Development and characterization of resveratrol fortified bread: effects on dough performance, bread quality, and *in vitro* starch digestibility

By

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DEDICATION

I dedicate this thesis to my beloved mother and father, Sumitra Karki and Ganesh Bahadur Karki, whose love has motivated me; my brother, Suman Karki, whose encouragement lifted me; and my dearest husband, Anish Karki, whose unwavering support makes every step possible.

Table of contents

Abstract

The global increase in type 2 diabetes mellitus (T2DM) has driven research in functional staple foods that reduce postprandial glycemic responses. Bread, a primary carbohydrate source, is characterized by rapid starch digestion and a high glycemic index. Resveratrol (RSV), a dietary polyphenol with antioxidant, anti-inflammatory, and enzyme-inhibitory effects, can lower starch digestibility; however, its sensitivity to heat, light, and oxygen makes baked-food fortification challenging. This thesis study aimed to (i) develop resveratrol-fortified wheat breads using cost-effective protective coatings to improve RSV retention, dough performance, and in-vitro digestibility, and (ii) determine whether RSV and amyloglucosidase (AMG), alone or in combination, can modulate starch digestion while maintaining bread quality.

Mixolab was used to determine thermomechanical characteristics (water absorption, development time, stability, weakening), whereas Rapid Visco Analyzer described pasting behavior. In vitro enzymatic hydrolysis (Box-Lucas modeling) and α-amylase inhibition were used to assess starch digestibility; bread quality and sensory acceptability were evaluated in parallel.

In the first section, RSV was either added as a free compound or encapsulated in gels of corn starch, pea starch, xanthan gum, or locust bean gum (0.5% w/w). Regardless of mixing speed, mixing was the breadmaking step with the highest RSV loss. RSV-enriched gels, especially xanthan gum and corn starch, increased RSV recovery in dough (44.01% and 39.04% vs. 34.97% for free RSV) and restored dough stability relative to free RSV. RSV addition slowed starch hydrolysis, and bread slices with RSV-enriched corn starch gels had lower hardness (1086 g vs 2030 g) and higher cohesiveness (0.84 vs 0.72) than those with free RSV.

The second study involved supplementing white and whole wheat flours with RSV, AMG, or RSV–AMG. In white flour, AMG reduced peak viscosity, but RSV partially reduced this impact and increased setback, indicating improved retrogradation. The combined RSV–AMG treatment had the lowest hydrolysis rate ($k = 0.56\ min^{-1}$), indicating an extensive starch–polyphenol network that limited enzymatic access. In whole-wheat systems, bran and fiber influenced the reaction, with RSV increasing starch retrogradation during cooling and the AMG–RSV combination softening white crumbs while reducing RSV-induced hardness in whole-wheat crumbs.

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List of Figures

List of Tables

List of abbreviations

Abbreviation	Full form
AMG	Amyloglucosidase
CVDs	Cardiovascular diseases
DGMO	Decaglycerol monooleate
EGCG	Epigallocatechin gallate
EU	European Union
FOS	Fructo-oligosaccharides
GI	Glycemic index
GL	Glycemic load
HbA1c	Glycated hemoglobin A1c
HDL	High-density lipoprotein
IDF	International Diabetes Federation
LBG	Locust bean gum
NaCas	Sodium caseinate
OSA	Octenyl succinic anhydride
RDS	Rapidly digestible starch
RS	Resistant starch
RSV	Resveratrol
SDS	Slowly digestible starch
T2DM	Type 2 diabetes mellitus
UV	Ultraviolet
V6/V7	V-type amylose single-helix inclusion complexes (six or seven glucose units)
W/O/W	Water-in-oil-in-water (double) emulsion
XG	Xanthan gum

Chapter 1

1.1 Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has been significantly increasing globally due to rising rates of obesity and sedentary lifestyles and is a serious health concern. T2DM is a set of metabolic diseases characterized by elevation of glucose levels in the blood which led to defective insulin secretion, resistance to insulin action, or both. According to the International Diabetes Federation (IDF) estimation, 589 million adults (20–79 y) live with diabetes worldwide (2024), a figure that is expected to increase to 853 million by 2050. According to Public Health Agency of Canada (2025), around 3.8 million people in Canada over a year old live with diagnosed diabetes (9.6% of the population) among which 10% are type 1 and 90% are T2DM. There is 3.3% average annual increase in prevalence. Most cases of T2DM are concentrated in low- and middle-income countries where access to care is uneven. These trends highlight the necessity for prevention and management approaches that are efficient, cost-effective, and simple to incorporate into daily routines.

Researchers have been only focusing on the development of novel medicinal interventions for the treatment of metabolic disorders like obesity, diabetes and cardiovascular disease. Dietary modification remains central to non-pharmacological management of metabolic disorders, emphasizing preferential selection of low–glycemic index (GI) and low–glycemic load (GL) carbohydrate sources while limiting rapidly absorbed, high-GI/GL carbohydrates that provoke disproportionate postprandial insulin responses (Banaszak et al., 2022). Hence there is a research gap in developing non-pharmacological strategies for the prevention of these disorders (Banaszak et al., 2022).Accordingly, novel dietary interventions accessible to everyone is needed in our daily

diet. Diet is one of the most effective non-pharmacological strategies for the prevention and management of metabolic diseases and can be accessible to all the public. Although fruits, vegetables, and grains which contain bioactive compounds have shown promise in alleviating metabolic disorders, many people are still unable to follow these dietary recommendations because of various factors like cost, accessibility, taste preferences, time constraints, and ingrained eating habits (Teodoro, 2019). Furthermore, it is challenging to classify specific food groups for specific diseases due to the wide variety of bioactive substances they contain. As a result, it is critical to recognize and incorporate valuable bioactive components which are effective to cure specific diseases in our regular diet. By doing so, people may make sure they are getting the most out of these advantageous substances, which may enhance their general health and reduce their chance of developing metabolic diseases.

Polyphenols are a prominent class of diet-derived bioactive that can influence both routes. Such as: they may bind carbohydrate-digesting enzymes to lower activity, and also reorganize starch microstructure during processing (Echave et al., 2024). The strongest dietary α-amylase inhibitors are tannins (such as tannic acid), which bind at or close to the catalytic pocket via hydrophobic interactions and H-bonding. Polyphenols frequently exhibit mixed/competitive kinetics with noticeable alterations in the conformation of the enzyme (Zhong et al., 2024). Cocoa and grape proanthocyanidin oligomers are similarly potent inhibitors, and their activity tends to increase with the degree of polymerization. Spectroscopy and docking investigations reveal that these oligomers occupy substrate subsites and form stable, ineffective complexes (Ćorković et al., 2022). By disrupting the enzyme's structure through non-competitive or multi-site processes, galloylated catechins like EGCG (Epigallocatechin Gallate) and quercetin inhibit human pancreatic α-

amylase, which is associated with decreased catalytic turnover *in vitro* and slowed starch digestion *in vivo* (Forester et al., 2012).

Among dietary bioactives, resveratrol (RSV), a stilbene present in grapes, berries, and peanuts, has garnered significant interest for its possible antidiabetic properties. Mechanistically, RSV may diminish post-meal glucose spikes by inhibiting carbohydrate-digesting enzymes: *in vitro* research indicates inhibition of α-glucosidase and, in more recent studies, human salivary and pancreatic α-amylases (Ma et al., 2023; Visvanathan et al., 2024). In addition, metabolically, Bhatt et al. (2012) examined if oral RSV can enhance the glycemic control of T2DM patients, and found beneficial effects with adaily dose of 250 mg resveratrol capsule for a period of 3 months. The results of this study indicate that taking resveratrol supplements orally can help with glycemic control and may even be used as an adjuvant to treat and manage diabetes. Furthermore, meta-analyses and systematic reviews indicate that RSV may improve insulin levels, insulin resistance, fasting glucose, and HbA1c in people with T2DM (Hoca et al., 2023; Zhu et al., 2017). Although the vast majority of clinical trials have showed beneficial metabolic effects of resveratrol, a few (trials) have reported neutral results, highlighting dose, length of the trial, and variation among populations as outcome influencing factors (Delpino & Figueiredo, 2022). Because postprandial glycemia is mainly determined by the rate at which starch is converted to glucose in the small intestine, combining an enzyme-modulating polyphenol like RSV with slowly digestible starch fractions is a viable dietary approach for lowering glycemic effect.

Incorporating RSV into food products faces challenge with regards to the stability of RSV because it is susceptible to isomerization and oxidation when exposed to heat and light. Currently, techniques like encapsulation or complexation can help (Đorđević et al., 2015; Zabot et al., 2022). In cereals, starch offers an inexpensive, food-grade matrix with potential to (i) shield RSV from

oxidation/UV and (ii) alter starch digestibility through matrix interactions. Recent theoretical and experimental studies show that hydrolyzed or gelatinized wheat starch can form complexes with RSV that protect it from degradation and may regulate its release during digestion; similar starch-polyphenol complexes are known to modify starch's physicochemical properties and reduce digestibility (Wu et al., 2022). Despite proven metabolic benefits, the use of resveratrol has been confined to nutraceutical supplement in the form of capsule. Enriching foods with RSV could be an excellent carrier for this bioactive compound. Ahmad and Gani (2021) incorporated nano encapsulated resveratrol (0.4%) in extruded snacks and assessed antidiabetic effect and anti-obesity effect. The result showed antidiabetic activity of 23.23–63.23% via α-glucosidase inhibition, anti-obesity activity of 24.86–44.46% via pancreatic lipase inhibition, and 59.58–74.10% via cholesterol esterase. Hence, its incorporation in everyday non-seasonal foods would be an inexpensive and accessible way of consuming resveratrol. Incorporating RSV into a bread matrix, such as with γ-cyclodextrin or zein nanoparticles, has been demonstrated to reduce loss and maintain bioaccessibility throughout baking (Silva et al., 2022).With development of such food products will open opportunities for marginalized communities and financially disadvantaged individuals to reap its health-promoting effects as part of their regular diet.

Bread is an excellent carrier for polyphenols since it is a staple and cost-effective foods which we eat in daily life. Its interior crumb remains below the boiling point during baking (the center usually stabilizes around 95–98°C), and its starch–protein structure can effectively trap and interact with phenolic which can contribute to both their retention and a slower digestion of starch (Kinner et al., 2021). These temperature conditions and structural properties are well established in baking science (the crumb reaches approximately 98°C at the end of baking, however the crust is significantly hotter) (Kinner et al., 2021). This suggests, crumbs are suitable for many polyphenols

that are sensitive to heat but remain fairly stable around the thermal plateau of the crumb. For example, breads fortified with grape seed extract demonstrated a higher antioxidant capacity, breads made with green tea polyphenols changed dough rheology properties and decreased the bread's *in vitro* glycaemic potential, and breads made with seaweed/plant polyphenols changed texture while increasing bioactive compounds (Peng et al., 2010; Wilcox et al., 2021). In practice, fortifying bread has repeatedly increased phenolic content/antioxidant activity and allowed matrix–polyphenol interactions that may also slow starch digestibility. Anthocyanin-rich wheats (purple/blue) preserve quantifiable pigments after baking (losses occur, but the product is still enriched) (Francavilla & Joye, 2022). Overall, these examples demonstrate why a familiar, everyday food like bread may supply polyphenols in important levels while preserving consumer-friendly quality.

1.2 Hypothesis

We hypothesize that RSV addition slows down *in-vitro* digestibility, and additionally, coating RSV with food-grade starches/hydrocolloids will protect RSV during breadmaking and improve dough rheology, digestibility and bread quality.

1.3 Research Objectives

1.3.1 General Objective

To develop resveratrol fortified bread and explore the resveratrol effect in the dough performances, bread quality and *in vitro* digestibility.

1.3.2 Specific Objectives

1. To compare different starch/hydrocolloid coatings of RSV on the thermomechanical and pasting properties, as well as the *in-vitro* starch digestibility, of the resulting wheat flour system.

2. To characterize the bread quality and sensory acceptability of resveratrol-fortified bread.
3. To evaluate the effect of RSV-hydrolyzed starch interaction on dough rheology, RSV retention, *in-vitro* digestibility, and the overall quality parameters of the finished bread product

1.4 Work plans

This study is organized into the following chapters:

Chapter 1: The introduction gives background information and context regarding the importance of resveratrol fortified bread. This chapter also reviews the broader field of study, highlights previous research, and identifies gaps that the current study aims to address.

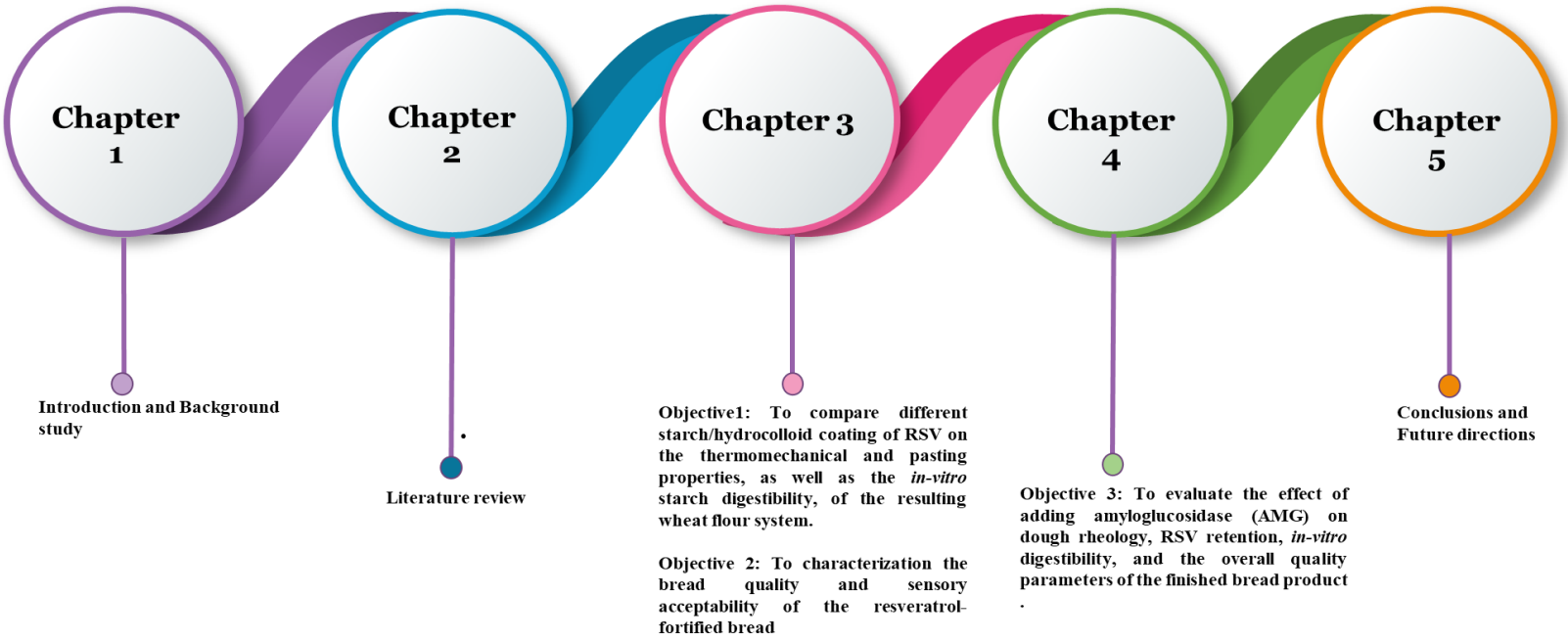
Chapter 2: The literature review explains the resveratrol's properties and health benefits. Moreover, this chapter explains the potential inhibitory effect of resveratrol and mechanism of this effect reported from existing research studies. Additionally, this chapter highlights significant research gaps in current knowledge that underscore the necessity for further research in this area.

Chapter 3: This chapter evaluates the impact of the physical constraints associated to the breadmaking process with RSV availability. In addition, the effectiveness of starch and hydrocolloids gels is evaluated by assessing the effects on dough mixing properties, bread quality, and *in vitro* starch hydrolysis. This chapter also highlights the sensory acceptability of resveratrol fortified bread.

Chapter 4: This investigation examines effect of addition of amyloglucosidase enzyme, and how its effect with RSV can increase RSV effectiveness as a bread ingredient. Additionally, it also determines enzyme-RSV effect in the pasting and *in-vitro* digestibility of breads.

Chapter 5: The final chapter summarizes the key findings and highlights the potential future research work.

A schematic and graphical representation of the research performed in this thesis is presented in the following flow diagram:



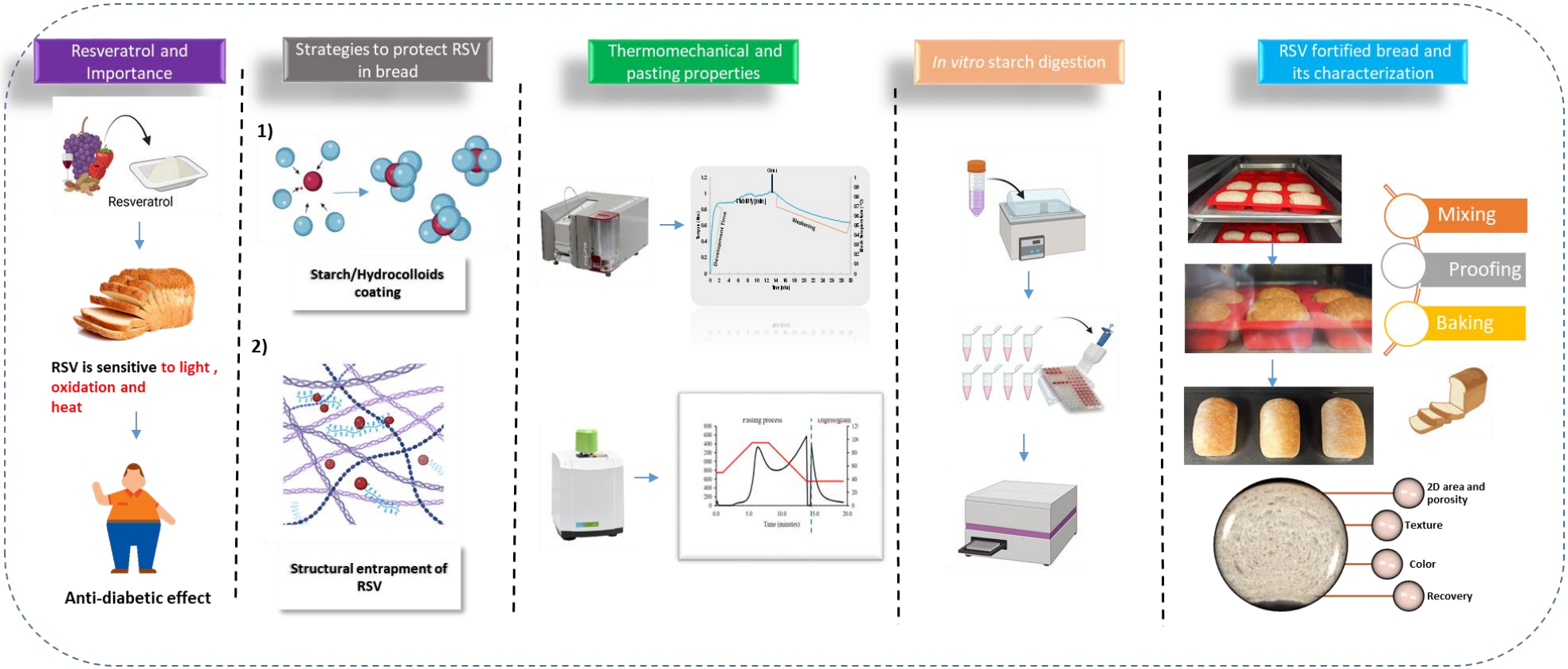


Fig 1.1 Overall graphical abstract of thesis. Created with BioRender.com

Chapter 2

2.1 Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) belongs to the category of non-flavonoid bioactive compounds called stilbenes. Stilbenes are characterized by having two phenol rings connected by an ethylene bridge as shown in Fig 2.1. This compound is a phenolic stilbenoid that plants synthesize as a phytoalexin when subjected to stresses such as fungal infection, wounds, or UV light. Resveratrol was first characterized in grapevine tissues and later confirmed across Vitis species (Langcake & Pryce, 1976), it is found predominantly in the skins of red grapes, red wine, peanuts, and other plants. Resveratrol has two isomeric forms: *trans* and *cis*, but trans-resveratrol is the most stable and biologically active. Trans-resveratrol has a molecular structure like diethylstilbestrol, a synthetic estrogen.

Trans form of RSV is predominant in terms of its prevalence, and distinct biological activities are assigned, particularly in producing cellular responses such as arrest of cell cycle transformation, apoptosis, and to boost cancer cell anti-proliferation (Akinwumi et al., 2018). Generally, *trans*-RSV demonstrates higher therapeutic characteristics than *cis*-RSV (Choi et al., 2022). Moreover, *trans*-resveratrol has been identified as an effective antiglycation agent and can be incorporated into functional foods or pharmaceuticals (Shen et al., 2017). The hydroxyl group in RSV improves insulin sensitivity and glucose metabolism in type 2 diabetes patients, enhancing glycemic control and cardiometabolic parameters (Ito-Nagahata et al., 2013).

2.2 Sources of resveratrol

Resveratrol is a polyphenolic chemical prevalent in plant families such as *Vitaceae, Dipterocarpaceae, Gnetaceae, Cyperaceae*, and *Leguminosae*, which include edible and non-edible species.

RSV can be found in a variety of foods, including grapes, wine, grape juice, mulberries, cranberries, cranberry juice, and peanuts which is shown in table 2.1. *Polygonum cuspidatum* is also called itadori or Japanese knotweed. Its roots are used in Eastern Asia as a medicinal tea (itadori tea in Japan) to cure inflammation, diarrhea, atherosclerosis, scalds, and hepatitis. RSV is among the key biological chemicals, along with emodin, piceid, and polydatin (Lei et al., 2016)

Table 2.1 Sources and content of resveratrol in different plant source.

Fruit Source	**Scientific Name**	**Content**	**Reference**
Common grapes	*Vitis vinifera*	36.6 μg g^{-1}	(B. Tian & Liu, 2020)
Red wine		0.27 mg per 100 ml	(B. Tian & Liu, 2020)
Mulberry	*Morus* sp.	7.95 μg g^{-1}	(Cione et al., 2019)
Peanut	*Arachis hypogaea*	0.4 μg g^{-1}	(Cione et al., 2019)
Blueberries	*Vaccinium* sect. *Cyanococcus*	6.7 μg g^{-1}	(Cione et al., 2019)

Phytoalexins play a role in plants' natural defense mechanisms against diseases. Researchers in plant biology have confirmed that RSV is one of the phytoalexins that aid plants in fighting off stress and infections. Many epidemiologic studies demonstrated that RSV is effective in the prevention of some diseases, such as CVDs and cancer. Moreover, different studies made in the laboratory and those carried out on individuals showed that RSV fights diseases such as cancer,

obesity, liver disorders, diabetes, cardiovascular disorders, Alzheimer's disease, and Parkinson's disease by activating redox/inflammatory/immune signaling pathways and its interactions on lipid and glucose metabolism pathways (Lin et al., 2014).

2.3 Health benefits of resveratrol

Resveratrol has been demonstrated to have cardioprotective effects (Raj et al., 2021), it also reversed hyperglycemia and hyperlipidemia in an animal model (Louis et al., 2012) by significantly reducing serum glucose level and serum triglycerides. Additionally, combining resveratrol supplementation with medication also prove to be advantageous for patients with T2DM as it lead to significant reductions in blood glucose levels, HbA1c levels, insulin levels and insulin resistance along with improvements in HDL levels (Movahed et al., 2013).

2.4 Safety dose of resveratrol

According to Brown et al. (2024), researchers have been using dose of resveratrol from 5 to 5000 mg for different clinical trials. However, studies have shown that consuming ≤1 g RSV once in a day is well-tolerable (Brown et al., 2024). According to the Natural Health Product by Health Canada (2025), the recommended level of resveratrol is 1g of RSV per day (Health Canada, 2025). In addition, as per the EU Commission Implementing Regulation (2021), the recommended level of the *trans*-resveratrol is not more than 150 mg/day. Overall, for the decrement in plasma glucose and insulin and reduce insulin sensitivity or in the management of T2DM, resveratrol supplementation at a dose of less than 1 g/day had showed significant effect (Hosseini et al., 2020).

2.5 Chemical properties of resveratrol

Resveratrol is chemically unstable, and heat and light accelerate its conversion from trans to cis, resulting in photo isomerization and additional oxidative processes (such as cyclization/closed-

ring products), necessitating heat and oxygen protection for any dietary use (Yoshinaga et al., 2024). Resveratrol is sensitive to light, pH, and increased temperature because of its unstable hydroxyls and C-C double bond. Resveratrol has an extremely poor water solubility (<0.03 mg/mL) and is moderately dissolved in dimethyl sulfoxide (16 mg/mL) and ethanol (50 mg/mL) (Choi et al., 2022). It has two benzene rings with a total of three hydroxyl groups attached. Moreover, it is extremely vulnerable to UV radiation, which is the process that converts *trans*-RSV to *cis*-RSV (Choi et al., 2022). Therefore, RSV has some disadvantages when it comes to providing health benefits during consumption, including being unstable against light, heat, and enzymes (Navarro et al., 2018). According to R. Silva et al. (2017), under air atmosphere and nitrogen atmosphere, resveratrol is thermally stable up to 240°C. After thermal degradation at 380°C in nitrogen atmosphere, the main residual products were reduced resveratrol and its dimers. Resveratrol is also susceptible to oxidation, leading to its loss of bioactivity. Resveratrol loss due to air oxidation was influenced by the protein carrier, with soy protein isolate showing the highest oxidability, impacting resveratrol stability during storage (Yin et al., 2022). Despite having excellent bioactive qualities, resveratrol is not widely used as a bioactive agent in the food or pharmaceutical industries for these reasons. One potential solution to this issue is the use of resveratrol in capsulated form. Research has been conducted on the encapsulation of resveratrol in different forms; however, none have examined the application of encapsulated resveratrol in the development of functional food products, nor how processing affects the bioactive properties of the component.

Besides its potential to incorporate RSV in different foods, there is a challenge in its retention in the foods because resveratrol has lower solubility and is highly prone to loss due to baking temperatures. So, to prevent its degradation and enhance the bioavailability, there is a necessity to

develop optimal delivery systems. There are different delivery options that have been developed like hydrogels, nanoparticles, emulsions etc., which are based on native protein, polysaccharides, or other food sources (Summerlin et al., 2015). These systems not only protect bioactive compounds from degradation but also facilitate delivery into the targeted food via sustained release. Hence carbohydrate matrix to encapsulate RSV seems a feasible alternative due to its non-toxic, biological compatibility and adhesive properties.

2.6 Protection of resveratrol

Yang et al. (2024) showed a dietary strategy for the RSV molecular inclusion using starch-derived hosts. Linear amylose can form V-type single-helix inclusion complexes (V6/V7) with RSV. As the amylose content increases, so does the level of inclusion and helical/crystalline order, creating a tightly packed hydrophobic environment that restricts O_2/light exposure and decreases molecular mobility. Hence, these effects are associated with lower *in-vitro* digestibility. Mechanistic calorimetry and simulations of short amylose-RSV show that CH-π and H-bonding form a V7 complex, which explains the improved thermal and photo-stability seen in cereal matrices (Yang et al., 2024). Incorporating RSV into porous corn-starch granules has also shown reduced oxygen penetration (by tortuous diffusion) and thermal/mechanical stress during mixing and baking; when combined with bread. The carrier withstands processing and facilitates controlled release during digestion (Wahab & Janaswamy, 2025). Pickering or emulsifier systems based on starch avoid coalescence and oxygen transfer at interfaces for oil-based delivery. Octenyl succinic anhydride-modified (OSA) starch particles (such as OSA-rice or quinoa starch) result in RSV-loaded emulsions with high encapsulation efficiency and great thermal/oxidative stability, making them ideal for batters and fillings (Matos et al., 2021).

Hydrocolloids enhance these dispersions: Xanthan + Locust bean gum (XG/LBG) mixes improve the physical stability of water-in-oil-in-water (W/O/W), double emulsions and preserve incorporated polyphenols from oxidation (H. Tian et al., 2021). Edible films and coatings create a barrier layer on the product surface, slowing O_2/light exposure and controlling release. Sodium-alginate films loaded with RSV (alone or in combination with thymol) prevent lipid oxidation while maintaining sensory quality during refrigerated storage, demonstrating that alginate networks are beneficial for antioxidant delivery and oxygen shielding (Hashemi et al., 2023). RSV nano-emulsions in yogurt improved physicochemical properties and maintained RSV through *in-vitro* digestion, illustrating how protein–lipid–hydrocolloid systems preserve this stilbene under realistic processing and storage conditions.

2.7 Resveratrol incorporation in food

Bread has been a leading product in baked foods, incorporating RSV via γ-cyclodextrin inclusion complexes or zein nanoparticles improved RSV stability and *in vitro* bioaccessibility compared to the free compound, thus validating bread as a feasible vehicle for stilbenes (Silva et al., 2022). A follow-up preclinical study by the same authors reported cardiac antifibrotic effects in fructose-fed rats using RSV-bread, thus demonstrating that technological retention can translate to biological signals *in vivo* (Silva et al., 2023). Snacks, crackers and cookies made with RSV-loaded emulsions and free RSV were developed and tested for consumer acceptance, and the results showed that RSV altered dough/crumb properties (P. M. Silva et al., 2024).

Addition of RSV to wheat flours in noodle (white-salted noodles) modified texture and enhanced functional attributes in the cooked product indicating compatibility with hydrated gluten-starch networks typical of Asian noodles. Also, 0.5% RSV addition led to a decrease in rapidly digestible starch and an increase in slowly digestible starch (He et al., 2020).

With regards to dairy and fermented foods, resveratrol-loaded nano-emulsions, especially those stabilized with sodium caseinate and decaglycerol monooleate (NaCas/DGMO) significantly improved resveratrol stability and bioaccessibility in yogurt. The fortified yogurts maintained desirable texture and nutritional profiles making them promising functional food products (Shi et al., 2024). In plant-based milk, resveratrol, curcumin and quercetin were individually encapsulated in soy milk, among these encapsulated resveratrol showed the high gastrointestinal stability and bioaccessibility, however encapsulated curcumin showed good encapsulation but lower bioaccessibility, while encapsulated quercetin was less stable during digestion when and making it effective for functional fortification (Zheng et al., 2023).

Beyond cultured milks, ready-to-drink lemon juice fortified with γ-cyclodextrin–RSV inclusion complexes showed improved photothermal stability during storage (A. F. R. Silva et al., 2020). In meat products, replacing fat with resveratrol-loaded myofibrillar protein-chitosan pickering emulsion increased the oxidative stability of pig patties without compromising pH or cooking yield. The emulsion increased antioxidant activity while improving meat texture, indicating a high potential as a fat substitute in low-fat meat products (Wang et al., 2025).

2.8 Polyphenols as α-amylase inhibitors

The most effective treatment for T2DM is to achieve ideal blood glucose levels after meals (post prandial glucose levels). Pancreatic α-amylase is an endo-hydrolase enzyme plays a crucial role in the starch hydrolyzing process by affecting 1, 4-glucosidic linkages in starch, glycogen, and various oligosaccharides. When simpler sugars are released by this enzymatic activity such as α-glucosidase, they are transformed into glucose for intestinal absorption. Consequently, inhibiting α-amylase activity leads to a reduction in the bioavailability of oligosaccharides and absorbable sugars, resulting in decreased postprandial hyperglycemia.

In recent times, investigation was conducted on plant source extracts to establish their potential in inhibiting starch hydrolyzing enzymes. In starchy foods, polyphenols inhibit the digestion of starch through two complementary mechanisms: direct inhibition of α-amylase and "matrix" effects that make starch physically more difficult for enzymes to access (Sun & Miao, 2020). When green tea catechins (0.45–2% w/w) were added to bread, the *in-vitro* glycemic potential was dramatically decreased, but most catechins were retained after baking or steaming, which is consistent with α-amylase/α-glucosidase inhibition as the main mechanism (Goh et al., 2015). Anthocyanins provide a second well-characterized example where both mechanisms operate: they can act as mixed-type α-amylase inhibitors and critically, form inclusion or surface complexes with starch that lower rapidly digestible starch (RDS) and raise slowly digestible starch (SDS) and resistant starch (RS) (Zhang et al., 2024). Condensed and hydrolyzable tannins, such as grape-seed proanthocyanidins also slow down digestion by binding α-amylase and co-structuring starch. In breadmaking, adding proanthocyanidins reinforced the dough or crumb and was linked to lower *in-vitro* starch digestibility, but cross-source comparisons reveal strong RS increase when starch complexes with these phenolics (Jiang et al., 2023).

2.9 Resveratrol as a potential α-amylase inhibitor

According to Aleixandre and Rosell (2022), phenolics which contain more than one hydroxyl group are more active against α-amylase, thus confirming that the quantity of hydroxyl groups within the phenolic structure is vital to the inhibition of digestion of starch. This study highlights that the RSV which has three hydroxyl groups can show higher inhibitory properties against α-amylase enzyme which is shown in Fig. 2.1.

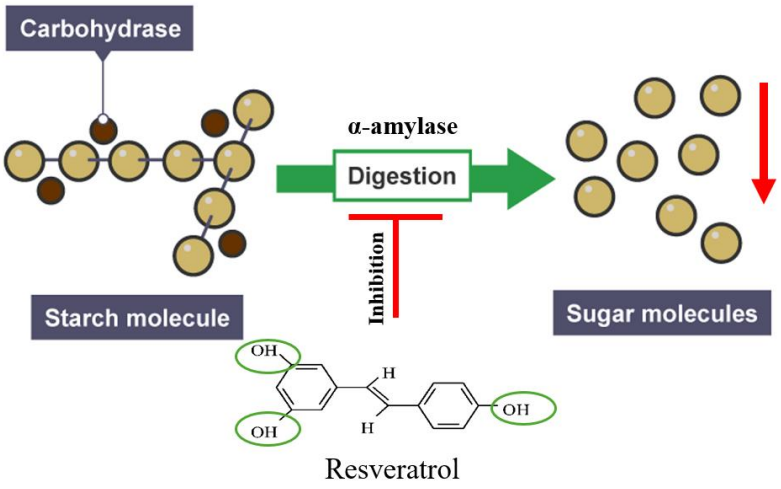


Fig 2.1 Schematic of α-amylase inhibition by resveratrol in starch digestion
Image adapted from BBC. (n.d.). https://ichef.bbci.co.uk/images/ic/976xn/p09pykz9.png

Resveratrol demonstrates promising effects against starch hydrolysis via two complementary pathways. Firstly, various oligomeric stilbenes sourced from RSV (such as vaticanol B and (−)-hopeaphenol) demonstrate moderate, competitive inhibition with low-micromolar effectiveness, although the RSV monomer typically acts as a weak direct inhibitor of human α-amylase. This shows strong evidence that stilbene structures can significantly inhibit amylase activity (Visvanathan et al., 2024). Secondly, and probably more pertinent in cereal products, RSV interacts with a matrix-structuring mechanism: it creates V-type inclusion complexes with amylose (including V7 motifs), which are stabilized by hydrophobic, CH–π, and hydrogen-bond interactions, thereby compressing starch packing, restricting enzyme access, and reducing *in-vitro* digestibility (Li et al., 2024; Yang et al., 2024).

In terms of food, cereal matrices can utilize these effects: RSV addition to white-salted noodles has been associated with reduced rapidly digestible starch and higher slowly digestible starch, alongside acceptable texture (He et al., 2020). Additionally, in bread formulations that embed RSV in protective carriers (e.g., porous starch granules or inclusion complexes) RDS shifts toward

SDS/RS compared with free RSV. This indicates a practical route to slow starch hydrolysis in starchy food products (Silva et al., 2022; Wahab & Janaswamy, 2025).

2.10 Bread: vehicle for nutrients and bioactive compounds

Bread is a suitable matrix for functional meals because it is widely consumed around the world. The widespread popularity of bread can be seen by the unprecedented production of nearly 180 million tons worldwide in 2020, with an average volume per human of 24 kg (Statista, 2022) . This massive production and consumption highlights bread's importance as a staple item in various societies and places around the world. Its accessibility, adaptability, and familiarity make it an ideal choice for integrating beneficial ingredients and improving its nutritional profile to meet a variety of health needs and tastes. Changes in lifestyles and innovative technologies have led bread to change from a traditional or local character to that of a handy food with a variety of nutritional or technological properties.

Bread has been used as carrier of different micronutrients. Pilz et al. (2018) recommended that the bread can be used as efficient vehicle for Vitamin D fortification. Additionally, Souza et al. (2022) suggested that incorporating vitamin D-fortified bread into diets could be an effective method for boosting both the average daily intake and serum levels of this essential nutrient. By fortifying bread with vitamin D, a commonly consumed staple in many diets, individuals may have an easier time meeting their daily requirements for this nutrient.

Micronutrient fortified breads have shown effectiveness in improving nutritional status and reducing the risk of chronic diseases among consumers. Fortification with essential micronutrients like calcium, sodium, and high-quality proteins enhances the nutritional quality of bread, making it a valuable dietary component for vulnerable groups (Bassett et al., 2022). Additionally, fortifying bread with critical micronutrients can effectively enhance nutritional status and reduce chronic

disease risks, aiding in transitioning towards healthier and more sustainable diets (Grasso et al., 2023). Flour fortification with essential micronutrients like vitamin A, iron, zinc, and B vitamins is crucial for improving public health in developing countries. . There are reports on calcium-fortified bread enriched with nutrients like phosphorus, selenium, zinc, and vitamin A (Nikitin et al., 2022), calcium from eggshell powder fortified bread (Platon et al., 2020) and calcium-fortified bread with high protein content (Bassett et al., 2022). These fortification strategies have shown improvements in bread quality, increased mineral content (such as calcium, iron, zinc), and enhanced protein levels. Incorporating micro-nutrients and bioactive compounds in staple foods like bread not only enhances its nutritional profile but also addresses deficiencies prevalent in certain targeted populations. The addition of these functional ingredients not only enhances the nutritional quality of bread but also contributes to reducing malnutrition and improving food security globally.

The ingredients that are necessary for baking bread are flour, water, yeast, and salt. These components enable the development of a visco-elastic dough that may be baked after being molded or shaped into the appropriate shape. Nevertheless, it must be considered that breadmaking involves thermal and biological constraints that might affect positively or negatively the structure and bioactivity of some compounds (Rosell, 2011). For instance, Morris and Morris (2012) reported that incorporation of fructo-oligosaccharides (FOS) and inulin lowered bread loaf volumes, increased crumb hardness and darker crust of bread which increased aroma characteristics. In addition, presence of polyphenols from *Ascophyllum nodosum* seaweeds in breads increased the hardness and chewiness and decreased the cohesiveness and resilience and reported increased in crumb hardening during storage (Montes et al., 2024).

Concluding remarks

Resveratrol is one of the most promising polyphenolic substances for the development of functional and health-promoting foods. Its unique biochemical structure with multiple hydroxyl groups provides antioxidant, anti-inflammatory, antiglycation, and glucose-lowering properties, making it especially relevant in the prevention and treatment of chronic diseases such as type 2 diabetes, cardiovascular disorders, and neurodegenerative conditions.

Despite its proven bioactivity, this compound's susceptibility to light, heat, and oxygen has limited its direct use in food systems. More recently, developments in encapsulation methods, as well as the utilization of starch- and protein-based delivery matrices, have shown that they can improve thermal and photo stability, bioaccessibility, and controlled release during digestion.

Bread emerges as a particularly suitable vehicle for the inclusion of resveratrol, combining widespread global use with the technological possibility of including protective carriers. The inclusion of resveratrol into bread and other cereal-based goods thus provides a feasible technique for creating functional foods that improve glycemic control and general health.

Future study should focus on optimizing encapsulation and processing conditions to enhance resveratrol stability and bioavailability, ensuring that this bioactive compound's health-promoting potential of this bioactive compound can be fully realized in practical food applications.

Chapter 3

DISCLAIMER: This chapter is based on the following published article: Karki S., et al. *Food Bioscience* (2025) Article 107796, https://doi.org/10.1016/j.fbio.2025.107796. The content has been adapted/expanded for the thesis, including additional sensory results.

Effect of hydrophilic colloids containing resveratrol on dough processing and bread quality

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Abstract

Resveratrol (RSV) is a bioactive polyphenol with proven health benefits including anti-diabetic effects, but its poor stability has limited food-based applications. This study aimed to examine the impact of the breadmaking constraints on RSV and to enhance RSV retention and functional efficacy in bread by protecting RSV with cost-effective gelling materials such as corn starch, pea starch, xanthan gum and locust bean gum. Free RSV or RSV-enriched gels were incorporated into flour (0.5% w/w) and mixed to check the RSV recovery. Furthermore, *in vitro* starch hydrolysis kinetics were assessed. Our results showed that mixing was the breadmaking stage with greatest impact on RSV recovery, and it was independent of the mixing speed. Mixolab analysis showed that RSV-enriched gels counteracted the dough stability reduction induced by free RSV. Corn starch and xanthan gum gels significantly improved RSV recovery from dough (44.01% and 39.04%, respectively), compared to free RSV containing dough (34.97%). Slower starch hydrolysis was observed when RSV was added in the dough. Importantly, RSV-enriched corn starch gels led to breads with reduced hardness (1086 g vs 2030 g) and higher cohesiveness (0.84 vs 0.72) compared to breads containing free RSV. This work highlights the potential of starch-hydrocolloid gels to stabilize RSV in bakery products and facilitate its incorporation into functional foods for metabolic health.

Keywords: Resveratrol; Stability; Bread; Starch; Hydrocolloid gels

3.1 Introduction

Resveratrol (RSV) is a naturally occurring polyphenolic compound known for its antioxidant, anti-inflammatory, anti-obesogenic, anti-diabetic, cardiovascular, and neuroprotective properties. These health benefits contribute to the prevention and management of various diseases through multiple biological mechanisms (Jabłońska et al., 2024; Yu et al., 2024). Furthermore, in pre-clinical and clinical studies, RSV has shown metabolic functions in managing type 2 diabetes by reducing fasting glucose, postprandial glycemic index, insulin, and improving lipid profiles (Ding et al., 2018; Khaleel et al., 2017; Ma & Zhang, 2022).

RSV consumption has been regulated by different health agencies, recommending a daily intake up to 150 mg of trans-RSV (EU Commission Implementing Regulation, 2021) or up to 1 g of RSV per day in the case of Health Canada (2021). However, RSV's inhibitory effect towards α-amylase enzyme has not been studied yet. α-Amylase is an endo-hydrolase enzyme which can play a crucial role in the starch hydrolyzing process, contributing to the released of sugars that in turn will increase the sugar level in blood and might results in hyperglycemia (diabetes) (Hua et. al., 2016). Consequently, inhibiting α-amylase activity leads to a reduction in the bioavailability of oligosaccharides and absorbable sugars, resulting in decreased postprandial hyperglycemia (Sun & Miao, 2019).

According to various authors, phenolic compounds can slow down and reduce the starch hydrolysis by the inhibitory action of hydroxyl groups within the phenolic structure against digestive enzyme (Figueiredo-González et al., 2019; Giuberti et al., 2020; Irondi et al., 2019). Aleixandre & Rosell (2022) reported that phenolic compounds containing more than one hydroxyl group are more effective hindering α-amylase activity, thus confirming that the number of hydroxyl groups within the phenolic structure plays a crucial role in modulating starch hydrolysis. However, no studies have been reported about RSV action on α-amylase activity.

Despite having excellent bioactive properties, RSV is highly susceptible to UV radiation, which induces the isomerization of *trans*-RSV to the less bioactive *cis*-form Choi et al. (2022). In addition, heat and enzymatic degradation make RSV molecule unstable with associated limitations in delivering health benefits upon consumption (Navarro et al., 2018). Consequently, its incorporation into food applications remains restricted. For this reason, RSV use has been confined to dietary supplements in various forms like tablets, capsules, and powders.

Lately, a growing body of research has focused on developing strategies to protect RSV from degradation during food processing. Alginate/chitosan-coating (Khan et al., 2019), γ-cyclodextrin and zein nanoparticles (Silva et al., 2022), and proniosomes (Shruthi et al., 2020) have been proposed to enhance RSV stability. Starch emulsions and hydroxypropyl methylcellulose (HPMC) (Silva and Neto, et al., 2024) and gelatinized or hydrolyzed wheat starch (Wu et al., 2022) have been also applied as RSV encapsulating materials for improving its chemical stability and bioavailability. These studies demonstrated that starch and hydrocolloids are promising candidates for RSV encapsulation due to their excellent film-forming properties, cost-effectiveness, and compliance with food-grade safety standards.

Currently, nano-encapsulated RSV fortified snacks (Ahmad & Gani, 2021), RSV-enriched noodles (He et al., 2020), cookies (Ou et al., 2018), and crackers (Cerqueira, et al., 2024) have been reported.

Incorporating RSV into every day, non-seasonal foods such as bread offers a promising strategy for delivering bioactive compounds through the regular diet. In fact, two studies have recently focused on the fortification of bread with RSV. Firstly, Silva et al. (2022) investigated bread fortified with both free and encapsulated RSV with γ-cyclodextrin and zein nanoparticles. The encapsulating system protected RSV from degradation during baking, enhancing its

bioaccessibility during digestion, and improving early symptoms of type 2 diabetes—such as reducing body weight loss and polydipsia in animal models (Silva et al., 2023). Secondly, (Wahab & Janaswamy, 2025) proposed the use of RSV encapsulated in porous (modified) starch for bread fortification, showing enhanced antioxidant activity of resveratrol, controlled intestinal release, and improved bread characteristics.

Previous mentioned studies have focused on encapsulation systems such as γ-cyclodextrin, zein nanoparticles, alginate/chitosan coatings, or modified starch carriers, primarily assessing antioxidant activity and bioaccessibility. However, to the best of the authors' knowledge, the effects of the breadmaking process on free RSV or RSV enriched gels, as well as its potential inhibitory activity against α-amylase, have not yet been investigated.

Our study addressed three main gaps: (i) the impact of breadmaking, particularly mixing and baking, on RSV stability and recovery; (ii) whether cost-effective starch and hydrocolloid gels can enhance RSV stability during processing; and (iii) the effect of RSV inclusion on bread quality. We hypothesized that mixing would be the most detrimental to RSV recovery, while starch/hydrocolloid gels could shield RSV from degradation, thereby improving recovery and functional properties. Therefore, this research aimed to evaluate the impact of the physical constraints associated to the breadmaking process on the availability of RSV. In addition, the effectiveness of starch and hydrocolloids gels (corn starch, pea starch, xanthan gum, locust bean gum) is evaluated by assessing the effects on dough mixing properties, bread quality, sensory quality and *in vitro* starch hydrolysis.

3.2 Materials and methods

3.2.1 Materials

RSV food grade powder (purity > 98%) was purchased from Split Science (Ottawa, Canada). Two starches were used in this study, corn starch, purchased from a local grocery store (Fleischmann's, Chicago, USA), and pea starch kindly provided by Roquette Ltd. (Portage la Prairie, MB, Canada). Xanthan gum (CP Kelco, Atlanta, USA) and locust bean gum (CP Kelco, Lille Skensved, Denmark) were employed as hydrocolloids for the preparation of RSV-based gels. Canadian Western Red Spring wheat flour (14.8% moisture content) was generously supplied by Cereals Canada (Winnipeg, Canada) and used for bread formulation.

3.2.2 Preparation of resveratrol enriched gels

Slurries were initially prepared with corn or pea starch (10% w/v) or different hydrocolloids (xanthan gum 0.5% w/v, locust bean gum 1% w/v). These concentrations were selected based on preliminary evaluations of solubility and gelling capacity. All dispersions were heated at 95-97°C for 15 minutes under constant stirring (500 rpm) to ensure uniformity. After cooling to 40°C, 1 g of RSV was incorporated and thoroughly mixed to achieve gelatinization and network formation. The resulting RSV-enriched gels were freeze-dried and stored at 4°C until further analysis.

3.2.3 Mixing properties and recovery of resveratrol

Mixing behavior of RSV incorporated flour during dough mixing was analyzed by using Mixolab (Chopin-Technologies, Villeneuve-la-Garenne, Cedex, France) and followed the time-temperature procedure as described by Rosell et al. (2007). All the Mixolab analysis were carried out using constant 60% water absorption (based on target consistency 1.1 Nm) at constant temperature 30°C using Chopin 'S' Protocol. RSV was incorporated into the dough at 0.5 g/100 g (w/w) based on flour weight, either in powder form or as an RSV-enriched gel. The impact of mixing speed (40,

80, and 200 rpm) and the use of different gel matrices for RSV encapsulation on its retention within the dough was evaluated. Dough thermomechanical properties, including water absorption (%), peak torque (Nm), dough development time (min), stability (min), and weakening (Nm), were assessed. Water absorption (%) refers to the percentage of water required for the dough to produce a torque of 1.1 Nm, peak torque is the highest torque indicating dough consistency, dough development time (min) is the time required for reaching that maximum dough consistency, stability (min) represents the time duration at which the sample sustains a steady torque, weakening (Nm) is the torque difference between the maximum torque and the torque after the 12 minutes of mixing. Samples were collected at the end of the mixing process (after 30 minutes) for RSV quantification (described in section 3.2.5).

3.2.4 Thermal properties and starch hydrolysis kinetics

The thermal properties and starch hydrolysis kinetics were analyzed using the Rapid Visco Analyzer (RVA) 4800 (PerkinElmer Inc., Shelton, CT 06484, U.S.A.) using different time-temperature profile settings as described by Santamaria et al. (2023). RVA simulates cooking process by applying controlled heating and shear to measure the pasting and viscosity properties of starch and flours. Three grams of RSV-fortified flour [wheat flour + 0.5 g/100 g RSV (w/w)], in either free or corn starch-coated form were placed into an aluminum canister and dispersed in 25 mL distilled water. Briefly, slurries were subjected to heating and cooling cycles following the device standard 1 protocol, but cooling down to 37°C, stopping for 30 s to add α-amylase solution (100 uL, 37°C), and further recording the viscosity for 5 min. Parameters recorded from the first part of the plot included apparent peak viscosity (maximum viscosity during heating, cP), trough viscosity (minimum viscosity after peak viscosity, cP), and setback viscosity (viscosity increase during cooling representing starch retrogradation, cP) and from the second part or digestogram,

the identified parameters included initial viscosity before adding the enzyme (cP), final viscosity (cP), and kinetic constant (*k*). These parameters were calculated using a Box-Lucas model (Eq. 1):

$$\mu = \mu_{\infty} + (\mu_{o} - \mu_{\infty})\, e^{-kt} \qquad \text{Eq. (1)}$$

Where, μ was the apparent viscosity (cP) at time t, μ_{∞} was the equilibrium viscosity, μ_{o} was the initial viscosity, *k* (min^{-1}) was the kinetic rate constant, and t (min) was the selected time point.

3.2.5 Quantification of resveratrol

RSV was quantified by following the method described by Ahmad & Gani (2021) with some modifications. Standard solutions were prepared by dissolving RSV in methanol (99.9% purity), followed by serial dilutions to obtain concentrations from 5 to 20 μg/mL. After getting the spectrum from 200 to 700 nm, the maximum wavelength (λmax) of 300 nm was selected to measure the absorbance and build the calibration curve.

For quantification of RSV in dough and bread samples, RSV was extracted by mixing the sample with methanol in a 1:10 ratio (w/v), followed by sonication in an ultrasonic bath (VWR International, Cortland, New York, USA) at 40 kHz for 10 minutes. The extracts were then centrifuged at 5000 rpm for 15 minutes using a centrifuge (Fisher AccuSpin Micro 17R, Germany), and the absorbance of the supernatant was measured in UV-Visible spectrophotometer (BioTek Epoch 2, Agilent Technologies, USA) at 300 nm.

The recovery percentage of RSV from the sample (dough or bread) was calculated using the following equation:

$$\text{RSV recovery (\%)} = \frac{\text{g of compound detected for 100 g of sample (dry weight basis)}}{\text{g of compound thereotically added for 100 g of flour (dry weight basis)}} \times 100$$

3.2.6 Breadmaking

Breadmaking was carried out following the scale-down procedure described by Espinosa-Ramírez et al. (2018). Bread recipe, based on flour content, consisted in 1 g/100 g dry yeast, 1.5 g/100 g

salt and 60 g/100 g water as determined by Mixolab. Wherever required, free RSV powder RSV and freeze-dried starch coated RSV were used at the concentration of 0.5 g/100 g (w/w). All the ingredients were mixed in a KitchenAid dough mixer (St. Joseph, Michigan, USA) at 25°C for 2 minutes at low speed followed by 5 minutes at medium speed. Dough portions (50 g) were transferred into mini pans and proofed at 30°C and 85% relative humidity (RH) in a proofing Chamber (AX-PR5, Axis, Italy)). Dough volume increase (ΔV) was recorded at 15-minute intervals over a 120-minute period. The proofing time was defined as the time required to reach 75% of the maximum dough expansion, as previously described by Beltrão Martins et al. (2022). Following proofing, the loaves were baked at 180°C for 15 minutes in an electric oven (CTB-1, GS Blodgett Corporation, USA).

3.2.7 Bread characterization

Breads were sliced to a thickness of 1 cm, prior to crumb color and texture analysis. Bread crumb color was analyzed by using a Croma Meter CR-400 (Konica-Minolta Sensing Americas, New Jersey, USA), following the procedure described by Espinosa-Ramírez et al. (2018). Color parameters L^* (lightness), a^* green (-) to red (+) and b^* blue (-) to yellow (+) were recorded according to the CIElab color space system. The difference in surface color (ΔE) of breadcrumbs was calculated, taking control bread as a reference.

The texture profile of crumb was determined using a texture analyzer (TA-XT-plus, Stable Micro Systems, Godalming, UK) equipped with a 5-kg load cell. A crumb disk of 20 mm diameter was compressed up to 50% using a 36 mm aluminum probe. A trigger force of 5 g was applied twice, with a pre-test speed of 1 mm/s, test and post-test speeds of 5 mm/s, and a 5-second pause between the two tests. The following parameters were calculated from the compression curve: hardness,

springiness, cohesiveness, chewiness, and resilience. Three bread slices from two different batches were used to analyze texture and color.

3.2.8 Sensory analysis of bread

Evaluation was conducted using a 9-point hedonic test (1 = dislike extremely; 9 = like extremely) by 60 untrained panelists (77% female, 23% male) for appearance, aroma, flavor, texture, and overall acceptability of two samples (Control and RSV_CS_Bread). Samples were blinded, presented in randomized order, and ratings were entered on tablets at partitioned workstations to minimize bias which is shown in Fig 3.1. All procedures complied with Research Ethics Board 2 (REB2), Protocol HE2025-0239 under the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2, 2022) and all participants provided written informed consent. To serve, loaves were taken out of the freezer, thawed, and portioned; full slices were 11 × 7.5 cm and were cut in half for tasting.

Fig 3.1 Sensory panel room

3.2.9 Statistical analysis

Statistical analysis of all samples was conducted using IBM SPSS statistics software (Version 20, Boston, USA). One-way analysis of variance (ANOVA) was used to determine significant

differences among samples at a 95% confidence interval, using Honestly Significant Difference test (HSD).

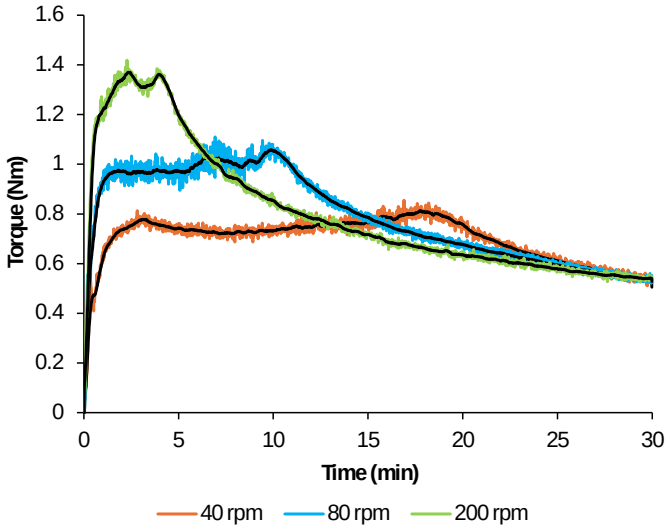
3.3 Results and Discussions

The integration of RSV into the breadmaking was systematically evaluated with particular focus on the impact of mechanical and thermal constraints on the RSV stability, besides the potential effect of RSV on the dough performance. Mixing stage allows the creation of the air bubbles inside the dough, including oxygen that could oxidase the RSV, whereas the thermal treatment during baking can decrease the availability of RSV. Because of that mixing at different speeds and thermal treatment of the dough were evaluated by analyzing dough properties and RSV recovery.

3.3.1 Impact of RSV addition to wheat flour and performance during mixing

Different mixing speeds were applied to obtain the dough in the presence of RSV and the final recovery after 30 min mixing was evaluated. The Mixolab plots of wheat flour with and without RSV at different speeds (40, 80 and 200 rpm) are shown in Fig 3.2 (A) and (B), respectively.

(A)



(B)

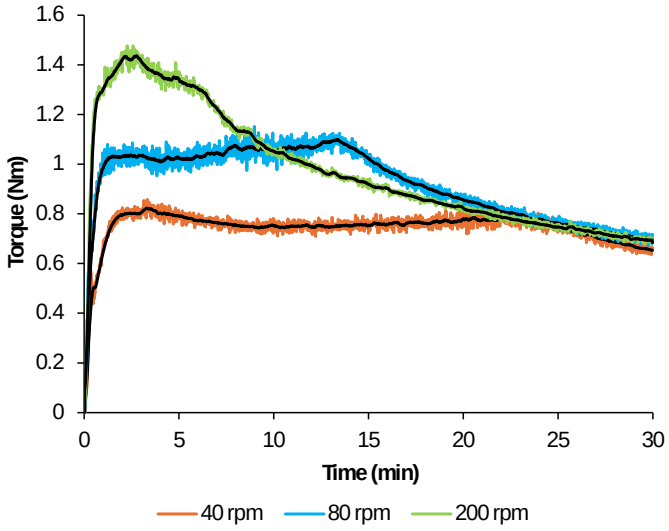


Fig 3.2 Impact mixing speeds on the dough performance in the absence (A) and the presence (B) of resveratrol (RSV) by using the Mixolab

As it was expected, mixing speed affected the dough consistency, reaching higher consistency with shorter development time when applying higher speed. The lowest mixing speed (40 rpm) allowed a smooth hydration of the constituents giving a longer stable consistency. Higher mixing speeds are leading to plots with different consistency peaks that reflect the diverse hydration rates of the flour constituents, particularly proteins and damaged starch. The inclusion of RSV did not modify the dough consistency but slightly changed the shape of the curves, with an additional prominent consistency peak after reaching the maximum consistency and a more pronounced decay of the consistency due to high mixing speed. This behavior suggests some interaction of the RSV with the dough constituents, particularly with gluten proteins. It has been reported that the addition of tea polyphenol (2%) decreases the formation of disulfide bonds which is essential for gluten network stability, resulting in weaker dough stability (Qin et al., 2022). This suggests that the hydroxyl group of RSV plays a key role in reducing gluten protein network stability.

The average RSV recovery after mixing was 33%, and not statistically significant (P>0.05) effect was observed with the mixing speed. The recovery reduction in the RSV may be attributed to the air incorporation during mixing, which can lead to the oxidation of RSV and its subsequent transformation into various metabolites. Lu et al. (2014) also reported a reduction in total phenolic content by 2 to 10% in a dough after mixing, depending upon the type of flour (refined and whole wheat). Agbadua et al. (2022) reported that RSV was highly prone to oxidation and readily reacts with reactive oxygen species, resulting in the formation of several oxidized derivatives such as dihydroresveratrol, viniferins, and 4′-dihydroxy-trans-stilbenes, thereby reducing the concentration of the parent compound. Likewise, the conformational changes in gluten proteins due to mixing may have exposed binding sites that facilitated interactions with the three hydroxyl

groups of RSV. This could have promoted strong gluten-RSV interactions, potentially hindering the recovery of RSV during the extraction process.

Initial mixing studies revealed the dramatic impact of mixing on RSV recovery, but since mixing speed did not have a significant effect, 80 rpm was selected for further studies. To improve the RSV stability, different hydrocolloids were tested for protecting RSV. The effect of RSV-enriched gels on the mixing parameters is illustrated in Table 3.1.

Table 3.1. Effect of the addition of free and coated RSV on dough mixing properties, determined with the Mixolab, and the RSV recovery after mixing.

Parameters	Control	RSV	RSV_CS	RSV_PS	RSV_XG	RSV_LBG
DDT (min)	3.17^{a}	3.85^{d}	3.78^{c}	3.60^{b}	4.30^{e}	3.68^{b}
Stability (min)	13.8^{b}	9.3^{a}	13.98^{b}	14.10^{c}	13.95^{b}	14.31^{d}
C-max (Nm)	1.099^{c}	1.06^{b}	1.03^{b}	1.03^{b}	0.98^{a}	0.96^{a}
Weakening (Nm)	0.34^{a}	0.41^{b}	0.33^{a}	0.34^{a}	0.32^{a}	0.33^{a}
RSV recovery (%)	0.01 ± 0.00^{a}	34.97 ± 0.86^{b}	44.01 ± 1.8^{d}	34.36 ± 0.71^{b}	39.04 ± 0.99^{c}	34.47 ± 0.55^{b}

DDT: Dough Development Time, RSV: Resveratrol, CS: Corn starch, PS; Pea starch, XG: Xanthan gum, LBG: Locust bean. Significant differences among means within each row are indicated by different letters, as determined by the HSD test.

Doughs made with RSV incorporated in xanthan gum gels (RSV_XG) exhibited a significantly ($P < 0.05$) longer dough development time (DDT) compared to the other formulations. This might be due to the strong water-holding capacity of xanthan gum which may reduce free water available for gluten hydration and thus slow the dough development process. The hydroxyl groups present in xanthan gum strongly interact with water, leading to increased water absorption which requires a longer time for gluten hydration and development (Rosell et al., 2001; Zannini et al., 2014). Interestingly, a tendency to strengthen the gluten network (stability) was observed when adding RSV-enriched gels as compared to free RSV, as indicated by the stability increase from 9.3 to 14.31 min. The inclusion of RSV within the hydrocolloids, might hinder its hydroxyl groups reducing the availability to interact with gluten. Th e results showed that doughs containing RSV-enriched locust bean gum (RSV_LBG) were characterized by higher stability ($P < 0.05$) as compared to other samples. A similar effect was observed in the study carried out by Li et al., (2019), where wheat dough containing locust bean gum showed the highest dough stability, followed by guar and xanthan gums, when all the hydrocolloids were added at concentrations of 0.2%, 0.6%, and 1%. In addition, a lower Cmax was observed in resveratrol (RSV) added samples compared to the control, which could be attributed to resveratrol's interaction with gluten or to the formation of a different structural network when included with hydrocolloids.

Further, longer mixing led to a decline in dough strength, which is likely attributed to gluten network degradation caused by excessive mechanical shear-induced protein weakening. Regarding weakening, the addition of RSV loaded starches or hydrocolloids brought about significantly higher ($P < 0.05$) dough consistency reducing the weakening of the dough observed in the presence of free RSV. Therefore, RSV-enriched gels led to wheat dough resisting the mechanical shear stress during overmixing as compared to control added with free RSV.

Regarding resveratrol recovery after mixing, RSV-loaded corn starch (RSV_CS) and xanthan gum (RSV_XG) showed significantly higher values, 44.01% (d.b.) and 39.04% (d.b.) respectively, as compared to RSV containing control. Corn starch provided effective protection for RSV, probably due to starch's known ability to shield RSV from environmental stressors such as light, heat, and oxygen. This protective effect might be attributed to physical barriers, hydrogen bonding, and hydrophobic interactions (Zhang et al., 2024), which contribute to the formation of stable matrices and inclusion complexes (such as V-type crystalline structures and nano-aggregates) that enhance RSV stability and reduce degradation (Yang et al., 2023).

3.3.2 Impact of temperature increase on the RSV stability and its effectivity as starch hydrolysis inhibitor

The RVA was used to evaluate the thermal treatment on the different RSV forms (uncoated and coated). Additionally, the standard RVA analysis was extended with the digestograms to examine the hindering action of the RSV on the starch hydrolysis Fig 3.3. The thermal impact of using different forms of RSV is shown in Table 3.3.

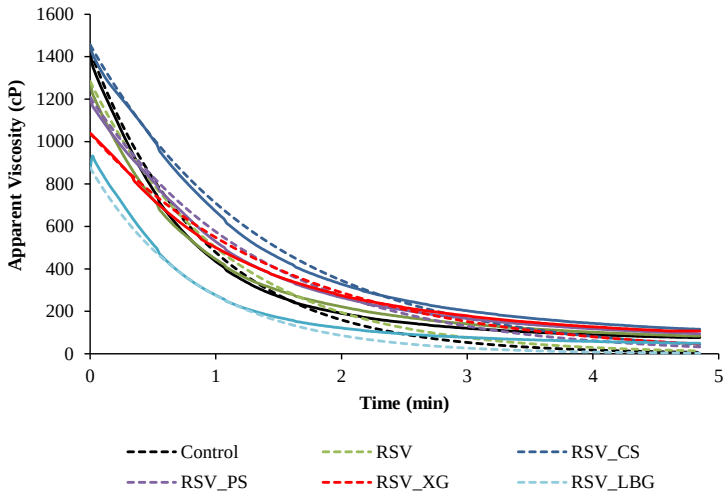


Fig 3.3 Digestogram plots, obtained with the RVA, of wheat flour supplemented with RSV coated using different hydrocolloids. Legend: RSV: Resveratrol, CS: Corn starch, PS; Pea starch, XG: Xanthan gum, LBG: Locust bean. Solid lines correspond to experimental data and dotted ones are resulting from applying Box Lucas model.

Table 3.2 Effect of free and coated RSV, with different hydrocolloids, on enzymatic hydrolysis kinetics of wheat flour.

Parameters	Control	RSV	RSV_CS	RSV_PS	RSV_XG	RSV_LBG
Apparent Peak Viscosity (cP)	1330^{f}	1236^{d}	1275^{e}	1203^{b}	1209^{c}	1134^{a}
Trough (cP)	799^{f}	739^{d}	760^{e}	661^{c}	642^{b}	630^{a}
Setback (cP)	775^{f}	695^{e}	693^{d}	581^{c}	542^{b}	506^{a}
μ_0 (cP)	1426^{e}	1283^{d}	1455^{f}	1208^{c}	1039^{b}	875^{a}
***k* (min^{-1})**	1.345^{d}	1.233^{c}	0.871^{a}	0.925^{b}	0.817^{a}	1.226^{c}
μ_∞ (cP)	89.2^{b}	103.4^{f}	98.6^{e}	90.7^{c}	91.1^{d}	50.8^{a}

RSV: Resveratrol, CS: Corn starch, PS; Pea starch, XG: Xanthan gum, LBG: Locust bean. Significant differences among means within each row are indicated by different letters, as determined by the HSD test.

RSV incorporation significantly decreased the apparent peak viscosity, and that reduction was only counteracted when RSV was coated with corn starch. RSV also promoted a significant reduction of trough and setback viscosities indicating the existence of some interaction of RSV and starch that reduce its gelatinization and amylose retrogradation. When coated with hydrocolloids, they were able, in different extent, to reduce that interaction. LBG was the hydrocolloid with the least hindering impact, being the corn starch the most effective coating hydrocolloid.

A similar trend of kinetic curves was observed for all samples containing differently coated RSV. The experimental apparent viscosity at the beginning and end of the digestograms obtained using the RVA significantly decreased for all samples, indicating that the α-amylase was degrading the starch, which was translated as a decrease in the viscosity. The RVA experimental data were fitted ($R^2 > 0.99$) to a Box-Lucas model, and the resulting parameters are presented in Table 3.3. The kinetic constant (k) values derived from the digestograms exhibited statistically significant differences ($P < 0.05$) among the samples, depending on the form of coated RSV applied. The highest hydrolysis rate ($k = 1.345$) was observed in the control sample, whereas all RSV-enriched samples demonstrated significantly lower hydrolysis rates. Interestingly, gels containing coated RSV showed lower hydrolysis rate than the one obtained with the free RSV, except for the locust bean gum. These results confirmed the effectivity of the RSV-enriched gels, and that the nature of the gels made a significant impact. Particularly, RSV-loaded corn starch (k=0.871) and xanthan gum (k=0.817) resulted in the lowest k values, followed by those obtained with pea starch (k=0.925). Therefore, our result suggests that starch can physically trap RSV via diffusion in its matrix, shielding it from stressors. The research also confirms that RSV is bound to starch via hydrogen bonding and hydrophobic interactions, which stabilizes it in an amorphous state, improving its bioaccessibility (Choi et al., 2022).

Results confirmed that both, starch (CS) and xanthan gum (XG), protected RSV from thermal degradation during the RVA heating phase, allowing more RSV to remain active and inhibit α-amylase activity. However, results from dough mixing indicated that RSV-loaded corn starch showed higher oxidative stability (greater recovery after mixing) compared to RSV-enriched xanthan gum. Based on this, RSV-enriched corn starch gels was prioritized and selected for further bread-making experiments.

3.3.3 Bread characteristics and resveratrol recovery

The effect on bread characteristics (texture and color) after the addition of RSV and its recovery in the final bread is presented in Table 3.3.

Table 3.3 Effect of RSV addition on bread characteristics

Parameter	Control bread	RSV bread	RSV_CS bread
			
Area (cm^2)	15.15 ± 0.54^a	15.84 ± 0.21^a	16.35 ± 0.21^a
Porosity (pores/cm^2)	26.10 ± 0.11^a	28.21 ± 0.40^a	35.71 ± 2.41^b
Color (ΔE*)	-	1.51±0.54	2.72±1.54
Hardness (g)	2059 ± 107^b	2030 ± 90^b	1086 ± 105^a
Springiness	1.30 ± 0.79^a	1.14 ± 0.42^a	1.00 ± 0.01^a
Cohesiveness	0.73 ± 0.05^a	0.72 ± 0.04^a	0.84 ± 0.02^b
Chewiness (g)	1475 ± 75^b	1488 ± 110^b	913 ± 88^a
Resilience	0.39 ± 0.03^a	0.39 ± 0.02^a	0.47 ± 0.02^b
RSV recovery (%)	0.53 ± 0.02^a	41.11 ± 0.14^b	45.56 ± 1.39^c

RSV: Resveratrol, CS: Corn starch, PS; Pea starch, XG: Xanthan gum, LBG: Locust bean.
Significant differences among means within each row are indicated by different letters, as determined by the HSD test.

Visually, the color of breads containing RSV or RSV-CS were similar to the creamy white color of the control. In fact, the addition of RSV and RSV_CS showed slight effect in the overall color of bread ($\Delta E^*>1$) as compared to the control bread. Silva et al. (2022) reported that adding 0.5% (w/w, based on dough weight) of either free RSV or RSV encapsulated in γ-cyclodextrin (γ-CD·RSV) did not alter the color of wheat bread, whereas the incorporation of RSV-loaded zein nanoparticles (nZ-RSV) resulted in noticeable color changes due to the encapsulated materials added. Similarly, while the slice area remained consistent across all samples, the RSV_CS bread exhibited significantly higher porosity *($P<0.05$)* compared to the other two samples.

Regarding textural properties of bread, the inclusion of RSV into the bread did not significantly modify the textural properties of the bread. However, hardness and chewiness were found to be significantly lower (*$P<0.05$*) in RSV_CS as compared to control and RSV bread, which showed that protecting the RSV was effective decreasing the hardness of bread as compared to free RSV samples. This softer, less chewy crumb might make it easier to chew and swallow, which could make it suitable for kids and older adults who frequently need or prefer softer bread textures. These findings are partially consistent with the study by Silva et al. (2022), in which both free-form RSV and RSV encapsulated in zein reduced bread hardness. However, in that study cyclodextrin RSV bread showed significantly higher hardness, indicating that the gel matrix plays a significant impact determining the RSV interactions. The enhanced softness observed in the RSV_CS bread could be attributed due to the physical barrier which limits direct contact between resveratrol and gluten proteins, thereby reducing strong RSV-gluten network strength. Polymeric polyphenols cross-link gluten proteins through hydrogen bonding and hydrophobic interactions, strengthening the gluten network strength (Girard & Awika, 2020). Regarding bread's cohesiveness and resilience, encapsulated RSV (CS_RSV) bread was significantly higher (*$P<0.05$*) than both the

free resveratrol (RSV_bread) and control bread, indicating improved textural performance. These results suggest that the use of encapsulated RSV outperformed those reported in previous studies (Wahab & Janaswamy, 2025), who observed no significant difference in cohesiveness and resilience between control and RSV encapsulated in porous starch fortified bread samples. Additionally, springiness remained statistically similar across all samples in our study, consistent with the findings of Silva et al., (2022); Wahab & Janaswamy, (2025).

The highest RSV recovery was observed in RSV_CS bread, with 45.56% (d.b.) of the added RSV retained in the sample, corresponding to 270 mg RSV per 100 g of dried bread. This was significantly higher ($P< 0.05$) than the recovery from RSV bread, which accounted for 41.11% (d.b.), equivalent to 250 mg RSV per 100 g of dried bread. This is lower than previous research led by Silva et al. (2022), who observed 89.0% retention (4.35 mg RSV/g bread) in bread fortified with free RSV, 62.8% (3.16 mg RSV/g bread) and 70.3% (3.38 mg RSV/g bread) in breads fortified with encapsulated RSV using γ-cyclodextrin and nanostructured zein, respectively. This might be due to either differences in the recipe, because yeast and salt was added in double amount, or the stage at which RSV was added during bread making. In their study, RSV was incorporated after dough formation, whereas in our approach, it was added at the beginning along with the other ingredients to ensure the uniform distribution of the RSV. The mixing process may have exposed reactive gluten sites that formed strong interactions with RSV, thereby limiting its recovery during the extraction process. However, starch-based gels have demonstrated a protective effect on RSV during processing. Hence, considering this higher RSV retention, lower cost of coating material (corn starch) and higher α-amylase inhibition of RSV_CS bread, a sensory analysis was conducted to determine its acceptability.

3.3.4 Sensory acceptability of the bread

The sensory evaluation of bread is presented in Fig 3.4. In a 9-point hedonic test, aroma, texture and appearance were significantly (*P>0.05*) similar between Control and RSV_CS. However, flavor was significantly lower with RSV_CS (5.6 ± 1.9) than control bread (6.9 ± 1.5), which impacted the overall acceptability, reducing from 6.8 ± 1.5 (control) to 6.0 ± 1.8 (RSV_CS). Nevertheless, scores fall under the "Like Slightly" category. There is no previous published work on sensory study of RSV fortified bread. Similar results were observed in RSV cookies/crackers (~4 mg RSV/g dough), where a large consumer test (110 participants) revealed controls as a most liked than the RSV cookies/crackers (encapsulated and non-encapsulated form). For both the fortified crackers and cookies, bitterness was observed (P. M. Silva et al., 2024), which was not identified in the bread of the our study.

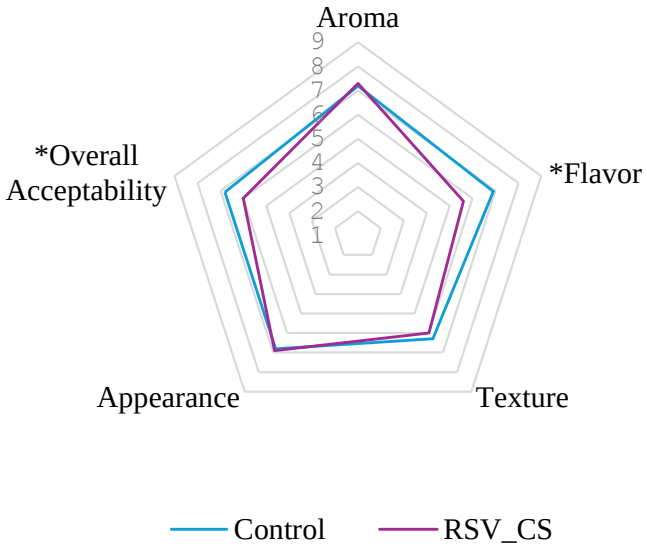


Fig 3.4 Radar chart of sensory evaluation test of control and RSV_CS bread. Star (*) indicates significant differences (p<0.05) between control and RSV_CS bread.

3.4 Conclusions

RSV can be incorporated into bread to obtain functional breads. This study provides insight about the impact of the breadmaking stages on the RSV recovery. Likewise, it highlights the potential use of corn starch and hydrocolloid-based matrices to protect and deliver RSV in bread. Results show that dough mixing causes significant loss of RSV in the final dough, but the baking temperature didn't show degradation to RSV. Corn starch and xanthan gum gels can be used to enhance RSV stability and inhibition ability towards the α-amylase activity. Nevertheless, corn starch as RSV (RSV_CS) loading material led to greater recovery after dough mixing. In line with these findings, RSV_CS bread had softer and more cohesive crumb than the wheat bread. Additionally, sensory study revealed a slightly less flavor and overall acceptability as compared to control bread. However, aroma, appearance and texture were similar and fell under acceptability rating score. Therefore, RSV-enriched corn starch gels offer a viable ingredient to obtain functional breads. Starch/hydrocolloid matrices provide a low-cost, food-grade strategy to stabilize resveratrol during conventional breadmaking and produce softened crumb without any change in color. This approach provides a functional bread with practical applications for controlling the postprandial glucose levels.

Future research will be focused on to assess the consumer acceptance of RSV enriched bread and to validate the *in-vitro* test with clinical trials to confirm the effect of this bread consumption on the postprandial responses. Furthermore, additional research will be required to understand the chemical and structural interaction of the RSV with the different gels.

CRediT Author contributions statement

Sunita Karki: Writing – original draft, Investigation; Formal analysis; Data curation**.**

Nicola Gasparre: Training; Writing – review & editing; Supervision; Conceptualization.

Thomas Netticadan: Writing – review & editing; Funding acquisition.

Cristina M. Rosell: Writing – review & editing; Conceptualization; Supervision; Investigation; Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 4

DISCLAIMER: This chapter is based on a manuscript submitted to the *Journal of Functional Foods* and currently under peer review.

Synergistic effect of amyloglucosidase and resveratrol on reducing *in vitro* starch digestibility

Abstract

Bread is a major dietary carbohydrate source, but its rapid starch digestion promotes a high glycemic index. Resveratrol (RSV), a plant polyphenol, and amyloglucosidase (AMG) can modify starch structure which could potentially alter the starch digestibility. This study examined whether the individual and combined use of RSV and AMG could lower starch digestibility while preserving bread quality. The pasting and hydrolysis behavior of white and whole-wheat flours enriched with RSV, AMG, or their combination were analyzed by Rapid Visco Analyzer and modeled with Box–Lucas kinetics. Breads were evaluated for texture, color, RSV recovery, and *in-vitro* starch digestibility. In white flour, AMG reduced peak viscosity, whereas RSV mitigated this effect and increased setback, indicating enhanced retrogradation. The combined RSV-AMG treatment yielded the lowest hydrolysis rate (k = 0.56 min^{-1}), suggesting a denser starch–polyphenol network that restricted enzymatic access. Whole-wheat flours, bran and fiber led to different effects, with RSV increasing starch retrogradation during cooling. The combination of the treatment led to softer white crumbs and reduced the RSV induced firmness of whole-wheat crumbs. In both breads, RSV shifted starch fractions from rapid digestible starch to slowly digestible starch in both matrices, slowing the *in vitro* starch hydrolysis. Overall, RSV and AMG acted synergistically to modulate starch chain length and matrix structure, reducing starch digestibility without compromising technological performance. These findings highlight a feasible strategy to design lower-glycemic breads through enzyme–polyphenol co-modulation of starch retrogradation and digestibility.

4.1 Introduction

Globally, an estimated 589 million adults (11.1% of those aged 20–79 years) are living with diabetes, and this number is projected to rise to about 853 million by 2050 (International Diabetes Federation, 2025). Chronic diabetes raises the risk of blindness, renal failure, cardiovascular disease, neuropathy, and other consequences, resulting in millions of deaths and a significant burden of disability globally. This complication is largely caused by unhealthy diets which leads to high glycemic index (GI) and sedentary lifestyles (World Health Organization, 2024). Bread is one of the most widely consumed foods worldwide and constitutes a nutrient dense source of vitamins and minerals. However, it is also rich in starch that after its consumption, it is quickly digested and absorbed, significantly increasing the GI (Mesta-Corral et al., 2024). Since bread is a key source of carbohydrates in the human diet, finding ways to reduce its glycemic impact is crucial for public health.

Multiple strategies have been identified to lower the glycemic index of bread. A primary approach is to incorporate fiber-rich flours or purified dietary fiber, which not only attenuates postprandial glycaemia but also increases overall fiber intake (Scazzina et al., 2013). Other alternatives include sourdough fermentation with lactic acid bacteria to reduce starch digestibility, and the incorporation of resistant starch to limit the availability of rapidly digestible carbohydrates (Roman & Martinez, 2019), or the use of high-amylose wheat flour, which further enhances resistant starch formation and slows enzymatic breakdown, contributing to marked reduction in glycemic response (Li et al., 2022). Beyond these, an emerging approach is the use of plant polyphenols to modulate starch digestibility by directly affecting/inhibiting the α-amylase activity (Sun & Miao, 2020). In bread, green-tea catechins have been shown to reduce the fraction of rapidly digestible starch (RDS) and glucose release, with 75–90% of catechins remaining intact after baking (Goh et al., 2015). Similarly, anthocyanin enrichment has been reported to reduce starch digestibility *in vitro*,

while human studies showed that anthocyanin-rich riceberry-rice bread blunted early postprandial glycemia compared to a white-rice bread (Chusak et al., 2020).

Among polyphenols, resveratrol is especially promising but chemically and structurally complex. Resveratrol (RSV) is a stilbene (3,5,4′-trihydroxystilbene) composed of two phenyl rings attached with three phenolic –OH groups at the 3, 5, and 4′ positions, which confer strong hydrogen-bonding capacity. These structural features underlie its well-known bioactivity (antioxidant, anti-inflammatory and anti-diabetic), as well as its ability to form non-covalent complexes with enzymes and starch polymer (Visvanathan et al., 2024). However, RSV is thermolabile and prone to oxidative/isomeric degradation (Zupančič et al., 2015), which could reduce its recovery after bread making unless the matrix offers protective interactions. By combining experiments and molecular simulations, Wu et al. (2022) demonstrated that RSV forms short strong hydrogen bonds with the glucose units in hydrolyzed wheat starch. These bonds resulted in a more stable RSV structure and lowered its susceptibility to UV and heat treatment, suggesting that complex formation between RSV and hydrolyzed starch could effectively protect RSV during demanding food processes like breadmaking.

Enzymes are widely used in breadmaking, particularly starch hydrolyzing enzymes like α-amylase and amyloglucosidase (Barrera et al., 2016). Amyloglucosidase, also named glucoamylase, is an enzyme involved in the breakdown of starch chains. It belongs to the class of exo-acting β-amylases that act on the non-reducing ends of starch polymer chains and produce β-D-glucose (Robyt, 2009). This mechanistic evidence suggests that incorporating AMG during dough making could generate glucose and dextrin molecules that interact with RSV, thereby enhancing its binding and stabilization within the bread matrix. These interactions would not only protect RSV during baking but also promote the formation of denser starch–polyphenol networks that limit enzymatic

accessibility and reduce starch digestibility. Despite these potential synergies, the joint application of resveratrol and amyloglucosidase in bread systems remains largely explored. Therefore, the aim of this study is to investigate whether amyloglucosidase-facilitated starch hydrolysis can promote the formation of glucose and dextrin capable of binding resveratrol, and thus improving its recovery and concurrently decreasing starch digestibility when incorporated in bread.

4.2 Materials and methods

4.2.1 Materials

Canadian Western Red Spring white wheat flour (14.8 g/100 g moisture content) and whole wheat flour (11.4 g/100 g moisture content) were supplied by Cereals Canada (Winnipeg, Canada). Human grade resveratrol (Purity>98%) was purchased from Split Science (Ottawa, Canada), GoldCrust®G (1650 AGU/g) from Novozymes (Bagsvaerd, Denmark) were used as a source of amyloglucosidase. α-Amylase (Sigma-Aldrich Co, St. Louis, Missouri, USA) solution (450 U/mL) were prepared using distilled water. Dried yeast, provided by Lallemand Inc. (Montreal, Quebec, Canada) and commercial salt were used for bread making.

4.2.2 Pasting properties and starch hydrolysis kinetics of wheat flours

The pasting properties of the white (WF) and whole wheat (WWF) flours and the starch hydrolysis kinetics were determined in the same assay by using the Rapid Visco Analyzer 4800 (PerkinElmer Inc., Shelton, CT 06484, U.S.A.) as described by Santamaria et al. (2025), who included the digestogram for the *in vitro* hydrolysis. Briefly, three grams of flour was added to 25 ml distilled water to make the slurry and time-temperature settings included: heating at 50°C for 1 min, heating from 50°C to 95°C in 4 min 30 s, holding at 95°C for 2 min 30 s, then cooling down to 37°C in 5 min 48 s and stopping at 37°C for 30 s to allow the addition of the α-amylase solution (450 U/mL), afterwards temperature was kept at 37°C for an additional 5 min. The rotational speed was 960

rpm for the first 10 seconds and then kept at 160 rpm for the rest of the test, except for 32 s in which the speed was stopped to allow the α-amylase addition. Whenever resveratrol was tested, 0.5 g/100 g (w/w of flour) was added to the slurry. Similarly, the impact of amyloglucosidase was tested by adding 30 µl of amyloglucosidase stock solution (10 mg AMG/mL), corresponding to 10 mg amyloglucosidase per 100 g flour (0.01% w/w, flour basis), to the initial flour slurry. Gels were freeze-dried to be used for the microstructure analysis. Two replicates were performed for each sample. Parameters recorded included: apparent peak viscosity (maximum viscosity during heating, cP), trough viscosity (minimum viscosity after peak viscosity, cP), breakdown (viscosity difference between peak viscosity and trough), setback viscosity (viscosity increase during cooling, cP), viscosity at 37°C, viscosity after adding α-amylase (cP) (μ_0) and viscosity at the end of the assay (μ_∞). Furthermore, experimental data from Rapid Visco Analyzer (RVA) digestogram were modeled using a non-linear Exponential model of Box-Lucas (Eq. 1)

$$\mu = \mu_\infty + (\mu_o - \mu_\infty)\, e^{-kt} \qquad \text{……………………………………………}(1)$$

where k (min^{-1}) was the kinetic constant for the hydrolysis and t was the selected time.

4.2.3 Scanning Electron Microscopy (SEM)

A SEM assessment of the freeze-dried wheat gels (both white and whole wheat), supplemented with 0.5 g/100 g RSV and 30 µl AMG, obtained from the RVA were used for the analysis. An ESEM (FEI Quanta FEG 650, Thermo Fisher Scientific, Hillsboro, Oregon, USA) was used to capture the images. A square section (~5 × 5 × 5 mm) was taken from the center of each sample using a sterile razor blade to reduce edge effects and ensuring a representative internal microstructure. Samples were mounted on aluminum stubs with conductive carbon adhesive and sputter-coated with a gold–palladium alloy (60% Au/40% Pd). Samples were sputter-coated at 50 millitorrs and 45 milliamps for 1 min. Images were captured at accelerating voltages of 10 kV,

working distance (WD) 12–14 mm, spot size of 3 in a high vacuum pressure chamber. SEM images were obtained for the samples at 100 × and 200 × magnifications to capture particle morphology and surface feature.

4.2.4 Preparation of breads

Breads were prepared according to the scale-down method outlined by Espinosa-Ramírez et al. (2018). The basic recipe consisted of 100 g flour, 1 g/100 g dry yeast, and 1.5 g/100 g salt. Resveratrol powder and amyloglucosidase were incorporated at concentrations of 0.5 g/100 g and 16.5 AGU/100 g of flour (corresponding to 10 mg amyloglucosidase per 100 g flour), respectively. All components were blended in a KitchenAid dough mixer (St. Joseph, Michigan, USA) at 25°C for 2 min on low speed, then for 5 min on medium speed. Dough portions (50 g) were placed into mini pans and proofed at 30°C with 85% relative humidity (RH) in a proofing chamber (AX-PR5, Axis, Italy). Dough volume change (ΔV) was measured every 15 min during a 120-min timeframe. The proofing duration was specified as the time necessary to achieve 75% of the maximum dough rise, as previously outlined by Beltrão Martins et al. (2022). Thereafter, the loaves were baked at 180°C for 15 min in an electric oven (CTB-1, GS Blodgett Corporation, USA). Two batches were performed for each recipe. Breads were kept packaged in plastic pouches before conducting the physical characterization. Notation used for referring to breads was control, RSV for breads containing resveratrol, AMG for breads containing amyloglucosidase and RSV-AMG for those breads containing resveratrol and amyloglucosidase.

4.2.5 Breads characterization

Breads were cut to a thickness of 1 cm before analyzing crumb color and texture. The color of the bread crumb was analyzed with a Croma Meter CR-400 (Konica-Minolta Sensing Americas, New Jersey, USA). Color parameters L^* (lightness), a^* from green (-) to red (+), and b^* from blue (-)

to yellow (+) were measured according to the CIE color space system. The difference in surface color (ΔE^*) of the breads was measured, using the control bread as a benchmark (Eq. 2).

$$\Delta E^* = \sqrt{(L_2{}^* - L_1{}^*)^2 + (a_2{}^* - a_1{}^*)^2 + (b_2{}^* - b_1{}^*)^2} \quad \text{.....(2)}$$

Where, $L_1{}^*, a_1{}^*, b_1{}^*$ are the CIELAB values of the control bread and $L_2{}^*, a_2{}^*, b_2{}^*$ are those of the sample

Image analysis was carried out to calculate 2D area (mm^2) and crumb porosity (%) using ImageJ software (Nationals Institutes of Health, USA, version 1.54), following the procedures described by Espinosa-Ramírez et al. (2018).

The texture profile of crumb was determined using a texture analyzer (TA-XT-plus, Stable Micro Systems, Godalming, UK) equipped with a 5-kg load cell. A bread slice having 20 mm diameter was compressed up to 50% using a 36 mm aluminum probe. The following parameters were calculated from the compression plot: hardness (g), springiness, cohesiveness, chewiness (g), and resilience. Three bread slices from two different batches were used to analyze texture and color.

Resveratrol recovery from the bread was quantified as described by Karki et al. (2025). Bread samples were subjected to drying and resveratrol was extracted in methanol (1:10, w/v), sonicated for 10 min at 40 kHz, centrifuged for 15 min at 5,000 rpm, and the supernatant was measured at 300 nm in UV-Vis Spectrophotometer. The RSV recovery percentage was calculated using Eq. 3.

$$\text{RSV recovery (\%)} = \frac{\text{g of compound detected for 100 g of dried bread/dough (dry weight basis)}}{\text{g of compound thereotically added for 100 g of flour (dry weight basis)}} \times 100 \quad \text{....(3)}$$

4.2.6 *In vitro* starch hydrolysis of breads

Two different methods were used to determine *in vitro* starch hydrolysis of breads. The first one consisted in the enzymatic hydrolysis of breads using porcine pancreatic enzyme as described by Santamaria et al. (2024). The parameters measured were rapidly digestible starch (RDS), indicating the starch fraction hydrolyzed in the first 20 min; slowly digestible starch (SDS), which

represents the fraction hydrolyzed between 20 and 120 min; digestible starch (DS); and resistant starch (RS), which indicate starch hydrolyzed or unhydrolyzed after 24 h of incubation respectively. Experimental data from *in vitro* enzymatic hydrolysis were modeled using a non-linear Exponential model of Box-Lucas (Eq. 4)

$$C = C_{\infty} (1 - e^{-kt}) \quad \text{……………………………………………}(4)$$

Where, C was the initial concentration of starch hydrolyzed at *t* time (min), C_{∞} was the maximum hydrolysis concentration, k (min^{-1}) was the kinetic constant and t was the selected time.

The second method evaluated the starch hydrolysis by using the RVA as previously described in section 4.2.2. Dry ground bread (4.5 g) was added to the RVA canister and dispersed in 25 mL of distilled water. Parameters recorded were viscosity at 37°C, viscosity after adding α-amylase (cP) (μ_0) and viscosity at the end of the assay (μ_{∞}). Data were modeled as described in section 4.2.2.

4.2.7 Statistical analysis

Statistical analysis of all samples was conducted using IBM SPSS statistics software (Version 20, Boston, USA). One-way analysis of variance (ANOVA) was used to determine significant differences among samples at a 95% confidence interval, using Honestly Significant Difference test (HSD).

4.3 Result and discussions

4.3.1 Pasting properties and starch hydrolysis kinetics of flours

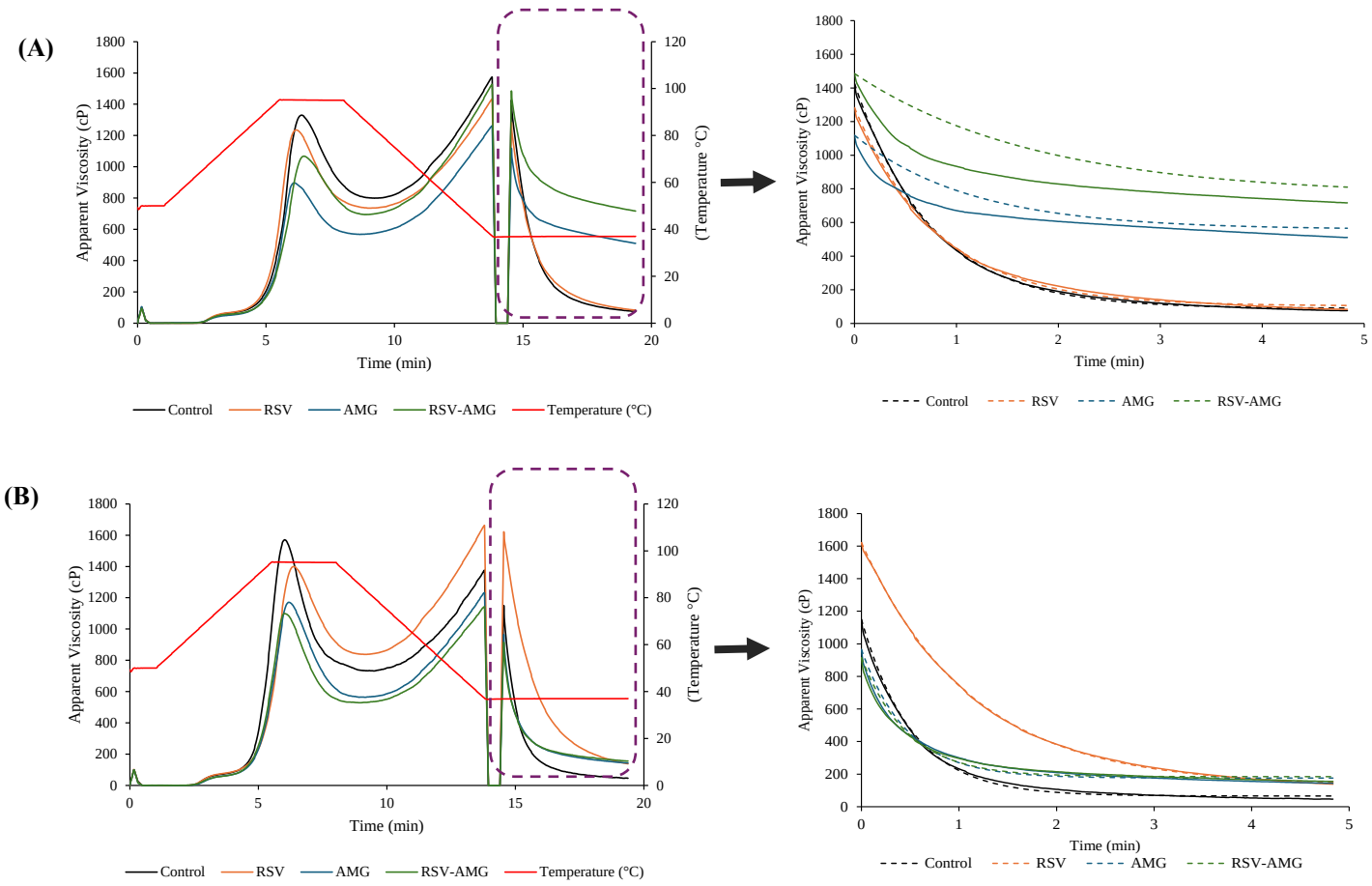
The pasting properties and the hydrolysis kinetics of the white and whole wheat flour with the addition of resveratrol and amyloglucosidase are presented in Table 4.1 and Fig 4.1.

Table 4.1: Effect of resveratrol and amyloglucosidase on pasting properties and enzymatic hydrolysis kinetics of white and whole wheat flour.

Process	Parameter	White wheat flour (WF)				Whole wheat flour (WWF)			
		Control	RSV	AMG	RSV-AMG	Control	RSV	AMG	RSV-AMG
Gelatinization	Apparent Peak Viscosity (cP)	1330^{d}	1236^{c}	898^{a}	1068^{b}	1570^{D}	1400^{C}	1171^{B}	1099^{A}
	Trough (cP)	799^{d}	736^{c}	568^{a}	603^{b}	732^{C}	838^{D}	564^{B}	529^{A}
	Breakdown (cP)	531^{d}	500^{c}	330^{a}	465^{b}	838^{D}	562^{A}	607^{C}	570^{B}
	Final Viscosity (cP)	1574^{d}	1434^{b}	1263^{a}	1527^{c}	1376^{C}	1663^{D}	1233^{B}	1144^{A}
	Setback (cP)	775^{c}	698^{b}	695^{a}	924^{d}	644^{B}	825^{D}	669^{C}	615^{A}
Hydrolysis	μ (cP)	1426^{c}	1283^{b}	1119^{a}	1485^{d}	1150^{C}	1622^{D}	965^{B}	915^{A}
	k (cP min^{-1})	1.34^{d}	1.23^{c}	0.88^{b}	0.56^{a}	1.93^{B}	0.88^{A}	2.12^{C}	2.12^{C}
	μ∞ (cP)	89.22^{a}	103.57^{b}	557.45^{c}	762.56^{d}	65.99^{A}	129.5^{B}	175.83^{C}	184.83^{D}

RSV: containing Resveratrol, AMG: Containing amyloglucosidase, RSV-AMG: Resveratrol with amyloglucosidase.
Significant differences among means within each row of white wheat and whole wheat flour are indicated by different letters, as determined by the HSD test.

Fig 4.1: Pasting and enzyme hydrolysis kinetic of white (A) and whole wheat flour (B) enriched with resveratrol (RSV) and amyloglucosidase (AMG). The RVA analysis included the addition of α-amylase after the cooling stage to record the viscosity decrease due to the enzymatic hydrolysis (dotted rectangular part) which is magnified separately in right hand side. Solid lines correspond to experimental data and dotted ones are resulting from applying Box Lucas model.



Resveratrol, amyloglucosidase and their combination, significantly altered the pasting behavior of white (WF) and whole (WWF) flours as compared to their respective controls (Fig. 4.1). As expected, viscosity of pastes along heating and cooling significantly decreased in the presence of amyloglucosidase, confirming the breaking down of the starch molecules into shorter chains caused by amyloglucosidase. Similarly, an apparent viscosity drop of corn starch after amyloglucosidase hydrolysis was observed by Dura et al. (2014).

In the WF, peak viscosity significantly decreased from 1330 cP in the control to 898 cP in amyloglucosidase, indicating starch hydrolysis by amyloglucosidase while resveratrol alone lowered this value to 1236 cP (Table 4.1). This could be due to phenolic-starch interactions that prevent granule swelling (Zheng et al., 2025). Resveratrol can form interaction with amylose and amylopectin via the hydrophobic interaction and the hydrogen bonds interactions (Wu et al., 2022). Interestingly, the combined treatment resveratrol- amyloglucosidase partially mitigated the peak viscosity reduction promoted by amyloglucosidase. This suggests that resveratrol either may have restricted amyloglucosidase access to starch substrates through competitive interactions with starch polymers, or alternatively, that resveratrol exerted a mild inhibitory effect on amyloglucosidase activity, leaving more intact starch available for swelling. In fact, Aleixandre & Rosell (2022) confirmed that inhibition by phenolic compounds occurs due to phenolic compounds-enzyme and phenolic compounds-starch interactions, although higher phenolic acid concentration is required for the latter interaction. Similar trend was observed for trough, breakdown, final viscosity and setback. Dura et al. (2014) reported that amyloglucosidase -treated starch showed higher setback viscosity because the short-chains released by the enzyme generates could still reassociate during cooling. Discrepancy with the present result might be explained by a more extensive hydrolysis of the starch, which released products could not form a strong

retrograded network. Notably, when resveratrol was added together with amyloglucosidase, the setback viscosity was higher than that observed in the individual treatments, indicating that resveratrol partly limited amyloglucosidase action and may have formed additional interactions with starch, thus leaving more structure available for re-association during cooling.

WWF responded differently to the same treatments which was likely due to its bran and fiber content. Specifically, resveratrol increased the trough, final viscosity, and setback compared to the WWF control, indicating that it promoted firmer paste formation, likely interactions resveratrol-starch were limited due to the lower starch content of the WWF compared to WF and the fiber interference. The combination of resveratrol and amyloglucosidase, thinned the system by reducing further the peak viscosity, trough, breakdown and final viscosity obtained with the individual addition of either resveratrol or amyloglucosidase, indicating a synergistic effect. These reductions suggest that in the presence of bran components, resveratrol may intensify interference with starch swelling and simultaneously facilitate enzymatic hydrolysis by amyloglucosidase.

Table 4.1 and Fig. 4.1 showed the findings relative to the amylase hydrolysis of the flour. The apparent viscosity at the beginning and end of the digestograms obtained using the RVA highlighted different kinetics for all WF and WWF samples. Therefore, the addition of resveratrol, amyloglucosidase and the type of flour had different effects on apparent viscosity decay (Fig. 4.1, right hand side). The hydrolysis rate (k) values derived from the digestograms exhibited statistically significant differences ($p< 0.05$) in both flours. In WF, the control showed the highest hydrolysis rate (k = 1.34 cP min^{-1}), and this was significantly reduced by resveratrol, which confirmed the inhibitory effect of resveratrol on α-amylase activity. Interestingly, amyloglucosidase addition further reduced the hydrolysis rate (k=0.88 cP min^{-1}). This could be due to the antagonistic interactions of amyloglucosidase and α-amylase, whereby both enzymes

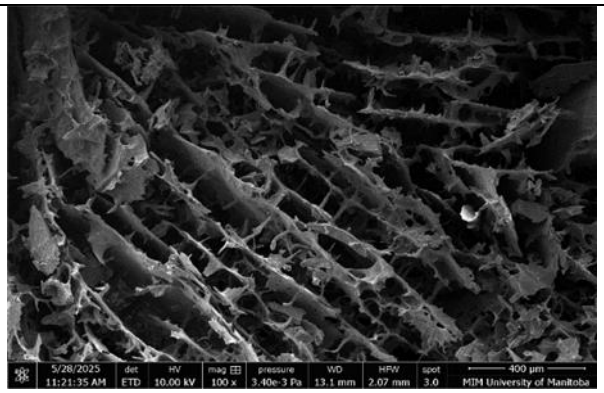
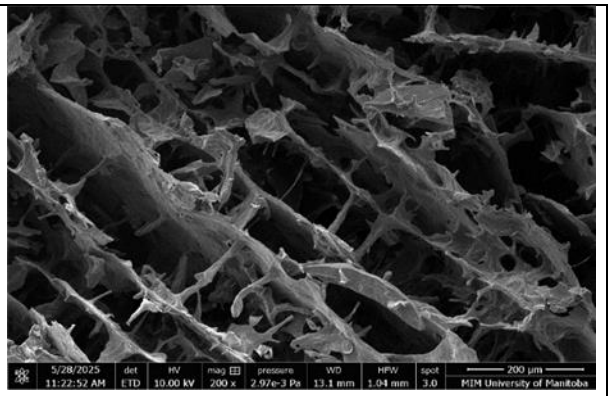
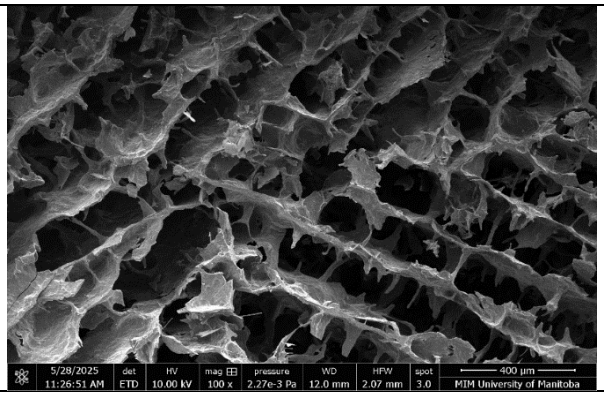
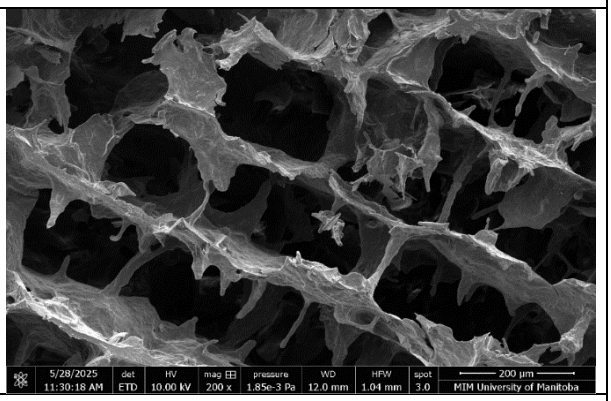
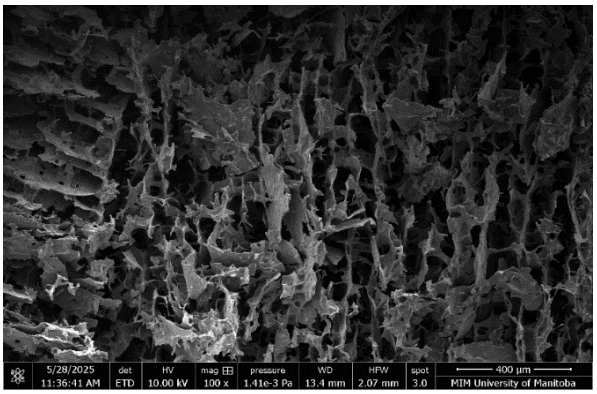
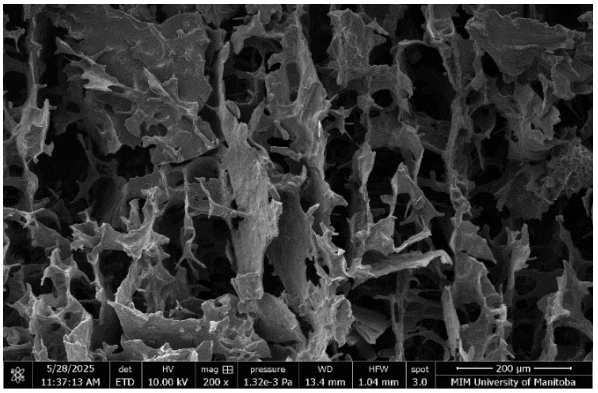
compete for the same starch chains and the trimming action of amyloglucosidase on non-reducing ends makes the substrate less favorable for α-amylase, resulting in a lower overall hydrolysis rate. Similar antagonism between these enzymes during the initial phase of starch hydrolysis (first 5 min) have been previously reported (Li et al., 2022). When resveratrol and amyloglucosidase were combined the hydrolysis rate declined even further (k=0.56 cP min^{-1}), which demonstrated an enhanced inhibitory effect of resveratrol against α-amylase in the mixed system. Again, the performance of WWF was different than the one observed with WF. The resveratrol addition lowered the hydrolysis rate of starch (k=0.88 cP min^{-1}) as compared to control (k=1.93 cP min^{-1}). However, the addition of amyloglucosidase, either alone or together with resveratrol, actually increased starch hydrolysis. This effect is likely due to the strong hydrolytic activity of amyloglucosidase being partially restricted by the crosslinking action of wheat bran arabinoxylans and resveratrol. As a result, less free amyloglucosidase and resveratrol are available to inhibit α-amylase, which may, in turn, enhance the overall rate of starch digestion. Zhou et al. (2025) reported cross-linked arabinoxylans with ferulic acid can inhibit α-amyloglucosidase activity via an anti-competitive mechanism, while having less effect on α-amylase.

4.3.2 Scanning electron microscopy (SEM) images of hydrolyzed wheat gels

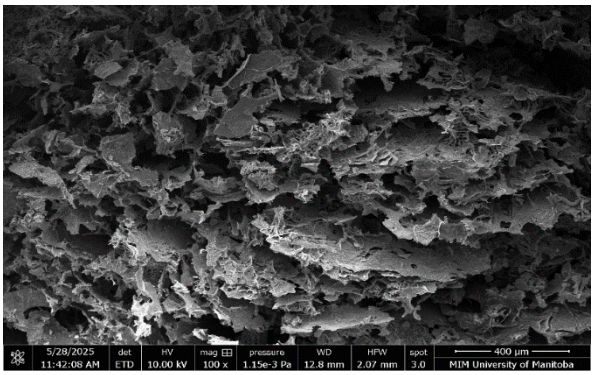
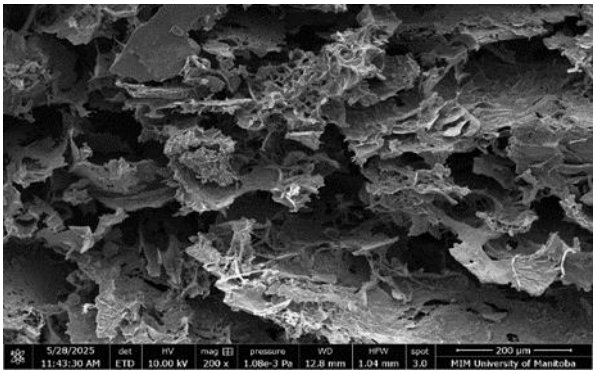
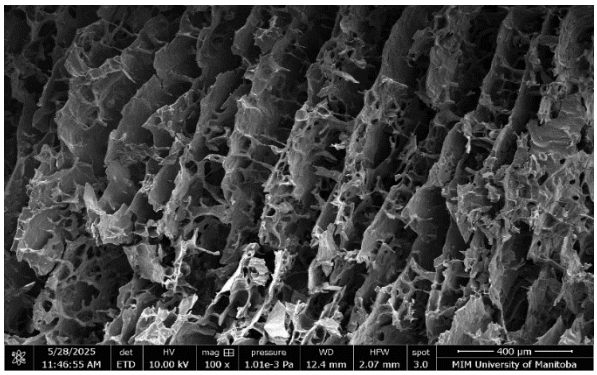
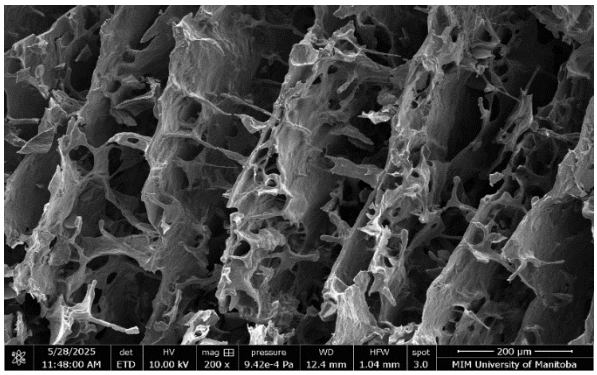
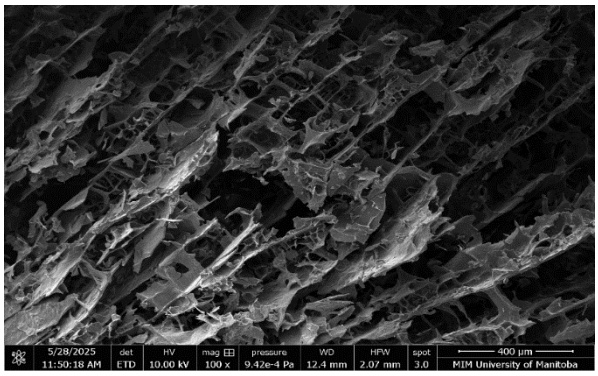
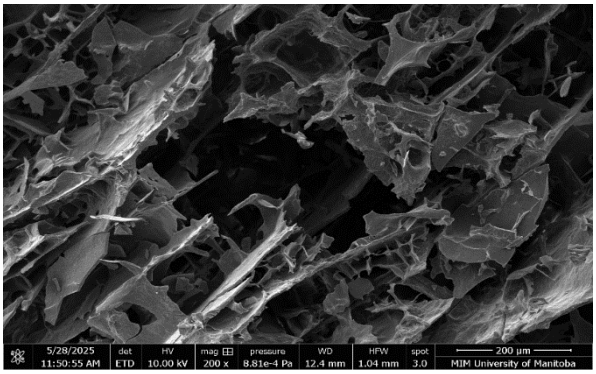
To further elucidate the structural modifications induced by resveratrol and amyloglucosidase on the wheat flours, the microstructure of the wheat gels was examined by SEM (Fig. 4.2)

Fig. 4.2: Scanning electron microscopy images of white (A) and whole wheat (B) pastes enriched with resveratrol (RSV) and combination of resveratrol and amyloglucosidase (RSV-AMG) obtained from the RVA after a heating-cooling-hydrolysis cycle.

(A)

		100 ×	200 ×
White wheat flour (WF)	Control		
	RSV		
	RSV-AMG		

(B)

Whole wheat flour (WWF)	Control		
	RSV		
	RSV-AMG		

The micrographs of white flour control (Fig. 4.2A) showed small voids or cavities, linearly distributed and separated by walls interconnected with tiny bridges. Similar honeycomb or sponge-like structure was previously observed in the wheat gels by Aleixandre et al. (2021). The observed morphology reflects entanglement between amylose and amylopectin chains forming a cohesive three-dimensional network. Nevertheless, visible differences were observed in the size distribution of the cavities and the wall thickness when resveratrol was added. Resveratrol gel led to looser open cavities, thicker walls and lesser connecting filaments as compared to control. This is also supported by previous findings about rice starch gels containing ferulic and gallic acid that resulted in looser porous gel matrix (Han et al., 2020). Additionally, Aleixandre et al. (2022) observed bigger voids in corn starch gels containing protocatechuic acid. Conversely, when amyloglucosidase was added, gels displayed a denser and more irregular morphology composed of compact aggregates and fragmented walls, reflecting extensive hydrolysis of starch into low-molecular-weight oligosaccharides. The disappearance of large hydrophobic voids and the collapse of the porous network support the enzymatic breakdown of the starch matrix (Wu et al., 2022).

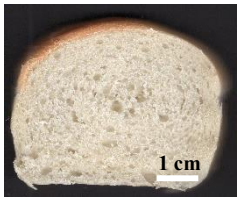
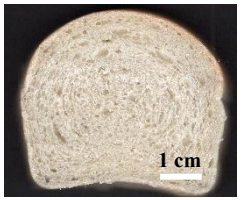
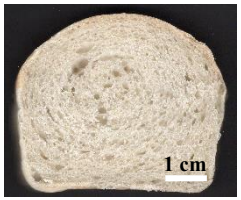
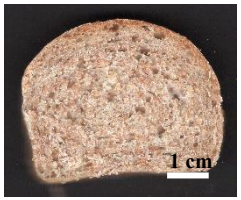
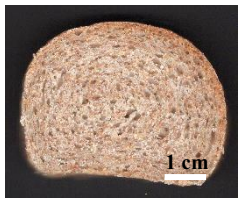
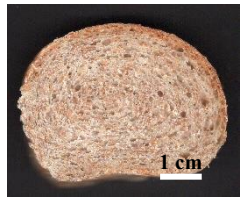
Whole-wheat flour (WWF) gels (Fig. 4.2B) exhibited distinct features due to the presence of bran and fiber components. The control samples showed uneven surfaces with fragmented flakes and discontinuous, flake-like structures, indicating the interference of insoluble fiber and cell-wall polysaccharides with starch gelatinization and network formation. Nevertheless, when RSV was added, the structure appeared smoother and well aligned. This more organized microstructure probably arises from phenolic-fiber interactions-either covalent grafting of resveratrol to arabinoxylans or non-covalent hydrogen bonding with cell-wall polysaccharides, that may have promoted tighter and more ordered arrangements within the gel matrix (Guo et al., 2021; Li et al.,

2023). The combination of resveratrol-amyloglucosidase led to a complex morphology comprising compact fibrillar bundles and remnants of ordered lamellae. This suggests that enzymatic hydrolysis fragmented starch domains, while resveratrol simultaneously stabilized certain regions through complexation, resulting in a partially degraded but denser structure.

3.3 Resveratrol enriched bread characteristics

Bread quality was not compromised by resveratrol and amyloglucosidase supplementation (Table 4.2), but significant differences were detected in some of the evaluated parameters.

Table 4.2: Effect of RSV and AMG addition on bread characteristics and RSV recovery.

Parameter	White wheat bread (WB)			Whole wheat bread (WWB)		
	Control	RSV	RSV-AMG	Control	RSV	RSV-AMG
						
2D area (cm^2)	15. 15±0.54^{a}	15.84±0.21^{a}	16.13±0.12^{a}	12.76±0.27^{A}	14.89±0.47^{B}	11.58±0.06^{A}
Porosity ($pores/cm^2$)	26.10±0.11^{a}	28.21±0.40^{a}	35.73±1.41^{c}	23.94±1.03^{A}	23.95±0.50^{A}	23.45±1.08^{A}
Color (ΔE*)	-	1.51±0.54	3.09±1.56	-	1.3±0.98	2.70±0.83
Hardness (g)	2059±107^{b}	2030±90^{b}	1074±106^{a}	2471±183^{B}	2854±189^{C}	1568±153^{A}
Springiness	1.30±0.79^{a}	1.14±0.42^{a}	1.00±0.01^{a}	0.97±0.043^{A}	0.996±0.005^{A}	1.00±0.01^{A}
Cohesiveness	0.73±0.05^{a}	0.72±0.04^{a}	0.84±0.02^{b}	0.71±0.011^{A}	0.73±0.038^{A}	0.83±0.01^{B}
Chewiness (g)	1475±75^{b}	1488±110^{b}	906±92^{a}	1699±169^{A}	2075±95^{B}	1598±126^{A}
Resilience	0.39±0.03^{a}	0.39±0.02^{a}	0.46±0.01^{b}	0.38±0.017^{A}	0.39±0.02^{A}	0.50±0.01^{B}
RSV recovery (%)	0.53±0.02^{a}	41.11±0.14^{b}	50.68±0.11^{c}	0.99±0.05^{A}	29.82±1.12^{B}	41.25±0.08^{C}

RSV: Resveratrol, AMG+RSV: Bread with amyloglucosidase and resveratrol
Scanned images of all the bread slices (scale bar = 1 cm)
Significant differences among means within each row of white and whole wheat bread are indicated separately by different letters, as determined by the HSD test.

In white breads, resveratrol enrichment in the absence or presence of amyloglucosidase did not significantly affect the area of the slice but significantly increased the crumb porosity in RSV-AMG bread. RSV showed enhanced color of the crumb compared to the control, but the difference was much more noticeable ($\Delta E^*>1$) in RSV-AMG. The impact was more pronounced in white breads, which suggests the RSV promotes the Maillard reaction and also white flour has fewer competing pigments thus, the color changes induced by RSV are more visible and yield higher ΔE^* values. In contrast, whole-wheat breads already contain bran-derived pigments and phenolics, and part of the RSV may be bound to fiber, so the relative color shift is smaller and ΔE^* is reduced compared with their white flour homologues. Although resveratrol enrichment did not significantly affect the crumb hardness, significantly softer crumbs were obtained in RSV-AMG breads ($p < 0.05$), and greater cohesiveness (0.84 versus 0.73, $p < 0.05$), which indicates more elastic structure during chewing. These modifications align with the role described for amylolytic enzymes in bread matrices, releasing low-molecular saccharides that enhance softness (Park et al., 2018).

In whole breads, no differences were observed in the porosity between the samples, however RSV bread showed slight increased area compared to control and RSV-AMG, and color was affected in lesser extent than that in the white bread. Resveratrol increased the hardness of the whole bread but a significant reduction was observed with the addition of amyloglucosidase, leading to breads softer than the control in line with the reported softening effect of amyloglucosidase-based enzyme systems on bread crumb (Barrera et al., 2016).

Enrichment in resveratrol was evident in the recovery amount; but the performance in white and whole bread was different. In white bread, high resveratrol recovery was observed, which was increased when combined with amyloglucosidase (RSV-AMG 50.68 ± 0.11%). In contrast, whole

bread showed low recovery (29.82 ± 1.12%) when solely resveratrol was added, which was significantly enhanced in RSV-AMG (41.25 ± 0.08%). This could be due to the hydrolysis products released from AMG activity that assisted resveratrol stability in the baking matrix. The complexes formed between polyphenols and hydrolyzed wheat starch through hydrogen bonds enhance both thermal and UV stability (Wu et al., 2024). Nevertheless, differences observed in the resveratrol recovery between white and whole breads might relate to the lower starch content in whole breads or the different interaction of resveratrol with starch in the presence of fibers.

4.3.4 *In vitro* digestion of bread

In vitro starch digestion was followed by both, direct and indirect methods (Table 4.3 and Fig. 4.3).

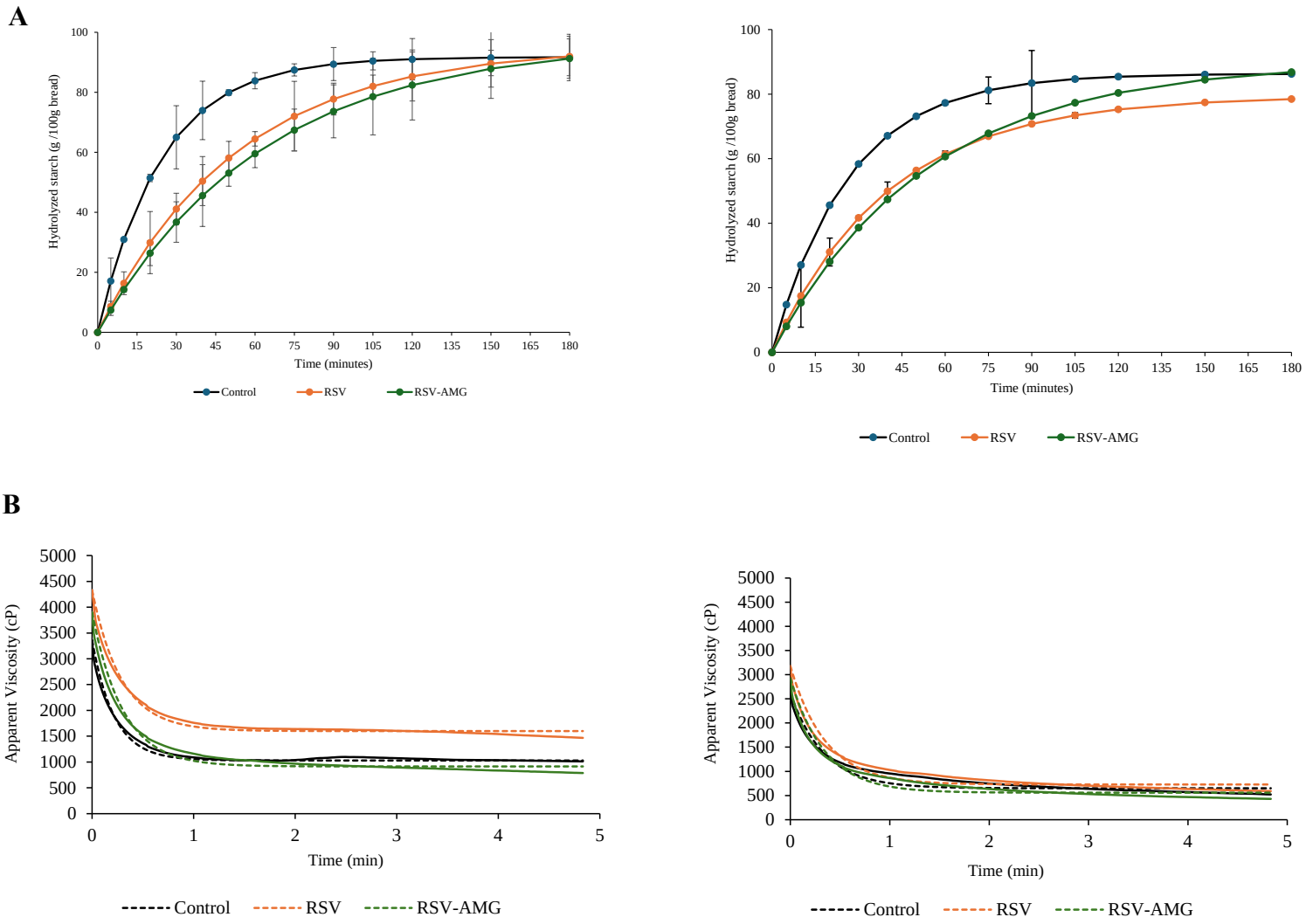
Table 3: Bread digestion parameters obtained when simulating the bread *in vitro* starch hydrolysis by either measuring the glucose release or the viscosity changes recorded by the Rapid Visco Analyzer (RVA).

Parameters	White wheat bread (WB)			Whole wheat bread (WWB)		
	Control	**RSV**	**RSV-AMG**	**Control**	**RSV**	**RSV-AMG**
***In vitro* enzymatic hydrolysis**						
RDS (g/100 g)	51.47 ± 9.05^{b}	29.91 ± 4.88^{a}	26.41 ± 3.22^{a}	45.6 ± 0.45^{B}	31.08 ± 2.42^{A}	28.07 ± 0.01^{A}
SDS (g/100 g)	39.59 ± 0.60^{a}	55.40 ± 9.54^{b}	56.05 ± 10.17^{b}	39.81 ± 1.44^{A}	44.19 ± 3.39^{AB}	52.33 ± 0.03^{B}
DS (g/100 g)	85.22 ± 0.05^{b}	82.15 ± 0.74^{a}	85.00 ± 0.28^{b}	76.88 ± 3.28^{A}	72.73 ± 1.90^{A}	70.37 ± 3.42^{A}
RS (g/100 g)	10.93 ± 0.42^{a}	12.69 ± 0.20^{b}	11.28 ± 0.64^{a}	12.83 ± 0.00^{A}	13.49 ± 0.56^{AB}	14.22 ± 0.40^{B}
***k* (min^{-1})**	0.04 ± 0.003^{b}	0.02 ± 0.000^{a}	0.0160 ± 0.000^{a}	0.038 ± 0.000^{B}	0.025 ± 0.003^{A}	0.019 ± 0.000^{A}
C_{∞}	91.76 ± 6.61^{a}	95.22 ± 11.57^{a}	96.86 ± 12.86^{a}	86.37 ± 1.41^{AB}	79.49 ± 2.07^{A}	89.93 ± 0.03^{B}
Rapid Visco Analyzer hydrolysis method						
k_{RVA} (min^{-1})	4.62 ± 0.71^{b}	3.46 ± 0.36^{a}	3.30 ± 0.09^{a}	2.91 ± 0.22^{A}	2.85 ± 0.06^{A}	2.92 ± 0.02^{A}
μ_{∞} (cP)	1054.90 ± 5.15^{b}	1598 ± 40.58^{c}	913.75 ± 36.61^{a}	648.55 ± 34.22^{AB}	728.23 ± 0.54^{B}	559.51 ± 17.06^{A}

Abbreviations: rapidly digestible starch (RDS), slowly digestible starch (SDS), digestible starch (DS), resistant starch (RS), kinetic constants (k), maximum hydrolysis concentration (C_{∞}), hydrolysis rate constant obtained from rapid visco analyzer (k_{RVA}) and the final viscosity (μ_{∞}).

Significant differences among means within each row of white and whole wheat bread are indicated separately by different letters, as determined by the HSD test

Fig. 4.3: *In vitro* enzymatic hydrolysis (A) and Rapid Visco Analyzer hydrolysis (B) kinetics of white bread (Left) and whole wheat bread (right). Solid lines in Fig B correspond to experimental data and dotted ones are resulting from applying Box Lucas model. Legend: RSV: Resveratrol fortified bread, RSV-AMG: Resveratrol fortified bread in the presence of amyloglucosidase.



Plots of the *in vitro* enzymatic hydrolysis clearly displayed the impact on the hydrolysis rate of resveratrol, slowing down the starch hydrolysis in both types of breads. Enzymatic hydrolysis plots show that breads containing resveratrol slowed down the starch hydrolysis, shifting the starch rapidly digestible starch (RDS) to slowly digestible starch (SDS) (Table 4.3). This trend was observed in both white and whole breads. Mechanistically, resveratrol-starch or resveratrol-non-starch carbohydrate interactions (H-bonding, hydrophobic interactions) reduce enzyme accessibility and the resveratrol-induced inhibition effect against α-amylase might be pushing digestible portions to SDS. Reported studies with phenolic acids enriched gels reveal that phenolic acids (multi-OH) lower both the rate and extent of starch hydrolysis, with inhibition increasing with hydroxylation degree of the phenolic compounds (protocatechuic acid) (Aleixandre & Rosell, 2022; Mao et al., 2023). Similarly, a decrease (24.43%) in the *in vitro* starch digestion rate and the predicted glycemic index (13.17%) was observed in green tea catechin fortified wheat bread versus control bread (Li et al., 2022). In addition, RSV-AMG further contributed to the effect of RSV, although to a lesser extent, and the values were not significantly different. Therefore, RSV appeared to be the main factor responsible for reducing RDS and enhancing SDS in both types of bread.

In white bread, RS was significantly higher in RSV (Table 3), likely due to the RSV–starch interactions might favor retrogradation of optimally sized linear chains (RS3), whereas the amyloglucosidase treatment over-shortens starch chains leading to less crystallizable material or RS forms (Villas-Boas et al., 2020). However, in whole breads, only RSV-AMG bread had significantly higher RS than the control, but there was not significant difference with RSV.

Fig. 4.3 shows that RSV slowed down the overall digestion rate (k) in both breads, but the combination of RSV-AMG did not promote further reduction of the kinetic rate observed in the RSV breads. The total digested starch remained constant in all the breads, except for the whole wheat enriched with resveratrol. Likely, RSV hindered early hydrolysis (RDS and SDS) by forming reversible, non-covalent H-bonding with hydrolyzed glucans. However, these interactions could be partially broken after 120 minutes.

Regarding maximum hydrolysis concentration (C_∞) in white breads, C_∞ did not differ significantly among control, RSV, and RSV-AMG, suggesting that treatments mainly influenced the early stage of hydrolysis rather than the final digestible starch fraction. However, in whole breads, C_∞ was significantly lower in RSV compared to RSV-AMG (Fig. 3A).

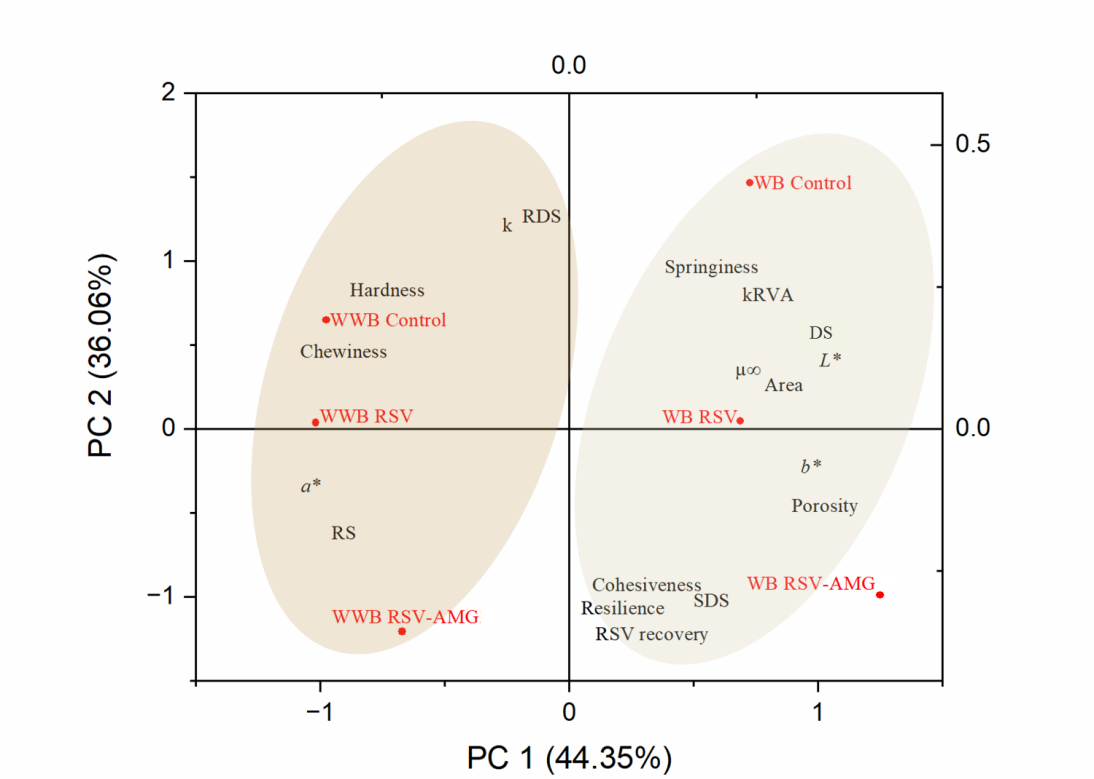
Enzymatic digestion was also indirectly evaluated, following changes in apparent viscosity of the breads, as reported by Santamaria et al., (2022). This methodology has been considered highly relevant, especially in the case of phenolic acids, because food matrix properties (composition, viscosity, structure, and starch nature) besides inhibition of digestive enzymes must be considered to explain the *in vitro* starch hydrolysis (Giuberti et al., 2020). Viscosity decay of bread slurries due to α-amylase showed the main effect of resveratrol, hindering the viscosity decrease, but only in white breads (Figure 4.3B). In opposition, whole breads digestion by RVA only showed subtle viscosity changes due to RSV. Regarding the digestion rate (k_{RVA}) in white breads, calculated from the viscosity plots modeling, showed that RSV significantly ($p<0.05$) dropped the hydrolysis rate, and this was further reduced in RSV-AMG bread, although the difference was not significant. In contrasts, no impact on the hydrolysis rate was observed in whole breads, probably due to RSV being absorbed by fibers.

Regarding the final viscosity (μ_∞) related to the extent of the starch hydrolysis, only resveratrol led to higher infinite viscosity, indicating lower hydrolysis extent of the starch. This effect was observed in both type of breads, although the impact was only significant in white breads. Conversely, RSV-AMG breads reached the lowest hydrolysis plateau in both matrices, indicating ultimately the largest hydrolysis of the starch. Likely, the amyloglucosidase action on starch led to a structure that can eventually be digested. Altogether, Table 4.3 and Figure 4.3 shows that resveratrol slows down the starch hydrolysis and shifts digestible fractions toward SDS, but its combination with amyloglucosidase, did not induce further improvement. Regarding the extent of the hydrolysis, in general, amyloglucosidase increased the extent of starch hydrolysis when combined with resveratrol.

3.5 Principal Component Analysis (PCA)

The multivariate structure of the dataset was resolved by PCA where PC1 and PC2 accounted for 80.41% of the variance (PC1 = 44.35% and PC2 = 36.06%). The biplot (Fig. 4.4) clearly distinguishes between samples, with PC1 mainly distinguishing the bread types: whole-wheat breads are positioned on the negative side while all white breads are positioned on the positive side which plainly shows that the type of flour is the main contributor to variability.

Fig. 4.4: Principal Component Analysis (PCA) of data collected from two different flour's bread of various formulations



Abbreviations: White bread (WB), whole wheat bread (WWB), resveratrol (RSV), amyloglucosidase and resveratrol added (RSV-AMG), lightness (L^*), redness (a^*), yellowness (b^*), rapidly digestible starch (RDS), slowly digestible starch (SDS), digestible starch (DS), resistant starch (RS), kinetic constants (k), hydrolysis rate constant from rapid visco analyzer (k_{RVA}) and the final viscosity (μ_∞).

PC2 represents the treatment gradient within each type of bread, arranging samples from control to RSV to RSV-AMG (with controls positioned higher on positive PC2, RSV on the middle and RSV-AMG situated lower on negative PC2). White breads were characterized by higher crumb porosity, slice area, lightness (L^*) and yellowness (b^*), springiness, and faster and more extensive starch hydrolysis (k_{RVA}, μ_∞, DS). High levels of RDS and faster starch hydrolysis constant (k) point slightly up/right, linking faster digestibility with the control bread. However, in the presence of resveratrol, the crumb area and porosity were increased, whereas RSV-AMG is mainly discriminated by the crumb cohesiveness, the higher amount of SDS and the level of RSV recovery.

A similar trend was observed in whole breads, although they exhibited higher crumb hardness, and high RS was obtained with RSV- AMG, indicating the firmer texture and greater resistant starch characteristic of whole-wheat bread.

4.4 Conclusions

This study provides novel insight into how phenolic–enzyme co-modulation can tailor starch digestibility in cereal matrices without compromising bread quality. The combination of resveratrol and amyloglucosidase is a viable way to retard starch of wheat breads while maintaining technological and nutritional quality. Resveratrol delayed α-amylase-driven hydrolysis and shifted starch from rapidly to slowly digestible fractions. RSV-AMG synergistically improved crumb softness and cohesiveness in white bread while also enhancing resveratrol recovery. However, in whole-wheat bread, it counteracted resveratrol-induced hardness and increased resveratrol recovery from a low baseline. SEM micrographs revealed a tighter, more ordered microstructure in the presence of resveratrol and denser clusters when amyloglucosidase was present, consistent with the kinetic results. Mechanistically, advantages are most likely due to reversible resveratrol-glucan contacts that prevent enzyme access and encourage reassociation, as well as amyloglucosidase-generated chain-length distributions that promote retrogradation. These actions work together to slow digestion and improve texture. These findings highlight a promising strategy to design lower-glycemic cereal products through controlled enzyme and polyphenol interactions influencing starch-polysaccharides structuring and retrogradation. Future research should evaluate *in vivo* glycemic responses, improve enzyme/polyphenol dose for various flours and processes, and investigate resveratrol-glucan complexation.

CRediT Author contributions statement

Sunita Karki: Writing – original draft, Investigation; Formal analysis; Data curation**.**

Nicola Gasparre: Training; Writing – review & editing; Supervision; Conceptualization; Funding acquisition.

Thomas Netticadan: Thomas Netticadan: Writing – review & editing; Funding acquisition.

Cristina M. Rosell: Writing – review & editing; Conceptualization; Supervision; Investigation; Funding acquisition; Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Final Conclusions and Future Directions

Conclusions

- Resveratrol (RSV) can be effectively incorporated into wheat-based bread to produce functional products by protecting RSV using carriers or starch hydrolysis products to entrap RSV.
- Breadmaking steps are responsible for RSV loss, being mixing the primary stage responsible for RSV degradation, while baking did not cause further RSV loss, indicating that mechanical forces and ingredient interactions are more damaging than heat.
- Corn starch (CS) and hydrocolloid-based matrices (especially xanthan gum gels) are effective, low-cost, food-grade carriers for RSV, improving its stability during mixing and preserving its α-amylase inhibitory activity. Among the carriers tested, RSV loaded into corn starch (RSV_CS) showed the highest RSV recovery following mixing.
- Bread made with RSV_CS demonstrated improved crumb softness and cohesiveness, without noticeable color changes—an important factor for consumer acceptance. Sensory evaluation showed only minor reductions in flavor and overall acceptability compared to the control bread, while aroma, appearance, and texture remained acceptable. This validates RSV-enriched corn starch gels as a practical option for functional bread development.
- Resveratrol–amyloglucosidase co-modulation offers a promising strategy to slow starch digestion in wheat breads by shifting starch toward slowly digestible fractions, improving resveratrol recovery, and enhancing bread texture without compromising technological or nutritional quality.
- A major finding was that adding amyloglucosidase (AMG) together with RSV significantly improved RSV recovery in white and whole-wheat breads. This improvement is likely because

AMG-generated hydrolysis products created a microenvironment that better protected or entrapped RSV.

- These observations align with known phenolic–polysaccharide interactions and support RSV's ability to bind starch and limit enzymatic access. Overall, the results reinforce that RSV-enhanced breads may help reduce postprandial glycemic response, contributing to the development of health-promoting functional bakery products.

Future Directions

- Future work should first validate these *in vitro* findings to *in vivo*, using acute postprandial human trials (or at least animal/postprandial models) comparing control bread, and RSV enriched bread to determine the actual effect on blood glucose and insulin.
- Our study clearly showed that mixing is a crucial stage for RSV loss. So, it is important to identify the reasons of mixing losses, it could be from oxidation or polymer binding.
- To identify the RSV-matrix interaction in the dough FTIR, DSC/XRD, and CLSM should be performed to track H-bonding, crystallinity/retrogradation, and spatial localization.
- The current study only found a slight decrease in flavor/overall liking with RSV_CS bread, but larger, more diverse panels and higher RSV doses are required to determine the maximum acceptable level, and to test strategies for flavor-masking or sweetness-balancing. This means that consumer-level sensory validation is required.
- A further strategy is to refine and expand the formulation. To find the optimal combination that maintains low mixing losses and great bread quality, a planned study adjusting the RSV level, AMG addition, and flour type (white vs. whole wheat) should be conducted. Comparing other food-grade carriers (such as pea starch, xanthan, LBG, and protein/cyclodextrin systems) is necessary to identify those that both protect RSV and promote retrogradation, which increases

resistant starch during storage. Since whole-wheat was the most difficult matrix, specific research on the interactions between fiber, phenol, and starch is required.

- Lastly, the same approach should be tested in gluten-free or high-GI breads and verified at the pilot or industrial level.

References

Agbadua, O. G., Kúsz, N., Berkecz, R., Gáti, T., Tóth, G., & Hunyadi, A. (2022). Oxidized resveratrol metabolites as potent antioxidants and xanthine oxidase inhibitors. *Antioxidants*, *11*(9), 1832. https://doi.org/10.3390/ANTIOX11091832/S1

Ahmad, M., & Gani, A. (2021). Development of novel functional snacks containing nano-encapsulated resveratrol with anti-diabetic, anti-obesity and antioxidant properties. *Food Chemistry*, *352*, 129323. https://doi.org/10.1016/J.FOODCHEM.2021.129323

Akinwumi, B. C., Bordun, K. M., & Anderson, H. D. (2018). Biological Activities of Stilbenoids. *Int J Mol Sci*, 19(3). doi: https://doi.org/10.3390/ijms19030792

Aleixandre, A., & Rosell, C. M. (2022). Starch gels enriched with phenolics: Effects on paste properties, structure and digestibility. *LWT*, *161*, 113350. https://doi.org/10.1016/J.LWT.2022.113350

Aleixandre, A., Benavent-Gil, Y., Moreira, R., & Rosell, C. M. (2021). In vitro digestibility of gels from different starches: Relationship between kinetic parameters and microstructure. *Food Hydrocolloids*, *120*, 106909. https://doi.org/10.1016/j.foodhyd.2021.106909

Banaszak, M., Górna, I., & Przysławski, J. (2022). Non-Pharmacological Treatments for Insulin Resistance: Effective Intervention of Plant-Based Diets—A Critical Review. *Nutrients,* 14(7), 1400. Retrieved from https://www.mdpi.com/2072-6643/14/7/1400

Barrera, G. N., Tadini, C. C., León, A. E., & Ribotta, P. D. (2016). Use of alpha-amylase and amyloglucosidase combinations to minimize the bread quality problems caused by high levels of damaged starch. *Journal of Food Science and Technology*, *53*(10), 3675–3684. https://doi.org/10.1007/s13197-016-2337-2

Bassett, N., Gutiérrez, A., Acuña, E., & Rossi, A. (2022). Development of Breads Fortified in Calcium and High Protein Content through the Use of Bean Flour and Regional Fruits. Paper presented at the Biology and Life Sciences Forum.

Beltrão Martins, R., Garzón, R., Peres, J. A., Barros, A. I. R. N. A., Raymundo, A., & Rosell, C. M. (2022). Acorn flour and sourdough: An innovative combination to improve gluten free bread characteristics. *Food Research and Technology, Springer*, *248*(6), 1691–1702. https://doi.org/10.1007/S00217-022-03996-Y

Bhatt, J. K., Thomas, S., & Nanjan, M. J. (2012). Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. *Nutrition Research,* 32(7), 537-541. doi: https://doi.org/10.1016/j.nutres.2012.06.003

Brown, K., Theofanous, D., Britton, R. G., Aburido, G., Pepper, C., Sri Undru, S., & Howells, L. (2024). Resveratrol for the Management of Human Health: How Far Have We Come? A Systematic Review of Resveratrol Clinical Trials to Highlight Gaps and Opportunities. *International Journal of Molecular Sciences*, 25(2), 747. https://pubmed.ncbi.nlm.nih.gov/38255828/

Choi, I., Li, N., & Zhong, Q. (2022). Enhancing bioaccessibility of resveratrol by loading in natural porous starch microparticles. *International Journal of Biological Macromolecules*, *194*, 982–992. https://doi.org/10.1016/J.IJBIOMAC.2021.11.157

Chusak, C., Pasukamonset, P., Chantarasinlapin, P., & Adisakwattana, S. (2020). Postprandial Glycemia, Insulinemia, and Antioxidant Status in Healthy Subjects after Ingestion of Bread made from Anthocyanin-Rich Riceberry Rice. *Nutrients*, *12*(3), 782. https://doi.org/10.3390/nu12030782

Cione, E., La Torre, C., Cannataro, R., Caroleo, M. C., Plastina, P., & Gallelli, L. (2019). Quercetin, Epigallocatechin Gallate, Curcumin, and Resveratrol: From Dietary Sources to Human MicroRNA Modulation. *Molecules*, 25(1). doi: https://doi.org/10.3390/molecules25010063

Ćorković, I., Gašo-Sokač, D., Pichler, A., Šimunović, J., & Kopjar, M. (2022). Dietary Polyphenols as Natural Inhibitors of α-Amylase and α-Glucosidase. *Life (Basel),* 12(11). doi: https://doi.org/10.3390/life12111692

Delpino, F. M., & Figueiredo, L. M. (2022). Resveratrol supplementation and type 2 diabetes: a systematic review and meta-analysis. *Crit Rev Food Sci Nutr*, 62(16), 4465-4480. doi: https://doi.org/10.1080/10408398.2021.1875980

Delpino, F. M., Figueiredo, L. M., da Silva, B. G. C., da Silva, T. G., Mintem, G. C., Bielemann, R. M., & Gigante, D. P. (2022). Omega-3 supplementation and diabetes: A systematic review and meta-analysis. *Critical Reviews in Food Science and Nutrition*, 62(16), 4435-4448. doi: https://doi.org/10.1080/10408398.2021.1875977

Ding, S., Jiang, J., Wang, Z., Zhang, G., Yin, J., Wang, X., Wang, S., & Yu, Z. (2018). Resveratrol reduces the inflammatory response in adipose tissue and improves adipose insulin signaling in high-fat diet-fed mice. *PeerJ*, *2018*(6), https://doi.org/10.7717/PEERJ.5173/SUPP-3

Dong, M., Yang, J., Fang, F., Li, Y., & Li, C. (2024). Effects of different preparation processes on the properties of resveratrol-wheat/rice starch complexes. *Journal of Food Measurement and Characterization*, *18*(7), 5243–5253. https://doi.org/10.1007/S11694-024-02554-2/METRICS

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., . . . Nedović, V. (2015). Trends in Encapsulation Technologies for Delivery of Food Bioactive

Compounds. Food Engineering Reviews, 7(4), 452-490. doi: https://doi.org/10.1007/s12393-014-9106-7

Dura, A., Błaszczak, W., & Rosell, C. M. (2014). Functionality of porous starch obtained by amylase or amyloglucosidase treatments. *Carbohydrate Polymers*, *101*, 837–845. https://doi.org/10.1016/j.carbpol.2013.10.013

Echave, J., Seyyedi-Mansour, S., Donn, P., Jorge, A. O. S., Cassani, L., Barros, L., & Prieto, M. A. (2024). Starch–Polyphenol Interactions: Impact on Food Structure and Starch Digestibility. Proceedings, 103(1), 63. Retrieved from https://www.mdpi.com/2504-3900/103/1/63

Espinosa-Ramírez, J., Garzon, R., Serna-Saldivar, S. O., & Rosell, C. M. (2018). Mimicking gluten functionality with β-conglycinin concentrate: Evaluation in gluten free yeast-leavened breads. *Food Research International*, *106*, 64–70. https://doi.org/10.1016/J.FOODRES.2017.12.055

Fan, H., Yao, X., Chen, Z., Ma, R., Wen, Y., Li, H., Wang, J., & Sun, B. (2023). Interaction of high amylose corn starch with polyphenols: Modulating the stability of polyphenols with different structure against thermal processing. *Food Chemistry*, *437*. https://doi.org/10.1016/J.FOODCHEM.2023.137708

Figueiredo-González, M., Reboredo-Rodríguez, P., González-Barreiro, C., Carrasco-Pancorbo, A., Cancho-Grande, B., & Simal-Gándara, J. (2019). The involvement of phenolic-rich extracts from Galician autochthonous extra-virgin olive oils against the α-glucosidase and α-amylase inhibition. *Food Research International*, *116*, 447–454. https://doi.org/10.1016/J.FOODRES.2018.08.060

Forester, S. C., Gu, Y., & Lambert, J. D. (2012). Inhibition of starch digestion by the green tea polyphenol, (−)-epigallocatechin-3-gallate. *Molecular Nutrition & Food Research*, 56(11), 1647-1654. doi: https://doi.org/10.1002/mnfr.201200206

Francavilla, A., & Joye, I. J. (2022). Anthocyanin Content of Crackers and Bread Made with Purple and Blue Wheat Varieties. *Molecules,* 27(21). doi: https://doi.org/10.3390/molecules27217180

Gasparre, N., Garzon, R., Santamaría, M., & Rosell, C. M. (2022). Rapid test to record the impact of bakery additives on starch gelatinization: Amylases, hydrocolloids and emulsifiers. *Journal of Cereal Science*, *107*, 103540. https://doi.org/10.1016/j.jcs.2022.103540

Girard, A. L., & Awika, J. M. (2020). Effects of edible plant polyphenols on gluten protein functionality and potential applications of polyphenol–gluten interactions. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 2164–2199. https://doi.org/10.1111/1541-4337.12572

Giuberti, G., Rocchetti, G., & Lucini, L. (2020). Interactions between phenolic compounds, amylolytic enzymes and starch: an updated overview. *Current Opinion in Food Science*, *31*, 102–113. https://doi.org/10.1016/J.COFS.2020.04.003

Goh, R., Gao, J., Ananingsih, V. K., Ranawana, V., Henry, C. J., & Zhou, W. (2015). Green tea catechins reduced the glycaemic potential of bread: An in vitro digestibility study. *Food Chemistry*, *180*, 203–210. https://doi.org/10.1016/j.foodchem.2015.02.054

Grasso, A. C., Besselink, J. J., Tyszler, M., & Bruins, M. J. (2023). The potential of food fortification as an enabler of more environmentally sustainable, nutritionally adequate diets. *Nutrients,* 15(11), 2473.

Gu, Y., Hurst, W. J., Stuart, D. A., & Lambert, J. D. (2011). Inhibition of Key Digestive Enzymes by Cocoa Extracts and Procyanidins. *Journal of Agricultural and Food Chemistry*, *59*(10), 5305–5311. https://doi.org/10.1021/jf200180n

Guo, Q., Xiao, X., Li, C., Kang, J., Liu, G., Goff, H. D., & Wang, C. (2021). Catechin-grafted arabinoxylan conjugate: Preparation, structural characterization and property investigation. *International Journal of Biological Macromolecules*, *182*, 796–805. https://doi.org/10.1016/j.ijbiomac.2021.03.190

Han, X., Zhang, M., Zhang, R., Huang, L., Jia, X., Huang, F., & Liu, L. (2020). Physicochemical interactions between rice starch and different polyphenols and structural characterization of their complexes. *LWT*, *125*, 109227. https://doi.org/10.1016/j.lwt.2020.109227

Hashemi, M., Aminzare, M., Hassanzadazar, H., Roohinejad, S., Tahergorabi, R., & Bekhit, A. E. A. (2023). Impact of sodium alginate-based film loaded with resveratrol and thymol on the shelf life of cooked sausage and the inoculated Listeria monocytogenes. *Food Sci Nutr*, 11(12), 7855-7869. doi: https://doi.org/10.1002/fsn3.3702

He, M., Xu, Z., Yao, S., Ma, M., Huang, W., Sui, Z., & Corke, H. (2020). Resveratrol alters texture and provides nutritional benefits in white-salted noodles. *International Journal of Food Science and Technology*, *55*(7), 2740–2750. https://doi.org/10.1111/IJFS.14527

Health Canada (2025), Natural Health Product: Resveratrol, Updated in 2025, January 10, https://webprod.hc-sc.gc.ca/nhpid-bdipsn/dbImages/mono_resveratrol_english.pdf

Hoca, M., Becer, E., & Vatansever, H. S. (2023). The role of resveratrol in diabetes and obesity associated with insulin resistance. *Archives of Physiology and Biochemistry,* 129(2), 555-561. doi: https://doi.org/10.1080/13813455.2021.1893338

Hosseini, H., Koushki, M., Khodabandehloo, H., Fathi, M., Panahi, G., Teimouri, M., . . . Meshkani, R. (2020). The effect of resveratrol supplementation on C-reactive protein (CRP) in type 2 diabetic patients: Results from a systematic review and meta-analysis of randomized controlled trials. *Complementary Therapies in Medicine*, 49, 102251.

Hou, C., Zhao, X., Tian, M., Zhou, Y., Yang, R., Gu, Z., & Wang, P. (2020). Impact of water extractable arabinoxylan with different molecular weight on the gelatinization and retrogradation behavior of wheat starch. *Food Chemistry*, *318*, 126477. https://doi.org/10.1016/j.foodchem.2020.126477

Hua, X., & Yang, R. (2016). Enzymes in starch processing. *Enzymes in food and beverage processing*, 139-170. https://doi.org/10.1201/b19408

International Diabetes Federation. (2025). *IDF Diabetes Atlas* (11th ed.). International Diabetes Federation. https://diabetesatlas.org

Irondi, E. A., Adegoke, B. M., Effion, E. S., Oyewo, S. O., Alamu, E. O., & Boligon, A. A. (2019). Enzymes inhibitory property, antioxidant activity and phenolics profile of raw and roasted red sorghum grains in vitro. *Food Science and Human Wellness*, *8*(2), 142–148. https://doi.org/10.1016/J.FSHW.2019.03.012

Ito-Nagahata, T., Kurihara, C., Hasebe, M., Ishii, A., Yamashita, K., Iwabuchi, M., . . . Fujiwara, Y. (2013). Stilbene Analogs of Resveratrol Improve Insulin Resistance through Activation of AMPK. Bioscience, *Biotechnology, and Biochemistry*, 77(6), 1229-1235. doi: https://doi.org/10.1271/bbb.121000

Iuga, C., Alvarez-Idaboy, J. R., & Russo, N. (2012). Antioxidant Activity of *trans* -Resveratrol toward Hydroxyl and Hydroperoxyl Radicals: A Quantum Chemical and Computational Kinetics Study. *The Journal of Organic Chemistry*, *77*(8), 3868–3877. https://doi.org/10.1021/jo3002134

Jabłońska, W., Wardęszkiewicz, M., Kasprzak, A., Markowiak, S., Świercz, M., Kubisz, M., Mańkowska, A., Truchta, M., Pejas, A., & Kolano, A. (2024). Resveratrol - its properties,

occurrence and health benefits. *Journal of Education, Health and Sport*, *51*, 116–128. https://doi.org/10.12775/JEHS.2024.51.009

Jiang, T., Wang, H., Xu, P., Yao, Y., Ma, Y., Wei, Z., . . . Zhao, D. (2023). Effect of grape seed proanthocyanidin on the structural and physicochemical properties of bread during bread fermentation stage. *Current Research in Food Science*, 7, 100559. doi: https://doi.org/10.1016/j.crfs.2023.100559

Karki, S., Gasparre, N., Netticadan, T., & Rosell, C. M. (2025). Effect of hydrophilic colloids containing resveratrol on dough processing and bread quality. *Food Bioscience*, *74*, 107796. https://doi.org/10.1016/j.fbio.2025.107796

Khaleel, E. F., Abdel-Aleem, G. A., & Mostafa, D. G. (2017). Resveratrol improves high-fat diet induced fatty liver and insulin resistance by concomitantly inhibiting proteolytic cleavage of sterol regulatory element-binding proteins, free fatty acid oxidation, and intestinal triglyceride absorption. *96*(2), 145–157. https://doi.org/10.1139/CJPP-2017-0001

Khan, M. A., Yue, C., Fang, Z., Hu, S., Cheng, H., Bakry, A. M., & Liang, L. (2019). Alginate/chitosan-coated zein nanoparticles for the delivery of resveratrol. *Journal of Food Engineering*, *258*, 45–53 https://doi.org/10.1016/J.JFOODENG.2019.04.010

Kinner, M., Rüegg, R., Weber, C. A., Buchli, J., Durrer, L., & Müller, N. (2021). Impact of selected baking and vacuum cooling parameters on the quality of toast bread. *J Food Sci Technol*, 58(12), 4578-4586. doi: https://doi.org/10.1007/s13197-020-04945-x

Langcake, P., & Pryce, R. J. (1976). The production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury. *Physiological Plant Pathology,* 9(1), 77-86. doi: https://doi.org/10.1016/0048-4059(76)90077-1

Lei, W., Peng, Q., Xiu-Feng, L., Zhang, S., Zhi-Gang, Z., & Yong-Qiang, T. (2016). Comparative analysis of chemical constituents, antimicrobial and antioxidant activities of ethylacetate extracts of Polygonum cuspidatum and its endophytic actinomycete, Streptomyces sp. A0916. *Chinese journal of natural medicines,* 14(2), 117-123.

Li, C., Dhital, S., & Gidley, M. J. (2022). High-amylose wheat bread with reduced in vitro digestion rate and enhanced resistant starch content. *Food Hydrocolloids*, *123*, 107181. https://doi.org/10.1016/j.foodhyd.2021.107181

Li, J., Zhang, J., Yu, W., Gao, H., Szeto, I. M. Y., Feng, H., Liu, X., Wang, Y., & Sun, L. (2023). Soluble dietary fibres decrease α-glucosidase inhibition of epigallocatechin gallate through affecting polyphenol-enzyme binding interactions. *Food Chemistry*, *409*, 135327. https://doi.org/10.1016/j.foodchem.2022.135327

Li, J., Zhu, Y., Yadav, M. P., & Li, J. (2019). Effect of various hydrocolloids on the physical and fermentation properties of dough. *Food Chemistry*, *271*, 165–173. https://doi.org/10.1016/J.FOODCHEM.2018.07.192

Li, L., Gao, J., Koh, H. S. A., & Zhou, W. (2022). Bioaccessibility and Bioavailability of (-)-Epigallocatechin Gallate in the Bread Matrix with Glycemic Reduction. *Foods*, *12*(1), 30. https://doi.org/10.3390/foods12010030

Li, S., Wu, W., Li, J., Zhu, S., Yang, X., & Sun, L. (2022). α-Amylase Changed the Catalytic Behaviors of Amyloglucosidase Regarding Starch Digestion Both in the Absence and Presence of Tannic Acid. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.817039

Li, X., Li, C., Feng, J., Li, T., Zhou, D., Wu, C., & Fan, G. (2024). Insights into formation and stability mechanism of V(7)-type short amylose-resveratrol complex using molecular

dynamics simulation and molecular docking. *Int J Biol Macromol*, 265(Pt 2), 130930. doi: https://doi.org/10.1016/j.ijbiomac.2024.130930

Lin, Y., Yngve, A., Lagergren, J., & Lu, Y. (2014). A dietary pattern rich in lignans, quercetin and resveratrol decreases the risk of oesophageal cancer. *British journal of nutrition*, 112(12), 2002-2009.

Louis, X. L., Thandapilly, S. J., MohanKumar, S. K., Yu, L., Taylor, C. G., Zahradka, P., & Netticadan, T. (2012). Treatment with low-dose resveratrol reverses cardiac impairment in obese prone but not in obese resistant rats. *J Nutr Biochem*, 23(9), 1163-1169. doi: https://doi.org/10.1016/j.jnutbio.2011.06.010

Lu, Y., Luthria, D., Fuerst, E. P., Kiszonas, A. M., Yu, L., & Morris, C. F. (2014). Effect of processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties. *Journal of Agricultural and Food Chemistry*, *62*(43).doi:https://doi.org/10.1021/JF501941R/ASSET/IMAGES/MEDIUM/JF-2014-01941R_0003.GIF

Ma, F., Ren, G., Zhou, M., Pan, R., & Zhang, B. a. F., Jinling. (2023). Exploring the Inhibitory Mechanism of Res on A-Amylase and A-Glucosidase Via Enzymatic Inhibition Assay, Multi-Spectroscopic Analysis and Molecular Docking. doi: https://doi.org/10.2139/ssrn.4405914

Ma, N., & Zhang, Y. (2022). Effects of resveratrol therapy on glucose metabolism, insulin resistance, inflammation, and renal function in the elderly patients with type 2 diabetes mellitus: A randomized controlled clinical trial protocol. *Medicine (United States)*, *101*(32), E30049. doi: https://doi.org/10.1097/MD.0000000000030049

Mao, S., Ren, Y., Ye, X., Kong, X., & Tian, J. (2023). Regulating the physicochemical, structural characteristics and digestibility of potato starch by complexing with different phenolic

acids. *International Journal of Biological Macromolecules*, *253*, 127474. https://doi.org/10.1016/j.ijbiomac.2023.127474

Matos, M., Marefati, A., Barrero, P., Rayner, M., & Gutiérrez, G. (2021). Resveratrol loaded Pickering emulsions stabilized by OSA modified rice starch granules. *Food Res Int*, 139, 109837. doi:10.1016/j.foodres.2020.109837

Mesta-Corral, M., Gómez-García, R., Balagurusamy, N., Torres-León, C., & Hernández-Almanza, A. Y. (2024). Technological and Nutritional Aspects of Bread Production: An Overview of Current Status and Future Challenges. *Foods*, *13*(13), 2062. https://doi.org/10.3390/foods13132062

Mkandawire, N. L., Kaufman, R. C., Bean, S. R., Weller, C. L., Jackson, D. S., & Rose, D. J. (2013). Effects of Sorghum (*Sorghum bicolor* (L.) Moench) Tannins on α-Amylase Activity and in Vitro Digestibility of Starch in Raw and Processed Flours. *Journal of Agricultural and Food Chemistry*, *61*(18), 4448–4454. https://doi.org/10.1021/jf400464j

Molina, M. T., Lamothe, L., Gunes, D. Z., Vaz, S. M., & Bouchon, P. (2021). The Effect of Arabinoxylan and Wheat Bran Incorporation on Dough Rheology and Thermal Processing of Rotary-Moulded Biscuits. *Foods*, *10*(10), 2335. https://doi.org/10.3390/foods10102335

Montes, L., Santamaria, M., Garzon, R., Rosell, C. M., & Moreira, R. (2024). Effect of polyphenols from Ascophyllum nodosum seaweeds on the rheology and digestion of corn starch gels and gluten-free bread features. *Heliyon*, 10(7).

Morris, C., & Morris, G. A. (2012). The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: A review. *Food Chemistry*, 133(2), 237-248. doi:https://doi.org/10.1016/j.foodchem.2012.01.027

Movahed, A., Nabipour, I., Lieben Louis, X., Thandapilly, S. J., Yu, L., Kalantarhormozi, M., . . . Netticadan, T. (2013). Antihyperglycemic Effects of Short Term Resveratrol Supplementation in Type 2 Diabetic Patients. *Evidence-Based Complementary and Alternative Medicine,* 2013, 851267. doi: https://doi.org/10.1155/2013/851267

Navarro, G., Martínez Pinilla, E., Ortiz, R., Noé, V., Ciudad, C. J., & Franco, R. (2018). Resveratrol and related stilbenoids, nutraceutical/dietary complements with health-promoting actions: industrial production, safety, and the search for mode of action. *Comprehensive Reviews in Food Science and Food Safety*, *17*(4), 808–826. doi: https://doi.org/10.1111/1541-4337.12359

Nikitin, I., Terentev, S. E., & Klokonos, M. V. (2022). Development of technology of rye wheat bread, enriched with calcium for consumers with a genetic predisposition to impaired bone mineral density. *Khleboproducty,* 31, 35-39. doi: https://doi.org/10.32462/0235-2508-2022-31-5-35-39

Peng, X., Ma, J., Cheng, K.-W., Jiang, Y., Chen, F., & Wang, M. (2010). The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. *Food Chemistry,* 119(1), 49-53. doi: https://doi.org/10.1016/j.foodchem.2009.05.083

Perrone, D., Fuggetta, M. P., Ardito, F., Cottarelli, A., de Filippis, A., Ravagnan, G., de Maria, S., & lo Muzio, L. (2017). Resveratrol (3,5,4′-trihydroxystilbene) and its properties in oral diseases. *Experimental and Therapeutic Medicine*, *14*(1), 3–9. https://doi.org/10.3892/etm.2017.4472

Pilz, S., März, W., Cashman, K. D., Kiely, M. E., Whiting, S. J., Holick, M. F., . . . Trummer, C. (2018). Rationale and plan for vitamin D food fortification: a review and guidance paper. *Frontiers in endocrinology*, 9, 373.

Platon, N., Arus, V.-A., Georgescu, A., Nistor, I. D., & Barsan, N. (2020). White bread fortified with calcium from eggshell powder. *Rev. Chim*, 71(7), 299-306.

Qin, W., Pi, J., & Zhang, G. (2022). The interaction between tea polyphenols and wheat gluten in dough formation and bread making. *Food & Function*, *13*(24), 12827–12835. https://doi.org/10.1039/D2FO02576K

Raj, P., Thandapilly, S. J., Wigle, J., Zieroth, S., & Netticadan, T. (2021). A Comprehensive Analysis of the Efficacy of Resveratrol in Atherosclerotic Cardiovascular Disease, Myocardial Infarction and Heart Failure. *Molecules,* 26(21), 6600. Retrieved from https://www.mdpi.com/1420-3049/26/21/6600

Robyt, J. F. (2009). Enzymes and Their Action on Starch. In Starch (pp. 237–292). Elsevier. https://doi.org/10.1016/B978-0-12-746275-2.00007-0

Roman, L., & Martinez, M. M. (2019). Structural Basis of Resistant Starch (RS) in Bread: Natural and Commercial Alternatives. *Foods*, *8*(7), 267. https://doi.org/10.3390/foods8070267

Rosell, C. M. (2011). The science of doughs and bread quality. In Flour and breads and their fortification in health and disease prevention (pp. 3-14): *Elsevier.* doi: https://doi.org/10.1016/B978-0-12-380886-8.10001-7

Rosell, C. M., Collar, C., & Haros, M. (2007). Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab. *Food Hydrocolloids, 21*(3), 452–462. https://doi.org/10.1016/j.foodhyd.2006.05.004

Rosell, C. M., Rojas, J. A., & Benedito de Barber, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids*, *15*(1), 75–81. https://doi.org/10.1016/S0268-005X(00)00054-0

Santamaria, M., Garzon, R., Skendi, A., Papageorgiou, M., & Rosell, C. M. (2025). Examining the role of polyphenols in the in vitro starch hydrolysis of gluten free enriched flatbreads. *Food Chemistry*, *486*, 144456. https://doi.org/10.1016/j.foodchem.2025.144456

Santamaria, M., Montes, L., Garzon, R., Moreira, R., & Rosell, C. M. (2023). Performance of Starch Gels on In Vitro Enzymatic Hydrolysis Assessed by Rheological Methodologies. *Starch/Staerke*, *75*(1–2). https://doi.org/10.1002/star.202200189

Santamaria, M., Ruiz, M., Garzon, R., & Rosell, C. M. (2024). Comparison of vegetable powders as ingredients of flatbreads: technological and nutritional properties. *International Journal of Food Science and Technology*, *59*(10), 7203–7212. https://doi.org/10.1111/ijfs.17441

Scazzina, F., Siebenhandl-Ehn, S., & Pellegrini, N. (2013). The effect of dietary fibre on reducing the glycaemic index of bread. *British Journal of Nutrition*, *109*(7), 1163–1174. https://doi.org/10.1017/S0007114513000032

Shen, Y., Xu, Z., & Sheng, Z. (2017). Ability of resveratrol to inhibit advanced glycation end product formation and carbohydrate-hydrolyzing enzyme activity, and to conjugate methylglyoxal. *Food Chemistry*, 216, 153-160. doi: https://doi.org/10.1016/j.foodchem.2016.08.034

Shi, Z., Chen, H., He, J., Zhang, W., & Lin, H. (2024). The Addition of Resveratrol-Loaded Emulsions to Yogurts: Physicochemical Characterization, *In Vitro* Bioaccessibility and NMR-Based Nutritional Profiles. *Foods*, 13(3), 426. Retrieved from https://www.mdpi.com/2304-8158/13/3/426

Shruthi, P. A., Pushpadass, H. A., Franklin, M. E. E., Battula, S. N., & Laxmana Naik, N. (2020). Resveratrol-loaded proniosomes: Formulation, characterization and fortification. *LWT*, *134*. https://doi.org/10.1016/J.LWT.2020.110127

Silva, A. F. R., Monteiro, M., Nunes, R., Baião, A., Braga, S. S., Sarmento, B., Coimbra, M. A., Silva, A. M. S., & Cardoso, S. M. (2022). Bread enriched with resveratrol: Influence of the delivery vehicles on its bioactivity. *Food Bioscience*, *49*, 101887. https://doi.org/10.1016/J.FBIO.2022.101887

Silva, A. F. R., Monteiro, M., Resende, D., Braga, S. S., Coimbra, M. A., Silva, A. M. S., & Cardoso, S. M. (2020). Inclusion Complex of Resveratrol with γ-Cyclodextrin as a Functional Ingredient for Lemon Juices. *Foods,* 10(1). doi: https://doi.org/10.3390/foods10010016

Silva, A. F. R., Silva-Reis, R., Ferreira, R., Oliveira, P. A., Faustino-Rocha, A. I., Pinto, M. de L., Coimbra, M. A., Silva, A. M. S., & Cardoso, S. M. (2023). The impact of resveratrol-enriched bread on cardiac remodeling in a preclinical model of diabetes. *Antioxidants*, *12*(5), 1066. https://doi.org/10.3390/ANTIOX12051066/S1

Silva, Monteiro, M., Nunes, R., Baião, A., Braga, S. S., Sarmento, B., . . . Cardoso, S. M. (2022). Bread enriched with resveratrol: Influence of the delivery vehicles on its bioactivity. *Food Bioscience*, 49, 101887. doi: https://doi.org/10.1016/j.fbio.2022.101887

Silva, P. M., Cerqueira, M. A., Pastrana, L., Coimbra, M. A., Vicente, A. A., Van Bockstaele, F., . . . Dewettinck, K. (2024). Development of functional foods: Consumer acceptance of resveratrol-loaded crackers and cookies. *Future Foods,* 10, 100459. doi: https://doi.org/10.1016/j.fufo.2024.100459

Silva, P. M., Neto, M. D., Cerqueira, M. A., Rodriguez, I., Bourbon, A. I., Azevedo, A. G., Pastrana, L. M., Coimbra, M. A., Vicente, A. A., & Gonçalves, C. (2024a). Resveratrol-loaded octenyl succinic anhydride modified starch emulsions and hydroxypropyl methylcellulose (HPMC) microparticles: Cytotoxicity and antioxidant bioactivity assessment after in vitro digestion.

International Journal of Biological Macromolecules, *259*, 129288. https://doi.org/10.1016/J.IJBIOMAC.2024.129288

Silva, R., Teixeira, J. A., Nunes, W. D. G., Zangaro, G. A. C., Pivatto, M., Caires, F. J., & Ionashiro, M. (2017). Resveratrol: A thermoanalytical study. *Food Chemistry*, 237, 561-565. doi: https://doi.org/10.1016/j.foodchem.2017.05.146

Silva, Silva-Reis, R., Ferreira, R., Oliveira, P. A., Faustino-Rocha, A. I., Pinto, M. d. L., . . . Cardoso, S. M. (2023). The Impact of Resveratrol-Enriched Bread on Cardiac Remodeling in a Preclinical Model of Diabetes. *Antioxidants,* 12(5), 1066.

Souza, S. V. S., Borges, N., & Vieira, E. F. (2022). Vitamin d-fortified bread: Systematic review of fortification approaches and clinical studies. *Food Chemistry*, 372, 131325. doi: https://doi.org/10.1016/j.foodchem.2021.131325

Statista. (2022). Bread. Retrieved from: https://www.statista.com/outlook/cmo/food/bread-cereal-products/bread/worldwide

Summerlin, N., Soo, E., Thakur, S., Qu, Z., Jambhrunkar, S., & Popat, A. (2015). Resveratrol nanoformulations: Challenges and opportunities. *International Journal of Pharmaceutics*, 479(2), 282-290. doi:https://doi.org/10.1016/j.ijpharm.2015.01.003

Sun, L., & Miao, M. (2020). Dietary polyphenols modulate starch digestion and glycaemic level: a review. *Critical Reviews in Food Science and Nutrition*, *60*(4), 541–555. https://doi.org/10.1080/10408398.2018.1544883

Sun, L., & Miao, M. (2020). Dietary polyphenols modulate starch digestion and glycaemic level: a review. *Crit Rev Food Sci Nutr,* 60(4), 541-555. doi: https://doi.org/10.1080/10408398.2018.1544883

Teodoro, A. J. (2019). Bioactive Compounds of Food: Their Role in the Prevention and Treatment of Diseases. *Oxid Med Cell Longev*, 2019, 3765986. doi: https://doi.org/10.1155/2019/3765986

Tian, B., & Liu, J. (2020). Resveratrol: a review of plant sources, synthesis, stability, modification and food application. *J Sci Food Agric,* 100(4), 1392-1404. doi: https://doi.org/10.1002/jsfa.10152

Tian, H., Xiang, D., & Li, C. (2021). Tea polyphenols encapsulated in W/O/W emulsions with xanthan gum-locust bean gum mixture: Evaluation of their stability and protection. *Int J Biol Macromol,* 175, 40-48. doi: https://doi.org/10.1016/j.ijbiomac.2021.01.161

Villas-Boas, F., Facchinatto, W. M., Colnago, L. A., Volanti, D. P., & Franco, C. M. L. (2020). Effect of amylolysis on the formation, the molecular, crystalline and thermal characteristics and the digestibility of retrograded starches. *International Journal of Biological Macromolecules*, *163*, 1333–1343. https://doi.org/10.1016/j.ijbiomac.2020.07.181

Visvanathan, R., Le, D. T., Dhital, S., Rali, T., Davis, R. A., & Williamson, G. (2024). Inhibition of Human Salivary and Pancreatic α-Amylase by Resveratrol Oligomers. *Journal of Medicinal Chemistry*, *67*(21), 18753–18763. https://doi.org/10.1021/acs.jmedchem.4c01042

Wahab, M., & Janaswamy, S. (2025). Porous starch granules loaded curcumin and resveratrol modulate starch digestibility of wheat bread: Toward developing novel functional foods. *Food Hydrocolloids*, *162*, 111006. https://doi.org/10.1016/J.FOODHYD.2024.111006

Wang, M., Ma, L., Xie, P., Li, C., Yang, X., & Lang, Y. (2025). Improved antioxidant properties of pork patties by replacing fat with resveratrol-loaded MP-CS complex stabilized pickering

emulsion. *Food Science and Technology International*, 31(3), 226-236. doi: https://doi.org/10.1177/10820132231196202

Wilcox, M. D., Cherry, P., Chater, P. I., Yang, X., Zulali, M., Okello, E. J., . . . Pearson, J. P. (2021). The effect of seaweed enriched bread on carbohydrate digestion and the release of glucose from food. *Journal of Functional Foods,* 87, 104747. doi: https://doi.org/10.1016/j.jff.2021.104747

World Health Organization. (2024, November 14). Diabetes. https://www.who.int/news-room/fact-sheets/detail/diabetes

Wu, Y., He, D., Zong, M., Wu, H., Li, L., Zhang, X., Xing, X., & Li, B. (2022). Improvement in the stability and bioavailability of trans-resveratrol with hydrolyzed wheat starch complexation: a theoretical and experimental study. *Food Structure*, *32*, 100267. https://doi.org/10.1016/j.foostr.2022.100267

Wu, Y., Liu, Y., Jia, Y., Zhang, H., & Ren, F. (2024). Formation and application of starch-polyphenol complexes: influencing factors and rapid screening based on chemometrics. *Foods (Basel, Switzerland)*, *13*(10), 1557–1557. https://doi.org/10.3390/FOODS13101557

Yan, W., Yin, L., Zhang, M., Zhang, M., & Jia, X. (2021). Gelatinization, Retrogradation and Gel Properties of Wheat Starch–Wheat Bran Arabinoxylan Complexes. *Gels*, *7*(4), 200. https://doi.org/10.3390/gels7040200

Yang, D., Guo, Q., Li, R., Chen, L., & Zheng, B. (2023). Amylose content controls the V-type structural formation and in vitro digestibility of maize starch-resveratrol complexes and their effect on human gut microbiota. *Carbohydrate Polymers*, *327*. https://doi.org/10.1016/J.CARBPOL.2023.121702

Yin, X., Cheng, H., Wusigale, Dong, H., Huang, W., & Liang, L. (2022). Resveratrol stabilization and loss by sodium caseinate, whey and soy protein isolates: Loading, antioxidant activity, oxidability. *Antioxidants,* 11(4), 647.

Yoshinaga, M., Toldo, J. M., Rocha, W. R., & Barbatti, M. (2024). Photoisomerization pathways of trans-resveratrol. *Phys Chem Chem Phys*, 26(36), 24179-24188. doi: https://doi.org/10.1039/d4cp02373k

Yu, X., Jia, Y., & Ren, F. (2024). Multidimensional biological activities of resveratrol and its prospects and challenges in the health field. *Frontiers in Nutrition*, *11*. https://doi.org/10.3389/fnut.2024.1408651

Zabot, G. L., Schaefer Rodrigues, F., Polano Ody, L., Vinícius Tres, M., Herrera, E., Palacin, H., . . . Olivera-Montenegro, L. (2022). Encapsulation of Bioactive Compounds for Food and Agricultural Applications. Polymers, 14(19), 4194. Retrieved from https://www.mdpi.com/2073-4360/14/19/4194

Zannini, E., Waters, D. M., & Arendt, E. K. (2014). The application of dextran compared to other hydrocolloids as a novel food ingredient to compensate for low protein in biscuit and wholemeal wheat flour. *European Food Research and Technology*, *238*(5), 763–771. https://doi.org/10.1007/S00217-014-2161-8/METRICS

Zhang, W., Kong, J., Zhang, M., Chen, Y., Yu, Q., Shen, M., & Xie, J. (2024). Starch-anthocyanins complex: Characterization, digestibility, and mechanism. *Food Hydrocolloids*, 151, 109797. doi: https://doi.org/10.1016/j.foodhyd.2024.109797

Zhang, Z., Meng, Y., Wang, J., Qiu, C., Miao, W., Lin, Q., Li, X., Sang, S., McClements, D. J., Jiao, A., & Jin, Z. (2024). Preparation and characterization of zein-based core-shell nanoparticles for encapsulation and delivery of hydrophobic nutrient molecules: Enhancing

environmental stress resistance and antioxidant activity. *Food Hydrocolloids*, *148*. https://doi.org/10.1016/J.FOODHYD.2023.109524

Zheng, B., Zhou, H., & McClements, D. J. (2023). Co-Encapsulation of Multiple Polyphenols in Plant-Based Milks: Formulation, Gastrointestinal Stability, and Bioaccessibility. Foods, 12(18). doi: https://doi.org/10.3390/foods12183432

Zheng, F., Ren, F., Zhu, X., Han, Z., Jia, Y., Liu, X., Chen, B., & Liu, H. (2025). The interaction between starch and phenolic acids: effects on starch physicochemical properties, digestibility and phenolic acids stability. *Food & Function*, *16*(11), 4202–4225. https://doi.org/10.1039/D5FO00855G

Zheng, K., Chen, Z., Fu, Y., Chen, L., Zhu, X., Chen, X., & Ding, W. (2022). Effect of tea polyphenols on the storage stability of non-fermented frozen dough: protein structures and state of water. *Foods 2023, Vol. 12, Page 80*, *12*(1), 80. https://doi.org/10.3390/FOODS12010080

Zhong, Y., Ni, D., Yang, Y., Li, Y., Wang, L., Tian, J., . . . Ye, X. (2024). Inhibitory effect and mechanism of tannic acid against two starch digestive enzymes. *Food Quality and Safety*, 8. doi: https://doi.org/10.1093/fqsafe/fyad057

Zhou, S., Zhang, Y., Xu, Z., Li, C., Gao, B., Xie, J., Li, B., & Zhang, X. (2025). Crosslinked arabinoxylan reduced the starch digestibility through inhibiting the enzyme activity of α-amyloglucosidase but not α-amylase. *Food Bioscience*, *67*, 106342. https://doi.org/10.1016/J.FBIO.2025.106342

Zhu, X., Wu, C., Qiu, S., Yuan, X., & Li, L. (2017). Effects of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: systematic review and meta-analysis. *Nutrition & Metabolism*, 14(1), 60. doi: https://doi.org/10.1186/s12986-017-0217-z

Zupančič, Š., Lavrič, Z., & Kristl, J. (2015). Stability and solubility of trans-resveratrol are strongly influenced by pH and temperature. *European Journal of Pharmaceutics and Biopharmaceutics*, *93*, 196–204. https://doi.org/10.1016/j