release. From the ancient wheat species, the highest glucose release was observed in emmer, which also had the highest content of RDS. *In vitro* protein digestibility (IVPD) was highest in refined flour (86.05%), with einkorn closely following at 84.29%. Both spelt and emmer exhibited lower IVPD values compared to common wheat samples and einkorn. Spelt was characterized by its significant level of bound phenolic acid content followed by einkorn. The variation in chemical composition, particularly in einkorn with its higher protein and phenolic acid content, combined with its favorable digestibility profile, highlights its potential health benefits. These findings emphasize the importance of further research on underutilized grains to fully comprehend their nutritional value.

Key words: ancient wheat, chemical composition, starch, protein, digestibility

3.1 Introduction

Wheat, scientifically known as *Triticum aestivum* L., plays a pivotal role in feeding the global population by serving as a staple food source in numerous regions across the world (Laugerotte et al., 2022). The anticipated global wheat production for 2023/24 is estimated to reach 793.4 million metric tons and this significant output feeds one-third of the world's population and contributes to approximately 20% of their caloric needs (USDA , 2023). To ensure global food security in the context of population growth and less favorable climate conditions, wheat production must be raised sustainably (Gaurav et al., 2022). Considering the current shift towards low environmental impact and sustainable farming practices, as well as the growing preference for healthy, nutrient-dense foods, it is thought that the earliest domesticated types of wheat may continue to have significance in terms of human consumption (Brandolini et al., 2008; Deyalage et al., 2024). The most well-known and widely studied ancient wheat species include diploid einkorn (*Triticum monococcum*), tetraploid emmer (*Triticum dicoccum*) and hexaploid spelt (*Triticum spelta*) (Shewry & Hey, 2015). These wheat species possess kernels with intact hulls and spikes that are not easily broken.

Apart from their positive environmental impacts, such as cultivation under low-input conditions, enhanced field biodiversity, climate adaptation, and disease resistance, which are linked to sustainable agricultural practices, the growing interest in ancient wheat species is largely attributed to the notable levels of protein, minerals, insoluble fiber and phytochemicals present in these grains (Bencze et al., 2020; Bonafaccia et al., 2000; Brandolini et al., 2008; Hidalgo & Brandolini, 2014; Lacko - Bartošová & Čurná, 2015). Despite the known nutritional benefits, there

is still a significant gap in the scientific literature regarding their potential impacts on health in terms of their detailed chemical composition and digestibility characteristics.

Starch holds significant importance as a glycemic carbohydrate. It is metabolized in the body through a series of digestive reactions resulting in disaccharides and monosaccharides, which leads to an increase in the glycemic index (Foster-Powell et al., 2009). In general, starch is categorized into three primary fractions of nutritional significance, namely rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) as per the classification proposed by Englyst et al. (1992). RDS is broken down in the small intestine and results in an immediate elevation of blood glucose level after consumption. SDS, an intermediary starch fraction between RDS and RS, is gradually digested providing a sustained and prolonged release of glucose with a low initial glycemic response. SDS is likely to be associated with favorable health outcomes, including glycemic control, reduced postprandial circulation of free fatty acids, and diminished oxidative stress (Lehmann & Robin, 2007; Zhang & Hamaker, 2009). RS fraction is not hydrolyzed after 120 fraction that is available for fermentation in the large bowel (Englyst et al., 1992). Therefore, assessing starch digestibility is of utmost importance as it underpins the understanding of glycemic control and health implications of starchy foods.

Furthermore, investigations into the extent of protein digestion of ancient wheat are frequently overlooked although protein serves as a crucial determinant in assessing the nutritional value of foods (Zhang et al., 2020). In addition to the digestibility characteristics, the synergy between other functional components in these grains, such as arabinoxylan and phenolic acids, may also be a contributing factor to the health benefits of these wheat species. Therefore, it is necessary to

evaluate the potential health benefits associated with the consumption of these ancient cereals in multiple fronts including starch and protein characteristics as well as other grain components. The underutilization of these wheat species can be attributed to the limited scientific understanding of the aforementioned characteristics. Hence, this study intends to address this gap by conducting an in-depth analysis of the composition of ancient wheat as well as digestibility characteristics to assess potential health benefits, while investigating the differences between ancient wheat species.

3.2 Materials and methods

Raw materials

Commercially available samples of Einkorn, emmer, and spelt flour harvested in 2021 were used in this work. Specifically, the einkorn and emmer were grown in Saskatchewan, while the spelt was grown in British Columbia. A common wheat sample [Canadian Western Red Spring wheat (CWRS)], representing grains from the Canadian prairies, was obtained from the Canadian Grain Commission (Winnipeg, MB). All ancient wheat grains were milled using an Ultra centrifugal mill [Type ZM 200] (Retsch GmbH, Haan, Retsch-Allee, Germany) [sieve:0.5mm] to obtain whole grain flour, while CWRS grains were processed into both whole grain (CWRS WW) and refined flour (CWRS RF), with whole grain produced using the Ultra centrifugal mill [Type ZM 200 and refined flour using Quadrumat Sr. (Brabender GmbH & Co.KG, Kulturstr, Germany).

3.2.1 Proximate composition

Ash, moisture and fat contents were measured following the dry ashing method: AOAC 923.03 (AOAC International, 2012), the vacuum oven method: AACCI 44-40.01) (AACC International, 2010) and the Soxhlet method (AOAC 920.39C), respectively. The nitrogen content of flours was determined by the combustion method (AACCI Method 46-30.01) using Rapid N Exceed

(Elementar Analysensysteme GmbH, Germany) (Conversion factor: 5.7). The total starch analysis was conducted using the Megazyme kit (Megazyme International, Ireland) K-TSTA-100A method (AOAC 996.11).

The total dietary fiber was analyzed using the Megazyme total dietary fiber analysis kit following AOAC Method 991.43.

3.2.2 Determination of the amino acid profile

The amino acid profiles of the flour samples were analyzed using the method published by Bailey et al. (2023). The majority of amino acids were quantified through acid hydrolysis in line with AOAC Official Method 982.30 (AOAC International, 2012), which involved a 24-hour hydrolysis using 6N HCl. This method leads to the deamidation of asparagine and glutamine, which are subsequently reported as part of the total aspartate and glutamate values, consistent with national nutrient database standards. Methionine and cysteine were analyzed following pre-oxidation with performic acid and subsequent acid hydrolysis, as described in AOAC Official Method 994.12. The tryptophan content was determined using alkaline hydrolysis, according to the protocol [13904] established by the International Organization for Standardization (2016) (International Organization for Standardization [ISO], 2016). Amino acid derivatization and separation were conducted using the AccQ-Tag Ultra Chemistry System (Waters Corporation, USA)) on a Shimadzu UPLC System (Shimadzu Corporation, Japan), equipped with an AccQ-Tag Ultra C18 column (1.7 μm) and an SIL-20AC autosampler. The amino acid contents were calculated using their respective hydrated molecular weights. The amino acid score (AAS) for each sample was calculated following the FAO/WHO protocol, the amino acid requirements of a 2–5-year-old child (Food and Agriculture Organization of the United Nations [FAO] & World Health Organization [WHO], 1991), using

reference values for indispensable amino acids (IAA) and selecting the lowest ratio as the final score. The reference values used were as follows: histidine, 19 mg/g; isoleucine, 28 mg/g; leucine, 66 mg/g; lysine, 58 mg/g; methionine + cysteine, 25 mg/g; phenylalanine + tyrosine, 63 mg/g; threonine, 34 mg/g; tryptophan, 11 mg/g; and valine, 35 mg/g.

3.2.3 Arabinoxylan (AX) quantification

The quantification of arabinoxylan (AX) in flour samples was conducted following the method outlined by Englyst and Cummings (1984). Initially, 100 mg of flour was mixed with 2 mL of DMSO was added to the sample to facilitate solubilization and dispersion of starch and then heated at 100°C for one hour in a boiling waterbath. Following this, 3 mL of sodium acetate buffer (pH 5.2) was added (maintained at 50°C). To initiate enzymatic hydrolysis, 3 mL of an enzyme solution was added, and the samples were incubated at 45°C for 16 hours in a magnetic water bath (HBR 4, IKA Werke GmbH & Co. KG, Germany). After incubation, 42 mL of 95% ethanol was introduced, and the mixture was inverted and left undisturbed at room temperature for one hour. The mixture was then centrifuged (Sorvall ST Plus series, Thermo Fisher Scientific, USA) at 2500 rpm for 10 minutes, and the supernatant was carefully decanted. The remaining residue was dried in an oven and the test tubes were systematically monitored for dryness at regular intervals and close attention was paid to any indications of sample caking, cracking, or detachment from the test tube walls. Once the samples achieved the desired level of dryness, the procedure advanced to the next step. 1 mL of 12M H₂SO₄ at 35°C was added to each sample to initiate the dissolution of any remaining residue. This step required continuous shaking at a controlled temperature of 35 °C over a duration of one hour with gentle vortexing. Following this, 6 mL of water and 5 mL of the internal standard (myo-inositol) were added, and the mixture was heated for 2 hours at 100°C. Sugar

standards, placed in two additional 50 ml test tubes, were also added, and then these test tubes were placed in an ice water bath. After cooling in an ice water bath, 5 μ L of octan-2-ol was added, and 1 mL of the sample was introduced to each tube and the tubes were kept at 40°C for one hour. Following the addition of 0.1 mL of acetic acid, 0.2 mL of N-Methyl Imidazole and 2 mL of acetic anhydride were introduced to 0.2 mL of the sample, which was then left at room temperature for 30 minutes. Finally, 5 mL of water and 1 mL of dichloromethane (CH_2Cl) were added, and the mixture was vigorously shaken while releasing the pressure. The CH_2Cl phase was transferred into a glass vial for quantification. The AX content was quantified using Gas Chromatography (GC) (6890N Network GC system, Agilent Technologies, USA) equipped with a Flame Ionization Detector (FID).

3.2.4 Phenolic acid profile

The extraction of phenolic acids was performed by assessing their free, conjugated, and bound forms, following the procedure outlined by Nicoletti et al. (2013) with some modifications. Initially, 1 g of dried, defatted flour was weighed and transferred into 50 mL glass tubes. The flour was mixed with 3 mL of an 80% ethanol-water solution. The mixture was subjected to sonication for 10 minutes, followed by vortexing and centrifugation at 3000 rpm for 10 minutes. The resulting supernatant was carefully transferred to a new glass tube, and this extraction was repeated two additional times; the supernatants from each extraction were then combined and subsequently dried under a nitrogen gas stream before being freeze-dried overnight.

Extraction of Free Phenolic Acids

The lyophilized sample was rehydrated with 2 mL of 2% aqueous acetic acid and adjusted to pH 2 with 12.1 N HCl. Diethyl ether (2 mL) was added to the sample, which was then vortexed and

centrifuged at 3000 rpm for 10 minutes. The upper organic layer was collected, and this extraction was repeated two more times. The supernatants were combined, evaporated to dryness under nitrogen, stored at -30°C, and reconstituted in 200 µL of an 80:20 methanol-water solution containing 0.1% acetic acid before UPLC analysis.

Extraction of Conjugated Phenolic Acids

The lyophilized sample was hydrolyzed with 2 mL of 2 M sodium hydroxide for 4 hours in a rotary mixer. After acidification to pH 2 with 12 N HCl, the conjugated PAs were extracted with diethyl ether and reconstituted as described in the previous section.

Extraction of Bound Phenolic Acids

The lyophilized pellet from the 80% ethanol-water extraction was ground into a fine powder and subjected to alkaline hydrolysis using 2 M sodium hydroxide in a rotary mixer for 4 hours. Following hydrolysis, the samples were acidified to pH 2 with 12.1 N HCl. Diethyl ether was subsequently added to the tubes, and the extraction and reconstitution procedures were carried out as previously described.

UPLC analysis was performed using a Acquity UPLC system (Waters Corporation, USA) equipped with a Acquity UPLC photodiode array detector (Waters Corporation, USA). Phenolic acids were separated on a Waters C18 LC column (50 x 2.1 mm, 1.7 µm) using a gradient of solvent A [water containing 0.1% (v/v) acetic acid] and solvent B [methanol containing 0.1% acetic acid] over 15 minutes at a flow rate of 0.75 mL/min. The gradient was: 99% A and 1% B initially, 98% A and 2% B at 5 minutes, 86% A and 14% B at 6 minutes, 85.6% A and 14.4% B at 10 minutes, 25% A and 75% B at 12 minutes, 99% A and 1% B at 13 minutes, and 99% A and 1% B at 15 minutes. Detection

was carried out at 260 nm, 280 nm, and 320 nm. The phenolic acids quantified included p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, o-coumaric acid, sinapic acid, ferulic acid, and p-coumaric acid, identified by comparing their retention times with those of phenolic acid standards.

Detection wavelengths for phenolic acids:

- 260 nm: p-hydroxybenzoic acid, vanillic acid, caffeic acid
- 280 nm: syringic acid, o-coumaric acid
- 320 nm: Sinapic acid, ferulic acid, p-coumaric acid

3.2.5 Assessing nutritional characteristics of ancient wheat species

3.3.5.1 Bread sample preparation

Bread samples were prepared according to AACCI 10–10.03 (AACC International, 2010) and it was conducted in triplicate for each sample. After preparation, the bread samples were freeze-dried, milled (Ultra centrifugal mill [Type ZM 200] (sieve size: 0.5mm), and stored at -°20C until further analysis.

3.3.5.2 In vitro starch digestibility

The experiment was conducted following the Megazyme (Megazyme International, Ireland) digestible and resistant starch assay protocol (K-DSTRS 11/19) based on a method described by Englyst et al. (1992). 1.0 g of the sample was mixed with 1 mL of 95% ethanol and 35 mL of maleate buffer, then equilibrated at 37°C in a waterbath for 5 minutes. Afterward, 5 mL of pancreatic α -amylase and amyloglucosidase (PAA/AMG) solution was added, and the reaction solutions were incubated at 37°C with continuous agitation. For the digestible starch determination, samples were taken at specified time intervals: 1.0 mL at 20 minutes for RDS measurement, 120 minutes for SDS

measurement, and 240 minutes for total digestible starch (TDS) measurement. These samples were mixed with 20 mL of 50 mM acetic acid solution, thoroughly mixed, and stored at 4°C awaiting analysis. Following this, 2 mL of each solution was transferred to 2.0 mL microfuge tubes and centrifuged at 13,000 rpm (Sorvall Legend Micro 21R, Thermo Fisher Scientific, USA) for 5 minutes. Then, 0.1 mL aliquots of the supernatant were transferred to glass test tubes, followed by the addition of 0.1 mL of diluted AMG (amyloglucosidase)(100 U/mL). After incubating at 50°C for 30 minutes, 3.0 mL of GOPOD(glucose oxidase/ peroxidase) reagent was added, and the mixture was further incubated at 50°C for 20 minutes. Absorbance readings at 510 nm were taken for both the sample and glucose standards against the reagent blank using a multi-mode microplate reader (Synergy LX, Agilent BioTek, USA).

For resistant starch, after a 240-minute incubation with PAA/AMG, 4.0 mL of the suspension was taken and transferred into a tube containing 4.0 mL of 95% ethanol . The tube was sealed, and the contents were thoroughly mixed by repeated inversion.

The tube was centrifuged at 4,000 rpm for 10 minutes, and the supernatant was carefully discarded. The pellet was re-suspended in 2 mL of 50% aqueous ethanol using a vortex. Another 6 mL of 50% aqueous ethanol was added, and the mixture was vortexed again. The tubes were centrifuged, and the pellets were recovered. Any remaining liquid was removed by inverting the tubes. This re-suspension and centrifugation step with 50% v/v aqueous ethanol (2 + 6 mL) was repeated again, ensuring the removal of excess liquid. A magnetic stirrer bar and 2 mL of cold 1.7 M NaOH were added to each tube. The pellets were re-suspended and the RS dissolved by stirring the contents in an ice/water bath over a magnetic stirrer for approximately 20 minute. Then, 8 mL of 1.0 M sodium acetate buffer (pH 3.8) was added while stirring. Immediately after, 0.1 mL of AMG

(3300 U/mL) was added, and the contents were thoroughly mixed. The tubes were placed in a water bath at 50°C and incubated for 30 minutes with intermittent mixing.

For samples with >10% RS content, the tube contents were quantitatively transferred to a 100 mL volumetric flask. The volume was adjusted to 100 mL with distilled water and mixed well. An aliquot (2 mL) of the solution was centrifuged at 13,000 rpm for 5 minutes. Next, 0.1 mL aliquots of either the centrifuged diluted or undiluted supernatants were transferred into glass test tubes. To each tube, 0.1 mL of 100 mM sodium acetate buffer (pH 4.5) was added, followed by 3.0 mL of GOPOD reagent. The tubes were incubated at 50°C for 20 minutes. The absorbance of the samples and glucose standards (prepared as described below) was measured at 510 nm against the reagent blank.

3.3.5.3 In vitro glucose determination

Measurement of *in-vitro* glucose release over time was carried out following the method reported by van Kempen et al. (2010). From each ground bread sample, 250 mg was weighed into 15 mL test tubes. Then, 2.5 mL of pepsin-guar gum solution (50mg of pepsin [Sigma cat P7000] and 50mg guar gum for every 10mL 0.05mol/L HCl) was added to each tube, and vortexed to ensure no clumps or dry spots were present. The tubes were subsequently incubated at 37°C for 30 minutes in a water bath (HBR 4, IKA Werke GmbH & Co. KG, Germany) with magnetic stirring. After incubation, samples were removed from the water bath and 2.5 mL of 0.25 mol/L sodium acetate solution and 1.25 mL of pancreatin-amyloglucosidase-invertase solution (concentration of this solution is equivalent to 0.7g pancreatin -50µL of 200U/mL of amyloglucosidase and 3mg invertase per 5mL solution) were added to the tubes. They were then vortexed and placed in a water bath maintained at 37°C. At 20-minute intervals, the first sample was removed, vortexed, and a 100 µL

aliquot was taken and added to a tube containing 5 mL of 100% ethanol. This process was repeated for all samples based on the time intervals of 40, 60, 120, 240 and 300 minutes. The vortexed sample-ethanol mixture was then centrifuged (Sorvall ST Plus series, Thermo Fisher Scientific, USA) at 300xg for 2 minutes. Subsequently, 100 µL of the sample was pipetted into 10 mL disposable glass tubes. To each tube, 3 mL of GOPOD was added, and the mixture was incubated at 50°C for 20 minutes. The blank, containing 100% ethanol + GOPOD, was used to zero the spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Sweden), and the absorbances at 510 nm were recorded. Additionally, 100 µL of 1 mg/mL glucose (Megazyme International, Ireland) was pipetted into a 10 mL disposable tube to obtain the absorbances for 100 µg of glucose. Glucose release at specific time intervals was quantified using the following formula.

$$\mu g \text{ of glucose} / 0.1 \text{ mL aliquot} = 100 \times \text{Abs of sample} / \text{Abs of } 100 \mu g \text{ glucose}$$

3.3.5.4 In vitro protein digestibility (IVPD)

The IVPD assay was performed on ground bread samples following the method described in Franczyk et al.(2024) in triplicates. Bread samples equal to 10 mg of nitrogen, were dissolved in 10 mL of deionized water, heated to 37°C, and allowed to solubilize for 1 hour. The pH of the solution was then adjusted to 8.0 ± 0.05 using 1 M NaOH or HCl.

A multienzyme solution was prepared with concentrations of 3.1 mg/mL chymotrypsin (bovine pancreas ≥ 40 units/mg protein), 1.6 mg/mL trypsin (porcine pancreas 13,000–20,000 BAEE units/mg protein), and 1.3 mg/mL protease (*Streptomyces griseus* ≥ 3.5 units/mg solid) in deionized water. This solution was adjusted to a pH of 8.0 ± 0.05 at 37°C before being transferred to an ice water bath at 4°C. Casein was included as a sample standard to ensure reproducibility between batches. To measure IVPD, 1 mL of the enzyme solution was added to the bread sample

solution. The pH change was recorded exactly 10 minutes after the enzyme addition, reflecting the release of carboxylic acids as proteins were hydrolyzed into peptides and amino acids.

Protein digestibility was calculated from the following regression equation

$$IVPD = 65.66 + 18.10(\Delta pH)$$

3.2.6 Statistical analysis

The Origin software (Origin 2023b, OriginLab Corporation, USA) was used to conduct a one-way analysis of variance (ANOVA) for five samples. Following this, the sample means were compared using Tukey pairwise comparison to determine significant differences at $p < 0.05$. All samples were analyzed at least in duplicate to ensure the accuracy and reliability of the results.

3.3 Results and discussion

3.3.1 Proximate composition

The chemical composition of flour samples is presented in **Table 3.1**. Starch, primarily derived from wheat and other cereal grains, serves as the primary source of dietary carbohydrates for humans (Shewry & Hey, 2015). Ancient wheat species had significantly lower ($p < 0.05$) starch content compared to refined flour of common wheat (CWRS RF). CWRS RF exhibited the highest starch content (66.39%), which can be attributed to the removal of bran and germ during processing, which concentrates the starchy endosperm. In contrast, einkorn had the lowest starch content at 52.15%. Emmer demonstrated the highest starch content among ancient wheat species, exceeding that of wholegrain common wheat; (CWRS WW) and this trend aligns with Kulathunga et al. (2021), who reported starch contents of 66% for emmer and 62% for common wheat. Multiple studies have reported lower content of starch in ancient wheat species (Brandolini et al.,

2008; Golea et al., 2023) and this could be a result of the yield dilution phenomenon which is associated with enhanced grain yield of common wheat, coupled with its higher ploidy level, which is indicative of increased starch accumulation due to higher cell size and biomass (Arzani & Ashraf, 2017).

In terms of protein content, einkorn demonstrated a significantly higher value (21.23%) ($p < 0.05$), surpassing all other samples. CWRS RF and CWRS WW show values of 14.40% and 14.92% respectively. This trend was also observed by Biel et al. (2021), who reported einkorn with 18.1% protein, followed by emmer at 15.4%, spelt at 12.8%, and common wheat at 11.0%. In contrast, Golea et al. (2023), observed the highest protein content in spelt at 18.95%, which was not significantly different from einkorn, with a value of 18.15%. The comparatively lower protein content in common wheat can be explained by its larger kernel size and heavier grains, leading to a higher proportion of starchy endosperm and consequently a lower protein content (Arzani & Ashraf, 2017). However, it is important to note that wheat protein content varies widely depending on genotype, environment and agricultural practices, where protein content at times can be as high as 18-20% (Khalid et al., 2023; Kulathunga et al., 2021).

In this study, einkorn demonstrated the highest dietary fiber content, accompanied by a notably higher level of insoluble fiber compared to all other samples. This finding aligns with previous literature, Biel et al. (2021), who also reported the highest fiber content in einkorn compared to common wheat. In contrast, Abdel-Aal et al. (1995) findings indicate the highest insoluble fiber content in common wheat, while einkorn showed the lowest among the samples. Both ash content and lipid content were also observed to be higher in all the ancient wheat samples analyzed in this study compared to common wheat, which is in agreement with previous studies (Abdel-Aal et al.,

1995; Biel et al., 2021; Golea et al., 2023). The lower levels of minerals in common wheat may result from mineral dilution due to higher grain yields, which are focused on maximizing milling output. Therefore, this characteristic could indicate the primitive nature of ancient wheat (Rasmusson et al., 1971). It is important to note that variations in chemical composition among these species can be attributed to differences in genotype and growing conditions (Massaux et al., 2008; Zencirci et al., 2022).

Table 3.1 Proximate composition of flour samples from different wheat species (% as-is basis)

Sample	Starch %	Protein%	Ash %	Fat %	Moisture %
Einkorn	52.15 ± 1.56 ^e	21.27 ± 0.08 ^a	1.78 ± 0.10 ^c	2.07 ± 0.24 ^a	7.78 ± 0.01 ^d
Emmer	63.78 ± 0.89 ^b	12.85 ± 0.26 ^e	1.86 ± 0.03 ^b	1.93 ± 0.05 ^{ab}	8.67 ± 0.10 ^c
Spelt	59.23 ± 0.37 ^c	15.41 ± 0.14 ^b	1.98 ± 0.01 ^a	2.17 ± 0.06 ^a	7.92 ± 0.06 ^d
CWRS WW	56.05 ± 0.31 ^d	14.92 ± 0.17 ^c	1.59 ± 0.01 ^d	1.57 ± 0.24 ^b	12.18 ± 0.08 ^b
CWRS RF	66.39 ± 1.01 ^a	14.40 ± 0.05 ^d	0.62 ± 0.01 ^e	0.89 ± 0.10 ^c	13.87 ± 0.14 ^a

Note: Values are presented as mean ± SD, Different letters in the same column indicate significant differences (p < 0.05)

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

3.3.2 Amino acid (AA) composition

The amino acid composition for all flour samples is presented in **Table 3.2**, while **Table 3.3** shows the corresponding amino acid scores. According to the AA profile, GLU was the most abundant AA fraction, which includes both glutamic acid and glutamine. This finding aligns with Abdel-Aal & Hucl (2002), who reported that wheat protein has higher levels of glutamine and proline, which are crucial for dough formation. Spelt stands out with the highest Indispensable Amino Acids (IAA) content among all samples, followed by emmer. However, Akar et al. (2019) found that einkorn

wheat had the highest IAA levels compared to emmer and durum wheat lines. Previous studies indicate that genotype plays a more significant role in the diversity of IAA and DAA contents than agricultural practices (Sulek et al., 2023). According to the findings of AAS (**Table 3.3**), lysine was the first limiting amino acid for all wheat species, which is consistent with the literature for cereal proteins (Sá & House, 2024; Sá et al., 2020) with AAS values ranging from 0.47 in einkorn, 0.53 in emmer, 0.53 in spelt, 0.49 in CWRS WW, and 0.42 in CWRS RF. Wheat protein is known to be low in certain indispensable amino acids, particularly lysine and, to a lesser extent, threonine, which is the second most deficient amino acid (Abdel-Aal & Hucl, 2002; Dimina et al., 2022). However, to the best of the authors' knowledge, very few studies have reported the AA composition of ancient wheat species, and the content may vary based on genotype and environmental conditions, as mentioned previously.

Table 3.2 Amino acid composition of flour samples from different wheat species (mg/g crude protein)

Sample	Einkorn	Emmer	Spelt	CWRS WW	CWRS RF
HIS	25.68±0.27	25.70±0.87	27.11± 0.73	25.70± 0.45	24.82± 1.12
SER	45.4± 0.66	48.16±1.37	48.45± 1.10	48.02± 1.47	49.51± 1.20
ARG	41.84 ± 0.34	45.46±1.10	46.68± 1.07	43.65± 0.15	37.24± 0.28
GLY	34.39 ± 0.37	34.60±0.61	38.88± 0.46	39.18± 0.32	34.92± 0.17
ASP	54.64± 0.59	54.34±1.20	54.64± 0.91	50.39± 0.73	43.79± 0.58
GLU	363.93±2.68	320.62±8.54	320.93± 3.42	330.6± 5.80	368.38± 3.09
THR	26.54±0.23	30.44±0.62	30.75± 0.32	29.18± 0.27	27.93± 0.20
ALA	31.69±0.07	37.43±0.68	36.95± 0.50	34.78± 0.45	30.80± 0.25
PRO	108.46±0.77	101.50±3.03	105.29± 0.69	108.16± 1.00	120.51±0.52
CYS	22.05±0.11	25.56±0.92	26.04± 0.10	23.90± 0.37	24.45± 0.77
LYS	26.97±0.25	30.80±1.15	30.48± 0.49	28.14± 0.18	24.21± 0.39
TYR	29.92 ±0.45	28.09±1.49	28.64± 2.07	30.04± 0.94	31.04± 1.20
MET	16.07± 0.32	17.69±0.66	18.21± 0.29	15.99± 0.07	15.67± 0.52
VAL	40.69± 0.40	45.32±0.99	46.37± 0.52	42.4± 0.50	41.25± 0.26

ILE	40.08± 0.55	38.86± 1.21	37.79± 0.43	35.92± 0.29	37.15± 0.20
LEU	65.50± 1.05	70.48± 2.35	71.91± 0.87	67.89± 0.71	69.36± 0.57
PHE	55.97± 0.94	49.18± 2.28	47.5± 1.27	48.91± 0.70	51.26± 0.58
TRP	9.87± 0.09	11.28± 0.14	14.52± 0.30	13.6± 0.26	11.77± 0.20
AAA	85.89	77.27	76.14	78.95	82.30
SAA	38.11	43.24	44.25	39.89	40.12
IAA	359.32 ^b	373.38 ^{ab}	379.32 ^a	361.68 ^b	358.91 ^b
DAA	680.34 ^a	642.11 ^b	651.81 ^b	654.78 ^b	685.15 ^a

Abbreviations: HIS = Histidine; SER = Serine; ARG = Arginine; GLY = Glycine; ASP = Aspartate + Asparagine; GLU = Glutamate + Glutamine; THR = Threonine; ALA = Alanine; PRO = Proline; CYS = Cysteine; LYS = Lysine; TYR = Tyrosine; MET = Methionine; VAL = Valine; ILE = Isoleucine; LEU = Leucine; PHE = Phenylalanine; TRP = Tryptophan

Note: Values are presented as mean ± SD, Different letters in the same row indicate significant differences ($p < 0.05$).

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour, IAA: Indispensable Amino Acids, DAA: Dispensable Amino Acids, AAA: Aromatic Amino Acids, SAA: Sulphur Amino Acids

Table 3.3 Amino acid score (AAS) of flour samples from different wheat species

Sample	19	28	66	58	25	63	34	11	35	AAS
	HIS	ILE	LEU	LYS	SAA	AAA	THR	TRP	VAL	
Einkorn	1.35	1.43	0.99	0.47	1.52	1.36	0.78	0.90	1.16	0.47
Emmer	1.35	1.39	1.07	0.53	1.73	1.23	0.90	1.03	1.30	0.53
Spelt	1.43	1.35	1.09	0.53	1.77	1.21	0.90	1.32	1.33	0.53
CWRS WW	1.35	1.29	1.03	0.49	1.60	1.25	0.86	1.24	1.21	0.49
CWRS RF	1.31	1.33	1.05	0.42	1.61	1.31	0.82	1.07	1.18	0.42

The amino acid scores were calculated using the FAO/WHO protocol, the amino acid requirements of a 2–5year-old child (FAO and WHO, 1991) reference pattern (mg/g protein): HIS 19, ILE 28, LEU 66, LYS 58, SAA (MET+CYS) 25, AAA (PHE+TYR) 63, THR 34, TRP 11, VAL 35.

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour, SAA: Sulfur-Containing Amino Acids, AAA: Aromatic Amino Acids

3.3.3 Arabinoxylan quantification

AXs offer numerous health benefits due to their ability to act as prebiotics and immunomodulatory compounds, while lowering cholesterol, enhancing the absorption of certain minerals, and promoting fecal bulking (Mendis & Simsek, 2014; Zannini et al., 2022). The data from this study (**Figure 3.1**) highlights a significant variability in AX content across wheat species, with common wheat (CWRS WW) and einkorn exhibiting the highest levels at 4.55% and 4.32% respectively. In contrast, AX content was lowest in CWRS RF, with a value close to 1.62%, as the bran layer was removed during milling. Among the ancient wheat species studied, einkorn had the highest AX content at 2.90%. However according to Gebruers et al. (2008) there were no significant differences observed between ancient wheat and common wheat in terms of AX content. Common wheat exhibited AX content ranging from 1.65% to 2.75%, einkorn from 1.45% to 2.35%, spelt from 1.60% to 2.15% and emmer from 1.40% to 1.95%. In contrast, Kulathunga & Simsek (2022) revealed considerable variations among the wheat species highlighting significant differences between ancient wheat and common wheat. Therefore, as per the findings of Kulathunga & Simsek (2022), common wheat demonstrated the highest variations in AX content, ranging from 2.4% to 4.28%. Following closely, einkorn displayed AX levels ranging from 2.9% to 4.07% while emmer had a range from 2.1 %- 2.6% and spelt ranged from 1.64% to 2.09% on a dry weight basis. The higher AX content observed in whole wheat samples compared to refined flour aligns with the well-documented variation in the distribution of AX throughout the wheat kernel. While AX constitutes only a small portion of the total endosperm, about 3–5%, they are more abundant in the aleurone and bran cell walls, making up approximately 60–70% of the total cell wall content (Lewko et al., 2023). Therefore, the inclusion of the bran fraction, known for its higher AX concentration, significantly contributes to the elevated AX levels observed in wholemeal flour

(Kiszonas et al., 2015). The variability in the AX content reported in different studies can be attributed to genotype x environment (GxE) effects (Tremmel-Bede et al., 2020). In this study, the composite nature of the samples, which combines grains from different environmental conditions, likely reflects the combined effects of genetic diversity and the environmental factors in which the grains were cultivated. Thus, further studies focusing on GxE are needed to fully understand how different ancient wheat genotypes respond to environmental conditions with reference to AX chemistry.

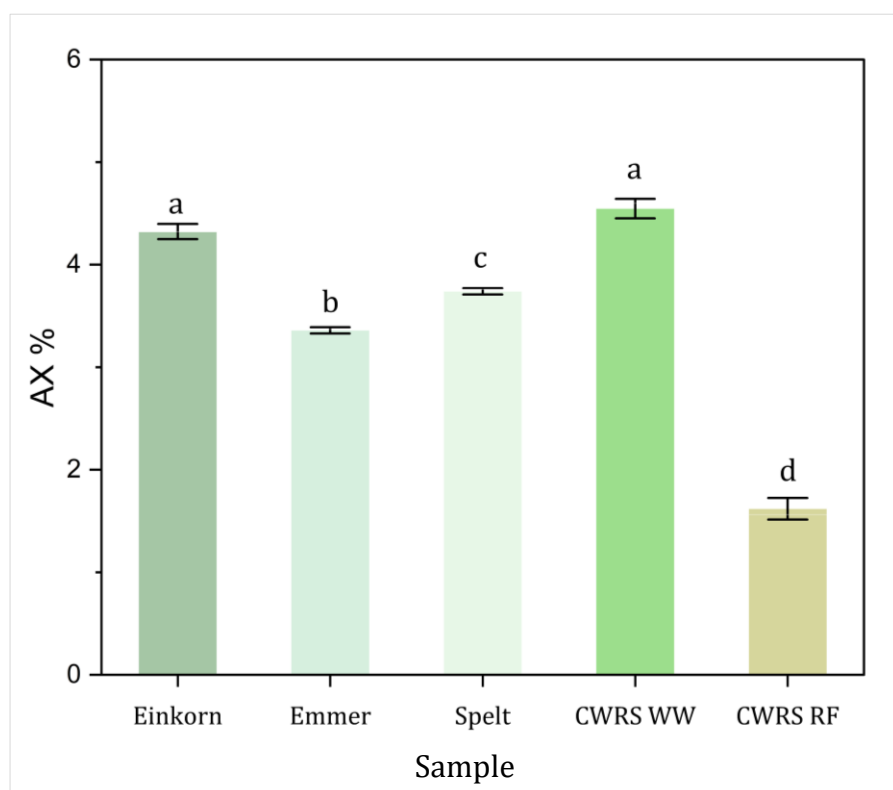


Figure 3.1 Arabinoxylan (AX) content of the flour samples from different wheat species (% as-is basis).

Mean value denoted by different letters specifies a significant difference at the $p < 0.05$

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

3.3.4 Phenolic acids profile

Phenolic acids (PAs) in wheat are present in three distinct forms: free, soluble conjugated, and insoluble bound. These forms vary significantly in their distribution and concentration across different wheat species (Nicoletti et al., 2013). **Table 3.4** details the quantities of free, conjugated and bound phenolic acids in ancient wheat species and common wheat. The total concentration of PAs was calculated by summing the individual PAs determined in each fraction. Significant variations ($p < 0.05$) in phenolic acid profiles were observed across different wheat species. Among the wheat species analyzed, einkorn exhibited the highest content of free phenolic acids (6.62 mg/kg), followed by spelt (5.24 mg/kg). The lowest content was observed in CWRS RF, with an average value of 1.14 mg/kg.

Notably, O-coumaric acid and sinapic acid were absent in the free form across all samples. Ferulic acid was the major free phenolic acid, exhibiting higher concentrations compared to other free phenolic acids in the soluble free fraction for the majority of the samples. This aligns with findings by Barański et al. (2020) who reported that ferulic acid accounts for over 72% of free PAs in wheat species. Lacko-Bartošová et al. (2023), also reported that ferulic acid was the predominant phenolic acid in all wheat cultivars, comprising 67.10% of the free phenolic acids in common wheat and between 65.76%-66.02% in emmer. Common wheat exhibited significantly lower concentrations of most free phenolic acids, except for ferulic acid. In this study, the percentage of free ferulic acid as a proportion of the total free phenolic acids was 28.5%, 48.3%, 57.9%, 47.1%, and 45.3% in einkorn, emmer, spelt, CWRS WW, and CWRS RF, respectively.

For the conjugated PAs, einkorn and spelt exhibited the highest total content, whereas CWRS RF had the least. Sinapic acid showed the highest concentrations in conjugated PA fraction, in both

einkorn and CWRS WW samples. However, its content was significantly lower in emmer, spelt, and CWRS RF. Ferulic acid was the second most abundant conjugated PA, with einkorn containing the highest concentration (24.25 mg/kg) and CWRS RF the lowest (6.26 mg/kg). Bound phenolic acids (PAs) contributed the most to the total PA content across all samples, with ferulic acid being the most abundant bound PA. Its concentration varied from 954.16 mg/kg in spelt to 49.22 mg/kg in CWRS RF. Most bound phenolic acids were highest in spelt, resulting in the highest total bound PA content among all samples. Consistent with the findings in the free and conjugated fractions, CWRS RF exhibited the lowest bound PA content. Overall, when considering all three fractions, spelt demonstrated the highest total PA content, followed by einkorn, CWRS WW, emmer, and CWRS RF. However, Barański et al. (2020) observed the content of PAs, encompassing total, bound, and free forms, was notably higher in einkorn compared to spelt and emmer which had lower concentrations. The concentration of phenolic acids in wheat is influenced by species, cultivars, and processing technologies (Barański et al., 2020; Gawlik-Dziki et al., 2012; Lacko-Bartošová et al., 2023; Tian et al., 2021). PAs are predominantly located in the bran and aleurone layers. Consequently, these components are less prevalent in refined flour, which contributes to significant variability in PA content between whole wheat and refined wheat flour, leading to a reduction in both nutrient and phytochemical content in refined grain products (Nicoletti et al., 2013). Based on the findings, the substantial diversity observed in PAs across the analyzed wheat species highlights that ancient wheat species, particularly einkorn and spelt, are characterized by a more favorable profile of phenolic acids. Selecting wheat species with higher phenolic content is therefore a promising approach to enhancing the nutritional properties of products made from these flours.

Table 3.4 Content of free, conjugated, and bound phenolic acids in flour samples from different wheat species (mg/kg)

Type of phenolic acid fraction	Phenolic acid	Einkorn	Emmer	Spelt	CWRS WW	CWRS RF
Free	P-hydroxybenzoic	0.35 ± 0.03 ^{ab}	0.29 ± 0.02 ^b	0.36 ± 0.00 ^{ab}	0.30 ± 0.01 ^{ab}	0.33 ± 0.01 ^{ab}
	Vanillic	1.25 ± 0.06 ^a	ND	0.88 ± 0.09 ^b	1.31 ± 0.06 ^a	0.30 ± 0.02 ^c
	Caffeic	2.27 ± 0.05 ^a	0.66 ± 0.03 ^b	0.81 ± 0.10 ^b	ND	ND
	Syringic	0.28 ± 0.04 ^b	0.27 ± 0.05 ^b	ND	0.59 ± 0.03 ^a	0.19 ± 0.00 ^b
	O-coumaric	ND	ND	ND	ND	ND
	Ferulic	1.89 ± 0.11 ^b	1.71 ± 0.05 ^b	2.82 ± 0.24 ^a	2.47 ± 0.07 ^a	0.68 ± 0.01 ^c
	Sinapic	ND	ND	ND	ND	ND
	P-coumaric	0.58 ± 0.00 ^{ab}	0.62 ± 0.02 ^a	ND	0.58 ± 0.01 ^b	ND
	Total	6.62 ^a	3.54 ^d	4.87 ^c	5.24 ^b	1.50 ^e
Conjugated	P-hydroxybenzoic	1.43 ± 0.03 ^c	2.15 ± 0.12 ^b	3.06 ± 0.08 ^a	2.17 ± 0.06 ^b	1.05 ± 0.01 ^d
	Vanillic	4.62 ± 0.02 ^b	5.57 ± 0.52 ^{ab}	6.19 ± 0.18 ^a	6.50 ± 0.56 ^a	2.45 ± 0.00 ^c
	Caffeic	0.48 ± 0.01 ^c	0.70 ± 0.02 ^b	1.02 ± 0.04 ^a	ND	ND
	Syringic	1.93 ± 0.01 ^b	1.46 ± 0.10 ^{bc}	3.39 ± 0.15 ^a	4.00 ± 0.33 ^a	1.14 ± 0.06 ^c
	O-coumaric	ND	ND	5.09 ± 0.14 ^a	ND	ND
	Ferulic	24.25 ± 0.60 ^a	16.72 ± 1.71 ^b	18.87 ± 0.49 ^b	19.98 ± 1.74 ^{ab}	6.26 ± 0.00 ^c
	Sinapic	29.30 ± 0.20 ^a	7.83 ± 0.57 ^b	6.90 ± 0.14 ^b	28.94 ± 3.18 ^a	6.08 ± 0.52 ^b
	P-coumaric	1.93 ± 0.00 ^a	1.50 ± 0.10 ^b	0.56 ± 0.01 ^c	1.57 ± 0.13 ^b	0.87 ± 0.08 ^c
	Total	63.93 ^a	35.93 ^b	45.10 ^b	63.16 ^a	17.85 ^c
Bound	P-hydroxybenzoic	2.40 ± 0.35 ^b	2.45 ± 0.25 ^b	6.09 ± 0.33 ^a	3.32 ± 0.17 ^b	0.78 ± 0.00 ^c
	Vanillic	7.31 ± 1.27 ^{ab}	5.10 ± 0.22 ^b	8.56 ± 0.62 ^a	8.54 ± 0.31 ^a	1.41 ± 0.06 ^c
	Caffeic	0.27 ± 0.06 ^c	0.88 ± 0.03 ^b	1.67 ± 0.07 ^a	0.35 ± 0.05 ^c	ND
	Syringic	4.82 ± 0.74 ^b	2.02 ± 0.08 ^b	37.47 ± 4.33 ^a	4.87 ± 0.38 ^b	2.92 ± 0.36 ^b
	O-coumaric	0.49 ± 0.13 ^{bc}	3.73 ± 0.39 ^a	1.17 ± 0.01 ^b	0.61 ± 0.04 ^{bc}	ND
	Ferulic	686.05 ± 86.41 ^b	488.48 ± 39.89 ^b	954.16 ± 48.29 ^a	645.28 ± 33.11 ^b	49.21 ± 4.88 ^c
	Sinapic	23.40 ± 4.80 ^a	5.37 ± 0.33 ^b	6.54 ± 0.65 ^b	16.50 ± 1.03 ^a	2.63 ± 0.17 ^b

P-coumaric	21.64 ± 3.22 ^a	17.97 ± 0.60 ^a	4.13 ± 0.60 ^c	11.74 ± 0.72 ^b	1.70 ± 0.10 ^c
Total PAs	746.39 ^b	526.01 ^b	1019.79 ^a	691.22 ^b	58.66 ^c

Note: Values are presented as mean ± SD. Different letters in the same row indicate significant differences (p < 0.05)

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

**ND: Not detected*

3.3.5 *In vitro* starch digestibility

Understanding the digestibility of starch in food is critical for promoting public health since starchy foods occupy a predominant place in the human diet. Excessive consumption can lead to adverse health outcomes such as type 2 diabetes, obesity and hyperglycemia (Yang et al., 2023). The release of glucose for energy and the rate of digestion are key physiological aspects of starch, and its nutritional value is heavily influenced by processing methods and its physical state (Englyst et al., 1992). The starch digestibility values observed for the bread samples of ancient wheat and common wheat are reported in **Table 3.5**. The findings of this study show significant variations ($p < 0.05$) in different starch fractions obtained following digestion. RDS, which represents starch that is quickly broken down into glucose, was found to be highest in CWRS RF bread and lowest in einkorn. SDS which is digested at a slower rate, exhibited considerable variations across wheat species with emmer and CWRS WW demonstrating the highest SDS contents followed by einkorn while spelt recorded the lowest level. This starch fraction offers a physiological advantage over RDS by providing a slow release of glucose and stabilizing blood glucose levels (Lehmann & Robin, 2007). The content of RS in emmer and spelt was significantly higher ($p < 0.05$) compared to CWRS WW. The findings of this study are partially consistent with previous literature, which Kulathunga and Simsek (2023) analyzed the starch digestibility of bread samples containing ancient wheat and hard red spring (HRS) common wheat. Their findings revealed that einkorn, emmer, and spelt had notably higher levels of SDS than HRS wheat. Emmer and spelt also had similar levels of RDS as HRS wheat, whereas einkorn bread had significantly lower RDS content. As per the findings of Skrabanja et al. (2001), refined spelt wheat bread (WSB) exhibited the highest rate of starch hydrolysis from all the samples analyzed. Within the first 30 minutes of incubation, WSB contained

significantly more RDS than wholemeal spelt wheat bread (WMSB) and later also more starch than reference bread prepared using refined flour. Furthermore, Świeca et al. (2014) highlighted significant variations in starch digestibility percentages (SD%) among different spelt grain varieties, ranging from 45.9% to 33.3%. This variation was accompanied by relatively high levels of (RS) in spelt grains, suggesting RS as a dominant factor influencing starch digestibility. In another study, cookies made from 100% common wheat and 100% einkorn displayed nearly identical digestion up to 120 minutes but differed after 180 minutes (Nakov et al., 2018). Phenolic compounds can reduce starch digestibility by inhibiting enzymes like amylase during starch hydrolysis and by binding to starch molecules (Schefer et al., 2021). This may explain the lower starch digestibility observed in einkorn and CWRS WW, which had higher levels of phenolic acids. In contrast, CWRS RF, with the lowest phenolic acid content, exhibited the highest starch digestibility.

The variations and inconsistent findings in starch digestibility could be attributed to various factors, including the starch source, granule size, amylose-to-amylopectin ratio, formation of amylose-lipid complexes, presence of antinutrients, and the presence of α -amylase inhibitors (Świeca et al., 2014; Yang et al., 2023). Considering the relationship between RDS and conditions such as metabolic syndrome, including obesity and diabetes, the higher amounts of slowly digestible starch observed in emmer and the higher level of resistant starch in spelt, combined with the lower levels of RDS observed in einkorn, indicate potential health benefits in addressing these conditions.

3.3.6 *In vitro* glucose determination

The *in vitro* glucose release over time of bread samples, ranging from 20 to 300 minutes, is depicted in **Figure 3.2**. The glucose concentration in the bread sample is determined by measuring the glucose available in the aliquot withdrawn at specific time intervals. As presented in the graph, extending the digestion time up to 240 minutes led to increased starch degradation and subsequently released more glucose into the medium. However, after 240 minutes, there was a downward trend in the release of glucose content. Among the samples, the quantity of glucose released at each time interval was highest in CWRS RF, primarily due to the removal of bran during the refining process. The refinement of flour makes starch more accessible to digestive enzyme, which can be associated with increased glucose release. On the contrary, einkorn displayed the lowest glucose release which is beneficial for glycemic control. The slow digestion property of endosperm starch in whole grain foods, when compared to refined flours derived from cereal grains, can be attributed to the complex matrix of whole grains which includes functional components like dietary fiber and phytochemicals, which play a role in slowing down starch digestion (Zhang & Hamaker, 2017). The penetration of α -amylase into the substrate is considered a critical step in digestion, yet interactions between starch and other food components can hinder the effective diffusion and adsorption of the enzyme (Lehmann & Robin, 2007). The presence of arabinoxylans leached from the bran in whole wheat samples during baking processes could contribute to a thicker food matrix, which acts as a physical barrier against digestive enzymes. Additionally, if arabinoxylans form a part of the matrix covering starch granules, they create a chemical barrier to starch digestion since human digestive enzymes do not hydrolyze arabinoxylans (Whitney & Simsek, 2017). This phenomenon is supported by the results of this

study, where higher glucose release was observed in refined flour samples, which exhibited lowest contents of arabinoxylan.

Overall, these findings contribute to the understanding of how different flours from ancient wheat species undergo starch digestibility and release glucose at various time intervals, providing insights into their nutritional properties and potential health implications, thereby highlighting their relevance for dietary and health considerations.

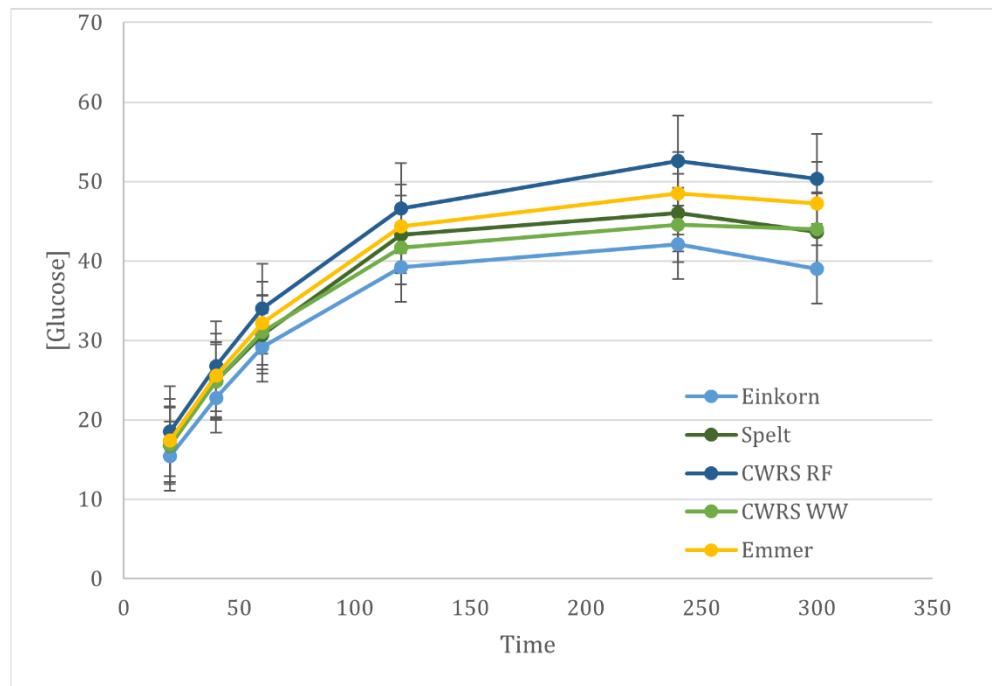


Figure 3.2 *In vitro* glucose determination curve of the bread samples of einkorn, emmer, spelt, and common wheat species

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

3.3.7 *In vitro* protein digestibility

In this study significant differences ($p < 0.05$) in the IVPD of bread samples were observed, with the standard casein exhibiting 91.6%. Notably, breads of CWRS RF demonstrated the highest IVPD

among all samples, highlighting the impact of refining processes on protein digestibility (**Table 5**). Among the ancient wheat samples, einkorn displayed the highest IVPD at 84.29%, while spelt had the lowest at 81.43%. However, CWRS WW fell in between with an IVPD of 82.71%. These findings indicate that bread made from ancient wheat species exhibits protein digestibility values closer to those of CWRS WW bread, although lower than those of CWRS RF bread. This further suggests that the use of ancient wheat species in breadmaking maintains a level of protein digestibility comparable to that of traditional whole wheat bread. The higher protein digestibility of the refined flour is likely due to the removal of the bran and germ during milling, which can impair protein digestibility (Joye, 2019). Additionally, starch digestion can enhance protein digestibility in bread, possibly due to the increased accessibility of gluten proteins to digestive enzymes resulting from the breakdown of starch granules. This influence stems from the presence of gluten-starch complexes in flour, which render flour proteins less accessible to digestive enzymes compared to purified protein fractions (Smith et al., 2015).

Abdel-Aal & Hucl (2002) investigated the *in vitro* protein digestibility of selected ancient wheat grains and their products. The IVPD of whole meals was 86.2% for einkorn, 86.9% for soft spelt, and 86.9% for common wheat. When analyzing the products, common wheat pita bread demonstrated an IVPD of 87.7%, while soft spelt pita bread obtained 86.4%. Ma & Baik (2021) studied the influences of grain and protein characteristics on the IVPD of ancient and common wheat species and they observed that the IVPD of cooked flour from 26 wheat varieties ranged from 86.5% to 92.3% with no significant differences in flour IVPD between bread and ancient wheat species. The variations in the protein digestibility observed in these wheat species can be attributed to their distinct protein composition and structure (Geisslitz et al., 2019). Furthermore,

the compact folding and aggregation of proteins during dough formation creates barriers that prevent enzymes from accessing cleavage sites, thereby impairing digestion. This structural complexity likely contributes to differences in digestibility compared to the Casein standard, especially in wheat bread samples that contain gluten (Ashkar & Wu, 2023; Joye, 2019).

Additionally, the presence of fiber and plant tissue structures has been observed to decrease protein digestibility, a phenomenon evident in both *in vitro* and *in vivo* studies. This effect is influenced by the specific type of fiber involved. Although whole-grain products offer superior health benefits compared to refined grains, their proteins are likely to exhibit reduced bioavailability (Hou et al., 2024; Poutanen et al., 2022). When dietary fiber, particularly soluble dietary fiber (SDF), is present in a food product, it can decrease protein digestibility due to its viscosity and swelling ability, resulting in lower digestibility compared to the absence of fiber (Lin et al., 2019). Gelatinized starch has been observed to interact with denatured proteins, thereby delaying the hydrolysis of proteins. Furthermore, studies indicate that lipids can inhibit protease activity by blocking their binding sites, leading to a decrease in protein digestibility (Zahir et al., 2018). Phenolic acids may further exacerbate the reduction in protein digestibility by hindering the active sites of proteins and blocking enzyme binding sites interacting with both digestive enzymes and proteins. This process inhibits the enzymes ability to hydrolyze peptide bonds and leads to a decrease in protein digestibility due to impaired enzymatic activity (Joye, 2019; Schefer et al., 2021). This characteristic explains the higher protein digestibility observed in CWRS RF bread in this study, as CWRS RF flour aligned with the trend of having the lowest phenolic acid content compared to all other flour types.

From a health perspective, the digestibility of proteins in food products plays a crucial role in determining amino acid bioavailability and nutrient absorption (Joye, 2019). Ancient wheat such as einkorn, spelt, and emmer generally exhibit lower IVPD compared to CWRS RF primarily due to the refining process. However, einkorn stands out with higher IVPD compared to whole grain common wheat (CWRS WW), indicating more efficient breakdown into absorbable amino acids during digestion and potentially offering enhanced nutritional uptake. Since the differences in values between CWRS WW and ancient grains are not substantial, there is an opportunity to blend various ancient grains to develop novel products with improved sensory qualities and nutritional benefits.

Table 3.5 *In vitro* starch digestibility and protein digestibility (IVPD) of the bread samples of einkorn, emmer, spelt, and common wheat species

Sample	RDS	SDS	RS	TDS	IVPD
Einkorn	48.38 ± 0.35 ^e	3.27 ± 0.17 ^b	0.38 ± 0.00 ^c	54.15 ± 0.03 ^e	84.29 ± 0.24 ^b
Emmer	59.96 ± 1.13 ^b	3.72 ± 0.05 ^a	0.83 ± 0.00 ^a	66.75 ± 0.20 ^b	80.60 ± 0.18 ^e
Spelt	55.02 ± 0.08 ^c	1.61 ± 0.00 ^d	0.93 ± 0.07 ^a	56.76 ± 0.54 ^d	81.43 ± 0.47 ^d
CWRS WW	51.18 ± 0.53 ^d	3.80 ± 0.01 ^a	0.63 ± 0.01 ^b	59.11 ± 0.72 ^c	82.71 ± 0.24 ^c
CWRS RF	68.88 ± 0.19 ^a	2.57 ± 0.10 ^c	0.55 ± 0.02 ^b	72.44 ± 0.75 ^a	86.05 ± 0.22 ^a

Note: Values are presented as mean ± SD. Different letters in the same column indicate significant differences (p < 0.05)

Abbreviations: CWRS WW: Canada Western Red Spring Whole Wheat, CWRS RF: Canada Western Red Spring Refined flour, RDS: Rapidly Digestible Starch, RS: Resistant Starch, SDS: Slowly Digestible Starch, TDS: Total Digestible Starch, IVPD: In vitro protein digestibility

3.4 Conclusion

The study revealed significant differences between ancient wheat species (einkorn, emmer, spelt) and common wheat (CWRS) in terms of their chemical composition and digestibility characteristics. According to the findings of the study, ancient wheat, particularly einkorn can be considered as a potential candidate to supplement the wheat industry with its unique chemical composition; significantly higher levels of protein along with phenolic acids. It also exhibited the lowest RDS value, followed by CWRS whole wheat, while refined CWRS flour had the highest RDS value, indicating a faster starch digestion rate. This suggests that einkorn may have a lower glycemic impact compared to both CWRS whole wheat and refined flour, highlighting its potential as a health-promoting food ingredient. In terms of protein digestibility, einkorn displayed a relatively high IVPD value, closely following refined CWRS flour. In contrast, both spelt and emmer exhibited lower IVPD values compared to CWRS whole wheat and einkorn.

With increasing consumer awareness of the health impact of diet, there's a rising demand for high-quality raw materials for developing food products. Consequently, ancient wheat presents an opportunity to complement the wheat industry when combined with other ingredients. However, further research should prioritize *in vivo* studies to validate the *in vitro* data, assess health impacts and elucidate underlying mechanisms. Additionally, lysine has been identified as the limiting amino acid in these wheat species, indicating that incorporating ancient wheat flour in combination with other grains or pulses could improve the overall protein nutritional value of the diet. There is also a need to develop new formulations that integrate these grains to create innovative food products.

3.5 References

- AACC International. (2010). *Approved methods of the American Association of Cereal Chemists* (11th ed.). Cereals & Grains Association, St. Paul, MN, U.S.A.
- Abdel-Aal, E. S. M., & Hucl, P. (2002). Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis*, 15(6), 737–747. <https://doi.org/10.1006/jfca.2002.1094>
- Abdel-Aal, E. S. M., Hucl, P., & Sosulski, F. W. (1995). Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chemistry*, 72(6), 621–624.
- Akar, T., Cengiz, M. F., & Tekin, M. (2019). A comparative study of protein and free amino acid contents in some important ancient wheat lines. *Quality Assurance and Safety of Crops and Foods*, 11(2), 191–200. <https://doi.org/10.3920/QAS2018.1382>
- AOAC International. (2012). *Official methods of analysis* (19th ed.). AOAC International, Gaithersburg, MD, USA.
- Arzani, A., & Ashraf, M. (2017). Cultivated Ancient Wheats (*Triticum* spp.): A Potential Source of Health-Beneficial Food Products. *Comprehensive Reviews in Food Science and Food Safety*, 16(3), 477–488. <https://doi.org/10.1111/1541-4337.12262>
- Ashkar, F., & Wu, J. (2023). Effects of Food Factors and Processing on Protein Digestibility and Gut Microbiota. *Journal of Agricultural and Food Chemistry*, 71(23), 8685–8698. <https://doi.org/10.1021/acs.jafc.3c00442>
- Bailey, T., Franczyk, A. J., Goldberg, E. M., & House, J. D. (2023). Impact of cooking on the protein quality of Russet potatoes. *Food Science and Nutrition*, 11(12), 8131–8142. <https://doi.org/10.1002/fsn3.3734>
- Barański, M., Lacko-Bartošová, M., Rembiałkowska, E., & Lacko-Bartošová, L. (2020). The Effect of Species and Cultivation Year on Phenolic Acids Content in Ancient Wheat. *Agronomy*, 10(5), 673. <https://doi.org/10.3390/agronomy10050673>
- Bencze, S., Makádi, M., Aranyos, T. J., Földi, M., Hertelendy, P., Mikó, P., Bosi, S., Negri, L., & Drexler, D. (2020). Re-introduction of ancient wheat cultivars into organic agriculture-Emmer and

Einkorn cultivation experiences under marginal conditions. *Sustainability (Switzerland)*, 12(4). <https://doi.org/10.3390/su12041584>

Biel, W., Jaroszewska, A., Stankowski, S., Sobolewska, M., & Kępińska-Pacelik, J. (2021). Comparison of yield, chemical composition and farinograph properties of common and ancient wheat grains. *European Food Research and Technology*, 247(6), 1525–1538. <https://doi.org/10.1007/s00217-021-03729-7>

Bonafaccia, G., Galli, V., Francisci, R., Mair, V., Skrabanja, V., & Kreft, I. (2000). Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chemistry*, 68(4), 437–441. [https://doi.org/10.1016/S0308-8146\(99\)00215-0](https://doi.org/10.1016/S0308-8146(99)00215-0)

Brandolini, A., Hidalgo, A., & Moscaritolo, S. (2008). Chemical composition and pasting properties of einkorn (*Triticum monococcum* L. subsp. *monococcum*) whole meal flour. *Journal of Cereal Science*, 47(3), 599–609. <https://doi.org/10.1016/j.jcs.2007.07.005>

Deyalage, S. T., House, J. D., Thandapilly, S. J., & Malalgoda, M. (2024). Nutritional characteristics and physicochemical properties of ancient wheat species for food applications. *Food Bioscience*, 62(November), 105397. <https://doi.org/10.1016/j.fbio.2024.105397>

Dimina, L., Rémond, D., Huneau, J. F., & Mariotti, F. (2022). Combining Plant Proteins to Achieve Amino Acid Profiles Adapted to Various Nutritional Objectives—An Exploratory Analysis Using Linear Programming. *Frontiers in Nutrition*, 8(February), 1–11. <https://doi.org/10.3389/fnut.2021.809685>

Englyst, H N, Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46 Suppl 2, S33-50. <http://www.ncbi.nlm.nih.gov/pubmed/1330528>

Englyst, Hans N., & Cummings, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *The Analyst*, 109(7), 937. <https://doi.org/10.1039/an9840900937>

Food and Agriculture Organization of the United Nations & World Health Organization.

(1991). *Protein quality evaluation: Report of the Joint FAO/WHO Expert Consultation, Bethesda, Md., USA 4-8 December 1989*. Rome: FAO.

Foster-Powell, K., Barclay, A., & Brand-Miller, J. (2009). Glycemic Health, Type 2 Diabetes, and Functional Foods. In *Nutraceuticals, Glycemic Health and Type 2 Diabetes* (pp. 87–95). <https://doi.org/10.1002/9780813804149.ch6>

Franczyk, A. J., Bui, N., Chen, J., Boyd, L., Wang, N., Sopiwnyk, E., Sarkar, A., Neufeld, J., Paliwal, J., Nickerson, M., & House, J. D. (2024). The impact of mechanical scouring and moisture conditioning on the in vitro protein digestibility and quality of roller-milled green lentil (*Lens culinaris*) and yellow pea (*Pisum sativum*). *Cereal Chemistry*, November 2023. <https://doi.org/10.1002/cche.10831>

Gaurav, K., Arora, S., Silva, P., Sánchez-Martín, J., Horsnell, R., Gao, L., Brar, G. S., Widrig, V., John Raupp, W., Singh, N., Wu, S., Kale, S. M., Chinoy, C., Nicholson, P., Quiroz-Chávez, J., Simmonds, J., Hayta, S., Smedley, M. A., Harwood, W., ... Wulff, B. B. H. (2022). Population genomic analysis of *Aegilops tauschii* identifies targets for bread wheat improvement. *Nature Biotechnology*, 40(3), 422–431. <https://doi.org/10.1038/s41587-021-01058-4>

Gawlik-Dziki, U., Świeca, M., & Dziki, D. (2012). Comparison of phenolic acids profile and antioxidant potential of six varieties of spelt (*Triticum spelta* L.). *Journal of Agricultural and Food Chemistry*, 60(18), 4603–4612. <https://doi.org/10.1021/jf3011239>

Gebruers, K., Dornez, E., Boros, D., Fraś, A., Dynkowska, W., Bedo, Z., Rakszegi, M., Delcour, J. A., & Courtin, C. M. (2008). Variation in the content of dietary fiber and components thereof in wheats in the healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9740–9749. <https://doi.org/10.1021/jf800975w>

Geisslitz, S., Longin, C. F. H., Scherf, K. A., & Koehler, P. (2019). Comparative Study on Gluten Protein Composition of Ancient (Einkorn, Emmer and Spelt) and Modern Wheat Species (Durum and Common Wheat). *Foods*, 8(9), 409. <https://doi.org/10.3390/foods8090409>

Golea, C. M., Stroe, S. G., Gâtlan, A. M., & Codină, G. G. (2023). Physicochemical Characteristics and Microstructure of Ancient and Common Wheat Grains Cultivated in Romania. *Plants*, 12(11).

<https://doi.org/10.3390/plants12112138>

Hidalgo, A., & Brandolini, A. (2014). Nutritional properties of einkorn wheat (*Triticum monococcum* L.). *Journal of the Science of Food and Agriculture*, 94(4), 601–612.

<https://doi.org/10.1002/jsfa.6382>

Hidalgo, A., Brandolini, A., & Ratti, S. (2009). Influence of genetic and environmental factors on selected nutritional traits of *Triticum monococcu*. *Journal of Agricultural and Food Chemistry*, 57(14), 6342–6348. <https://doi.org/10.1021/jf901180q>

Hou, Y., Wei, M., Wu, Y., & Ouyang, J. (2024). In vitro digestibility of starch and protein in cooked wheat and oat whole flours: A comparative study. *Food Chemistry*, 440(December 2023), 138203. <https://doi.org/10.1016/j.foodchem.2023.138203>

International Organization for Standardization. (2016). *Animal feeding stuffs – Determination of tryptophan content* (ISO 13904).

Joye, I. (2019). Protein digestibility of cereal products. *Foods*, 8(6), 1–14.

<https://doi.org/10.3390/foods8060199>

Khalid, A., Hameed, A., & Tahir, M. F. (2023). Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*, 10(February), 1–14.

<https://doi.org/10.3389/fnut.2023.1053196>

Kiszonas, A. M., Fuerst, E. P., Luthria, D., & Morris, C. F. (2015). Arabinoxylan content and characterisation throughout the bread-baking process. *International Journal of Food Science and Technology*, 50(8), 1911–1921. <https://doi.org/10.1111/ijfs.12829>

Kulathunga, J., Reuhs, B. L., Zwinger, S., & Simsek, S. (2021). Comparative study on kernel quality and chemical composition of ancient and modern wheat species: Einkorn, emmer, spelt and hard red spring wheat. *Foods*, 10(4). <https://doi.org/10.3390/foods10040761>

Kulathunga, J., & Simsek, S. (2022). Dietary fiber variation in ancient and modern wheat species: Einkorn, emmer, spelt and hard red spring wheat. *Journal of Cereal Science*, 104(October 2021), 103420. <https://doi.org/10.1016/j.jcs.2022.103420>

- Kulathunga, J., & Simsek, S. (2023). Pasting properties, baking quality, and starch digestibility of einkorn, emmer, spelt, and hard red spring wheat. *Cereal Chemistry*, October 2022. <https://doi.org/10.1002/cche.10644>
- Lacko-Bartošová, M., Lacko-Bartošová, L., Kobida, L., Kaur, A., & Moudrý, J. (2023). Phenolic Acids Profiles and Phenolic Concentrations of Emmer Cultivars in Response to Growing Year under Organic Management. *Foods*, 12(7). <https://doi.org/10.3390/foods12071480>
- Lacko - Bartošová, M., & Čurná, V. (2015). Nutritional Characteristics of Emmer Wheat Varieties. *Journal of Microbiology, Biotechnology and Food Sciences*, 4(special issue 3 (Food Sciences)), 95–98. <https://doi.org/10.15414/jmbfs.2015.4.special3.95-98>
- Laugerotte, J., Baumann, U., & Sourdille, P. (2022). Genetic control of compatibility in crosses between wheat and its wild or cultivated relatives. *Plant Biotechnology Journal*, 20(5), 812–832. <https://doi.org/10.1111/pbi.13784>
- Lehmann, U., & Robin, F. (2007). Slowly digestible starch - its structure and health implications: a review. *Trends in Food Science and Technology*, 18(7), 346–355. <https://doi.org/10.1016/j.tifs.2007.02.009>
- Lewko, P., Wójtowicz, A., & Gancarz, M. (2023). Distribution of Arabinoxylans and Their Relationship with Physiochemical and Rheological Properties in Wheat Flour Mill Streams as an Effective Way to Predict Flour Functionality. *Applied Sciences (Switzerland)*, 13(9). <https://doi.org/10.3390/app13095458>
- Lin, Y., Chen, K., Tu, D., Yu, X., Dai, Z., & Shen, Q. (2019). Characterization of dietary fiber from wheat bran (*Triticum aestivum* L.) and its effect on the digestion of surimi protein. *LWT*, 102(149), 106–112. <https://doi.org/10.1016/j.lwt.2018.12.024>
- Ma, F., & Baik, B. K. (2021). Influences of grain and protein characteristics on in vitro protein digestibility of modern and ancient wheat species. *Journal of the Science of Food and Agriculture*, 101(11), 4578–4584. <https://doi.org/10.1002/jsfa.11100>
- Massaux, C., Sindic, M., Lenartz, J., Sinnaeve, G., Bodson, B., Falisse, A., Dardenne, P., & Deroanne, C. (2008). Variations in physicochemical and functional properties of starches extracted from

European soft wheat (*Triticum aestivum* L.): The importance to preserve the varietal identity. *Carbohydrate Polymers*, 71(1), 32–41.
<https://doi.org/10.1016/j.carbpol.2007.05.012>

Mendis, M., & Simsek, S. (2014). Arabinoxylans and human health. *Food Hydrocolloids*, 42(P2), 239–243. <https://doi.org/10.1016/j.foodhyd.2013.07.022>

Nakov, G., Brandolini, A., Ivanova, N., Dimov, I., & Stamatovska, V. (2018). The effect of einkorn (*Triticum monococcum* L.) whole meal flour addition on physico-chemical characteristics, biological active compounds and in vitro starch digestion of cookies. *Journal of Cereal Science*, 83(August), 116–122. <https://doi.org/10.1016/j.jcs.2018.08.004>

Nicoletti, I., Martini, D., De Rossi, A., Taddei, F., D'Egidio, M. G., & Corradini, D. (2013). Identification and quantification of soluble free, soluble conjugated, and insoluble bound phenolic acids in durum wheat (*triticum turgidum* L. var. durum) and derived products by RP-HPLC on a semimicro separation scale. *Journal of Agricultural and Food Chemistry*, 61(48), 11800–11807. <https://doi.org/10.1021/jf403568c>

Poutanen, K. S., Kårlund, A. O., Gómez-Gallego, C., Johansson, D. P., Scheers, N. M., Marklinder, I. M., Eriksen, A. K., Silventoinen, P. C., Nordlund, E., Sozer, N., Hanhineva, K. J., Kolehmainen, M., & Landberg, R. (2022). Grains – a major source of sustainable protein for health. *Nutrition Reviews*, 80(6), 1648–1663. <https://doi.org/10.1093/nutrit/nuab084>

Rasmusson, D. C., Hester, A. J., Fick, G. N., & Byrne, I. (1971). Breeding for Mineral Content in Wheat and Barley 1. *Crop Science*, 11(5), 623–626.
<https://doi.org/10.2135/cropsci1971.0011183x001100050005x>

Sá, Amanda G.A., & House, J. D. (2024). Protein quality of cereals: Digestibility determination and processing impacts. *Journal of Cereal Science*, 117(November 2023), 103892.
<https://doi.org/10.1016/j.jcs.2024.103892>

Sá, Amanda Gomes Almeida, Moreno, Y. M. F., & Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. *Trends in Food Science & Technology*, 97(December 2019), 170–184. <https://doi.org/10.1016/j.tifs.2020.01.011>

- Schefer, S., Oest, M., & Rohn, S. (2021). Interactions between phenolic acids, proteins, and carbohydrates—Influence on dough and bread properties. *Foods*, 10(11), 2798.
- Shewry, P. R., & Hey, S. (2015a). Do “ancient” wheat species differ from modern bread wheat in their contents of bioactive components? *Journal of Cereal Science*, 65, 236–243.
<https://doi.org/10.1016/j.jcs.2015.07.014>
- Shewry, P. R., & Hey, S. J. (2015b). The contribution of wheat to human diet and health. *Food and Energy Security*, 4(3), 178–202. <https://doi.org/10.1002/FES3.64>
- Skrabanja, V., Kovac, B., Golob, T., Liljeberg Elmståhl, H. G. M., Björck, I. M. E., & Kreft, I. (2001). Effect of spelt wheat flour and kernel on bread composition and nutritional characteristics. *Journal of Agricultural and Food Chemistry*, 49(1), 497–500.
<https://doi.org/10.1021/jf000819w>
- Smith, F., Pan, X., Bellido, V., Toole, G. A., Gates, F. K., Wickham, M. S. J., Shewry, P. R., Bakalis, S., Padfield, P., & Mills, E. N. C. (2015). Digestibility of gluten proteins is reduced by baking and enhanced by starch digestion. *Molecular Nutrition and Food Research*, 59(10), 2034–2043.
<https://doi.org/10.1002/mnfr.201500262>
- Sulek, A., Cacak-Pietrzak, G., Rózewicz, M., Nieróbca, A., Grabiński, J., Studnicki, M., Sujka, K., & Dziki, D. (2023). Effect of Production Technology Intensity on the Grain Yield, Protein Content and Amino Acid Profile in Common and Durum Wheat Grain. *Plants*, 12(2).
<https://doi.org/10.3390/plants12020364>
- Świeca, M., Dziki, D., Gawlik-Dziki, U., Różyło, R., Andruszczak, S., Kraska, P., Kowalczyk, D., Pałys, E., & Baraniak, B. (2014). Grinding and nutritional properties of six spelt (*triticum aestivum* ssp. *spelta* L.) cultivars. *Cereal Chemistry*, 91(3), 247–254. <https://doi.org/10.1094/CCHEM-02-13-0019-R>
- Tian, W., Wilson, T. L., Chen, G., Guttieri, M. J., Nelson, N. O., Fritz, A., Smith, G., & Li, Y. (2021). Effects of environment, nitrogen, and sulfur on total phenolic content and phenolic acid composition of winter wheat grain. *Cereal Chemistry*, 98(4), 903–911.
<https://doi.org/10.1002/cche.10432>

- Tremmel-Bede, K., Szentmiklóssy, M., Tömösközi, S., Török, K., Lovegrove, A., Shewry, P. R., Láng, L., Bedő, Z., Vida, G., & Rakszegi, M. (2020). Stability analysis of wheat lines with increased level of arabinoxylan. *PLoS ONE*, 15(5), 1–15.
<https://doi.org/10.1371/journal.pone.0232892>
- USDA. (2023). *Wheat Outlook : August 2023 Domestic Changes at a Glance : August*.
- van Kempen, T. A. T. G., Regmi, P. R., Matte, J. J., & Zijlstra, R. T. (2010). In Vitro Starch Digestion Kinetics, Corrected for Estimated Gastric Emptying, Predict Portal Glucose Appearance in Pigs ., *The Journal of Nutrition*, 140(7), 1227–1233. <https://doi.org/10.3945/jn.109.120584>
- Whitney, K., & Simsek, S. (2017). Reduced gelatinization, hydrolysis, and digestibility in whole wheat bread in comparison to white bread. *Cereal Chemistry*, 94(6), 991–1000.
<https://doi.org/10.1094/CCHEM-05-17-0116-R>
- Yang, Z., Zhang, Y., Wu, Y., & Ouyang, J. (2023). Factors influencing the starch digestibility of starchy foods: A review. *Food Chemistry*, 406(September 2022), 135009.
<https://doi.org/10.1016/j.foodchem.2022.135009>
- Zahir, M., Fogliano, V., & Capuano, E. (2018). Food matrix and processing modulate in vitro protein digestibility in soybeans. *Food & Function*, 9(12), 6326–6336.
<https://doi.org/10.1039/C8FO01385C>
- Zannini, E., Núñez, Á. B., Sahin, A. W., & Arendt, E. K. (2022). Arabinoxylans as Functional Food Ingredients: A Review. *Foods*, 11(7), 1–28. <https://doi.org/10.3390/foods11071026>
- Zencirci, N., Ulukan, H., Baloch, F. S., Mansoor, S., & Rasheed, A. (2022). Ancient Wheats. In N. Zencirci, H. Ulukan, F. S. Baloch, S. Mansoor, & A. Rasheed (Eds.), *Ancient Wheats*. Springer International Publishing. <https://doi.org/10.1007/978-3-031-07285-7>
- Zhang, G., & Hamaker, B. R. (2009). Slowly Digestible Starch: Concept, Mechanism, and Proposed Extended Glycemic Index. *Critical Reviews in Food Science and Nutrition*, 49(10), 852–867.
<https://doi.org/10.1080/10408390903372466>
- Zhang, G., & Hamaker, B. R. (2017). The nutritional property of endosperm starch and its

contribution to the health benefits of whole grain foods. *Critical Reviews in Food Science and Nutrition*, 57(18), 3807–3817. <https://doi.org/10.1080/10408398.2015.1130685>

Zhang, K., Zhang, Y., Xu, N., Yang, X., Zhang, G., & Zhang, Y. (2020). Study of the protein , antioxidant activity , and starch during in vitro simulated digestion of green wheat and wheat cooked flours. *International Journal of Food Properties*, 23(1), 722–735. <https://doi.org/https://doi.org/10.1080/10942912.2020.1754234>

Chapter 4 Comparative Evaluation of Starch and Protein Characteristics in Ancient Wheat species

Abstract

Driven by increasing consumer demand for nutrient-dense and more sustainable food options, ancient wheat species present a promising opportunity due to their environmental adaptability and perceived health benefits. Therefore, this study evaluates the starch and protein characteristics of ancient wheat species, einkorn, emmer, and spelt, providing insights into their functionality for potential food applications. Einkorn exhibited the smallest median granule size, emmer the largest, and spelt intermediate sizes, signifying variations in granule size distribution. Thermal behavior revealed that spelt exhibited the highest thermal stability with high transition temperatures, while emmer required less energy for gelatinization. Amylose% and amylopectin% showed no significant differences among these wheat species. Protein composition analysis indicated significant variations, mainly in gliadin and glutenin fractions, among common wheat and ancient wheat species. The SDS unextractable glutenin levels were significantly lower in the ancient wheat compared to common wheat suggesting that processing modifications may be required to optimize the use of ancient wheat in bakery applications. Additionally, the variations in the chemistry of starch and protein can be associated with nutritional value, emphasizing the need for further research to explore these differences in greater detail.

Keywords: Ancient wheat, Starch, Protein, Physicochemical properties

4.1 Introduction

A sustainable and wholesome food supply has become increasingly important in recent years, driving a renewed interest in ancient wheat species (Arzani & Ashraf, 2017). This trend towards ancient wheat has been further enhanced by consumer awareness of healthy grain-based products, as these grains are increasingly recognized for their nutritional benefits, particularly as whole grains that offer high amounts of fiber, micronutrients and bioactive compounds (Akar et al., 2019; Costanzo et al., 2019). Farmers are drawn to these species for the potential to diversify their crops, as they require less fertilizer, adapt well to a range of conditions, and exhibit resilience against environmental stresses (Zencirci et al., 2022). Meanwhile, bakers appreciate ancient wheat for their ability to produce specialty products that enhance both organoleptic qualities and nutritional value, enabling them to compete effectively in the market (Belcar et al., 2021; Kohajdová & Karovičová, 2008).

Ancient wheat species, referred to as hulled wheat due to their strong glume attached to the kernel, are ancestors of common wheat (Arzani & Ashraf, 2017). Einkorn wheat [*Triticum monococcum* L.] is recognized as the earliest domesticated wheat species and is classified as diploid. Emmer wheat [*Triticum dicoccum* (Schrank)] Schuebl is a tetraploid species, while spelt wheat [*Triticum spelta* L.] is a hexaploid species (Tran et al., 2020). These ancient wheat species offer notable health benefits due to their diverse mineral composition, higher dietary fiber, and protein levels (Kaplan Evlice et al., 2022; Roumia et al., 2023). They also contain antioxidants such as ferulic acid and lutein, which contribute to reduced oxidative stress (Barański et al., 2020; Li et al., 2008; Şahin et al., 2017). Studies suggest that the consumption of these grains may enhance inflammatory and antioxidant profiles, improve glycemic

control, and positively affect lipid levels, thereby potentially reducing the risk of type 2 diabetes and cardiovascular diseases (Dinu et al., 2018; Thorup et al., 2014).

In recent years, there has been a growing effort within the research community to introduce ancient wheat flours into various bakery products. Given that the quality and functionality of flour are linked to its physicochemical properties, which are influenced by the principal biomacromolecules; starch and protein—and their behavior, it is crucial to assess these components in these non-traditional flours. Wheat flour typically comprises 65% to 73% starch, primarily consisting of large A-granules and smaller B-granules, with the potential formation of very small C-granules during grain development. The distribution of these granules is critical for determining bread-making quality, as it significantly affects the physicochemical and functional properties of starch (Simsek et al., 2020). Furthermore, gluten proteins, categorized into gliadins and glutenins, are essential for baking performance as variations in gluten protein composition are associated with differences in dough rheology, hence baking performance (Geisslitz et al., 2019). However, there is a lack of knowledge and research focused on assessing the starch and protein physicochemical properties of ancient wheat species. Due to the aforementioned association between chemistry and end-use functionality, this information is critical in determining the food applications for ancient grains. Therefore, this study seeks to address this by providing a comprehensive analysis of the starch and protein profiles, as well as their related physicochemical parameters of underutilized ancient wheat species.

4.2 Materials and methods

Raw materials

Ancient wheat species, including einkorn, emmer and spelt along with common hexaploid wheat (Canada Western Red Spring, CWRS), were selected for this study. The ancient wheat samples were sourced from a commercial supplier and represented grains harvested in 2021. Einkorn and emmer were cultivated in Saskatchewan, while spelt was grown in British Columbia. A sample of CWRS wheat, representative of grains produced in the Canadian prairies, was provided by the Canadian Grain Commission (Winnipeg, MB).

To prepare the samples for analysis, all ancient wheat grains were milled using the Ultra Centrifugal Mill [Type ZM 200] (Retsch GmbH, Haan, Retsch-Allee, Germany), [0.5mm sieve] to produce whole grain flour. The CWRS wheat was processed to yield both whole grain flour (CWRS WW), milled with the Ultra Centrifugal Mill [Type ZM 200], and refined flour (CWRS RF), obtained using the Quadrumat Sr. (Brabender GmbH & Co.KG, Kulturstr, Germany).

4.2.1 Scanning electron microscopy (SEM) analysis of kernel morphology

Kernels were sectioned longitudinally with a razor blade, and the resulting transverse slices were fixed to cylindrical aluminum mounts using double-sided adhesive tape. The samples were then coated with a thin conductive layer of gold using a Denton Vacuum, Desk II sputter coater (Denton Vacuum, Desk II, NJ, USA). The transverse sections, including the endosperm and outer layers of the kernel, were imaged using a Quanta FEG 650 scanning electron microscope (FEI, Hillsboro, OR, USA) at an accelerating voltage of 10 kV.

4.2.2 Starch extraction

Starch was extracted from flour samples prior to conducting further analyses on starch characteristics. The extraction method was based on the procedure outlined by Ee et al. (2020) with some modifications. The process involved mixing flour samples with a 0.25% (w/v) NaOH solution at a 1:3 ratio, which was then left to stand at ambient temperature for 60 minutes. Following this, the mixture underwent centrifugation at 1000 g for 5 minutes. The resulting white precipitate was collected and subjected to a series of washing steps using distilled water and a mesh sieve (150 μm). The filtrate was subjected to another round of centrifugation (Sorvall RC 6 Plus, Thermo Fisher Scientific, MA, USA) at 1000 g for 5 minutes, and this washing and centrifugation cycle was continued until the resulting precipitate was visibly clean, with no grey material or discoloration remaining. The purified starch precipitate was subsequently re-suspended in water and neutralized with a 1M HCl solution. Following neutralization, the mixture was centrifuged once more, and the resulting pellet was dried at 40°C overnight. After drying, the starch pellets were crushed into a fine powder using a mortar and pestle and then passed through a 106 μm mesh sieve to ensure uniform particle size and prepare the samples for further analysis.

4.2.3 Scanning electron microscopy for starch granule visualization

Starch samples were mounted on cylindrical aluminum stubs using double-sided adhesive tape, and any excess material was removed using an airflow. The samples were then coated with a gold-palladium alloy using a Denton Vacuum, Desk II sputter coater (Denton Vacuum, Desk II, NJ, USA) at a thickness of 20 nm (Denton Vacuum, Desk II, NJ, USA). Imaging was

conducted with a Quanta FEG 650 scanning electron microscope (FEI, Hillsboro, OR, USA) at a magnification of 500× and an accelerating voltage of 7 kV.

4.2.4 Granule size distribution

The particle size distribution of starch samples was analyzed using a Mastersizer 2000 laser scattering instrument (Malvern Panalytical Ltd., Malvern, UK). Each starch sample was analyzed in triplicate. The resulting data was then processed using Mastersizer v3.8.5 software. This software generated cumulative particle size percentiles, specifically D10, D50, and D90. These percentiles represent the particle size thresholds beneath which 10%, 50%, and 90% of the total particle population fall, respectively.

4.2.5 Characterization of amylose and amylopectin

Amylose and amylopectin content were assessed using size exclusion high-performance liquid chromatography (SE-HPLC), as outlined by Simsek et al. (2013). Flour samples (30 mg) were measured into glass test tubes with screw caps, and 2.5 mL of methanol was added to each tube. The mixture was vortexed thoroughly before being heated at 100 °C for 30 minutes to facilitate extraction. Following centrifugation, the supernatant was carefully removed, and the solid residues were dried overnight. The dried material was treated with 0.9 mL of a 1 M KOH solution and 0.1 mL of 6 M urea to disperse the starch. This mixture was again heated at 100 °C for 15 minutes. Ethanol (2 mL) was introduced while vortexing to precipitate starch, followed by an additional 4 mL of ethanol to ensure complete precipitation. The resulting suspension was centrifuged, the supernatant was discarded, and the final starch pellet was dried overnight.

For the SE-HPLC analysis, the starch samples were dissolved in a 1:10 (v/v) solution of 6 M urea and 1 M KOH, then subjected to heating at 100 °C for 90 minutes to ensure thorough solubilization. After cooling, the solutions were neutralized using 1 M HCl, filtered through 0.45- μ m nylon syringe filters, and prepared for chromatography. The analysis was conducted with an Agilent 1200 HPLC system equipped with a refractive index detector (Agilent Technologies, Inc., Santa Clara, CA). Separation of starch fractions was achieved using a Waters ((Waters Corporation, MA,USA) Ultrahydrogel guard column and a size exclusion column. The columns and detector were kept at a constant temperature of 40 °C, with HPLC-grade water used as the mobile phase at a flow rate of 0.4 mL/min. A 20 μ L injection volume was used for each sample.

4.2.6 Thermal properties of starch gelatinization

A Differential Scanning Calorimeter (DSC) (Q200 Series™DSC; TA Instruments, NC, DE, USA), calibrated with indium, was used to determine the gelatinization temperature range of the starch. Starch samples weighing 3 mg were placed in Tzero stainless-steel pressure pans, with water added at a 1:3 starch-to-water ratio. The pans were hermetically sealed and allowed to hydrate at room temperature for 1 hour.

The sealed pans were then positioned in the DSC cell, alongside an empty reference pan, under a nitrogen atmosphere. The samples were equilibrated at 25 °C and heated to 100 °C at a rate of 5 °C per minute. Each sample was analyzed in triplicate to determine the onset temperature (T_o), peak temperature (T_p), and enthalpy change (ΔH) of gelatinization, with data processed using TA Universal Analysis 2000 software (version 4.7A, TA Instruments Waters LLC, NC, USA).

4.2.7 Swelling power of starch

The swelling and solubility characteristics of starch were assessed following the methodology outlined by Ee et al. (2020) with modifications. A starch sample weighing 1 g was mixed with distilled water in a 50-mL centrifuge tube, bringing the total volume to 40 mL. The mixture was gently agitated and then subjected to heat treatment in a water bath at 85 °C for 30 minutes, with continuous shaking throughout. Following the heating process, the centrifuge tube was left to cool down to ambient temperature. Once cooled, the sample underwent centrifugation (Sorvall RC 6 Plus superspeed centrifuge, Thermo Fisher Scientific, MA, USA) at 520 g for a duration of 15 minutes. The resulting supernatant was carefully transferred to a pan and placed in an oven (Heratherm, Thermo Fisher Scientific, MA, USA) set at 105 °C for a period of 4 hours to dry. The weights of the dried residue (DR) and sediment paste (SP) were recorded to calculate the swelling power (g/g) using the formula provided below.

$$\text{Swelling power} = \frac{SP}{1g - DR}$$

4.2.8 Protein composition analysis

Protein extraction for SE-HPLC was carried out following a modified protocol based on Malalgoda et al. (2018). For the preparation of the SDS-extractable protein fraction, 10 mg of flour (adjusted to 14% moisture) was combined with 1 mL of an extraction buffer containing 0.5% sodium dodecyl sulfate (SDS) and 0.05 M sodium phosphate at pH 6.9. The mixture was thoroughly vortexed at 2500 rpm for 5 mins, after which it was centrifuged at 17000 g. The supernatant was filtered through a 0.45 µm polyvinylidene fluoride (PVDF)

membrane filter (Sun Sri, Rockwood, TN, USA). The filtered solution was heated at 80 °C for 2 mins in a water bath, then cooled to room temperature. To isolate the SDS-unextractable proteins, the residue from the initial centrifugation was resuspended in 1 mL of the extraction buffer. This suspension was subjected to 30 secs of sonication at a power setting of 10 W using a Sonic Dismembrator 100 (Thermo Fisher Scientific, MA, USA), followed by centrifugation. The resulting supernatant was filtered in the same way as the SDS-extractable proteins.

SE-HPLC separation of both protein fractions was performed individually using an Agilent 1100 series narrow bore column (300 × 4.5 mm) with a BIOSEP SEC S4000 guard cartridge (Phenomenex, Torrance, CA, USA). A 10 µL injection volume was used, with the mobile phase comprising 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) in water. The analysis was conducted at a flow rate of 0.5 mL/min, and solutes were detected at 214 nm using an Agilent 1200 photodiode array detector (Agilent Technologies, Waldbronn, Germany).

The data generated from SE-HPLC were analyzed using MATLAB (2015, The MathWorks, Natick, MA, USA) and a custom program described by Malalgoda et al. (2018). Protein fractions were categorized into four groups based on retention times: fraction 1 (F1) at 3.5–4.7 mins, fraction 2 (F2) at 4.7–5.1 mins, fraction 3 (F3) at 5.1–5.8 mins, and fraction 4 (F4) at 5.8–7.4 mins. The relative protein content of each fraction was calculated from the area percentage of its SE-HPLC profile. For example, a species with 14% total protein content was assigned this value as the total area (extractable + unextractable). If the SDS-extractable fraction 1 (EF1) accounted for 10% of the total area, its corresponding protein percentage was calculated as 1.4%.

4.3 Results and discussion

4.3.1 Scanning electron microscopy analysis of kernel morphology

A. Kernel structure

Figure 4.1 presents transverse sections of the kernels from the different wheat species analyzed. The overall morphology of wheat grains is typically oval, characterized by a prominent longitudinal furrow, or crease, on the ventral side, which extends almost to the center of the kernel (Bechtel et al., 2009; Owuoche, 2017). According to the visual observation, einkorn kernels exhibit an elongated and distinct shape, being thinner and shrunken in the middle portion, with a shape that differs from all the other wheat types. These kernels appear less full, suggesting a relatively lower proportion of endosperm. Previous literature indicates that einkorn grains have a lower endosperm content, with 74% compared to 81% in common hexaploid wheat, along with differences in bran content (Hidalgo & Brandolini, 2019). In contrast, minimal differences were observed between emmer and spelt kernels in terms of overall kernel shape. Both emmer and spelt kernels exhibit a more regular, plump structure with a better-developed endosperm than einkorn, although they are not as full as CWRS kernels. CWRS kernels display a round, well-developed appearance with a higher proportion of endosperm, resulting in higher flour yield. Seed size significantly affects various compositional and qualitative characteristics, as larger, heavier kernels contain a greater proportion of starchy endosperm and less of the external pericarp and aleurone layers (Hidalgo & Brandolini, 2019). However, when analyzing the kernels of ancient wheat species, it is essential to distinguish inherent grain size variations, which arise from genetic or positional factors (the position of the grain within the inflorescence), from

size reductions due to environmental stress, nutrient deficiencies, or disease. Grain shriveling, often an indicator of suboptimal endosperm development, contrasts with naturally smaller grains in certain species, which maintain endosperm proportions comparable to those in larger grains (Evers & Millar, 2002).

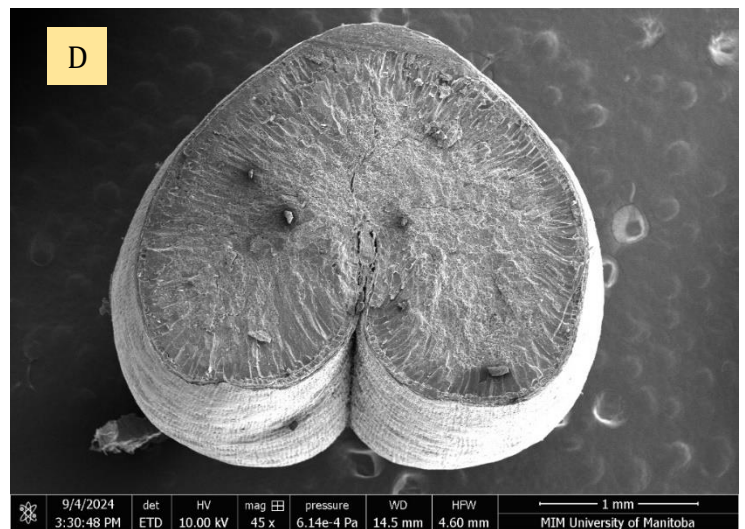
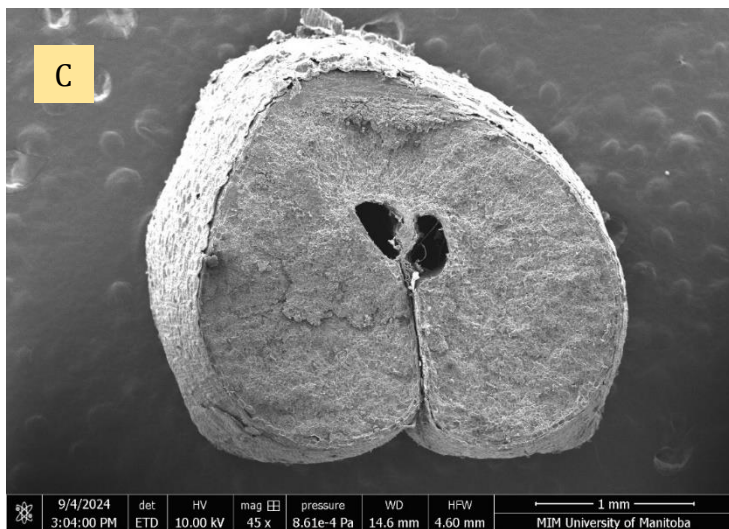
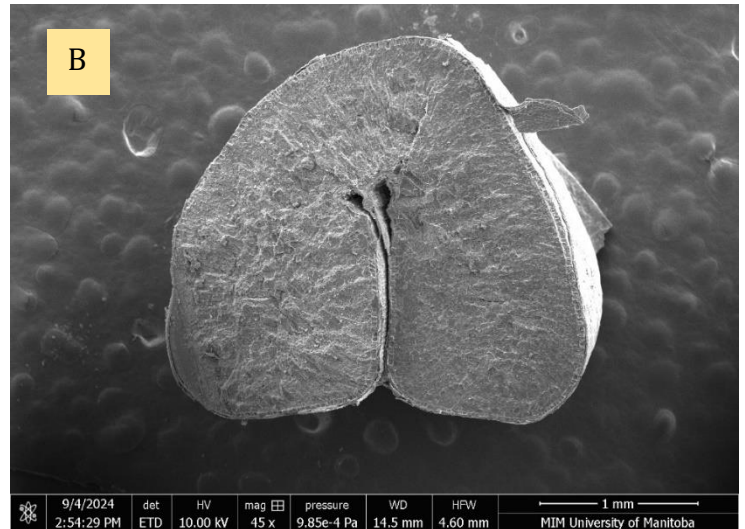
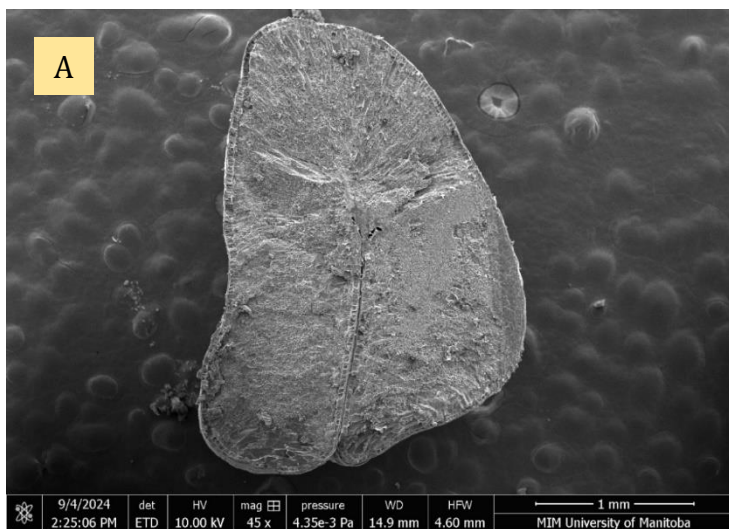


Figure 4.1 The microstructure of the transverse section of wheat. A) Einkorn, B) Emmer, C) Spelt, D) Common wheat.

B. Endosperm

Figures 4.2 and **4.3** display the internal structure of the endosperm and aleurone layers in various wheat species. In mature wheat grain, the endosperm is surrounded by a single layer of aleurone cells and is the primary morphological component for nutrient storage, consisting of starch and protein. The endosperm constitutes a solid mass at the core of the grain, representing the largest and most valuable structure in cereals grains (Evers & Millar, 2002).

The mature endosperm structure has starch granules embedded within a continuous protein matrix, which compresses cytoplasmic remnants into irregular shapes, forming a cohesive network (Bechtel et al., 2009) . The endosperm typically contains two distinct populations of starch granules; A-type and B-type in wheat, which differ in size and shape. A-type granules are lenticular in form and generally larger than 10 μm in diameter, while B-type granules are typically spherical and measure under 10 μm (Yin et al., 2012). However, there are reports indicating the existence of an additional group, C-type granules, which are sometimes classified as B-type granules. C-type granules are typically irregular or cuboidal in shape and measure 2–3 μm in diameter (Shevkani et al., 2016).

In **Figure 4.2**, the aleurone layers of the wheat species einkorn, emmer, spelt, and common wheat are presented, highlighting their structural differences. The aleurone layer, is removed along with the outer grain layers during the milling process. The aleurone cells have a block-shaped appearance and contain spherical particles called aleurone grains, which are protein storage vacuoles surrounded by lipid droplets (Lebert et al., 2022; Meziani et al., 2021; Tian et al., 2021). Both einkorn and emmer have comparatively small cube-shaped

aleurone cells, with a clear visual distinction from those in spelt and CWRS. The aleurone cells in spelt and CWRS appear more elongated, wider and block-shaped, showing less of the cubic structure seen in einkorn and emmer. Additionally, spherical protein bodies, or aleurone grains, are visible in the CWRS cells.

Based on the images of endosperm structure (**Figure 4.3**), einkorn displays two distinct populations of starch granules: larger, lenticular-shaped granules and smaller, spherical ones. These granules are lightly bound within the protein matrix, resulting in a less integrated starch-protein structure compared to other wheat species. In contrast, emmer starch granules are densely packed and fully cemented within a compact protein matrix, reflecting a more cohesive structure. In spelt, starch granules are more loosely associated with the protein matrix than those of emmer and CWRS wheat, but more integrated than those in einkorn. In CWRS wheat, a strong and cohesive network of starch and protein is visible, with starch granules exhibiting strong adhesion to the protein matrix, confirming the more tightly bound nature of the starch-protein matrix.

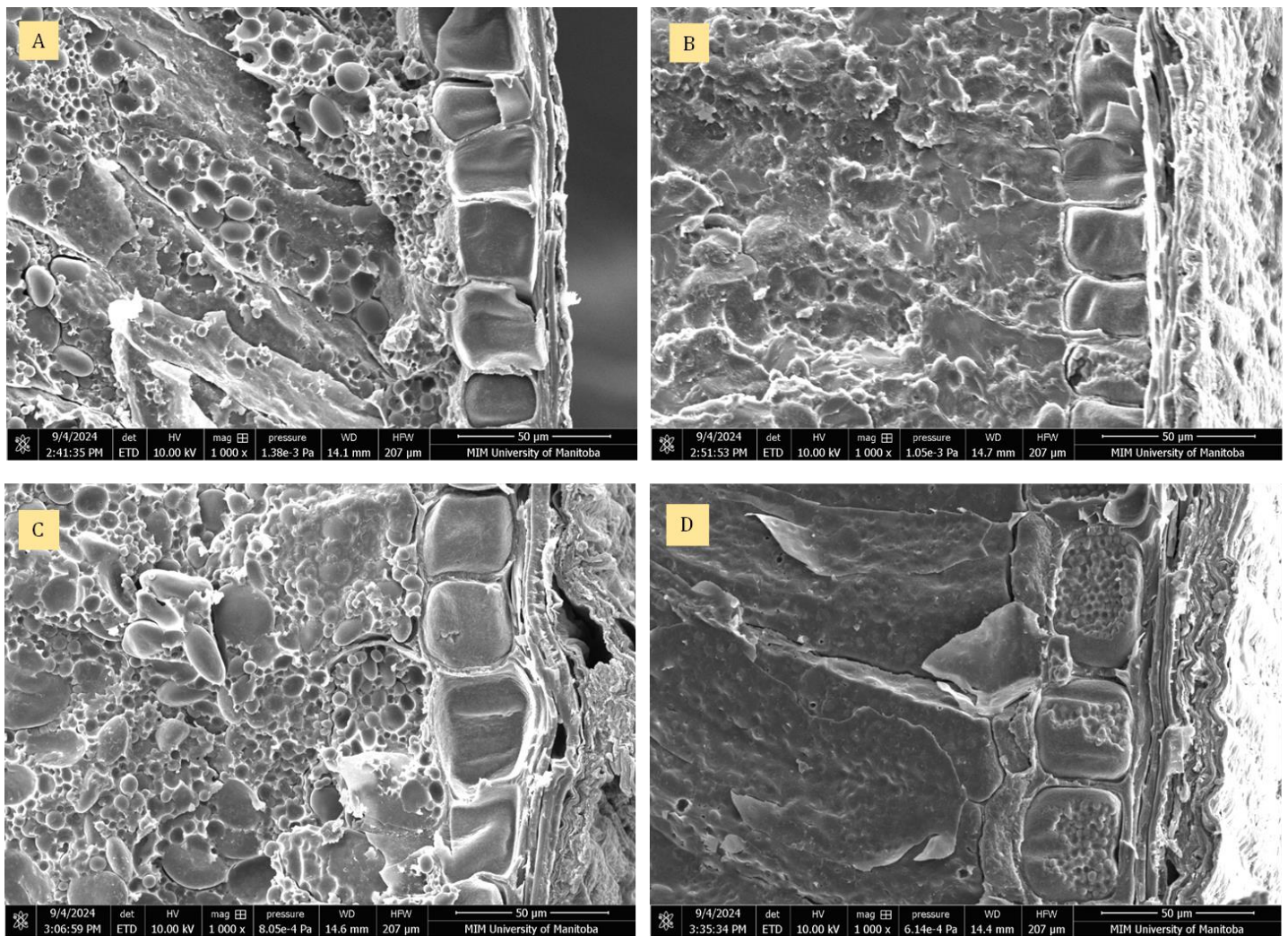


Figure 4.2 The microstructure of Aleurone layers. A) Einkorn, B) Emmer, C) Spelt, D) Common wheat

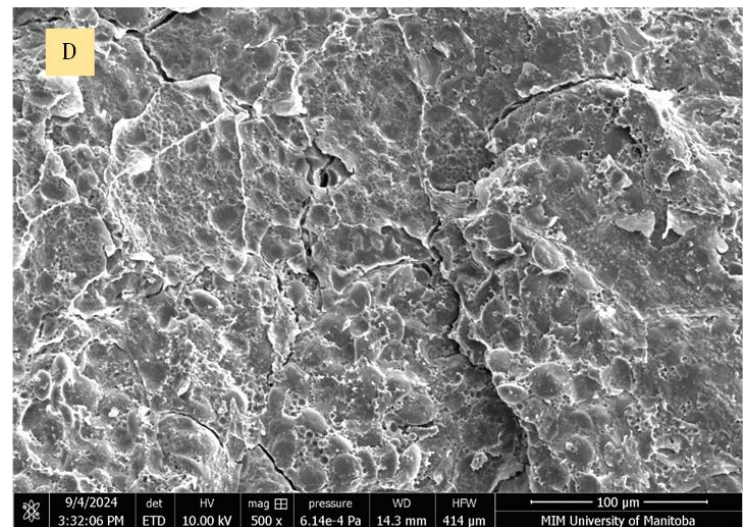
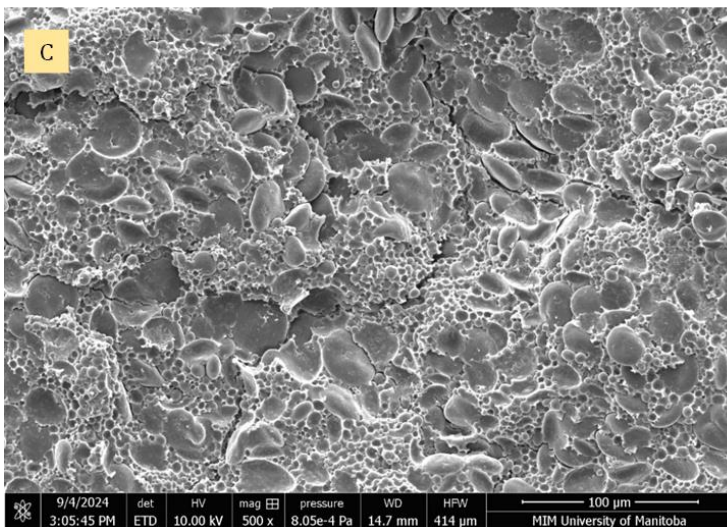
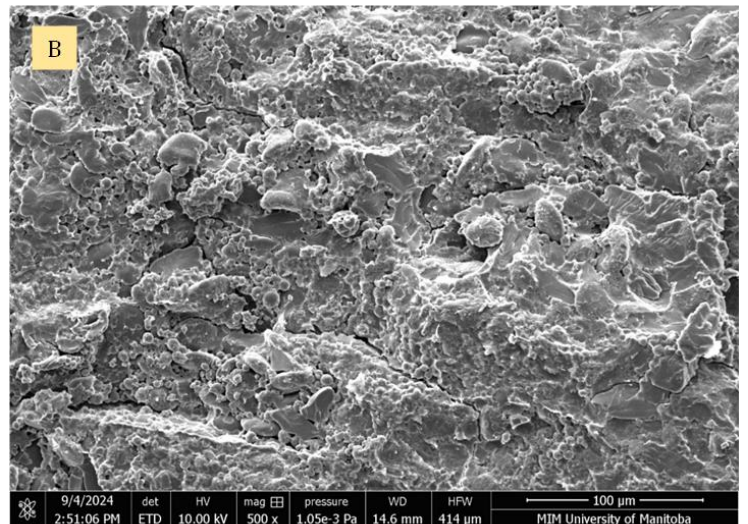
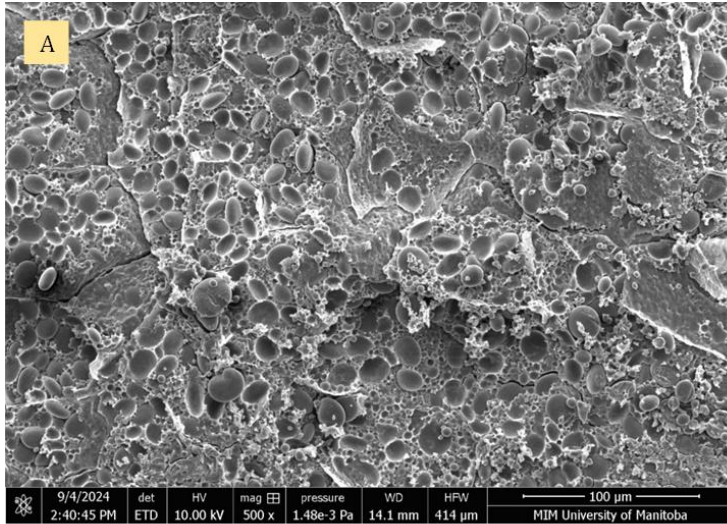


Figure 4.3 The microstructure of the endosperm of wheat kernels. A) Einkorn, B) Emmer, C) Spelt, D) Common wheat

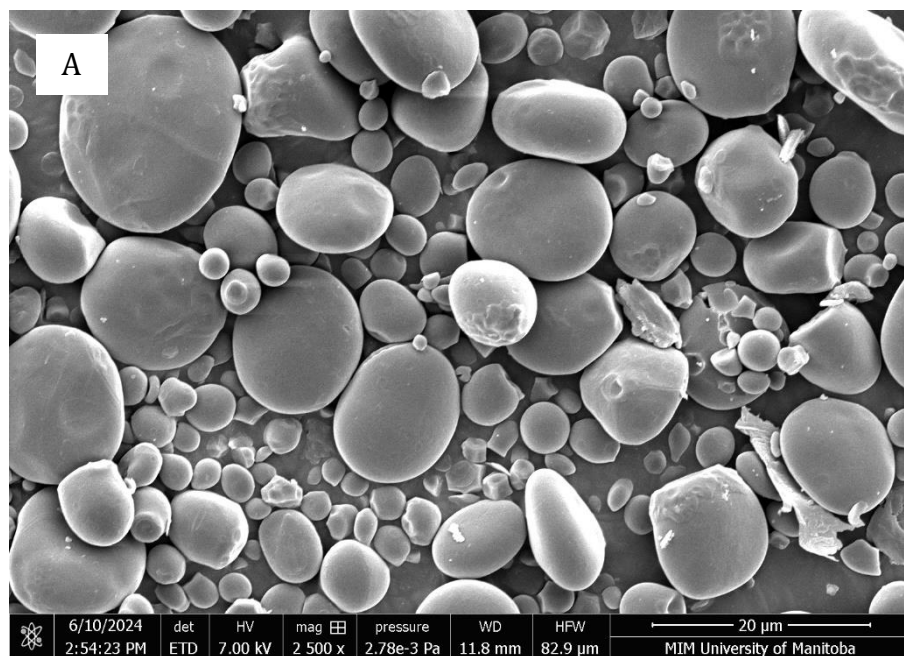
4.3.2 Scanning electron microscopy for starch granule visualization

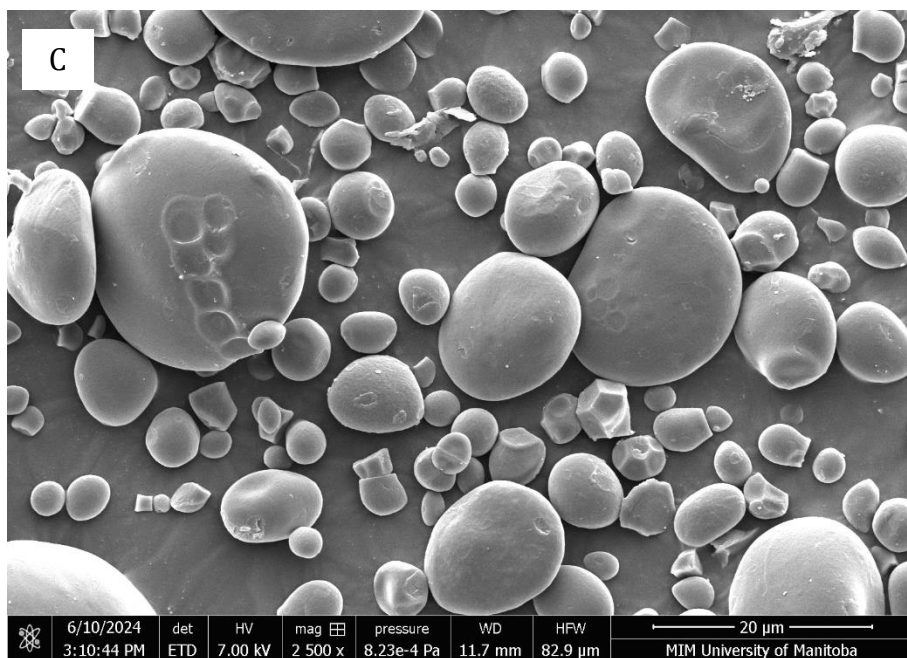
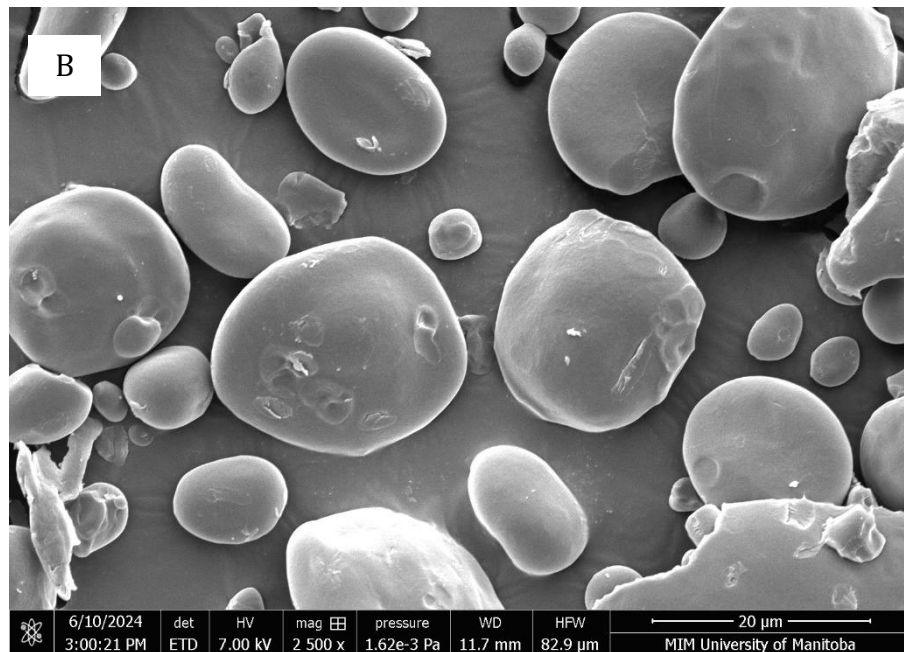
The starch granule visualization presented in **Figure 4.4** displays the unique morphology of starch extracted from einkorn, emmer, spelt, CWRS WW and CWRS RF. As mentioned previously, wheat starch exhibits mainly large lenticular-shaped granules (A-granules), which account for approximately 75% by weight (or about 5% by number), and smaller round granules (B-granules), which make up around 25% by weight (or about 95% by number) (Maningat & Seib, 2010).

The surface of wheat starch granules is usually smooth; however, certain A-type granules display grooves or indentations. These surface features are thought to arise from the dense arrangement of starch granules within the endosperm, where the close packing of B-type granules against A-type granules creates these impressions (Shevkani et al., 2016). Einkorn displayed a higher prevalence of smaller B-type granules compared to the other wheat species. In contrast, emmer starch had a lower proportion of small granules and a more prominent population of medium-sized A-type granules. Additionally, emmer starch demonstrated a higher concentration of larger granules, occupying a greater area of the sample relative to other ancient wheats. The starch extracted from CWRS common wheat, both in whole wheat (CWRS WW) and refined flour (CWRS RF) forms, displayed a higher occurrence of visually larger A-type granules. As stated in the previous literature, Stoddard (1999) reported that einkorn exhibited the smallest A-type granules, with a diameter of 13.2 μm , compared to 15.3 μm in durum wheat and 23.8 μm in common wheat. Regarding B-type granules, einkorn had a higher frequency compared to durum wheat, but similar to common wheat (38–40% vs. 22–32% vs. 34–42%, respectively). These observations are consistent

with the findings for CWRs common wheat, where A-type granules were more visually prominent and larger in size compared to those in einkorn and emmer. Additionally, Golea et al. (2023) investigated the endosperm structure of common wheat, einkorn, and spelt, with granule measurements. Their findings reported that the diameters of type A starch granules were $16.63 \pm 3.53 \mu\text{m}$ in common wheat, $12.59 \pm 4.99 \mu\text{m}$ in einkorn, and $16.39 \pm 5.4 \mu\text{m}$ in spelt. In addition, the diameters of type B starch granules were $4.38 \pm 2.25 \mu\text{m}$ in common wheat, $3.85 \pm 1.69 \mu\text{m}$ in einkorn, and $3.23 \pm 1.45 \mu\text{m}$ in spelt.

These morphological distinctions highlight the diversity in granule size and distribution among ancient and common wheat species, suggesting implications for their functional properties in food applications (Kumar et al., 2023).





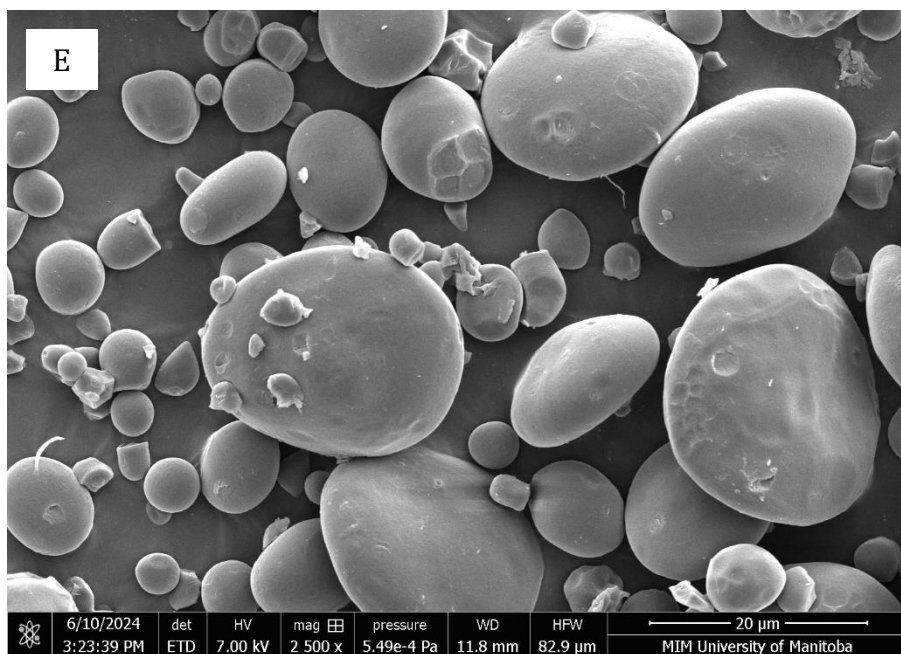
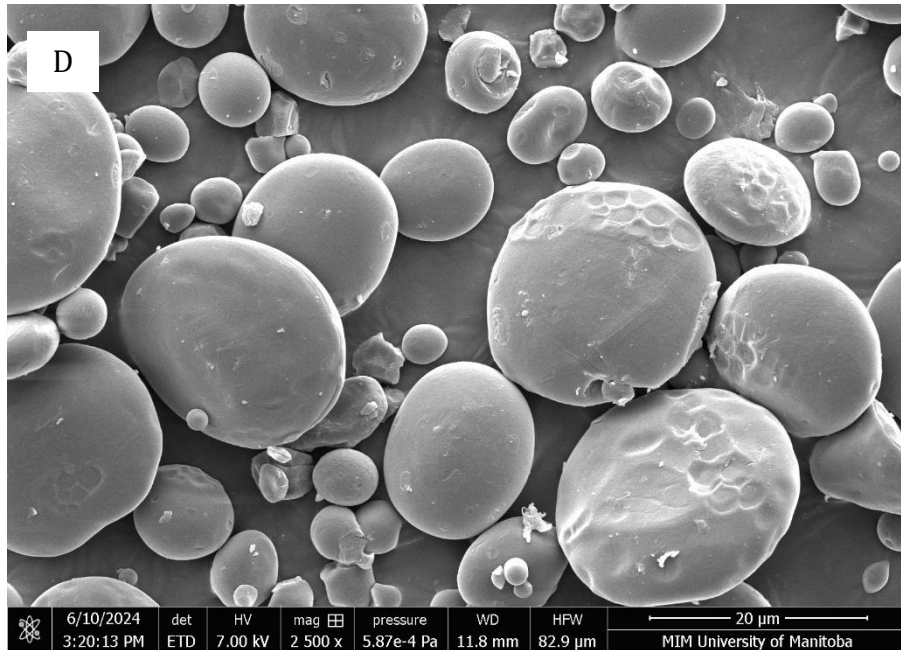


Figure 4.4 Scanning electron microscope images of starch extracted from different wheat species: A) Einkorn, B) Emmer, C) Spelt, D) Common wheat :CWRS Whole wheat, and E) Common wheat :CWRS Refined flour

4.3.3 Granule size distribution

Figure 4.5 and **Table 4.1** presents the granule size distribution graphs, displaying the volume density percentage of starch samples across different size classes for each wheat species studied. The graphs highlight the variations in starch granule sizes, emphasizing the distribution at key percentiles (DV10, DV50, and DV90) and showcasing the differences in granule size profiles among the species. Einkorn wheat demonstrates a unique granule size profile with the smallest DV (10) and DV (50) values at 4.06 μm and 12.1 μm , respectively, among all samples. The DV (10) value confirms the presence of smaller B-type granules, and it is likely that type C granules, smaller than 4.06 μm in diameter, are also present in einkorn. Emmer wheat demonstrates the largest granule sizes across all percentiles among the ancient wheat species studied with a highest DV (10) value of 9.46 μm indicating a significant portion of its granules are larger even at the lower end of the size distribution. Additionally, its DV(50) value, which reflects the median particle size, is the highest at 21.13 μm , among all samples confirming a tendency towards larger granules. This observation is further supported by SEM analysis of the emmer starch sample (**Figure 4.4**), which demonstrated a greater prominence of larger granules compared to other ancient wheat species.

Spelt wheat shows intermediate granule sizes with a DV (10) of 5.47 μm , DV(50) of 17.6 μm , and a DV(90) of 42.37 μm , indicating smaller variations in the size of larger granules compared to other samples. CWRS WW and CWRS RF had moderate characteristics for DV 10 and DV 50 values. CWRS RF has the highest DV (90) value (90.93 μm) along with emmer. This indicates that while the majority of its particles are of moderate size of A granules based on the median granule size, there is a significant proportion of very large type A granules

and that these samples have a wider distribution of different sizes of starch granules compared to other samples. The median size of starch granules and the proportion of each granule type are strongly influenced by environmental factors. Higher temperatures have been linked to a reduction in B-type granules, shifting the ratio in favor of A-type granules (Stoddard, 1999). Therefore, the variability in granule size distribution among different wheat species can be attributed to genetic differences, as well as the influence of environmental conditions and soil types on the enzymes involved in starch biosynthesis. This highlights the complex interplay between genetics and environmental factors in shaping starch characteristics in different wheat species (Shevkani et al., 2016). Overall, the findings of the study indicate that each wheat species studied has a unique starch granule size distribution, which is likely to affect its functional properties in food processing. Granule size plays a critical role in determining various starch characteristics, including gelatinization, pasting behavior, enzyme susceptibility, crystallinity, swelling, and solubility (Lindeboom et al., 2004).

Table 4.1 Granule size distribution of starch extracted from different wheat species.

Sample	DV (10), μm	DV (50), μm	DV (90), μm
Einkorn	4.06 ± 0.05^e	12.13 ± 0.06^e	78.23 ± 2.89^b
Emmer	9.46 ± 0.02^a	21.13 ± 0.06^a	86.93 ± 1.40^a
Spelt	5.47 ± 0.04^d	17.60 ± 0.00^d	42.37 ± 0.15^c
CWRS WW	7.43 ± 0.12^b	18.20 ± 0.00^c	74.40 ± 1.84^b
CWRS RF	6.97 ± 0.08^c	19.30 ± 0.10^b	90.93 ± 1.36^a

Values are presented as mean $\pm$ SD. Different letters in the same column indicate significant differences ($p < 0.05$)

Abbreviations: CWRs WW: Canada Western Red Spring Whole wheat, CWRs RF: Canada Western Red Spring Refined flour

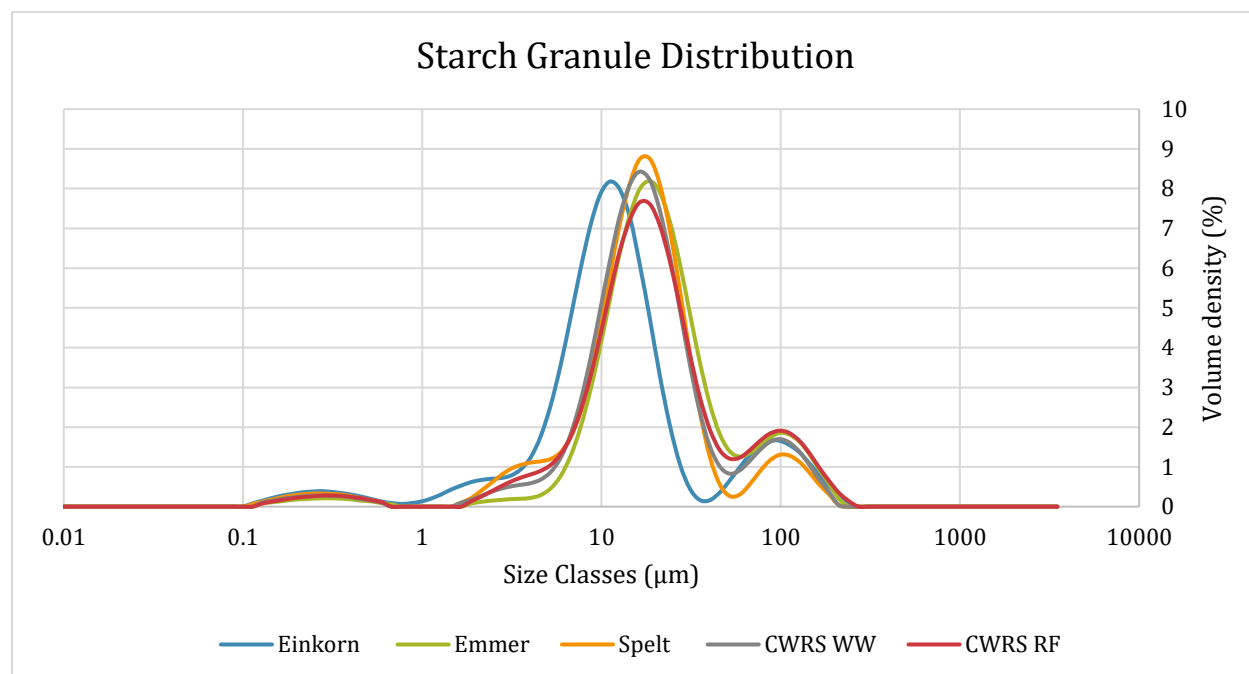


Figure 4.5 Granule size distribution of starch extracted from different wheat species

Abbreviations: CWRs WW: Canada Western Red Spring Whole wheat, CWRs RF: Canada Western Red Spring Refined flour

4.3.4 Characterization of amylose and amylopectin

The amylose and amylopectin content values for starch from ancient wheat and common wheat (CWRs WW and CWRs RF) are reported in **Table 4.2**. No significant differences ($p < 0.05$) were observed among the samples in terms of both amylose and amylopectin percentages. The amylose content ranged from 25.93% to 25.95% in ancient wheat species, while common wheat exhibited values of 25.44% (CWRs WW) and 25.55% (CWRs RF). Amylopectin content demonstrated similar consistency, ranging from 73.44% to 74.07% in ancient wheat, with CWRs WW and CWRs RF reporting 74.56% and 74.45%, respectively,

For einkorn, the amylose content observed in this study (25.93%) aligns closely with the findings of Brandolini et al. (2008), who reported an average amylose content of 25.7%, with values ranging from 23.2% to 28.6%. However, in another study, a higher average amylose content of 30.0% was reported in einkorn, with values ranging from 23.1% to 34.7% among 21 accessions (Rodriguez-Quijano et al., 2004). Mangan et al. (2022) investigated amylose content in emmer accessions over two years, 2018 and 2019. In 2018, 17 emmer accessions exhibited normal amylose levels (20–30%), while 7 accessions had amylose content of $\geq 30\%$. Similarly, in 2019, 20 emmer accessions displayed normal amylose content, with 6 accessions exceeding 30%. This variation highlights the diversity within emmer wheat and suggests that environmental factors may also influence amylose content. For spelt, the amylose content observed in this study (25.55%) falls within the range reported by Ie et al. (2012), who identified amylose content ranging from 23.0% to 29.8%. However, Wilson et al. (2008) reported higher amylose levels in spelt starch, with values between 30% and 33%. These differences could be attributed to genetic variability among spelt cultivars or differences in environment and growing conditions.

Even though there were no significant differences in amylose% and amylopectin% among the analyzed starches, variations in their functionality can still be anticipated due to differences in their fine molecular structures (Li et al., 2020). Especially, The chain length distribution of amylopectin plays a critical role in determining the relative crystallinity of starches, which, in turn, directly impacts their functional properties during processing and application (Shevkani et al., 2016).

Table 4.2 Amylose and amylose/ amylopectin (% as-is basis) of starch extracted from different wheat species.

Sample	Amylose (%)	Amylopectin (%)
Einkorn	25.93 ± 0.80 ^a	74.07 ± 0.80 ^a
Emmer	26.56 ± 0.26 ^a	73.44 ± 0.26 ^a
Spelt	25.95 ± 0.76 ^a	74.05 ± 0.76 ^a
CWRS WW	25.44 ± 0.55 ^a	74.56 ± 0.55 ^a
CWRS RF	25.55 ± 0.30 ^a	74.45 ± 0.30 ^a

Values are presented as mean ± SD. Different letters in the columns indicate significant differences ($p < 0.05$)

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

4.3.5 Thermal properties of starch gelatinization

DSC is a thermoanalytical technique commonly used to study gelatinization by measuring the heat flow associated with structural changes in starch (Schirmer et al., 2015). During starch gelatinization, starch granules transition from an ordered, crystalline state to a disordered, amorphous state when heated in the presence of water. This transformation disrupts the semicrystalline structure of the starch, causing the granules to swell and break down, ultimately forming a viscous solution. Gelatinization greatly influences the texture, structure, and palatability of starch-based foods (Ratnayake & Jackson, 2008). DSC assesses the extent of hydrogen bond disruption within the starch granules and measures the heat energy required, represented as enthalpy (Liu et al., 2019). Therefore, investigating the onset temperature (T_0), peak temperature (T_p), and enthalpy (ΔH) of gelatinization provides insight into the thermal behavior and structural properties of starch from different wheat species.

In this study, significant differences ($p < 0.05$) in onset temperature, peak temperature, and enthalpy were observed across samples (**Table 4.3**), revealing unique thermal behaviors. Spelt starch exhibited the highest T_0 (58.74 ± 0.07 °C) and T_p (62.01 ± 0.09 °C), indicating a greater thermal stability compared to other wheat types. Transition temperatures are closely related to the quality of the crystallites, with higher values typically reflecting a greater degree of crystalline perfection (Shevkani et al., 2016). The elevated transition temperatures observed in spelt starch may be attributed to a higher proportion of tightly packed, ordered crystalline regions that are more resistant to thermal disruption. These

higher temperatures are generally linked to an increased presence of longer-chain amylopectin, which requires more thermal energy for dissociation. The longer amylopectin chains promote the formation of more extensive double-helical structures, resulting in larger and more stable crystalline regions (Cooke & Gidley, 1992; Ie et al., 2012; Li, 2022). The gelatinization properties of starch are primarily determined by the molecular structure of amylopectin (double-helical order) and the amylopectin-to-amylose ratio (Chakraborty et al., 2022).

Emmer starch, in contrast, displayed the lowest T_0 (54.12 ± 0.12 °C) and T_p (58.38 ± 0.06 °C), along with the lowest ΔH (3.97 ± 0.04 J/g), suggesting it gelatinizes more readily due to less compact granule structures that require less energy to gelatinize. Einkorn starch demonstrated an intermediate onset and peak temperatures (55.70 ± 0.14 °C and 59.57 ± 0.34 °C, respectively) but differed significantly ($p < 0.05$) from all the samples with the highest enthalpy suggesting a unique thermal profile among the samples analyzed. CWRS WW and CWRS RF were not significantly different ($p < 0.05$) in terms of peak temperature and enthalpy value required for gelatinization. However, from all the samples including common wheat, highest and lowest gelatinization enthalpies were recorded in ancient wheat starches, einkorn and emmer respectively.

According to Warechowska et al. (2023), significant differences in gelatinization temperatures were observed between spelt cultivars and common wheat. Common wheat exhibited the highest T_0 (61.0 °C) and T_p (65.1 °C) temperatures, indicating a greater thermal requirement for gelatinization compared to spelt. The gelatinization enthalpy for spelt ranged from 5.3 J/g to 6.3 J/g, whereas common wheat exhibited a value of 5.4 J/g. As

reported by Dzhivoderova-Zarcheva et al. (2023), emmer starch exhibited a T_0 of 49.1 °C and a T_p of 76.3 °C.

There is limited literature available to facilitate proper comparisons of the current results with previous studies. Based on the values reported in a few existing studies, slight differences are noted in comparison to the values obtained in the present research. The ability to detect changes in enthalpy during DSC is influenced by several factors, including the scanning rate, moisture content, the hydration state of the starch granules, and the sensitivity of the equipment. Furthermore, when samples are subjected to a gradual increase in heat during "direct scanning," accurately identifying the enthalpic changes that occur at specific temperatures poses a challenge (Dzhivoderova-Zarcheva et al., 2023; Ratnayake & Jackson, 2008).

Table 4.3 Thermal behaviour of starch extracted from different wheat species.

Sample	Onset Temperature (°C)	Peak Temperature (°C)	Enthalpy (J/g)
Einkorn	55.70 ± 0.14 ^d	59.57 ± 0.34 ^c	7.13 ± 0.11 ^a
Emmer	54.12 ± 0.12 ^e	58.38 ± 0.06 ^d	3.97 ± 0.04 ^d
Spelt	58.74 ± 0.07 ^a	62.01 ± 0.09 ^a	5.49 ± 0.20 ^b
CWRS WW	56.68 ± 0.05 ^b	60.88 ± 0.07 ^b	5.10 ± 0.09 ^c
CWRS RF	56.22 ± 0.21 ^c	60.92 ± 0.28 ^b	5.06 ± 0.19 ^c

Note: Values are presented as mean ± SD, Different letters in the same column indicate significant differences ($p < 0.05$)

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

4.3.6 Swelling power of starch

The swelling power of the starch samples is presented in **Table 4.4**. Starch swelling power and solubility are key physicochemical properties that indicate the extent of starch interaction with water (Wang et al., 2023). Based on the findings, the swelling power of ancient wheat species ranged from 10.10 to 11.52 g/g. The lowest swelling power among all the samples was observed in spelt, with a value of 10.10 g/g. In contrast, einkorn, emmer, and the common wheat samples (CWRS WW and CWRS RF) exhibited similar swelling power values, with no significant differences ($p < 0.05$) observed. Starch swelling is the process by which starch granules absorb water and expand, leading to a softening and fragility of the granules. This behavior significantly impacts the texture, taste, functionality, and nutritional properties of starch-based foods (Jia et al., 2023). The extent of swelling is influenced by the interactions between starch chains within the amorphous and crystalline regions of the granule. Factors such as the amylose-to-amylopectin ratio, molecular weight, branching, and conformation of both amylose and amylopectin components play a crucial role in determining the swelling behavior (Hoover, 2001). Starches with higher crystallinity generally exhibit lower swelling power, as a more ordered crystalline structure restricts granule expansion. The DSC and swelling power data in this study are consistent with the observed structural and functional characteristics of spelt starch. The reduced swelling power of spelt starch is likely due to its high degree of crystalline perfection, which results in higher transition temperatures. These elevated transition temperatures are indicative of a more stable and well-organized crystalline structure (Sandeep et al., 2010).

Table 4.4 Swelling power values amylose and amylose/ amylopectin (% as-is basis) of starch extracted from different wheat species.

Sample	Swelling Power (g/g)
Einkorn	11.52 ± 0.31 ^a
Emmer	11.20 ± 0.16 ^a
Spelt	10.10 ± 0.34 ^b
CWRS WW	11.14 ± 0.20 ^a
CWRS RF	10.93 ± 0.44 ^a

Values are presented as mean ± SD. Different letters in the columns indicate significant differences ($p < 0.05$)

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

4.3.7 Protein composition analysis

The protein structure in wheat flour plays a critical role in determining its processing capabilities and suitability for diverse food products. Specially, the storage proteins that form a three-dimensional gluten network are essential for the viscoelastic properties of the dough, directly impacting its functionality in food processing applications (Lafiandra & Shewry, 2022). Analyzing the protein characteristics within ancient wheat species offer insights into their potential in food applications and provides further understanding of how they can influence modifications in processing.

In this study, the HPLC chromatogram is divided into four fractions: F1 (3.5-4.7 min), F2 (4.7-5.1 min), F3 (5.1-5.8 min), and F4 (5.8-7.4 min). According to previous literature, these fractions correspond to different types of proteins: F1 contains high molecular weight polymeric proteins (HMW-GLU), F2 is composed of low molecular weight polymeric proteins

(LMW-GLU), F3 includes gliadin proteins (GLI), and F4 is made up of albumin and globulin (Malalgoda et al., 2020).

Based on the findings of the current study **Table 4.5**, unique profiles were detected for SDS extractable (EP) and SDS unextractable (UP) fractions. For EP1, which contains HMW-GLU, emmer (18.04 ± 0.52) exhibited the highest area percent, significantly higher ($p < 0.05$) than both CWRS WW (12.23 ± 0.24) and CWRS RF (14.79 ± 0.19). Einkorn (14.28 ± 0.18) and spelt (15.11 ± 1.12) displayed intermediate levels. This suggests that emmer contains a higher proportion of HMW-GLU proteins compared to all the other samples. For EP2, which corresponds to LMW-GLU, emmer (8.59 ± 0.07) again had the highest area percent followed by einkorn (8.15 ± 0.03), which was also significantly higher ($p < 0.05$) than spelt (7.04 ± 0.26), CWRS WW (7.15 ± 0.28), and CWRS RF (7.05 ± 0.37). For EP3, which corresponds to GLI proteins, einkorn (41.05 ± 0.14) had the highest protein content, significantly surpassing all other samples, with CWRS RF (36.17 ± 1.72) also showing high levels. Finally, for EP4, which corresponds to albumin and globulin (F4), emmer (16.07 ± 0.21) exhibited the highest area percent, significantly ($p < 0.05$) higher than CWRS RF (12.58 ± 1.53), while einkorn (13.79 ± 0.15) and spelt (15.87 ± 1.23) were not significantly different ($p < 0.05$) from each other.

In terms of SDS unextractable protein fractions, all ancient wheat species reported significantly lower values ($p < 0.05$) for the F1 fraction compared to common wheat, with emmer having the lowest value (8.25 ± 0.26), followed by einkorn (11.18 ± 0.06) and spelt (13.15 ± 0.81). Common wheat (CWRS WW and CWRS RF) exhibited significantly higher values ($p < 0.05$) in this fraction, indicating a higher content of HMW-GLU proteins. For the

UP2 fraction, both einkorn and emmer had the lowest values (2.80 ± 0.03 and 2.84 ± 0.02 , respectively), which were significantly lower ($p < 0.05$) than the common wheat samples.

For the UP3 fraction, spelt had the highest value, which was not significantly different ($p < 0.05$) from CWRS WW and emmer. CWRS RF and einkorn had the lowest levels for the UP3 fraction, indicating a lower content of GLI proteins in the SDS unextracted fraction of these species. Spelt demonstrated the highest levels for the UP4 fraction 4.69, followed by CWRS WW.

Overall, among the ancient wheat species, emmer had a unique protein profile, with higher levels of GLU in the SDS extractable fractions, which typically favors the development of a strong gluten network. However, in the SDS unextractable fractions, emmer did not show this characteristic; instead, it had lower levels of GLU, which may hinder the development of a proper gluten network. As reported in previous studies, SDS-unextractable HMW proteins in HRS wheat were positively correlated with farinograph peak time, stability, and loaf volume, while SDS-extractable proteins showed negative correlations with these traits (Malalgoda et al., 2018; Ohm et al., 2010). Additionally, a higher percentage of monomeric gliadin leads to a weaker gluten with improved extensibility (Zou et al., 2022).

Protein composition data for ancient wheat species remains limited. However, available studies have provided valuable insights into the gluten protein profiles of both ancient and common wheat. Geisslitz et al. (2019), investigated the gluten protein composition, specifically the GLI/GLU, which is crucial for assessing breadmaking quality, particularly in relation to loaf volume. Their findings indicated that common wheat exhibited a lower GLI/GLU ratio compared to ancient wheat species. The GLU content decreased progressively

from common wheat and spelt to einkorn, with durum wheat and emmer exhibiting intermediate levels. Similarly, Wieser (2000) reported that common wheat contained the highest levels of total GLU and HMW-GLU, while the ratio of GLI/GLU was lower in common wheat relative to ancient wheat species. The variations in protein composition observed in the sample set are consistent with what is expected, as these factors are known to be influenced by the species, cultivars, and growing conditions (Geisslitz et al., 2019; Konvalina et al., 2013). Overall, the protein HPLC data indicated that ancient wheat species are generally lower in SDS unextractable glutenin proteins, which are essential for breadmaking, indicating the need for process modifications or additional ingredients to enhance the end-use quality of bakery products developed using ancient grains.

Table 4.5 Protein composition of protein extracted from different wheat species as analyzed by SE-HPLC.

Sample	SDS extractable				SDS unextractable			
	EP1	EP2	EP3	EP4	UP1	UP2	UP3	UP4
Einkorn	14.28 ± 0.18 ^b	8.15 ± 0.03 ^a	41.05 ± 0.14 ^a	13.79 ± 0.15 ^{ab}	11.18 ± 0.06 ^b	2.80 ± 0.03 ^c	6.00 ± 0.16 ^b	2.75 ± 0.06 ^c
Emmer	18.04 ± 0.52 ^a	8.59 ± 0.07 ^a	34.63 ± 0.21 ^{b c}	16.07 ± 0.21 ^a	8.25 ± 0.26 ^c	2.84 ± 0.02 ^c	7.83 ± 0.40 ^a	3.75 ± 0.15 ^b
Spelt	15.11 ± 1.12 ^b	7.04 ± 0.26 ^b	31.95 ± 0.97 ^c	15.87 ± 1.23 ^a	13.15 ± 0.81 ^b	3.81 ± 0.24 ^{ab}	8.38 ± 0.34 ^a	4.69 ± 0.29 ^a
CWRS WW	12.23 ± 0.24 ^c	7.15 ± 0.28 ^b	33.41 ± 1.14 ^{b c}	14.19 ± 1.01 ^{ab}	16.12 ± 0.73 ^a	4.42 ± 0.26 ^a	8.16 ± 0.82 ^a	4.31 ± 0.38 ^{ab}
CWRS RF	14.79 ± 0.19 ^b	7.05 ± 0.37 ^b	36.17 ± 1.72 ^b	12.58 ± 1.53 ^b	17.44 ± 1.88 ^a	3.60 ± 0.37 ^b	5.56 ± 1.03 ^b	2.82 ± 0.45 ^c

Note: Values are presented as mean ± SD, Different letters in the same column indicate significant differences (p < 0.05)

Protein composition profiles for SDS-extractable fractions (EP1–EP4); EP1: high molecular weight glutenin (HMW-GLU), EP2: low molecular weight glutenin (LMW-GLU), EP3: gliadin (GLI), and EP4: albumin and globulin fractions.

Protein composition profiles for SDS-unextractable fractions (UP1–UP4) follow the same order: UP1:high molecular weight glutenin (HMW-GLU), UP2:low molecular weight glutenin (LMW-GLU), UP3: gliadin (GLI), and UP4: albumin and globulin fractions.

Abbreviations: CWRS WW: Canada Western Red Spring Whole wheat, CWRS RF: Canada Western Red Spring Refined flour

4.4 Conclusion

Overall, the evaluation of starch and protein characteristics of einkorn, emmer, and spelt reveals distinct differences compared to common wheat, with significant implications for their potential use in food applications. Visual observations of kernel size, endosperm morphology, and starch granule morphology also highlighted differences among ancient wheat species. Einkorn exhibited thinner kernels, reflecting its more primitive nature, while both emmer and spelt had rounder kernels, though not to the extent of common wheat. Common wheat kernels were more rounded and fully developed with a higher endosperm content. Variations in granule size distribution, starch gelatinization behavior, and protein composition suggest that ancient wheat species possess unique functional properties that may influence their suitability for specific food products. While amylose and amylopectin content showed no significant differences, variations in other starch functional characteristics indicate that the fine structure of starch also affects its behavior. The major differences in gliadin and glutenin fractions between common wheat and ancient wheat suggest potential challenges in achieving desirable dough properties. These findings emphasize the importance of considering the functional properties of ancient wheat when exploring its use in bakery products and highlight the need for processing modifications or

blending with common wheat types to optimize its potential. Further research into the processing techniques and development of new formulations using ancient wheat species could offer new opportunities to improve the utilization of ancient wheat and expand its market potential.

4.5 References

- Akar, T., Cengiz, M. F., & Tekin, M. (2019). A comparative study of protein and free amino acid contents in some important ancient wheat lines. *Quality Assurance and Safety of Crops and Foods*, 11(2), 191–200. <https://doi.org/10.3920/QAS2018.1382>
- Arzani, A., & Ashraf, M. (2017). Cultivated Ancient Wheats (*Triticum* spp.): A Potential Source of Health-Beneficial Food Products. *Comprehensive Reviews in Food Science and Food Safety*, 16(3), 477–488. <https://doi.org/10.1111/1541-4337.12262>
- Barański, M., Lacko-Bartošová, M., Rembiałkowska, E., & Lacko-Bartošová, L. (2020). The Effect of Species and Cultivation Year on Phenolic Acids Content in Ancient Wheat. *Agronomy*, 10(5), 673. <https://doi.org/10.3390/agronomy10050673>
- Bechtel, D. B., Abecassis, J., Shewry, P. R., & Evers, A. D. (2009). Development, Structure, and Mechanical Properties of the Wheat Grain. In *Wheat: Chemistry and Technology: Fourth Edition* (pp. 51–95). Elsevier Inc. <https://doi.org/10.1016/B978-1-891127-55-7.50010-0>
- Belcar, J., Sobczyk, A., Sekutowski, T. R., Stankowski, S., & Gorzelany, J. (2021). Evaluation of Flours from Ancient Varieties of Wheat (Einkorn, Emmer, Spelt) used in Production of Bread. *Acta Universitatis Cibiniensis. Series E: Food Technology*, 25(1), 53–66. <https://doi.org/10.2478/auaft-2021-0005>
- Brandolini, A., Hidalgo, A., & Moscaritolo, S. (2008). Chemical composition and pasting properties of einkorn (*Triticum monococcum* L. subsp. *monococcum*) whole meal flour. *Journal of Cereal Science*, 47(3), 599–609. <https://doi.org/10.1016/j.jcs.2007.07.005>
- Chakraborty, I., N, P., Mal, S. S., Paul, U. C., Rahman, Md. H., & Mazumder, N. (2022). An Insight into the Gelatinization Properties Influencing the Modified Starches Used in Food Industry: A review. *Food and Bioprocess Technology*, 15(6), 1195–1223. <https://doi.org/10.1007/s11947-022-02761-z>
- Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydrate Research*, 227, 103–112. [https://doi.org/10.1016/0008-6215\(92\)85063-6](https://doi.org/10.1016/0008-6215(92)85063-6)

- Costanzo, A., Amos, D. C., Dinelli, G., Sferrazza, R. E., Accorsi, G., Negri, L., & Bosi, S. (2019). Performance and nutritional properties of Einkorn, Emmer and Rivet Wheat in response to different rotational position and soil tillage. *Sustainability (Switzerland)*, 11(22). <https://doi.org/10.3390/su11226304>
- Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., & Sofi, F. (2018). Ancient wheat species and human health: Biochemical and clinical implications. *Journal of Nutritional Biochemistry*, 52, 1–9. <https://doi.org/10.1016/j.jnutbio.2017.09.001>
- Dzhivoderova-Zarcheva, M. M., Shopska, V. N., Kostov, G. A., & Denkova-Kostova, R. S. (2023). Comparative analysis of the gelling, retrograding and rheological properties of emmer starch compared to other cereal starches. *Acta Periodica Technologica*, 54, 265–275. <https://doi.org/10.2298/APT2354265D>
- Ee, K. Y., Eng, M. K., & Lee, M. L. (2020). Physicochemical, thermal and rheological properties of commercial wheat flours and corresponding starches. *Food Science and Technology (Brazil)*, 40, 51–59. <https://doi.org/10.1590/fst.39718>
- Evers, T., & Millar, S. (2002a). Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science*, 36(3), 261–284. <https://doi.org/10.1006/jcrs.2002.0435>
- Evers, T., & Millar, S. (2002b). Cereal grain structure and development: Some implications for quality. *Journal of Cereal Science*, 36(3), 261–284. <https://doi.org/10.1006/jcrs.2002.0435>
- Geisslitz, S., Longin, C. F. H., Scherf, K. A., & Koehler, P. (2019). Comparative Study on Gluten Protein Composition of Ancient (Einkorn, Emmer and Spelt) and Modern Wheat Species (Durum and Common Wheat). *Foods*, 8(9), 409. <https://doi.org/10.3390/foods8090409>
- Golea, C. M., Stroe, S. G., Gâtlan, A. M., & Codină, G. G. (2023). Physicochemical Characteristics and Microstructure of Ancient and Common Wheat Grains Cultivated in Romania. *Plants*, 12(11). <https://doi.org/10.3390/plants12112138>
- Hidalgo, A., & Brandolini, A. (2019). Nutritional, Technological, and Health Aspects of Einkorn Flour and Bread. In *Flour and Breads and their Fortification in Health and Disease Prevention* (2nd ed., pp. 99–110). Elsevier. <https://doi.org/10.1016/B978-0-12-814639-2.00008-3>
- Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers*, 45(3), 253–267. [https://doi.org/10.1016/S0144-8617\(00\)00260-5](https://doi.org/10.1016/S0144-8617(00)00260-5)
- Ie, P. S., Petros, D., Stinner, D. H., Phelan, P. L., Hamaker, B., Koelling, K. W., & Vodovotz, Y. (2012). Comparison of the gelatinization behavior of organic and conventional spelt starches assessed by thermal and rheological analyses. *Journal of Agricultural and Food Chemistry*, 60(36), 9229–9235. <https://doi.org/10.1021/jf301171j>

- Jia, R., Cui, C., Gao, L., Qin, Y., Ji, N., Dai, L., Wang, Y., Xiong, L., Shi, R., & Sun, Q. (2023). A review of starch swelling behavior: Its mechanism, determination methods, influencing factors, and influence on food quality. In *Carbohydrate Polymers* (Vol. 321). Elsevier Ltd. <https://doi.org/10.1016/j.carbpol.2023.121260>
- Kaplan Evlice, A., Pehlivan, A., Keçeli, A., Şanal, T., Karabak, S., Zencirci, N., & Yaman, H. M. (2022). Nutritional and Technological Aspects of Ancient Wheat. In N. Zencirci, H. Ulukan, F. S. Baloch, S. Mansoor, & A. Rasheed (Eds.), *Ancient Wheats* (pp. 147–179). Springer International Publishing. https://doi.org/10.1007/978-3-031-07285-7_7
- Kohajdová, Z., & Karovičová, J. (2008). Nutritional value and baking applications of spelt wheat *. In *ACTA Acta Sci. Pol., Technol. Aliment* (Vol. 7, Issue 3).
- Konvalina, P., Bradová, J., Capouchová, I., Stehno, Z., & Moudry, J. (2013). Baking Quality and High Molecular Weight Glutenin Subunit Composition Of Emmer Wheat, Old and New Varieties of Bread Wheat. In *Romanian agricultural research* (Issue 30).
- Kumar, R., Singh, N., & Khatkar, B. S. (2023). Effects of A- and B-type starch granules on composition, structural, thermal, morphological, and pasting properties of starches from diverse wheat varieties. *Food Bioengineering*, 2(4), 373–383. <https://doi.org/10.1002/fbe2.12068>
- Lafiandra, D., & Shewry, P. R. (2022). Wheat Glutenin polymers 2. The role of wheat glutenin subunits in polymer formation and dough quality. *Journal of Cereal Science*, 106(July). <https://doi.org/10.1016/j.jcs.2022.103487>
- Lebert, L., Buche, F., Sorin, A., & Aussenac, T. (2022). The Wheat Aleurone Layer: Optimisation of Its Benefits and Application to Bakery Products. In *Foods* (Vol. 11, Issue 22). MDPI. <https://doi.org/10.3390/foods11223552>
- Li, C. (2022). Recent progress in understanding starch gelatinization - An important property determining food quality. In *Carbohydrate Polymers* (Vol. 293). Elsevier Ltd. <https://doi.org/10.1016/j.carbpol.2022.119735>
- Li, C., Hu, Y., Huang, T., Gong, B., & Yu, W.-W. (2020). A combined action of amylose and amylopectin fine molecular structures in determining the starch pasting and retrogradation property. *International Journal of Biological Macromolecules*, 164, 2717–2725. <https://doi.org/10.1016/j.ijbiomac.2020.08.123>
- Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the healthgrain diversity screen. *Journal of Agricultural and Food Chemistry*, 56(21), 9732–9739. <https://doi.org/10.1021/jf801069s>
- Liu, Y., Yu, J., Copeland, L., Wang, S., & Wang, S. (2019). Gelatinization behavior of starch: Reflecting beyond the endotherm measured by differential scanning calorimetry. *Food Chemistry*, 284, 53–59. <https://doi.org/10.1016/j.foodchem.2019.01.095>

- Malalgoda, M., Ohm, J. B., Howatt, K. A., Green, A., & Simsek, S. (2020). Effects of pre-harvest glyphosate use on protein composition and shikimic acid accumulation in spring wheat. *Food Chemistry*, 332, 127422. <https://doi.org/10.1016/j.foodchem.2020.127422>
- Malalgoda, M., Ohm, J. B., Meinhardt, S., & Simsek, S. (2018). Association between gluten protein composition and breadmaking quality characteristics in historical and modern spring wheat: *Cereal Chemistry*, 95(2), 226–238. <https://doi.org/10.1002/cche.10014>
- Maningat, C. C., & Seib, P. A. (2010). Understanding the physicochemical and functional properties of wheat starch in various foods. *Cereal Chemistry*, 87(4), 305–314. <https://doi.org/10.1094/CCHEM-87-4-0305>
- Meziani, S., Nadaud, I., Tasleem-Tahir, A., Nurit, E., Benguella, R., & Branlard, G. (2021). Wheat aleurone layer: A site enriched with nutrients and bioactive molecules with potential nutritional opportunities for breeding. In *Journal of Cereal Science* (Vol. 100). Academic Press. <https://doi.org/10.1016/j.jcs.2021.103225>
- Nisa Mangan, B., Lashari, M. S., Baloch, A. W., Kandhro, M. N., & Bhutta, Z. M. (2022). Starch Properties Ratio among Wheat Species Originated from different Regions of the World. *Pakistan Journal of Biochemistry and Biotechnology*, 3(1), 49–63. <https://doi.org/10.52700/pjbb.v3i1.77>
- Ohm, J., Hareland, G., Simsek, S., Seabourn, B., Maghirang, E., & Dowell, F. (2010). Molecular Weight Distribution of Proteins in Hard Red Spring Wheat: Relationship to Quality Parameters and Intrasample Uniformity. *Cereal Chemistry*, 87(6), 553–560. <https://doi.org/10.1094/CCHEM-06-10-0088>
- Owuoche, J. (2017). Wheat Improvement, Management and Utilization. In R. Wanyera & J. Owuoche (Eds.), *Wheat Improvement, Management and Utilization*. InTech. <https://doi.org/10.5772/63694>
- Ratnayake, W. S., & Jackson, D. S. (2008). Chapter 5 Starch Gelatinization. In *Advances in Food and Nutrition Research* (Vol. 55, pp. 221–268). [https://doi.org/10.1016/S1043-4526\(08\)00405-1](https://doi.org/10.1016/S1043-4526(08)00405-1)
- Rodriguez-Quijano, M., Vázquez, J. F., & Carrillo, J. M. (2004). Waxy proteins and amylose content in diploid Triticeae species with genomes A, S and D. *Plant Breeding*, 123(3), 294–296. <https://doi.org/10.1111/j.1439-0523.2004.00883.x>
- Roumia, H., Kókai, Z., Mihály-Langó, B., Csobod, É. C., & Benedek, C. (2023). Ancient Wheats—A Nutritional and Sensory Analysis Review. *Foods*, 12(12), 2411. <https://doi.org/10.3390/foods12122411>
- Şahin, Y., Yıldırım, A., Yücesan, B., Zencirci, N., Erbayram, Ş., & Gürel, E. (2017). Phytochemical content and antioxidant activity of einkorn (*Triticum monococcum* ssp. *monococcum*),

- bread (*Triticum aestivum* L.), and durum (*Triticum durum* Desf.) wheat. *Progress in Nutrition*, 19(4), 450–459. <https://doi.org/10.23751/pn.v19i4.5847>
- Sandeep, S., Singh, N., Isono, N., & Noda, T. (2010). Relationship of granule size distribution and amylopectin structure with pasting, thermal, and retrogradation properties in wheat starch. *Journal of Agricultural and Food Chemistry*, 58(2), 1180–1188. <https://doi.org/10.1021/jf902753f>
- Schirmer, M., Jekle, M., & Becker, T. (2015). Starch gelatinization and its complexity for analysis. In *Starch/Staerke* (Vol. 67, Issues 1–2, pp. 30–41). Wiley-VCH Verlag. <https://doi.org/10.1002/star.201400071>
- Shevkani, K., Singh, N., Bajaj, R., & Kaur, A. (2016). Wheat starch production, structure, functionality, and applications—a review. In *International Journal of Food Science and Technology* (Vol. 52, Issue 1, pp. 38–58). <https://doi.org/10.1111/ijfs.13266>
- Simsek, S., Budak, B., Schwebach, C. S., & Ovando-Martínez, M. (2020). Starch digestibility properties of bread from hard red spring wheat cultivars released in the last 100 years. *Cereal Chemistry*, 97(1), 138–148. <https://doi.org/10.1002/cche.10211>
- Simsek, S., Whitney, K., & Ohm, J. B. (2013). Analysis of Cereal Starches by High-Performance Size Exclusion Chromatography. *Food Analytical Methods*, 6(1), 181–190. <https://doi.org/10.1007/s12161-012-9424-4>
- Stoddard, F. L. (1999). Survey of starch particle-size distribution in wheat and related species. *Cereal Chemistry*, 76(1), 145–149. <https://doi.org/10.1094/CCHEM.1999.76.1.145>
- Thorup, A. C. athrine, Gregersen, S., & Jeppesen, P. B. endix. (2014). Ancient Wheat Diet Delays Diabetes Development in a Type 2 Diabetes Animal Model. *The Review of Diabetic Studies: RDS*, 11(3–4), 245–257. <https://doi.org/10.1900/RDS.2014.11.245>
- Tian, X., Wang, Z., Yang, S., Wang, X., Li, L., Sun, B., Ma, S., & Zheng, S. (2021). Microstructure observation of multilayers separated from wheat bran. *Grain and Oil Science and Technology*, 4(4), 165–173. <https://doi.org/10.1016/j.gaost.2021.10.002>
- Tran, K. D., Konvalina, P., Capouchova, I., Janovska, D., Lacko-Bartosova, M., Kopecky, M., & Tran, P. X. T. (2020). Comparative Study on Protein Quality and Rheological Behavior of Different Wheat Species. *Agronomy*, 10(11), 1763. <https://doi.org/10.3390/agronomy10111763>
- Wang, W., Hu, A., Liu, S., Yan, J., Li, Y., & Zheng, J. (2023). Physicochemical properties and structure of starch ester/copolymer/complex synthesized by combination of microwave and acid (anhydride): A review. In *Trends in Food Science and Technology* (Vol. 138, pp. 539–550). Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2023.06.033>
- Warechowska, M., Anders, A., Warechowski, J., Bramowicz, M., Markowska-Mendik, A., Rejmer, W., Tyburski, J., & Kulesza, S. (2023). The endosperm microstructure, physical, thermal

properties, and specific milling energy of spelt (*Triticum aestivum* ssp. *spelta*) grain and flour. *Scientific Reports*, 13(1). <https://doi.org/10.1038/s41598-023-30285-9>

Wieser, H. (2000). Comparative investigations of gluten proteins from different wheat species. *European Food Research and Technology*, 211(4), 262–268. <https://doi.org/10.1007/s002170000165>

Wilson, J. D., Bechtel, D. B., Wilson, G. W. T., & Seib, P. A. (2008). Bread quality of spelt wheat and its starch. *Cereal Chemistry*, 85(5), 629–638. <https://doi.org/10.1094/CCHEM-85-5-0629>

Yin, Y. an, Qi, J. cang, Li, W. hua, Cao, L. pu, & Wang, Z. bu. (2012). Formation and Developmental Characteristics of A- and B-Type Starch Granules in Wheat Endosperm. *Journal of Integrative Agriculture*, 11(1), 73–81. [https://doi.org/10.1016/S1671-2927\(12\)60784-6](https://doi.org/10.1016/S1671-2927(12)60784-6)

Zencirci, N., Ulukan, H., Baloch, F. S., Mansoor, S., & Rasheed, A. (2022). Ancient Wheats. In N. Zencirci, H. Ulukan, F. S. Baloch, S. Mansoor, & A. Rasheed (Eds.), *Ancient Wheats*. Springer International Publishing. <https://doi.org/10.1007/978-3-031-07285-7>

Zou, X., Wang, X., Li, L., Peng, P., Ma, Q., Hu, X., & Appels, R. (2022). Effects of Composition and Strength of Wheat Gluten on Starch Structure, Digestion Properties and the Underlying Mechanism. *Foods*, 11(21). <https://doi.org/10.3390/foods11213432>

Chapter 5 Conclusion and Future Direction

5.1 Conclusion

This thesis is composed of two chapters. The first study investigated the nutritional characteristics of the ancient wheat species einkorn, emmer, and spelt, comparing them with Canada Western Red Spring (CWRS) wheat. The second study focused on understanding the physicochemical properties and differences between ancient wheat species and CWRS wheat.

***All ancient wheat samples analyzed were whole grains, while the common wheat (CWRS) samples included both whole wheat (CWRS WW) and refined flour (CWRS RF).*

The findings from the first study can be summarized as follows:

Chemical Composition

1. **Proximate Composition:** Protein content was highest in einkorn, while starch content was highest in CWRS RF, with emmer exhibiting the highest starch content among the ancient wheat species.
2. **Arabinoxylan Content:** CWRS WW had significantly higher AX content ($p < 0.05$), followed by einkorn, with CWRS RF containing no detectable AX content.
3. **Amino Acid Profile:** Lysine was identified as the limiting amino acid across all samples.
4. **Phenolic Acid (PA) Content:**
 - Einkorn exhibited significantly higher free PA content (6.62 mg/kg) ($p < 0.05$), while CWRS RF had the least amount (1.50 mg/kg).

- Conjugated PA content was significantly higher ($p<0.05$) in einkorn (63.93 mg/kg) and CWRS WW (63.16 mg/kg), compared to CWRS RF, which had the lowest value.
- Spelt exhibited the highest bound PA content (1019.79 mg/kg), followed by einkorn (749.39 mg/kg). In contrast, CWRS RF had the lowest bound PA content (58.66 mg/kg), consistent with the trend observed across all PA fractions, likely due to the removal of the bran during the refining process.

Digestibility Characteristics

1. *In vitro* Starch Digestibility:

- Rapidly Digestible Starch (RDS) was highest in CWRS RF bread, followed by emmer bread, while einkorn bread had the lowest RDS content. This trend is consistent with the initial starch content observed in the base flours.
- Slowly digestible starch (SDS) content was significantly higher ($p<0.05$) in CWRS WW and emmer breads, while spelt bread had the lowest SDS content.

- ### 2. *In Vitro* Glucose Release:
- CWRS RF bread had significantly higher glucose concentrations ($p<0.05$) at specific time intervals, whereas einkorn bread exhibited the lowest glucose release at the same time intervals. Emmer bread had the highest glucose release among the ancient wheat bread samples.

3. ***In vitro* Protein Digestibility (IVPD):** CWRS RF bread exhibited the highest IVPD (86.05), significantly higher ($p < 0.05$) than all other samples, followed by einkorn bread (84.29). Emmer bread displayed the lowest IVPD (80.60).

The findings from the second study can be summarized as follows:

1. **Kernal Morphology:** Einkorn kernels exhibited primitive characteristics, with a visually lower proportion of endosperm and an elongated shape. In contrast, emmer and spelt kernels displayed more developed endosperms than einkorn but were outperformed by common wheat (CWRS), which exhibited round, well-developed kernels with a higher endosperm proportion, supporting higher milling output.

2. Starch Properties

- Einkorn starch demonstrated the smallest median granule size (DV 50), reflecting a predominance of smaller granules. In contrast, emmer exhibited the largest median granule size, indicating a higher proportion of larger A-type granules. Spelt presented intermediate granule sizes, while CWRS samples displayed moderate granule size characteristics, highlighting the variability in starch granule distribution among wheat species.
- No significant differences ($p < 0.05$) were observed in the amylose and amylopectin ratios across the starch samples from all wheat samples.
- DSC analysis highlighted distinct thermal profiles among the wheat species. Spelt exhibited the highest onset (T_0) and peak (T_p) temperatures, likely attributed to its higher starch crystallinity, which requires greater energy for thermal disruption. In

contrast, emmer demonstrated the lowest enthalpy. CWRS WW and CWRS RF displayed moderate peak temperatures and enthalpy values for gelatinization, positioned between those of einkorn and emmer.

- The swelling power of starch samples ranged from 10.29 to 11.40 g/g. Spelt exhibited the lowest swelling power (10.29 g/g), which may be attributed to its ordered crystalline structure that restricts granule expansion. In contrast, einkorn, emmer, and CWRS samples displayed comparable swelling power, with no significant differences ($p < 0.05$)

3. Protein Composition

- Emmer exhibited the highest SDS-extractable HMW-GLU and LMW-GLU levels. Einkorn had the highest SDS-extractable gliadin content, contributing to a weaker but more extensible gluten network. Spelt had intermediate levels of gluten proteins, with notably higher gliadin and albumin/globulin levels in the SDS-unextractable fraction. SDS-unextractable HMW-GLU levels were significantly lower ($p < 0.05$) in the ancient wheat species compared to CWRS, which had higher levels critical for breadmaking. These findings suggest that processing modifications or blending may be necessary to optimize ancient wheat for bakery products.

The findings from Chapters 01 and 02 highlight the relationship between nutritional and physicochemical properties in ancient wheat species. Nutritional characteristics, such as starch digestibility are closely connected to physicochemical traits such as granule size distribution and thermal properties. These connections illustrate how chemical composition

and structural attributes influence functional and nutritional outcomes, emphasizing the importance of further investigations to deepen understanding and explore their applications in food systems.

5.2 Future work

The present study serves as a comprehensive assessment of ancient wheat species in terms of their nutritional characteristics and physicochemical properties, with a focus on understanding the variations between these species and common hexaploid wheat. Building on these findings, future research should expand to include genotype studies, with particular attention to genotype-by-environment interactions and their influence on key parameters. Such studies, conducted over multiple years and locations, will help identify cultivars with favorable traits for incorporation into breeding programs.

Given that this study employed *in vitro* experimental techniques, including starch digestibility and *in vitro* protein digestibility, there is a need for further validation through *in vivo* and clinical studies. These studies will be essential to better assess the health impacts and further validate the nutritional benefits of ancient wheat species.

Future research should also explore the allergenicity of ancient wheat species. Understanding their allergenic potential is crucial for ensuring consumer safety and unlocking their full potential in the food industry, particularly for individuals with wheat sensitivities and other related conditions.

Future research should prioritize the development of innovative product formulations that incorporate ancient wheat, potentially in combination with other cereals. These formulations could support protein complementation, meeting the increasing consumer

demand for nutritionally balanced and health-promoting food products. Optimizing technological processing to align with the unique compositional and morphological traits of ancient wheat will further enhance its applications in the food industry, enabling the creation of novel food products. Additionally, sensory evaluation will be critical to assess consumer acceptance, as sensory attributes such as taste, texture, and appearance play a significant role in determining market success.

Author Contribution

Chapter 2

Nutritional Characteristics and Physicochemical Properties of Ancient Wheat Species for Food Applications

A version of this chapter (section 2.1-2.8) has been published in Food Bioscience

Deyalage, S. T., House, J. D., Thandapilly, S. J., & Malalgoda, M. (2024). Nutritional Characteristics and Physicochemical Properties of Ancient Wheat Species for Food Applications. Food Bioscience, 105397.

<https://doi.org/10.1016/j.fbio.2024.105397>

Creative Commons Attribution 4.0 International License

Sathsara Thakshani Deyalage: Writing – original draft, **James Duncan House:** Writing – review & editing. **Sijo Joseph Thandapilly:** Writing – review & editing. **Maneka Malalgoda:** Writing – review & editing, supervision, resources, project administration, investigation, funding acquisition, conceptualization.

Chapter 3

Determining the Nutritional Potential of ancient wheat species: A closer look at starch and protein digestibility, and phenolic acid content

A version of this is currently in progress for future publication.

Sathsara Deyalage: Methodology, data collection, data curation, and formal analysis, writing-original draft, **Sijo Joseph Thandapilly:** Writing – review & editing, **James Duncan House:** Writing – review & editing, **Adam Franczyk:** data collection, writing – review &

editing, **Amanda Gomes Almeida Sá**: Writing – review & editing, **Maneka Malalgoda**: Writing – review & editing, supervision, resources, project administration, investigation, funding acquisition, conceptualization.

Chapter 4

Comparative Evaluation of Starch and Protein Characteristics in Ancient Wheat species

A version of this is currently in progress for future publication.

Sathsara Deyalage: Methodology, data collection, data curation, and formal analysis, writing-original draft, **Jae-Bom Ohm**: Data collection, writing – review & editing, **Kristin Whitney**: Data collection, Writing – review & editing, **Senay Simsek**: Data collection, writing – review & editing, **Maneka Malalgoda**: Writing – review & editing, supervision, resources, project administration, investigation, funding acquisition, conceptualization.