Impact of Modified and Native Starch on the Quality Attributes of Gluten-Free Bread

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2025

MASTER THESIS

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Effects of Wheat, Cross-Linked Tapioca, Fractioned and Acetylated Potato Starches on Volume, Texture, Staling, Retrogradation, and Sensory Attributes of Gluten-Free Bread

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Published by
Department of Process and Life Science Engineering
Faculty of Engineering LTH, Lund University
P.O. Box 118, SE-221 00 Lund, Sweden

Subject: KLTM02 Degree Project in Food Engineering, Nutrition and Food Chemistry

Division: Food and Pharma
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June 2025

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Why Gluten-Free Bread Is So Tricky to Get Right — And How Starch Might Be the Secret Ingredient

Gluten-free bread has come a long way, but anyone who's tried baking or buying it knows it's still a tricky product. Without gluten, the protein that gives regular bread its soft, stretchy, and moist texture, gluten-free bread can easily turn dry, crumbly, or tough. For many people with gluten allergies or intolerances, having tasty, fresh bread isn't just a luxury it's a daily necessity. Yet, creating gluten-free bread that stays soft and fresh over time remains a challenge for bakers and food scientists alike.

This study focused on one of the most important ingredients in gluten-free bread: starch. Starches take on much of the job gluten normally does, helping bread hold its shape, trap air bubbles, and keep that desirable soft texture. But starches differ widely, some come straight from grains or tubers, while others are chemically modified to improve their performance. Four types of starch were tested: natural wheat starch, two types of potato starch (one chemically modified and one refined to remove larger starch particles), and a special chemically strengthened tapioca starch.

The type of starch used had a huge impact on how the bread turned out! from the size of the loaf and its texture to how quickly it went dry and tough (stale).
Wheat starch naturally produced the biggest and softest loaves. Its balance of softness and firmness made for bread that was pleasant to eat fresh and reasonably stable over time.
The chemically modified potato starch helped the bread stay moist and soft longer by slowing down the staling process. However, it made smaller loaves, likely because its thicker, stickier dough limited how much the bread could rise during baking and proofing.
The potato starch, with its bigger particles removed, created bread with a good size however, it came at the cost of disappointing texture. The bread quickly became dry, hard, and crumbly, the opposite of what is wanted for gluten-free bread.

The strengthened tapioca starch had the most unique properties: it made a chewy, springy bread that resisted drying out for longer, never becoming crumbly, but the loaves were smaller and denser. This unique texture split opinions in the taste panel: some loved the springiness, others found it too chewy.

Bread going stale usually happens when starch molecules "tangles up" like roots twisting together, making bread hard and dry. But the tapioca bread hardened even without this "root tangling," suggesting other factors like moisture moving around or the crumb structure tightening also affect staling.

This study provides valuable insights for future research and for bakers and producers aiming to make gluten-free bread softer, fresher, and more enjoyable. Since bread is a daily staple for people with gluten allergies, improving its quality is important. Future efforts combining different starches could unlock the ideal balance of loaf size, texture, and shelf life bringing gluten-free bread closer than ever to what people really want.

Abstract

This study investigated the influence of various native and modified starches on the quality and staling behaviour of gluten-free bread. Four starch types: native wheat starch, cross-linked tapioca starch, fractionated native potato starch (FPS), and acetylated potato starch (Amylacetate) were evaluated by incorporating them into a standardized gluten-free bread formulation as the main starch source. Several gluten-free bread quality parameters were assessed, including pasting properties (RVA), gelatinisation and retrogradation characteristics (DSC), specific loaf volume, baking loss, crumb colour (L*a*b*), texture profile analysis (TPA), crumb and crust moisture content, and sensory perception through a ranked attribute test.

The results demonstrated that the type of starch significantly affected all measured bread quality attributes. Wheat starch produced loaves with the highest specific volume and showed balanced textural properties with moderate firmness and cohesiveness. Amylacetate yielded similar texture and retrogradation behaviour to wheat, with lower firmness and retrogradation enthalpy than FPS, but its higher paste viscosity likely inhibited gas expansion during baking, resulting in lower loaf volume. FPS, despite achieving high specific volume, exhibited the most unfavourable textural qualities: high crumb firmness, low cohesiveness, and high retrogradation enthalpy, indicating rapid staling due to amylopectin recrystallization and moisture immobilization. Tapioca starch, while yielding low-volume bread, showed excellent resistance to staling, with the lowest crumb firmness and highest cohesiveness, though its unique chewy texture received mixed sensory ratings.

A general correlation was observed between higher retrogradation enthalpy, crumb firmness, dryness, and poor sensory outcomes, supporting the role of amylopectin recrystallization in staling dynamics. However, exceptions such as the increased firmness in Tapioca bread without corresponding retrogradation enthalpy increases, suggested additional staling mechanisms, possibly linked to moisture redistribution or matrix densification. Additionally, Crumb moisture content did not directly correlate with firmness, highlighting that moisture availability for crumb plasticisation, rather than total moisture, influences texture. Overall, the findings underscore the importance of starch physiochemical properties and modification on both initial bread quality and shelf-life performance in gluten-free baking applications.

Acknowledgement

I would like to express my heartfelt gratitude to my family, my friends, and my boyfriend for their unwavering support throughout this journey. Their encouragement, patience, and care, both emotionally and in keeping me well-fed, have been essential in helping me stay grounded and focused.

I am especially grateful to René Schrøder from KMC for his generosity and invaluable assistance. His guidance in the production of gluten-free bread, provision of raw materials, and deep knowledge of baking were crucial to the successful execution of this project.

I would like to sincerely thank my supervisor, Dr. Jeanette Purhagen, for her dedicated support throughout this thesis. Her insightful feedback, constructive corrections, and guidance have been deeply appreciated and instrumental in shaping the final work

I would also like to thank my fellow students and PhD colleagues for creating a collaborative and enjoyable atmosphere in the lab. The many hours spent working were made far more rewarding through their kindness and company.

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1 Introduction

Gluten-free bread is primarily targeted at individuals with gluten allergies or intolerances. However, its formulation poses a significant challenge due to the absence of gluten, a structural protein essential for providing elasticity and moisture retention to the dough. In gluten-free bread, these functionalities must be compensated for by using alternative ingredients such as starches and functional ingredients.

A problem with gluten-free bread is its tendency to rapidly stale, resulting in crumb firming and loss of freshness. The process of staling involves, among other things, the migration of water from the crumb to the crust as well as retrogradation of the starch molecules, leading to undesirable textural changes (Horstmann, Lynch and Arendt, 2017). The extent and rate of staling vary with the type of starch used, as different sources such as wheat, tapioca, and potato exhibit distinct functional properties. These differences arise from intrinsic characteristics such as crystallinity, amylopectin fine structure, non-starch components, and granule morphology (Horstmann, Lynch and Arendt, 2017; Hellsten, 2019).

To further combat these issues, modified starch can be incorporated to modify the interaction between starch molecules, thereby slowing down the reassociation, improving storage stability and overall quality of the gluten-free bread (Hellsten, 2019).

1.1 Aim

This project aimed to investigate the impact of different starch types, both native and modified on quality attributes, including loaf volume, texture, staling behaviour, retrogradation, and sensory attributes in gluten-free bread. This was achieved by modifying a base recipe and evaluating the effects of each starch on. The study focused on four different starches: native wheat starch (Wheat), cross-linked tapioca starch (Tapioca), fractionated potato starch (FPS), and acetylated potato starch (Amylacetate).

2 Background and Theory

2.1 Gluten and Its Role in Breadmaking

The need for Gluten-free bread stems from the presence of individuals who have either Coeliac disease, sensitivity to gluten, or gluten allergy (Di Sabatino and Corazza, 2009). Coeliac disease is one of the most common food-related autoimmune disorders, with an estimated and increasing prevalence of approximately 1.6%, particularly in Northern Europe. (Roberts *et al.*, 2021). It is triggered by the ingestion of prolamin and glutamine-rich proteins found in wheat, barley, rye, and likely oats. The ingestion causes immune-mediated inflammation leading to injury of the small intestine villi and the mucosa as seen in Figure 1. This subsequently worsens the absorption of nutrients and can prompt gastrointestinal disorders (Di Sabatino and Corazza, 2009; WANG *et al.*, 2017).

This food sensitivity is seen in both children and adults, and currently the only way to mediate the symptoms is to comply with a strict, life-long gluten-free diet, as even small amounts can be detrimental to their health (Wieser, 2007; Di Sabatino and Corazza, 2009). As more people choose gluten-free products, whether due to dietary sensitivities or lifestyle trends, the demand for high-quality gluten-free bread continues to grow, making its development more important than ever (Hassan *et al.*, 2024).

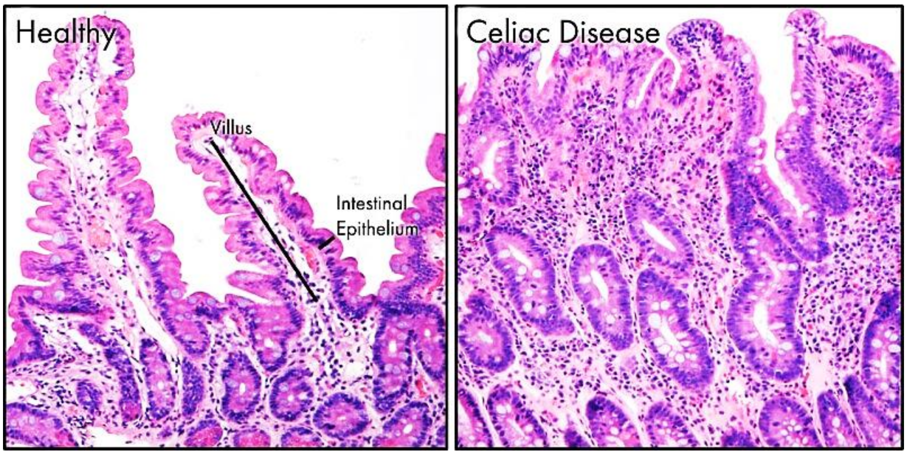

Figure 1 Visual representation of intestinal change in Celiac Disease, highlighting the mucosal damage and villous atrophy (Daley and Haseeb, 2025)

Gluten is primarily composed of two key proteins, gliadins, which are mainly monomeric, and glutenins, which are polymers stabilized by inter-chain disulfide bonds. The former contributes viscosity and extensibility, and the latter contributes to cohesiveness and the elasticity of a dough (Brandner, Becker and Jekle, 2019; Shewry and Lafiandra, 2022). When flour and water is kneaded together these proteins form disulfide and hydrogen bonds, forming a cohesive viscoelastic matrix (WANG *et al.*, 2017; Yano, 2019).

In the context of bread making, this gluten matrix is responsible for gas retention during fermentation. It traps gas, allowing the dough to expand, resulting in a desired volume and crumb structure, which is a critical quality aspect of baked bread. The absence of gluten is particularly significant in gluten-free formulations, as doughs made with gluten-free flours typically lack the viscoelastic properties necessary for effective gas retention during proofing and baking.

This leads to reduced structural stability and impacts the crumb texture, often resulting in loaves that are denser and less cohesive.

Most gluten-free formulations result in doughs with a liquid, batter-like consistency due to the absence of structure-forming proteins. This lack of structure necessitates the inclusion of hydrocolloids and other sources of starch and protein to improve dough structure, enhance gas retention, and increase bread volume. As a result, pans are essential to provide external support during proofing and baking, which influences crust formation and the overall appearance of the final product (Sciarini *et al.*, 2010).

The preparation process also differs, particularly in terms of water content and mixing. Gluten-free doughs typically require higher water content, leading to a viscous batter that promotes starch hydration and swelling, thereby improving dough structure during proofing and baking. Kneading is generally omitted, and mixing time is minimized to just hydrate and homogenize the ingredients, as there is no gluten network to develop. Proofing agents may be chemical, yeast-based, or a combination of both, used to compensate for the dough's limited gas retention capacity. Consequently, achieving a desirable crumb structure depends on several factors, which differ significantly from those in traditional gluten-containing bread (Cappelli, Oliva and Cini, 2020).

2.2 Starch

Different starches from gluten-free sources such as potato and tapioca have been utilized in gluten-free bread formulations (McCarthy *et al.*, 2005; Milde, Ramallo and Puppo, 2012; Horstmann *et al.*, 2016). Furthermore, recent studies have shown that the use of purified gluten-free wheat starch is safe for individuals with gluten sensitivities, as it does not harm the intestinal mucosa, enabling its use for gluten-free bread development (Mäki, 2014; Walter, Wieser and Koehler, 2014).

Native starch, present as discrete granules with alternating crystalline and amorphous growth rings within. Their size, shape, composition, properties, and inner molecular arrangement all depend on their botanical origin and is affected by the climatic conditions and soil type during growth (Singh *et al.*, 2003). A summary of the characteristics of the three different starches used in this study is seen below in Table 1.

Table 1. Composition and morphology of commonly used gluten-free native starches: wheat, tapioca, potato

Starch	Amylose [%]	Moisture [%]	Protein [%]	Lipids [%]	Size [µm]	Shape
Wheat	18-28.8[1,2,4]	12.8-13.0[3,2]	0.4-0.6[1,2,4]	0.38-0.78[4]	0.5-45[6]	Round, lenticular[2]
Tapioca	17-19.8[1,2,5]	13.5-13.7[5,3]	0.04-0.1[1,2,3]	0.10-0.12[2,5]	4-35[6]	Truncated oval[2]
Potato	21-25.2[1,2,5]	13.1-18.0[5,3]	0.02-0.1[1,2,3]	0.05-0.20[2,5]	5-100[6]	Oval, round[2]

1:(BeMiller, 2019) 2: (Swinkels, 1985) 3:(Horstmann et al.*, 2016), 4:(Shevkani et al., 2016), 5:(Gunaratne and Hoover, 2002), 6: (Molenda* et al.*, 2006). *%Amylose refers to the* *proportion of amylose in a starch sample, while the remaining percentage is assumed to be amylopectin.*

Starch granules are composed of two types of alpha-glucans, amylose and amylopectin, which represent approximately 98–99% of the dry weight (Tester, Karkalas and Qi, 2004). Amylose consists mostly of unbranched linear chains of α-1,4-linked d-glucopyranose units with a few branches of α-1,6 linkages, while the highly branched amylopectin is composed of both α-1,4 and α-1,6 branch linkages. Both are illustrated in Figure 2. The larger branched amylopectin is mainly responsible for the crystalline double-helix structures within the starch framework. Amylose, on the other hand, is smaller and represents the amorphous region (BeMiller, 2019).

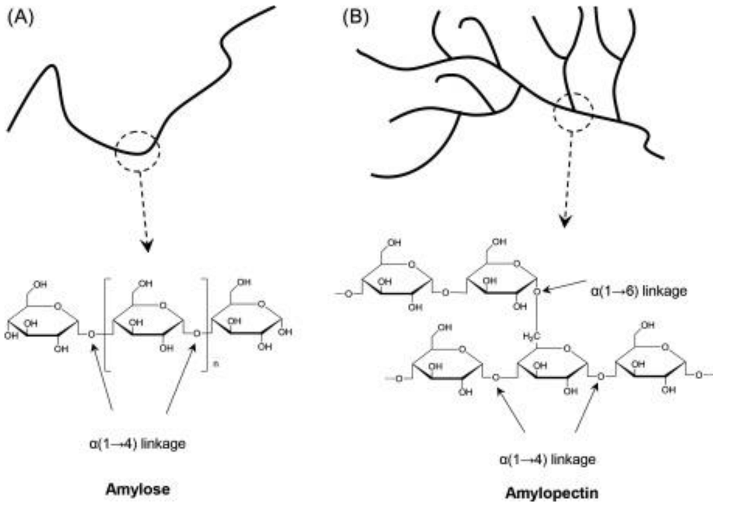

Figure 2 The two macromolecules of starch amylose (A) and amylopectin (B)(Xie, Zhang and Wang, 2017).

2.2.1 Crystalline Structure

Evidence of starch's crystalline structure and birefringence becomes apparent under polarized light, where the granules exhibit a characteristic polarization, called the Maltese Cross, when observed. Granules of different botanical origin have different X-ray diffraction patterns, illustrated in Figure 2 (Pérez, Baldwin and Gallant, 2009; BeMiller, 2019; Dome *et al.*, 2020).

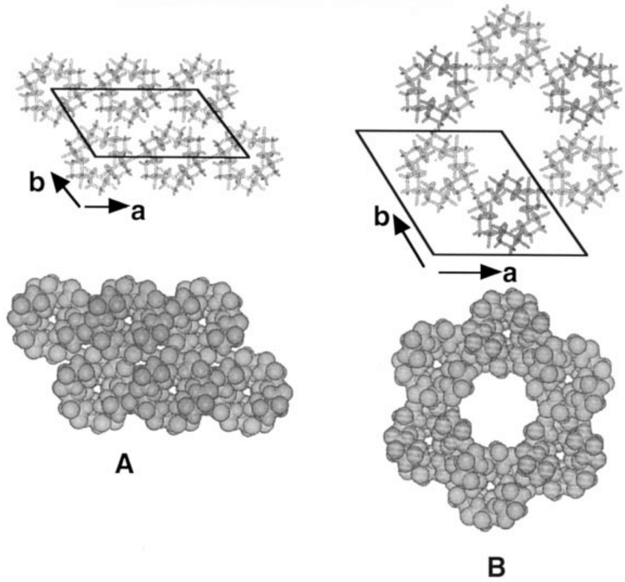

Figure 2. Type A and type B crystalline structure (Buléon et al.*, 1998).*

Wheat starch exhibits mainly the A-type crystalline structure, which is characteristic of cereal starches. This structure is composed of parallel double helices separated by interstitial water and is identified by a specific X-ray diffraction pattern. The packing of amylose and amylopectin in wheat starch reflects this arrangement, which results in a relatively dense crystalline organisation compared to other starch types (Pérez, Baldwin and Gallant, 2009; BeMiller, 2019; Dome *et al.*, 2020).

Potato starch has a B-type crystalline structure. This form is typical of high-amylose starches derived from tubers. In the B-type configuration, six left-handed parallel-stranded double-helices are arranged within a loosely packed hexagonal unit cell, with water molecules occupying interstitial spaces. This results in a looser packing of the helices in the B-type structure (BeMiller, 2019; Dome *et al.*, 2020).

Starch from cassava, also known as tapioca starch, has had both A- and C-pattern reported due to sensitivity to processing as well as variability depending on geographical and environmental origin (da Cruz Francisco *et al.*, 1996; Dome *et al.*, 2020; Patty and Wattimena, 2023). The C-type is considered a hybrid of the A- and B-type crystalline orders. This mixed structural arrangement is commonly found in starches extracted from legumes and some plant roots (Pérez, Baldwin and Gallant, 2009).

There is a significant correlation between the crystalline structure of starch granules and the fine structure of amylopectin. Research has shown that the average chain length of amylopectins varies according to starch type: amylopectins from A-type starches possess shorter chain lengths compared to those from B-type starches, while C-type starches exhibit intermediate chain lengths. In particular, cereal amylopectins tend to associate over shorter segment lengths, which contributes to their reduced rate of retrogradation (Kalichevsky, Orford and Ring, 1990; Vamadevan and Bertoft, 2018). Additionally, a study found that tapioca starch did not retrograde at room temperature (Gomand *et al.*, 2010).

2.2.2 Chemical composition and Functional Properties

Several differences beyond crystalline X-ray patterns were seen between these starches in terms of structure, composition, and physical properties.

Cereal starch amylose molecules, such as those found in wheat, are smaller compared to those found in tuber or root starches like tapioca and potato. Wheat starch generally contains polar lipids in the range of 0.5–1.2%, which tend to form inclusion complexes with amylose and long amylopectin chains, limiting the granule's swelling capacity. Unlike tuber and root starches, wheat starch contains very low levels of phosphorus, mainly present as phospholipids rather than esterified groups on starch polymers. Additionally, wheat starch is chemically characterised by low protein and ash content, bright white colour, and a neutral taste and odour. Its relatively higher starch damage levels may be due to more intensive mechanical extraction processes used in cereal starch isolation (Maningat *et al.*, 2009; Gunaratne and Corke, 2016).

One of the most distinctive features of potato starch is its high swelling power, which is due in part to its large granule size and the presence of covalently bound phosphate groups on amylopectin. The phosphate content in potato amylopectin can range from 200 to 1000 ppm, substantially higher than in cereal starches. Potato starch contains essentially no lipids and exhibits a higher ash and moisture content compared to cereal starches, with the ash content largely attributed to its higher phosphorus levels. The higher moisture content is also believed to be linked to the starch's B-type polymorphic form. Furthermore, potato starch undergoes a gentler extraction process, resulting in lower starch damage levels (Noemi Sivak and Preiss, 1998; Grommers and van der Krogt, 2009, p. 11; BeMiller, 2019; Kovrlija, Goubin and Rondeau-Mouro, 2020).

Tapioca starch shares several characteristics with potato starch, especially in terms of swelling behaviour. Tapioca amylose, like that of potato, has a higher molecular weight than cereal amylose, though its branching pattern is similar to that of wheat. Tapioca starch contains minimal to no lipids and very low levels of phosphate. Like potato starch, tapioca shows high swelling power, but typically to a lesser extent, partially related to smaller granule size. This and the fact that the small amount of phosphorus in tapioca starch is not bound as the phosphate ester, like in potato starch, is likely the reason (Noemi Sivak and Preiss, 1998; Breuninger, Piyachomkwan and Sriroth, 2009).

2.2.3 Granule morphology and Size Distribution

Figure 3 illustrates the morphology of the three starches analysed in this study.

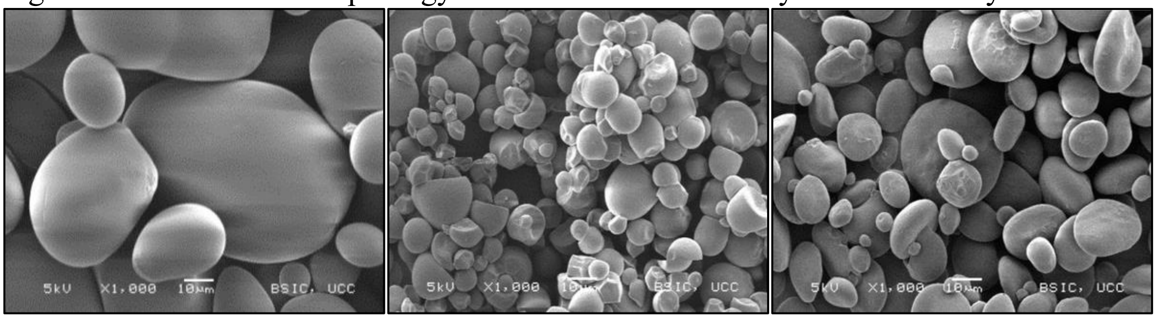

Figure 3. Micrographs (from left to right) of potato starch, tapioca starch, and wheat starch (Magnification 1000x) (Horstmann et al.*, 2016).*

A study on the microstructure of starch granules analysed their size distribution and morphology (Molenda *et al.*, 2006). Potato starch exhibits predominantly oval and spherical granules, which are notably larger than those of tapioca and wheat, with diameters ranging from 5 to 100 µm and a median size of 41.5 µm. Wheat starch granules, which are round and lenticular in shape, range from 0.5 to 45 µm, with a median size of 20.2 µm. In contrast, tapioca starch granules display a truncated round to oval morphology, with a size distribution similar to that of wheat, ranging from 4 to 35 µm and a mean diameter of 5.6 µm. While tapioca and potato granules are unimodally distributed, wheat starch exhibits a bimodal size distribution consisting of lenticular A-type granules (>10 µm) and small, spherical B-type granules (1–5 µm) (BeMiller, 2019).

2.3 Gelatinization and retrogradation

As previously addressed, starch in its native form exists as insoluble semi-crystalline granules. When heating native starch in excess water, the endothermic reaction called gelatinization occurs. The initial steps involve granule swelling, breaking of the hydrogen bonds in amorph regions, melting of the native crystalline structure, which results in the loss of birefringence, and finally solubilization of the starch molecules. The time and temperature at which the gelatinisation happens is related to the degree of crystallinity, amylose content as well as the presence of other non-starch components (Xie, Zhang and Wang, 2017).

In the bread matrix, starch is heated with limited water and often without shear. Under these conditions, starch swelling is reduced, requiring higher temperatures to increase molecular mobility and disrupt crystalline regions. Due to the low moisture content of the dough, many starch granules may remain ungelatinized in some baked goods (BeMiller, 2019).

After the gelatinisation and cooling, the two starch polymers return to an ordered structure not too dissimilar to the original structure within the native starch granules. This return to a more insoluble state is called retrogradation. Opposite to the original structure, however, is the fact that amylose is now the main contributor to the crystalline structure. The initial crystallisation is dominated by amylose chains, and within hours, they combine into a double-helix formation. Over several days, amylopectin slowly reassociates, slower than amylose due to the physical constrictions of the branched chains delaying the formation of the double-helices. Bread's initial setting and hardening are due to amylose, but later the staling and shelf-life in general is closely linked to the reassociation of the amylopectin fraction as well as the migration of water from the crumb to the crust (Hug-Iten, Escher and Conde-Petit, 2003; Patel *et al.*, 2017). Other variables that influence retrogradation are temperature, starch concentration, and the presence and concentration of other non-starch ingredients (BeMiller, 2019).

2.4 Modified starch

Modified starches are a category of starches that have been altered through physical or chemical processes to enhance their functional properties. These modifications are crucial for overcoming limitations in native starches, such as high retrogradation, poor shear stability, and inadequate thermal resistance, which may not meet the specific demands of food processing (Atichokudomchai and Varavinit, 2003; Miyazaki *et al.*, 2006; Dominguez-Ayala *et al.*, 2023).

Cross-linking is one of the most commonly used chemical modification methods for starch, typically involving phosphate ester cross-linking within the starch granule. Cross-linked starch results from the formation of ether or ester bonds between hydroxyl groups of natural starch under the action of the cross-linking reagent, such as sodium trimetaphosphate (STMP) or phosphoryl chloride ($POCl_3$) (BeMiller, 2019; Zhao *et al.*, 2024). This modification strengthens the granule and reduces both the rate and extent of swelling and disintegration during heat treatment. At low levels of cross-linking, the enhanced granule stability allows for greater swelling without rupture, leading to larger granules. This increased granule size contributes to a higher peak viscosity (BeMiller, 2019).

Another common chemical modification is acetylation, where starch is treated with acetic anhydride, partly substituting hydroxyl groups in the granules with acetyl esters (FAO/WHO Expert Committee on Food Additives (JECFA), 2016). The product of this modification results in starch acetate. Starch acetates have lower gelatinisation and pasting temperatures, as well as providing resistance to retrogradation. Additionally, the modified starch also has a higher peak viscosity and a lower viscosity of the cooled paste, indicating lower retrogradation compared to its non-modified counterparts (BeMiller, 2019).

Starch can also be modified by physical means. Potato starch, known for its wide range of granule sizes, can have its properties tailored by selectively removing larger granules. A study has shown that the smaller fraction of potato starch granules exhibits improved functional characteristics such as higher swelling power, lower transition and gelatinisation temperatures, reduced breakdown during heating, and reduced retrogradation during storage (Singh *et al.*, 2003).

Cold-swelling starch, a form of physically modified starch, undergoes a pre-cooking or pregelatinisation process that enables the starch granules to swell significantly in the absence of heat or under conditions of moderate heat and/or pressure. Despite this treatment, the granules largely retain their structural integrity, though they become swollen, deformed, and often exhibit wrinkled surfaces. This modification results in a loss or significant reduction of birefringence. Cold-swelling starches are characterized by enhanced swelling capacity and water solubility, while typically demonstrating lower paste viscosity and reduced retrogradation compared to their native counterparts (Majzoobi and Farahnaky, 2021).

2.5 Lipids

Lipids are well-recognized for their functional and technological benefits in traditional baked products. In gluten-free bread formulations, they serve a critical role in improving texture and delaying starch retrogradation, in part due to their ability to form complexes with amylose. Moreover, lipids contribute to increased bread volume, primarily through their capacity to support gas retention during proofing and baking. The functional performance of lipids in gluten-free systems is influenced by specific characteristics such as the degree of unsaturation and the length of the fatty acid chains, which affect their physical behaviour (Waziiroh *et al.*, 2023).

Amylose–lipid complexes (ALCs) are formed both upon biosynthesis and during gelatinization (Kwasniewska-Karolak, Rosicka-Kaczmarek and Ewa, 2010). In the presence of polar lipids, amylose transitions from a random coil to a helical structure that envelops lipid molecules. This structural transformation affects both the initial gelatinization process and the subsequent retrogradation. ALCs, along with free lipids, create an external barrier around starch granules, which limits water diffusion and granule swelling. As a result, the mobility of amylose is reduced, slowing gelatinization, decreasing swelling, and lowering the water-binding capacity. These effects, in turn, alter the pasting properties of the starch. In addition, the complexes formed between amylose and the outer branches of amylopectin hinder chain entanglement and molecular interactions, thereby inhibiting the formation of retrograded starch (Eliasson, 2020; Lee *et al.*, 2023; Cui *et al.*, 2024). Two thermally distinct types of amylose–lipid complexes have been identified: Form I, an amorphous structure with a melting transition in the DSC near 100 °C, and Form II (subdivided into IIa and IIb), crystalline forms with higher melting transitions at approximately 115 °C and 125 °C, respectively (Copeland *et al.*, 2009).

2.6 Other functional ingredients

In the absence of gluten, the protein providing elasticity and structure poses significant challenges when creating a gluten-free bread. Gluten-free formulations often rely heavily on starch, which can lead to undesirable textural properties. The high starch content can result in a dry, crumbly mouthfeel, and the lack of gluten accelerates staling, reducing the bread's shelf-life. To address these issues, a variety of additives are often incorporated to mimic the viscoelastic properties of gluten and improve the overall quality of the bread (Horstmann, Lynch and Arendt, 2017; Hellsten, 2019).

Among the most commonly used ingredients in gluten-free bread formulations are whey protein, sodium stearoyl lactylate (SSL), xanthan gum, guar gum, fibres, hydroxypropyl methylcellulose (HPMC), and pregelatinized starch. Each of these plays a unique role in enhancing the dough and final product.

Whey protein, derived from milk, contributes to the dough's structure and elasticity, helping compensate for the lack of gluten while also improving moisture retention and boosting the bread's protein content (Houben, Höchstötter and Becker, 2012; Vatansever and Hall, 2021).

SSL, an emulsifier, in addition to contributing an overall higher viscosity it also facilitates dough handling, enhances gas retention, and promotes a finer crumb structure, ultimately increasing the loaf's volume and improving texture (Nunes *et al.*, 2009; BeMiller, 2019).

Xanthan gum, a polysaccharide, enhances the dough's viscosity, stabilizes the batter, and supports gas retention during fermentation, while also extending shelf-life by helping retain moisture. Guar gum, from the guar bean, binds water, softens the crumb, and reduces staling, helping the bread stay fresh longer. HPMC, a cellulose derivative, affects the rheological properties of the dough, improving its processing and handling, while also contributing to a desirable crumb structure and prolonging freshness (Muzaffar *et al.*, 2021; Vatansever and Hall, 2021; Rifna, Dwivedi and Kulshrestha, 2022).

Potato fibre, like other dietary fibres, can play an important role in gluten-free bread formulations due to its functional properties. It enhances water retention, increases dough viscosity, and reduces starch gelatinization by competing for available water. However, due to its high water-holding capacity, it often requires an increase in the water content of the dough. This ensures the appropriate viscosity for proper starch gelatinization and protein denaturation, crucial for achieving the desired texture and structure in the bread (Capriles and Arêas, 2014; Muzaffar *et al.*, 2021).

2.7 Sensory aspects

In gluten-free bread development, sensory analysis plays a crucial role in understanding how different recipes perform in terms of taste, texture, and overall consumer satisfaction. Since gluten-free products often face challenges with texture and structure, sensory testing helps identify which variations are most preferred by consumers.

Sensory analysis is not only about taste, but it also provides a way to compare different recipe variations through human perception. This is especially valuable in the gluten-free market, where texture and mouthfeel are often the main concerns.

Recent studies, such as (Tóth, Kaszab and Lambert-Meretei, 2022), emphasize the importance of combining sensory evaluation with objective measurements, like Texture Profile Analysis (TPA), to assess and improve product quality. While sensory analysis helps capture consumer preferences, TPA provides more precise, scientific data on key attributes such as firmness, chewiness, and springiness. These objective measurements can be used to validate and complement sensory feedback, ensuring that the bread not only meets consumer expectations but also performs well in terms of its physical characteristics (Tóth, Kaszab and Lambert-Meretei, 2022).

Attribute ranking is a methodology where either trained or untrained panellists rank products based on the perceived intensities of specific sensory attributes, such as appearance, smell/taste, and texture. One advantage of ranking is that it requires minimal training compared to more detailed descriptive analysis, as assessors only need to understand the task and agree on the sensory attributes involved (Cleaver, 2018; Tóth, Kaszab and Lambert-Meretei, 2022).

However, a limitation is that ranking does not provide a measure of the size of the difference in intensity between products or assign overall scores along a reference scale. Despite this, ranking allows for relative comparisons that provide insights into product preferences (Cleaver, 2018).

3 Materials and Method

3.1 Raw materials

The following baking ingredients were used for the base recipe (% of wet weight): Tap water (42.3%); Starch either native wheat starch, acetylated potato starch, fractioned potato starch or cross-linked tapioca starch (41.4%); rapeseed-oil (5.6%); whey powder (5.3%); dry yeast (1.3%); sugar (0.8%); cold water soluble potato starch (CWS) (0.8%); physillium husk (0.8%); salt (0.6%); potato fibre (0.4%); guar gum (0.4%); Xanthan gum (0.2%); hydroxypropyl methylcellulose (HPMC) (0.2%); Sodium stearoyl lactylate (SSL) (0.1%).

3.2 Screening phase

The initial phase of the project involved test-baking, to optimize the recipe, cooking and proofing times, and identifying which of the ten starches showed the most potential for gluten-free bread. The selection of four starches was based on key attributes such as volume, texture, crumbliness, and perceived dryness after one day of storage. While these factors are subjective, they proved effective in efficiently narrowing down the most promising candidates for further study.

3.3 Experimental design

The experiment involved four types of gluten-free bread formulations, made with four different starches: wheat starch, cross-linked tapioca, fractioned potato starch and acetylated potato starch. For each formulation, nine breads were baked, with three breads analysed per day across three separate days (day 1, day 2, and day 4) following a standardized baking day (day 0). To ensure consistency, the position of the breads in both the rising chamber and the oven was controlled and kept identical across all starch types. Each formulation was prepared and analysed over a separate week.

3.4 Dough preparation & Baking

Dry ingredients were weighed and pre-mixed prior to the addition of wet ingredients (water and oil). Mixing was performed using a planetary mixer (Electrolux, Sweden) using middle-low speed, beginning with 1 minute of mixing, followed by side-scraping, then an additional 3 minutes of mixing, and a final 1-minute mix. The dough was then weighed and portioned into greased bread tins, with 575 grams allocated to each tin. Precautions were taken to ensure consistent tin placement on baking trays within the proofing cabinet and oven (Combination Oven Sveba Dahlen, Sweden).

Proofing was conducted at 37 °C and 80% relative humidity for 25 minutes. Immediately following proofing, the breads were baked in a preheated oven at 220 °C for 17 minutes, with a 4-second burst of steam applied at the beginning of the bake. Upon completion, the loaves were removed from the tins and allowed to cool on perforated baking trays for 1.5 hours. Breads were then weighed to determine baking loss, sealed in labelled plastic bags, and stored in a closed container until further analysis.

3.5 Starch moisture content

The moisture content of starch was determined according to ISO 1666:1996, which specifies an oven-drying method under atmospheric pressure.

Approximately 5.00 ± 0.25 g of homogenized starch sample was weighed into a metal dish with a tight-fitting lid. Samples were placed in a preheated oven at 130°C and dried for 1 hour and 30 minutes. Following drying, the dish was rapidly covered, transferred to a desiccator, and cooled to room temperature for 45 minutes before final weighing. Three independent determinations were carried out per sample. The result was reported as the arithmetic mean.

3.6 Pasting properties

The pasting properties of the starch samples were evaluated using a Rapid Visco Analyser (RVA 4500, Perten Instruments, Australia), following a modified version of AACC International Method 76-21.01 and ICC Standard No. 162. Distilled water (25.0 g, adjusted to a 14% moisture basis) was weighed into standard RVA aluminium canisters, and 2.0 g of starch sample was added. A general 91 °C pasting profile was applied in accordance with RVA Method 44.01 (Perten Instruments); details of the temperature-time profile are provided in Table 2.

Table 2. Perten Instruments RVA 91C Method 44.01.

Time	Type	Value
00:00:00	Temp	50ºC
00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm
00:10:00	Temp	50ºC
00:04:42	Temp	91ºC
00:07:12	Temp	91ºC
00:11:00	Temp	50ºC
00:13:00	End	

Idle Temperature: 50 ± 1ºC
Time Between Readings: 4 s

The following pasting parameters were analysed:

Peak viscosity [cP]: the maximum viscosity during heating, indicating the water-binding capacity and swelling power of the starch granules.

Trough viscosity [cP]: the minimum viscosity after peak, representing the ability of swollen granules to withstand shear and heat.

Breakdown viscosity [cP]: the difference between peak and trough viscosity, reflecting the paste's stability under heat and shear.

Final viscosity [cP]: the viscosity at the end of the cooling stage, related to retrogradation and re-association of starch molecules.

Setback viscosity [cP]: the difference between final and trough viscosity, indicating the tendency of the starch to retrograde or gel upon cooling.

Pasting temperature [°C]: the temperature at which viscosity first increases, associated with the initial gelatinization of starch granules.

3.7 Dough mixing characteristics

The rheological characteristics of the dough samples were analysed using a DoughLab (Perten Instruments, Australia) in accordance with the AACC International Method 54-21.02. A 50 g sample, adjusted to 14% moisture content, was prepared for each analysis. Yeast was excluded from the formulations to focus on dough development properties. Oil was manually added after the pre-mixing phase. The mixing profile began with a 60-second premixing phase at 63 rpm, followed by a continuous mixing phase at 120 rpm for a total test duration of 10 minutes.
The following parameters were recorded and evaluated:

Peak resistance [mNm]: the maximum resistance during mixing, representing the dough's strength and its ability to form a cohesive structure.

Stability [min]: the duration the dough maintains its maximum resistance, indicating tolerance to mixing and protein strength.

Bandwidth at peak [mNm]: the width of the curve at peak resistance, reflecting the dough's tolerance to overmixing and consistency during kneading.

Water absorption [%]: the amount of water required to achieve optimum dough consistency (500 mNm), which relates to the water-binding capacity of the dough.

Development time [min]: the time required for the dough to reach peak resistance, indicating the speed of dough formation and hydration rate.

Softening [mNm]: the reduction in dough resistance after peak mixing, providing insight into dough weakening and protein matrix stability.

Peak energy [Wh/kg]: the total energy required to reach peak resistance, related to dough elasticity and workability during mechanical processing.

3.8 Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) measurements were conducted using a EXSTAR6000 DSC (Seiko Instruments Inc., Japan) to evaluate gelatinisation in the starch samples and retrogradation in bread samples.

For the gelatinisation measurement, approximately 2.5 mg of starch was weighed into a coated aluminium pan, followed by the addition of three times the weight in Milli-Q distilled water. The pans were hermetically sealed and analysed immediately using differential scanning calorimetry (DSC), with a temperature scan from 20 °C to 130 °C at a heating rate of 10 °C/min under a continuous nitrogen flush. The pans were measured without further pretreatment and measured against a sealed, empty reference pan. The gelatinization temperature and associated thermal transitions were analysed with DSC software (SII EXSTAR6000 Muse, Shizuoka, Japan). Six replicates were conducted for each starch type.

The bread crumb analysis consisted of samples collected on Days 1, 2, and 4 of storage. Following the texture profile analysis (TPA), two samples (~11 mg each) were taken from the inner slice of each loaf, spaced approximately 1 cm apart. Samples were scanned from 20 °C to 180 °C. After analysis, the pans for both gelatinisation and retrogradation were punctured and dried overnight at 110 °C to determine dry matter content. Melting enthalpy was expressed in joules per gram of dried sample.

The following thermal properties related to starch gelatinisation and retrogradation were recording and evaluated: The onset temperature (T_o, °C), representing the beginning of the phase transition, the peak temperature (T_p, °C), indicating the point of maximum energy absorption, and the conclusion temperature (T_c, °C), marking the end of the transition, were recorded to characterise the thermal transition range. The enthalpy change (ΔH, J/g) was calculated from the area under the endothermic peak, indicating the total energy required for disrupting the crystalline structure within the granule (gelatinisation), or melting of the recrystallised amylopectin and ALC (retrogradation).

3.9 Baking loss, bread volume measurement and Colour

Baking loss was determined by first taring the bread tins and weighing the dough prior to proofing. After baking and cooling, the bread was weighed again. The difference in weight was used to calculate baking loss (%).

Specific bread volume (cm^3/g) was measured using a Bread Volume Measurement (BVM) instrument, model BVM L 370 (Perten Instruments, Australia). Measurements were conducted on day 0, the day of baking, after the loaves had fully cooled. The same loaves were later used for additional analyses (e.g., TPA, DSC). The instrument utilizes a laser sensor in combination with a flat rectangular support plate (50 × 70 mm). Each of the nine loaves was measured in triplicate. A calibration procedure was performed before each set of measurements to ensure accuracy.

A portable Spectrophotometer (VTLCM-700, Konica Minolta, Japan) with a capture cone (1 cm diameter) measured the surface colour of the bread crumb. From each loaf, one slice was taken just before the three inner slices were used for texture analysis, resulting in one colour measurement per loaf. The CIE L*a*b* (CIELAB) colour model was used to quantify surface colour characteristics, where L* indicates lightness and a* and b* represent chromaticity coordinates.

3.10 Texture profile analysis

Texture Profile Analysis (TPA) was performed using a TexVol TVT-300 XP texture analyser (Perten Instruments, Sweden) equipped with a 7 kg load cell. A cylindrical aluminium probe with rounded edges (36 mm diameter, 45 mm height) was used for the measurements. Bread samples were sliced using a bread slicer with centimetre-scale adjustment to obtain slices of uniform thickness (2 cm). From each loaf, three slices were taken from the centre, and measurements were conducted on days 1, 2, and 4 post-baking.

Texture Profile Analysis (TPA) was used to evaluate textural parameters. Firmness was determined by the height of the first compression peak, representing the force required to deform the sample. Cohesiveness was calculated as the ratio of the area under the second compression curve to that of the first, indicating the internal bonding strength of the sample.
The samples underwent a double compression with the setting parameters seen in Table 3.

Table 3. Bread crumb firmness compression modified AACCI 74-09.01 method.

Parameter	Value	Unit
Sample height	20.0	mm
Starting distance	5.0	mm
Compression	40.0	%
Initial speed	1.0	mm/s
Test speed	1.7	mm/s
Retract speed	10.0	mm/s
Trigger force	5.0	g
Data rate	250	pps

3.11 Bread moisture content

For each set of three breads, three inner slices were collected. On days 1, 2, and 4 post-baking, the slices were subjected to TPA and DSC analysis. Following analysis, the upper 1 cm of each slice was cut and designated as the crust, while the remaining portion was considered the crumb. Both crust and crumb were weighed, individually wrapped in aluminium foil, and dried overnight at 110 °C to determine their respective dry matter content after they were cooled in a desiccator. This was done to determine the migration of moisture from the crumb to the crust.

3.12 Sensory evaluation

Seven loaves of each bread type were baked; six were used for sensory evaluation, while the seventh was reserved for analysis (BVM and TPA). The analysis served to verify consistency with previously produced batches and ensure there were no significant differences.

The sensory evaluation took place a day after the baking (day 1). The four types of bread samples were sliced to a thickness of 2 cm and cut into uniform shapes using a circular pastry cutter. Each sample plate was labelled with a unique three-digit random number. 35 untrained panellists were recruited amongst students and staff of Lund University.

All samples were presented simultaneously, with their positions rotated for each participant based on William's Latin square design to minimize positional bias. Participants were asked to evaluate the samples in the following order: appearance, aroma/flavour, and texture. Finally, they provided an overall impression of each sample using a nine-point hedonic scale measuring likeability (1 = dislike extremely, 9 = like extremely). Space was provided for general comments after each evaluation. The sensory evaluation sheet can be seen in Appendix I.

3.13 Statistical analysis

All statistical analyses were performed using R (version 4.5.0) in the RStudio environment (version 2025.05.0).

3.13.1 Raw material analysis

Data for gelatinisation and pasting properties of raw starches, analysed with DSC and RVA, respectively, were screened for outliers using the Tukey method (1.5 × IQR rule). Observations identified as outliers were excluded from further analysis. Levene's test was used to assess the homogeneity of variances across starch types. One-way ANOVA was then conducted to evaluate differences in gelatinisation and pasting parameter values among starch types. Where the ANOVA indicated a significant effect ($p = 0.05$), post-hoc pairwise comparisons were performed using Tukey's Honestly Significant Difference (HSD) corrected for family-wise error rate.

3.13.2 Bread analysis

Sensory evaluation data were analysed a using non-parametric Friedman test ($p=0.05$), pairwise comparisons were conducted using the Wilcoxon signed-rank test, with Bonferroni correction was applied to adjust the significance level to control family-wise error rate. Effect sizes were calculated using Kendall's coefficient of concordance (W), which measures the degree of agreement among panellists. Kendall's W values range from 0 (no agreement) to 1 (perfect agreement), with higher values indicating stronger consensus.

Data from TPA, crumb and crust moisture and DSC were averaged per bread sample. Levene's test was conducted to evaluate the homogeneity of variances across different starch types. A two-way ANOVA was then performed with starch type and storage day (Day 1, Day 2, Day 4) as fixed factors. Where the ANOVA revealed statistically significant effects ($p < 0.05$), post hoc pairwise comparisons were carried out using Tukey's Honestly Significant Difference (HSD) test, with correction for the family-wise error rate.

Specific volume, baking loss, and LAB values were analysed using the same approach, except that a one-way ANOVA was used (starch type as the sole factor). Specific volume and LAB values were averaged per bread prior to analysis.

4 Results

This section presents the results of analyses on gluten-free bread and their raw starch materials.

4.1 Starch raw material analysis

FPS exhibited the highest moisture content, followed by Amylacetate, Tapioca and Wheat showing the lowest value, as can be seen in Table 4.

Table 4. Moisture content of Wheat, Tapioca, FPS, and Amylacetate.

Starch	Moisture content [%]
Wheat	11.2±0.05
Tapioca	11.4±0.06
FPS	17.0±0.06
Amylacetate	15.9±0.04

Values are presented as mean ± standard deviation (SD); n = 3

The pasting properties of the different starches can be seen in Table 5 and Figure 4.

Table 5. Pasting properties of Wheat, Tapioca, FPS, and Amylacetate.

Sample	Peak viscosity [cP]	Trough [cP]	Break down [cP]	Final viscosity [cP]	Setback viscosity [cP]	Pasting temperature [°C]
Wheat	$331±12^{d}$	$265±9^{d}$	$66±3^{c}$	$373±14^{d}$	$108±5^{d}$	$76±0.4^{a}$
Tapioca	$1151±57^{c}$	$1120±57^{b}$	$31±1^{c}$	$1966±89^{c}$	$846±32^{b}$	$68±0.4^{b}$
FPS	$5968±4^{b}$	$2461±14^{a}$	$3497±13^{b}$	$2751±33^{a}$	$290±19^{c}$	$67±0.5^{b}$
Amylacetate	$6353±37^{a}$	$909±8^{c}$	$5444±29^{a}$	$2567±80^{b}$	$1658±85^{a}$	$64±0.4^{c}$

Values are presented as mean ± standard deviation (SD); n = 3. Means across a single column with different superscripts are significantly different ($p < 0.05$).

Amylacetate showed a significantly higher peak viscosity compared to all other starches, followed by FPS, Tapioca, and Wheat. The trough values showed that the viscosity of Amylacetate decreased significantly compared to the other starches. As the breakdown is related to the trough, Amylacetate also exhibited the highest breakdown in viscosity, followed by FPS, while Wheat and Tapioca showed no significant difference.

FPS had the highest final viscosity, followed by Amylacetate, Tapioca, and Wheat. Amylacetate had a significantly higher setback viscosity than all other starches, followed by Tapioca, FPS, and Wheat. Wheat starch had the highest pasting temperature, followed by Tapioca and FPS, which were similar, and finally Amylacetate which had the lowest pasting temperature.

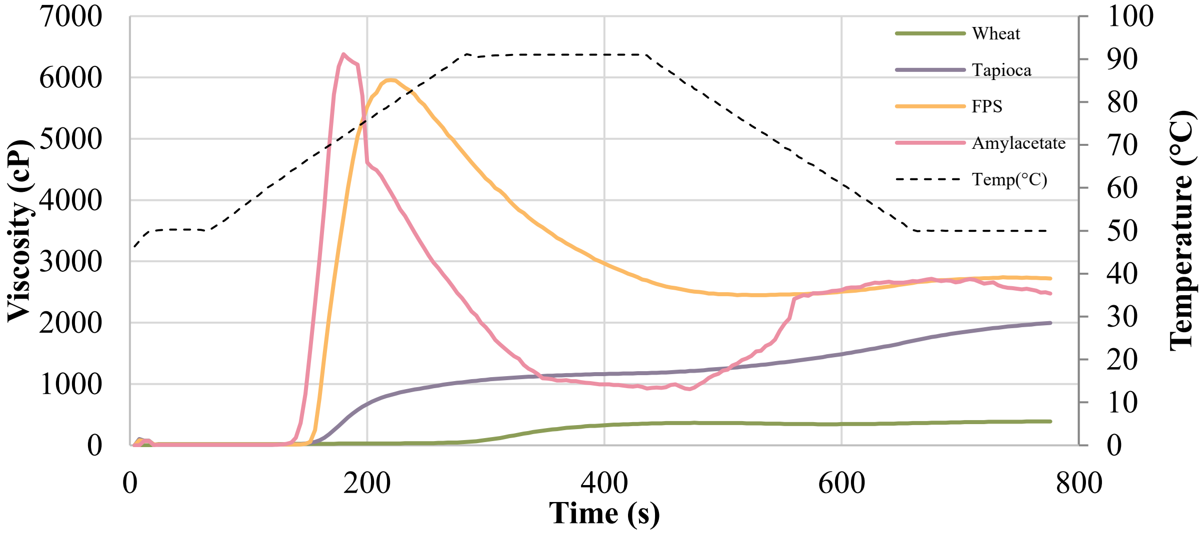

Figure 4. RVA pasting behaviour of Wheat, Tapioca, FPS, Amylacetate starch.

Figure 4 illustrates the apparent viscosity over time during heat treatment and mechanical stress, as measured by the RVA. Wheat and tapioca produced less distinct curves with limited visible viscosity development. FPS displayed a clear and well-defined viscosity curve. In contrast, Amylacetate showed an irregular and less smooth curve, likely due to an excessive sample amount in the RVA test, which resulted in overloading and abnormal viscosity behaviour.
Doughlab results for the different starches are seen in Table 6.

Table 6. Doughlab values for doughs created with Wheat, Tapioca, FPS, Amylacetate

Sample	Peak (mNm)	Stability (min)	Bandwidth at peak (mNm)	Water absorption (%)	Development time (min)	Softening (mNm)	Peak energy (Wh/kg)
Wheat	1195±12	0,5±0	112±5	64±0	1±0	305±1	1,9±0
Tapioca	1150±35	0,7±0	124±3	66±0	1,1±0	311±2	2±0
FPS	481±32	7±0	137±5	58±0	4±0	75±2	3,2±0
Amylacetate	530±4	10±0	108±3	62±0	10±0	X	11±0

Values are presented as mean ± standard deviation (SD); n = 2

The doughs made with Wheat and Tapioca showed similar values across all DoughLab parameters. In contrast, FPS and Amylacetate also appeared similar, generally exhibiting lower values across all parameters compared to Wheat and Tapioca. Peak energy was highest for Amylacetate followed by a lower value of FPS, not too dissimilar to Wheat and Tapioca. No value for softening was seen for Amylacetate due to the lack of moisture in the dough.
Table 7 summarises the DSC results for the different starches.

Table 7. Starch samples analysed by DSC showing mean To onset temp, Tp peak temp, Tc concluding temp, △H endothermic enthalpy values.

Sample	**T_o [°C]**	**T_p [°C]**	**T_c [°C]**	**△H [J/g]**
Wheat	54.8 ± 2^{b}	$60.2 \pm 0{,}4^{c}$	66.1 ± 0.4^{c}	8.58 ± 1.6^{c}
Tapioca	54.5 ± 0.7^{b}	$61.5 \pm 0{,}6^{b}$	69.0 ± 0.8^{b}	9.70 ± 1.2^{bc}
FPS	58.9 ± 0.2^{a}	64.4 ± 0.3^{a}	70.2 ± 0.7^{a}	12.3 ± 2.1^{b}
Amylacetate	54.4 ± 0.8^{b}	62 ± 0.2^{b}	69.2 ± 0.7^{ab}	16.2 ± 2.1^{a}

Values are presented as mean ± standard deviation (SD); n = 6. Means across a single column with different superscripts are significantly different ($p < 0.05$).

Among the starches analysed, FPS exhibited a significantly higher onset temperature (T_o) compared to all other samples. In terms of peak temperature (T_p), both FPS and Wheat starch showed significantly different values from Amylacetate and Tapioca starches, with FPS having a higher T_p and Wheat a lower one. Amylacetate and Tapioca did not differ significantly from one another in T_p.

Concluding temperature (T_c) also varied notably between starches. Wheat starch had a significantly lower T_c than all other samples. FPS exhibited a significantly higher T_c than both Tapioca and Wheat starches, while Amylacetate showed a significantly higher T_c than Wheat. Amylacetate presented the highest enthalpy of melting (ΔH) across all starches, being significantly greater than the rest. FPS also showed a significantly higher ΔH compared to Wheat starch.

4.2 Gluten-free bread results

Each of the four gluten-free bread types was baked and sampled across three storage days (Day 1, 2, and 4). From each loaf, four slices were taken: one inner slice (taken before the three middle slices) was used for photographic documentation and color, while three middle slices were used for analysis.

Figure 5. Gluten-free bread slices top going left to right: Wheat, Tapioca, FPS, Amylacetate. Each slice is the middle slice from the second bread produced for each day.

Figure 5 illustrates the differences in crumb and crust structure as well as overall appearance and colour, which vary between slices and starch type.

4.3 Simple Ranking Test

The consumer ranking test was done by an untrained panel (n=35). The judges evaluated attributes across several categories. Summarised in Table 8, are the visual sensory crumb attributes (whiteness, uniformity, crumbliness). Smell and taste attributes (bread-like smell, off-taste) are presented in Table 9. Textural attributes (softness, chewiness, dryness) are detailed in Table 10. Finally, the overall impression is reported in Table 11. Higher values reflect a greater perceived intensity of each attribute by the panel, with higher overall impression scores indicating increased favourability.

To ensure that the breads produced for sensory were significantly the same as the breads produced in batches for analysis, BVM and TPA were performed. BVM showed no significant difference between the original and sensory Tapioca bread, however, the other bread types were significantly different. Despite statistical significance, the actual mean differences were small, therefore, the results should still be comparable. The TPA measurements were compared. Cohesiveness values were consistent across new and original bread; however, the new measurements showed significantly higher firmness, although the relation between the starches remained the same, and the mean differences were small.

Table 8. Visual attributes, whiteness, uniformity, and crumbliness of the gluten-free crumb.

Starch Type	Wheat	Tapioca	FPS	Amylacetate
Whiteness	75^{c}	50^{d}	103^{b}	122^{a}
Uniformity	97^{ab}	72^{b}	82^{ab}	99^{a}
Crumbliness	102^{ab}	42^{c}	113^{a}	93^{b}

Values are presented as R-sum; n = 35. Means across a single row with different superscripts are significantly different (p < 0.05).

There was a significant difference in the perceived whiteness of the crumb among all gluten-free bread samples, with strong agreement between judges (Kendall's W = 0.489). For crumb uniformity, a significant difference was found only between Tapioca and Amylacetate, although agreement among judges was weak (W = 0.081). Regarding perceived crumbliness, Tapioca was rated significantly different from all other starches, and a significant difference was also found between Amylacetate and FPS, with strong inter-judge agreement (W = 0.483). Boxplots of Whiteness and Crumb uniformity can be seen in Appendix II Figure A and B, where they illustrate the agreement among judges.

Table 9. Smell and taste attributes, bread-like smell, and off-taste of gluten-free bread crumb.

Starch Type	Wheat	Tapioka	FPS	Amylacetate
Bread-like smell	94^{a}	82^{a}	89^{a}	85^{a}
Off-Taste	92^{a}	80^{a}	100^{a}	78^{a}

Values are presented as R-sum; n = 35. Means across a single row with different superscripts are significantly different (p < 0.05).

There were no significant differences in the perceived bread-like smell or off-taste among the gluten-free bread samples. Agreement among judges was also low for both attributes, as reflected by Kendall's W values of 0.0132 and 0.0527, respectively.

Table 10. Textural attributes, softness, chewiness, and dryness of gluten-free bread crumb.

Starch Type	Wheat	Tapioka	FPS	Amylacetate
Softness	88^{a}	83^{a}	87^{a}	92^{a}
Chewiness	83^{b}	133^{a}	46^{c}	88^{b}
Dryness	90^{b}	39^{c}	135^{a}	90^{b}

Values are presented as R-sum; n = 35. Means across a single row with different superscripts are significantly different (p < 0.05).

No significant difference was observed in the perceived softness of the gluten-free bread, and agreement among judges was very low (W = 0.0067). In contrast, significant differences were found for both chewiness and dryness of the crumb. Tapioca exhibited the highest chewiness and was significantly different from all other starch types. Amylacetate and wheat followed, showing similar chewiness values with no significant difference between them.

FPS exhibited the lowest chewiness and the highest dryness scores, differing significantly from all other starches. In contrast, Tapioca showed the lowest dryness. No significant differences were observed between Amylacetate and Wheat in terms of dryness.

Table 11. Overall impression of gluten-free bread crumb.

Starch Type	Wheat	Tapioka	FPS	Amylacetate
Overall impression	184^{a}	191^{a}	118^{b}	193^{a}

Values are presented as R-sum; n = 35. Means across a single row with different superscripts are significantly different (p < 0.05).

FPS had the significantly lowest overall impression rating compared to all other starches. The rest of the starches were not significantly different, with low agreement among judges (Kendall's W = 0.239). Amylacetate had the highest sum, followed by Tapioca, Wheat and lastly FPS.

4.4 Gluten-free bread analysis

The results from bakeloss, Specific volume, CIE L*a*b* coordinates of the different gluten-free bread are summarised in Table 12. In Addition to the CIE coordinates a visualisation of the colour is also illustrated in the table.

*Table 12. Results from bakeloss, specific volume, and CIE L*a*b coordinates (and visual) of gluten-free bread.*

Starch	Bakeloss (%)	Specific Volume (cm^3/g)	L*	a*	b*	Colour
Wheat	9.12±0.3^{a}	2.15±0.03^{a}	72±3^{a}	0.94±0.3^{b}	19±1^{a}	
Tapioca	9.10±0.3^{a}	1.97±0.04^{c}	62±4^{c}	1.7±0.5^{a}	18±0.8^{b}	
FPS	8.83±0.2^{a}	2.06±0.01^{b}	73±1^{a}	1.1±0.3^{b}	18±1^{ab}	
Amylacetate	9.06±0.3^{a}	1.98±0.02^{c}	66±2^{b}	1.1±0.3^{b}	15±0.5^{c}	

*Values are presented as mean ± standard deviation (SD); bakeloss n = 3, specific volume n = 9, L*a*b* n = 9. Means across a single row with different superscripts are significantly different ($p < 0.05$).*

Baking loss showed no starch dependent effect, indicating comparable water retention after baking. Specific volume measurements demonstrated strong starch-type dependence, with the wheat starch formulation having the highest mean volume followed by FPS, Amylacetate and lastly Tapioca. There was a significant difference between all the starches except for Tapioca and Amylacetate.

Colorimetric analysis indicated significant starch-dependent variation in lightness (L*) values. FPS and wheat exhibited comparable lightness, both significantly lighter than Amylacetate. Tapioca presented the lowest L* values, indicating the darkest appearance, and differed significantly from all other starch formulations. For the chromatic parameter *a (red-green axis), tapioca showed the highest mean values and was the only formulation to differ significantly from the others. Analysis of the *b parameter (yellow-blue axis) revealed the highest yellowness in wheat, followed by tapioca and FPS, with Amylacetate exhibiting the lowest *b values. Significant differences in *b were observed between FPS and Amylacetate, Tapioca and Amylacetate, Wheat and Amylacetate, as well as Wheat and Tapioca.

The results from the day 1 and day 4 TPA graph of the different gluten-free breads is visualised in Figure 6 and Figure 7, respectively.

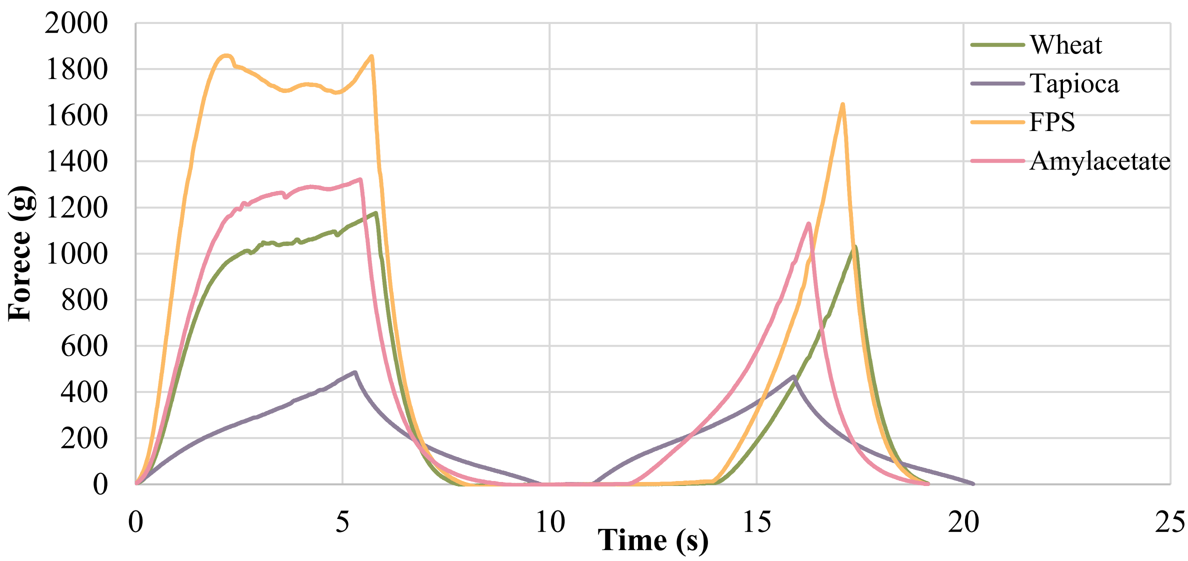

Figure 6. TPA graph for day 1 gluten-free bread.

Tapioca exhibited typical bread-like behaviour, showing a single clear peak during the first compression. In contrast, the curves of the other gluten-free breads indicate the presence of a hard outer crumb layer, visualised by an initial peak in the first compression. The subsequent reduction in firmness corresponds to the collapse of the inner crumb structure under compression. A small increase just before probe retraction reflects the crumb's build-up of break resistance. In breads with strong structure, the first peak is larger but similar in shape to the second peak; this pattern was not observed in any of the gluten-free breads. While Tapioca showed similar curves for both compressions, the other gluten-free breads differed between compressions.

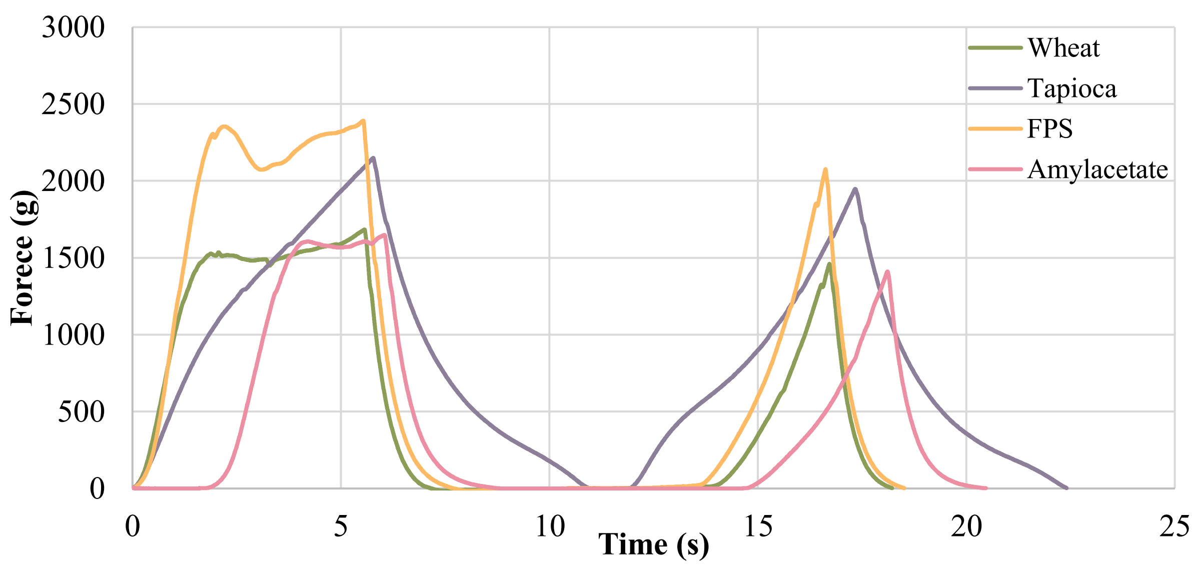

Figure 7. TPA graph for day 4 gluten-free bread.

An increase in firmness was noted for all bread types. Tapioca surpassed but Wheat and Amylacetate in firmness on day 4. The presence of the crust layer on the crumb is even more evident on this day.

Firmness and cohesiveness values from the TPA as well as moisture content of both the crumb and crust are presented in Table 13.

Table 13. Results from TPA (firmness, cohesiveness) and moisture analysis of crumb and crust, respectively.

Sample	**Day**	**Texture Profile Analysis**		**Crust Moisture (%)**	**Crumb Moisture (%)**
		Firmness (g)	Cohesiveness (-)		
Wheat	1	1199±24^{bA}	0.24±0.004^{cA}	39.3±1.9^{bB}	44.3±0^{bB}
	2	1433±31^{bB}	0.23±0.002^{cB}	42.4±0.2^{aA}	46.5±0.4^{aA}
	4	1780±42^{cC}	0.19±0.005^{cC}	42±0.2^{abB}	44.8±0.1^{bB}
Tapioca	1	482±13^{cA}	0.58±0.004^{aA}	40.58±0.1^{abA}	44.3±0.1^{bA}
	2	890±10^{cB}	0.56±0.003^{aA}	39.5±0.5^{bA}	44.7±0^{bA}
	4	2126±73^{bC}	0.51±0.012^{aB}	39.3±0.2^{bA}	44.3±0.1^{bA}
FPS	1	1898±48^{aA}	0.20±0.015^{dA}	42.41±2^{aA}	47.1±0.8^{aA}
	2	2254±101^{aB}	0.20±0.005^{cA}	41.6±0.7^{abA}	46.7±0.5^{aA}
	4	2436±90^{aC}	0.20±0.008^{cA}	42.5±0.3^{aA}	47.0±0.6^{aA}
Amylacetate	1	1280±61^{bA}	0.27±0.004^{bA}	40.9±2^{abA}	46.4±0.3^{aA}
	2	1516±79^{bB}	0.24±0^{bB}	42±0.2^{abA}	46.0±0.1^{aA}
	4	1799±75^{cC}	0.23±0.025^{bB}	42.1±3^{aA}	46.0±0.2^{aA}
Two-way ANOVA-p					
Factor A (Starch)		<0,001	<0,001	<0,001	<0,001
Factor B (day)		<0,001	<0,001	<0,001	<0,05
Factor AxB		<0,001	<0,001	<0,001	<0,001

Values are presented as mean ± standard deviation (SD); n = 3. with different lowercase superscripts are significantly different (p < 0.05) between starches on the same day. Means across a single row with different uppercase superscripts indicate significant difference (p<0.05) within starch between days.

FPS exhibited the highest overall mean firmness across all days. On both day 1 and day 2, FPS was followed in descending order by Amylacetate, Wheat, and Tapioca. FPS was significantly harder than all other starches. Amylacetate and Wheat showed intermediate firmness values and did not differ significantly from each other. Tapioca consistently exhibited the lowest firmness values and was significantly softer than all other starch types. On day 4, the earlier significant differences between starches remained; however, Tapioca showed a marked increase in firmness, surpassing both Wheat and Amylacetate, also seen in Figure 7**Error! Reference source not found.**. All starches exhibited a significant increase in firmness over time.

In terms of cohesiveness, Tapioca exhibited the highest mean values across all days, followed by Amylacetate, Wheat, and FPS. On day 1, all starch types were significantly different from one another. A similar ranking was observed on day 2; however, Wheat and FPS did not differ significantly on this day. By day 4, FPS slightly surpassed Wheat in mean cohesiveness, though the difference remained non-significant. FPS did not exhibit any significant changes in cohesiveness over the four days, and no significant difference was observed for Tapioca between day 1 and day 2. Additionally, Amylacetate showed no significant change in cohesiveness from day 2 to day 4. Overall, there was a general trend of decreasing cohesiveness over time, with

Tapioca consistently maintaining notably higher cohesiveness compared to the other starch types.

Crumb moisture on day 1 followed the mean order: FPS > Amylacetate > Wheat > Tapioca. FPS and Amylacetate had significantly higher moisture content than Wheat and Tapioca. On day 2, the order shifted slightly to: FPS > Wheat > Amylacetate > Tapioca. Tapioca exhibited significantly lower moisture than all other starch types, while no significant differences were observed among the remaining three. By day 4, the ranking returned to the same pattern as day 1. Although FPS and Amylacetate still showed higher crumb moisture than Wheat and Tapioca, the difference between FPS and Amylacetate was only marginal.

Crust moisture on day 1 followed the mean order: FPS > Amylacetate > Tapioca > Wheat. FPS had a significantly higher moisture content compared to Wheat, but there was no significant difference between the remaining starches. On day 2, the mean order shifted slightly to: Wheat > Amylacetate > FPS > Tapioca. Wheat had significantly higher moisture levels compared to Tapioca, but no significant differences were observed between the rest of the starches. The order of means once again shifted on day 4, Wheat > Amylacetate > FPS > Tapioca. FPS and Amylacetate had significantly higher moisture levels compared to Tapioca. There was no difference seen between Wheat and Tapioca, but it was marginally significant. Across the storage period, crumb moisture remained statistically unchanged for FPS, Amylacetate, and Tapioca. In contrast, Wheat exhibited a significant change in moisture across days 1 and 2.

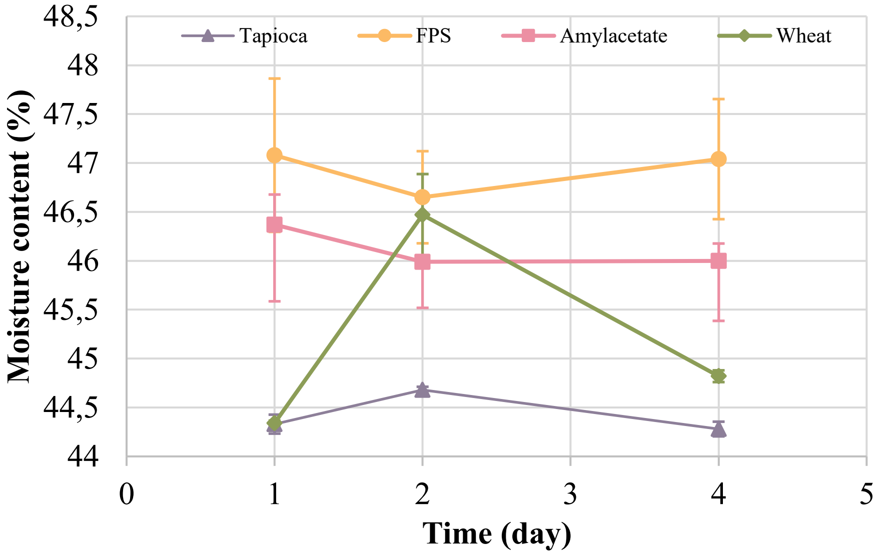

Figure 8. Moisture content of gluten-free bread crumb across storage days 1,2, and 4. Values are presented as mean ± standard deviation (SD) error bars, n = 3.

It was difficult to identify a consistent trend in moisture content over time, as some samples increased while others decreased, as shown in Figure 8. Tapioca consistently exhibited the lowest moisture levels overall, both in the crumb and the crust. In contrast, FPS had the highest average crumb moisture, followed by Amylacetate and then Wheat. For the crust, no clear trend was observed across starch types, except for Wheat, which showed a notable increase in moisture content across the first two days.

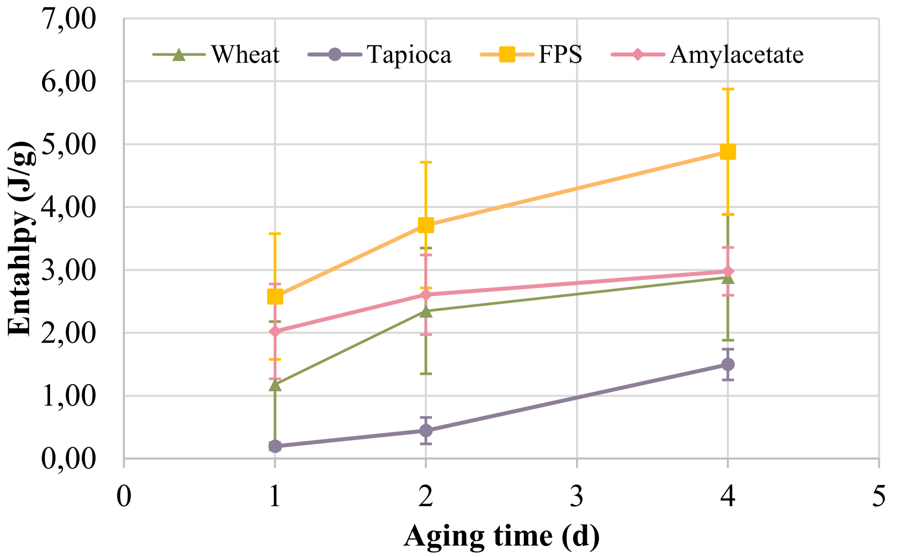

Figure 9. Gluten-free bread DSC ΔH retrogradation/amylopectin melting enthalpy values across storage days 1,2, and 4.
Values are presented as mean ± standard deviation (SD) error bars, n = 3.

As illustrated in Figure 9, the amylopectin melting enthalpy increased over time for all starch types, with FPS showing the highest values, followed by Amylacetate and Wheat (which were similar), and Tapioca exhibiting the lowest values.

The DSC result values are shown in Table 14, showing the difference between gluten-free bread across day 1, 2 and, 4.

Table 14. Gluten-free bread samples analysed by DSC across day 1,2 and, 4 showing mean To onset temp, Tp peak temp, Tc concluding temp, ΔH retrogradation/amylopectin melting enthalpy values.

Sample	Day	T_o(°C)	T_p(°C)	T_c(°C)	ΔH (J/g)
Wheat	1	49.2±2.8	63.7±0.9	72.2±2.7	$1.18±0.11^{abB}$
	2	49.3±4.0	63.5±1.7	70.8±0.9	$2.34±0.14^{aAB}$
	4	47.2±0.7	61.6±1.2	71.8±0.9	$2.88±0.11^{bcA}$
Tapioca	1	54.7±5.5	62.9±3.9	65.3±5.1	$0.20±0.06^{bA}$
	2	54.1±1.8	68.2±0.7	72.5±2.3	$0.44±0.21^{bA}$
	4	50.3±3.5	66.6±2.6	72.3±2.8	$1.50±0.24^{cA}$
FPS	1	49.4±3.2	73.7±0.3	77.5±2.0	$2.58±1.08^{aB}$
	2	48.1±2.7	69.0±3.4	76.5±5.5	$3.71±0.19^{aAB}$
	4	49.5±0.9	70.3±2.5	77.7±4.7	$4.88±0.36^{aA}$
Amylacetate	1	50.6±2.8	73.1±1.9	77.3±3.4	$2.02±0.75^{aA}$
	2	47.1±2.3	74.4±7.9	78.8±5.7	$2.61±0.63^{aA}$
	4	50.9±2.9	72.1±2.1	78.0±2.0	$2.98±0.38^{bA}$

Values are presented as mean ± standard deviation (SD); n = 3. with different lowercase superscripts are significantly different ($p < 0.05$) between starches on the same day. Means across a single row with different uppercase superscripts indicate significant difference ($p<0.05$) within starch between days.

The melting enthalpy analysis revealed consistent differences among starch types across all experimental days, with mean values consistently following the order: FPS > Amylacetate > Wheat > Tapioca. Day one that Tapioca exhibited significantly lower values compared to both FPS and Amylacetate, while no significant difference was observed between Wheat and Tapioca. Notably, on day 2, Tapioca was the only starch type showing highly significant differences from all other samples. By day 4, FPS displayed significantly higher melting enthalpy than the remaining starches. Tapioca was significantly different from both FPS and Amylacetate, while its values remained statistically comparable to Wheat.

Across the storage period, no significant changes in melting enthalpy were observed for Amylacetate and Tapioca. Although no significant change was observed there was a tendency to have an increasing enthalpy as seen in Figure 9. A significant difference was observed between days for FPS and Wheat where day 4 had significantly higher melting enthalpy values compared to day 1.

Table 15 summarises the DSC results for the melting of the amylose lipid complex across days 1,2 and, 4.

Table 15. Gluten-free bread samples analysed by DSC across days 1,2 and, 4 showing mean values of Amylose Lipid Complex ALCo onset temp, ALCp peak temp, ALCf final temp, △H endothermic enthalpy numbers.

Sample	Day	ALC_0(°C)	ALC_p(°C)	ALC_f(°C)
Wheat	1	109.0±2.3	115.8±4.0	120.3±5.0
	2	112.6±3.8	112.6±2.8	118.8±3.2
	4	114.1±7.3	120.8±4.5	126.4±3.7
Tapioca	1	98.7±11.6	105.1±11.7	110.2±13.5
	2	10.8±4.6	110.67±4.8	114.2±5.9
	4	114.3±4.3	120.1±5.9	122.7±6.1
FPS	1	110.1±5.1	117.0±4.5	120.5±4.8
	2	114.3±4.2	120.0±3.7	121.8±7.0
	4	112.7±3.1	116.7±2.9	120.1±2.4
Amylacetate	1	101.1±6.9	108.5±3.6	111.4±4.0
	2	106.3±4.2	111.8±5.1	114.4±4.1
	4	115.9±16.2	120.0±14.1	123.2±12.9

Values are presented as mean ± standard deviation (SD); n = 3.

There seems to be a tendency for a higher ALC_0 melting temperature over a longer storage time of the gluten-free bread. The ALC peak temperature (ALCp) was pushed higher over time in all samples. FPS consistently had the highest peak temperature (especially on day 1), whereas Tapioca and Amylacetate started with lower onset and peak temperatures but showed strong increases.

5 Discussion

Starch thermal properties

DSC measures the thermal energy required to disrupt crystalline regions within the starch granules, capturing the onset of molecular transitions under controlled heating. The enthalpy of gelatinization provides a general measure of crystallinity, reflecting both its quality and quantity and serves as an indicator of the disruption of molecular order within the granule (Singh *et al.*, 2003).

It was assumed that Amylacetate, the acetylated potato starch, would have a lower enthalpy needed to melt the crystalline structure compared to the native fractioned potato starch, FPS. This is due to the loosened molecular and crystalline packing arrangements and damage to ordered structures within starch granules due to the modification (Choi and Kim, 2022). Hereby lowering the energy needed to melt the structures. However, that was not the case when comparing the two potato starches, since Amylacetate had a significantly higher enthalpy than FPS. Perhaps this could be a user error related to the analysis or an indicator that the composition of the smaller granules, present in FPS, are different from those in the chemically modified Amylacetate. This was indicative of a lower degree of crystallinity. A study done by (Singh and Kaur, 2004) also confirms that the smaller-sized fraction of potato starch granules had lower gelatinisation enthalpies compared to the bigger fractions, as well as a higher gelatinisation temperature.

The gelatinisation enthalpy values for Amylacetate and FPS fell within the expected values seen in studies however, both Wheat and cross-linked Tapioca had lower values (Singh *et al.*, 2003a; Thirathumthavorn and Trisuth, 2008; Wickramasinghe *et al.*, 2009; Sriprablom *et al.*, 2023). Perhaps the lower enthalpy value for Wheat could be attributed to damaged starch granules. In general, the DSC method was the method with the most outliers among all the analyses.

Starch pasting properties

The peak viscosity had the following order of highest to lowest: Amylacetate>FPS>Tapioca>Wheat. The two potato starches had the expected highest peak viscosities due to their larger granule size, B-type crystallinity as well as their phosphate-rich amylopectin (Noemi Sivak and Preiss, 1998; Grommers and van der Krogt, 2009). The difference between Amylacetate and FPS was expected as acetylation of starch has been found to increase peak viscosity (BeMiller, 2019).

The cross-linked Tapioca starch had a quite low peak viscosity, which can be due to a high level of cross-linking. This would constrict the granules and reduce the swelling due to the enhanced stability from the covalent cross-linking.

Wheat had an unexpectedly high pasting temperature. While the Wheat starch is legally gluten-free, it might still contain traces of gluten, and other proteins as well as lipids. The content of these would, according to literature, suppress the swelling and increase the gelatinization temperature, while also lowering the viscosity of wheat starch (Guo *et al.*, 2023).

Ranked test - Sensory

An additional loaf was produced from the batch used for sensory analysis to allow comparison with previously collected TPA and BVM data. BVM results showed no significant difference between the original and sensory Tapioca bread, while the other starch types differed significantly. However, the observed mean differences were minor, indicating a high degree of comparability between batches.

TPA measurements indicated consistent cohesiveness across batches. Although firmness increased in the new breads, the relative ranking of starch types remained unchanged, with Tapioca the softest and FPS the hardest. The absolute differences were minor, suggesting limited practical significance despite statistical significance. Observed variations may stem from the smaller sample size and baking variability.

Visual attribute whiteness showed a significant difference between all the different starches. Where Amylacetate had the whitest appearance and Tapioca the darkest. This can be explained by the different appearance and composition of the starches, as well as the different gelatinisation properties leading to a more or less white crumb.

Despite low inter-judge agreement, ANOVA indicated a significant effect for crumb uniformity, with Amylacetate scoring higher than Tapioca. However, this result is weakened by the untrained panel and potentially insufficient definition of the attribute. Visual inspection also revealed inconsistencies in the bread slices, suggesting uneven air cell distribution, even within the same bread.

FPS showed the highest crumbliness, significantly greater than Tapioca, which exhibited the lowest and was described by the panel as spongy and crumb-free. This aligns with the known retrogradation behaviour of Type-B potato starches longer amylopectin chains contributing to retrogradation versus the minimal retrogradation of Tapioca. Additionally, the cross-linked structure of Tapioca, while lowering peak viscosity, stabilises granules against disintegration, reducing starch molecule dispersion and subsequent reassociation (Roman *et al.*, 2020).

No significant differences were found in bread-like smell or off-taste across starch types, with low inter-judge agreement suggesting unclear parameter definitions or weak attribute intensity. This underscores texture as the primary focus of the sensory analysis, consistent with its central role in gluten-free bread quality. Other attributes, such as whiteness, crumbliness, and crumb uniformity, were included mainly as supporting measures.

Softness, the first texture-related attribute assessed, showed no significant differences between starch types and low inter-judge agreement. One panellist noted that Tapioca compressed easily without breaking down, while FPS was firmer but disintegrated more readily. Several also described Tapioca as texturally distinct. These findings suggest potential confusion around the softness definition, which combined compression and breakdown, which may have led to inconsistent interpretations among the panel.

As anticipated, chewiness and dryness exhibited an inverse relationship: Tapioca demonstrated the highest chewiness and the lowest dryness and crumbliness, whereas FPS showed the opposite trend. This is related to the available moisture as well as the degree of retrogradation, which will be explained under TPA.

The overall impression ratings showed a clear preference for Amylacetate, Wheat, and Tapioca, which all scored significantly higher than FPS. Panel comments indicated that FPS's high dryness and crumbliness were the main factors contributing to its low score. Although Tapioca was among the most favoured, its unique texture divided opinions. Some panellists enjoyed the moist, elastic crumb, while others found it overly chewy.

Gluten-free bread bake loss, specific volume, L*a*b*

It was expected that, due to the different physical and chemical properties of the starches, that the bake loss would be significantly different, but that was not the case. However, although not significantly different FPS had the lowest mean bake loss of all the starch types. Overall, the bake loss was very low compared to other studies using different gluten-free recipes, which ranged from 18-20% loss compared the 8,8-9% loss in this experiment (Horstmann *et al.*, 2016; Lu, Donner and Liu, 2021). The addition of the functional ingredients such as the whey protein, hydrocolloids and complex carbohydrates such as fibres, must have had a significant impact on the water-holding capacity of the bread after baking, diminishing the effect of the starches.

Wheat starch produced the highest specific volume among all starches, consistent with previous findings on gluten-free bread made with various starch sources (Horstmann *et al.*, 2016; Roman, Gomez and Martinez, 2021). The study attributed Wheat's higher capacity for gas retention to the morphology and size of the granules, which allow for better self-packing. This self-packing mechanism may also help explain why FPS achieved a volume comparable to Wheat, as its starch fraction contained a higher proportion of smaller granules, due to the exclusion of larger ones during the fractionation process.

According to a previous study, peak viscosity was correlated positively with gluten-free bread volume (Alvarez-Jubete *et al.*, 2010). These results do not seem to show a clear relation between bread volume and peak viscosity. Wheat starch with the lowest viscosity produced the highest volume bread, while Amylacetate with the highest viscosity had the lowest volume, together with Tapioca.

A minimum dough viscosity is required to support gas retention and expansion during proofing and baking. Excessive viscosity can hinder these processes by limiting dough expansion and reducing gas cell stability. This may explain why FPS, with its lower viscosity, achieved a significantly higher volume than Amylacetate. This difference is also reflected visually: after baking, both FPS and Amylacetate breads had rounded tops, but upon cooling, their crusts deflated and formed indents. In contrast, Wheat and Tapioca maintained a consistently rounded crust even after cooling, indicating better structural stability.

Nonetheless, viscosity alone does not fully account for volume differences. For example, Tapioca exhibited lower viscosity than FPS but produced a lower bread volume. This could be attributed to the strong molecular interactions in cross-linked starches and Tapioca's limited tendency to retrograde, potentially resulting in an under-set crumb. Such a structure may result in more water accessible to plasticise the matrix, leading to a more elastic type of bread.

The relationship between peak viscosity and specific volume is complex, as various ingredients within the formulation compete for water in an already limited hydration matrix.

Overall, the specific volume of the gluten-free bread produced in this study was lower than that reported in previous research (Kim, Yun and Jeong, 2015; Horstmann, Foschia and Arendt, 2017, 2017; Pico *et al.*, 2019; Lu, Donner and Liu, 2021) and commercial bread (Cappelli, Oliva and Cini, 2020; Monteiro *et al.*, 2021). Many breads in these studies had simpler formulations, often lacking nutritional components such as protein and fibre, which may contribute to higher specific volumes due to lower dough viscosity and a thinner consistency. Although large volume is an important quality attribute for gluten-free bread, maintaining adequate protein and fibre content is essential for nutritional value.

The colour analysis yielded results partly consistent with the sensory evaluation. Both methods agreed that Tapioca had the darkest crumb and that FPS appeared lighter than Wheat, though not significantly so. However, Amylacetate, rated lightest in the sensory test, showed a lower lightness value on the CIE L*a*b* scale and did not differ significantly from Wheat or FPS. This discrepancy may be attributed to sensory panel comments noting warm, yellow-toned lighting, which could have influenced perceived crumb colour.

Gluten-free bread Staling characteristics: TPA, Crumb Moisture, Retrogradation

Specific volume did not seem to directly correlate to firmness, and crumb moisture seemed to oppositely correlated to firmness. Among the starches evaluated, bread formulated with FPS exhibited the highest crumb moisture as well as the highest firmness, which corresponded with the highest perceived dryness and crumbliness, as reported by the sensory panel. This firmness is also reflected in the highest mean retrogradation enthalpy seen for FPS. Confirming the relationship between amylopectin retrogradation enthalpy and crumb firmness.

Amylacetate had a lower crumb firmness, melting enthalpy values and was comparable with Wheat in moisture content. This is also reflected in the dryness, crumbliness and chewiness attributes in the sensory evaluation, where they ranked significantly similarly.

Potato starch, like mentioned earlier, has a higher tendency to re-associate due to various reasons: fine amylopectin structure (i.e. longer chains), high phosphorus content, and granules with higher swelling capacity are also more sensitive to rupturing and leaking. The difference seen between Amylacetate and FPS can be contributed partly to the chemical modification. The addition of acetyl groups on the starch could have stabilised the internal structures, leading to more controlled swelling and gelatinisation, which results in lower dispersion of starch molecules contributing to the staling promoting-aggregates (Roman, Gomez and Martinez, 2021). The acetyl groups in the starch have also been shown to be a steric hindrance preventing the alignment of starch chains (Roman *et al.*, 2020; Zhang *et al.*, 2020), reducing retrogradation, which explains the lower melting enthalpy.

Tapioca stood out as having the lowest firmness, lowest dryness and crumbliness, and the lowest moisture content of the crumb. This aligns with the known retrogradation behaviour of Tapioca previously explained. Similar to the acetylated starch, the cross-linked structure of the Tapioca starch also protects the granule against disintegration, reducing starch molecule dispersion and subsequent reassociation (Roman *et al.*, 2020).

The observed cohesiveness trends suggest that starch type plays a significant role in maintaining crumb integrity over time. Tapioca consistently exhibited the highest cohesiveness, indicating a more elastic and structurally resilient crumb, which is also reflected in the results from chewiness in the sensory evaluation. In contrast, FPS showed the lowest cohesiveness across most time points and was the only starch not to exhibit a significant decrease over time. This stability, despite a lower starting point, could reflect a more rigid and brittle crumb structure that is less affected by staling dynamics. It could also mean that FPS had already exhibited its major retrogradation during the first day of storage. Amylacetate and Wheat displayed significantly similar intermediate behaviour. However, Amylacetate maintained a slightly (not significantly) higher cohesiveness than Wheat across all days, also reflected in the slightly higher value seen for chewiness in the sensory evaluation. The overall decline in cohesiveness with storage time aligns with expected staling effects, likely driven by moisture loss and starch retrogradation.

Firmness and enthalpy values increasing over time, as well as cohesiveness decreasing over time, was generally in agreement with the expected staling dynamics. However, the distinct increase in firmness for Tapioca on day 4, was not reflected in the enthalpy value. This could be due to the fact that, while DSC measures the enthalpy change associated with amylopectin retrogradation, firmness in gluten-free bread may be influenced by additional factors such as moisture loss, crumb structure tightening, or interactions with other bread components, which do not necessarily involve crystallization detectable by DSC but still affect the staling dynamic. This also makes sense in the fact that the cross-linked Tapioca would have less leaking of starch molecules, reducing the mechanics related to retrogradation.

One likely explanation regarding the moisture content and its relation to firmness, as well as the differences between starches, is as follows. The initial moisture content of the potato-based starches was higher, due to properties such as B-type crystalline structure, phosphorus content and bigger granules, which could contribute to their elevated crumb moisture levels post-baking. The assumption is that higher moisture would be a less dry crumb, however it has more to do with the mobilisation of water, if the water is bound within a structure, there is less water available to plasticize the structures within the bread leading to a more brittle and drier crumb. This could explain the reason why higher moisture did not contribute to lower firmness. Although a general decrease in crumb moisture over time was anticipated, this pattern was not consistently observed across any starch types, the reason for this will be explained under limitations.

The peaks seen for ALC seem to show a tendency for a higher initial ALC melting temperature across days, reflecting that the main melting point of the complex became more thermally stable. FPS consistently had the highest peak temperature (especially on day 1), indicating inherently more stable ALCs at earlier stages. whereas Tapioca and Amylacetate started with lower onset and peak temperatures but showed strong increases. suggesting slower complex development. The difference overall in mean was not drastic, perhaps due to the uncertainty of the method.

Improvements and limitations

Differential Scanning Calorimetry (DSC) exhibited the highest number of outliers among all analytical methods used in this study. Consequently, only gelatinization and retrogradation melting enthalpy values were considered for discussion.

Limitations in both RVA and DoughLab methods arose due to the stark differences in water absorption and swelling capacity among the starches, making it challenging to apply a single standardized method for all samples. The RVA produced the most distinct pasting profiles using 3.5 g samples for Tapioca and Wheat, while Amylacetate exhibited its clearest pasting behaviour with only 1.2 g. 2g seemed to be the best middle ground to show the different properties of the starches, although it slightly exceeded the optimal dosage for Amylacetate. The use of the

DoughLab proved suboptimal for analysing this gluten-free bread formulation, largely due to the intrinsic variability in the functional properties of the starches, particularly swelling capacity and water absorption. Achieving comparable torque values required method optimisation, as the standard protocol did not yield usable or consistent results across samples. In future applications, torque measurements should be standardized to ensure equivalent dough consistency across formulations. Prior to this, only differences in water absorption should be compared, as starches vary significantly in their hydration capacity. Once a standardised torque is achieved, comparisons can then be made based on performance under equivalent mechanical conditions.

Although a general decline in crumb moisture over time was expected, this trend was not consistently observed across the different starch types. It is important to note that bread volume measurements (BVM) were conducted on the day of baking using a method that involved physical puncturing of the loaves. This may have compromised crust integrity, which is known to play a critical role in limiting moisture loss. Despite storage in sealed plastic bags, the initial disruption of the crust likely influenced moisture migration during storage. Additionally, inconsistencies in sample handling during moisture analysis, such as variations in the duration that slices were exposed to ambient conditions prior to measurement, may have introduced further variability. For more accurate assessment of starch-dependent moisture retention, future analyses should be conducted on untouched loaves that have not undergone prior testing. This approach would help isolate the effects of starch type and minimize confounding variables, thereby enhancing the reliability of the results.

It is worth noting that the number of sensory attributes included in the evaluation may have been excessive for an untrained panel, despite the panel size exceeding the recommended minimum by nearly threefold. This could have affected the consistency and interpretation of certain parameters, particularly those that were ambiguously defined or overlapped conceptually. For future studies, it is recommended to refine and limit the number of sensory attributes, with a stronger focus on texture and clearer definitions to ensure consistent understanding among panellists. This would improve both the reliability and interpretability of the results, ensuring agreement among judges and stronger evidence.

6 Conclusion

The quality of gluten-free bread, including staling-related textural properties, retrogradation behaviour, moisture content, specific volume, and sensory attributes, was significantly influenced by the type of starch used.

Wheat starch delivered the highest specific volume and favourable texture characteristics, including moderate firmness and cohesiveness. Its crumb moisture was lower than the potato starches, yet it retained acceptable textural properties. Wheat starch's ability to produce high-volume bread with good overall acceptability reaffirms its functional advantage in gluten-free bread, despite possible minor gluten contamination risks.

Tapioca, a cross-linked starch, consistently showed the lowest crumb firmness, highest cohesiveness, and lowest crumbliness and dryness, both from the bread analysis and according to sensory evaluation. Its minimal retrogradation, due to the protective effect of cross-linking, preserved the initial crumb integrity. Although it had one of the lowest specific volumes, its unique spongy and elastic texture was generally well-received, albeit polarising to some panellists due to the chewy texture.

The fractionated native potato starch FPS, produced gluten-bread with high volume, similar to Wheat, but also the highest firmness, lowest cohesiveness, and highest crumb moisture. Despite its high moisture content, the bread was perceived as the driest and most crumbly by the sensory panel, likely due to the water being immobilized, rendering it unavailable for crumb plasticisation. This was further supported by its high retrogradation enthalpy, reflecting strong amylopectin reassociation, which contributed to rapid staling and textural degradation. The low sensory ratings for FPS bread further underscored its inferior quality from the panel's perspective, and the importance of bread firmness.

Amylacetate, the chemically modified acetylated potato starch, displayed improved performance relative to FPS and was similar in both textural and retrogradation properties to Wheat starch. Although both FPS and Amylacetate are derived from potato starch, acetylation appeared to reduce retrogradation through steric hindrance and internal structure stabilisation, leading to lower crumb firmness and retrogradation enthalpy. Amylacetate maintained moderate cohesiveness and had significantly higher sensory preference scores. Despite its low specific volume, likely a consequence of its higher paste viscosity, which may have limited gas cell expansion during baking, its superior texture-related properties, particularly reduced dryness and crumbliness, suggest it is a more favourable starch option for gluten-free bread formulations.

Firmness, cohesiveness, dryness, crumbliness, and retrogradation enthalpy were generally correlated, aligning with the expected staling dynamics: progressive crumb firming and brittleness over time, driven by increased amylopectin recrystallisation and moisture immobilisation. However, a notable increase in firmness was observed for Tapioca over time, which did not correspond with a significant rise in retrogradation enthalpy, suggesting that factors other than amylopectin recrystallisation, such as moisture redistribution or structural, tightening may have contributed to its staling behaviour.

7 Future perspectives and studies

Meta-analyses of gluten-free bread development support the idea that combining different starches can improve loaf volume and overall product quality (Cappelli, Oliva and Cini, 2020; Monteiro *et al.*, 2021; Roman, Gomez and Martinez, 2021). Notably, in most formulations, modified or functional starches are not used as complete replacements for native starches; rather, they typically constitute only a partial substitution, often around 20% of the total starch content (Roman *et al.*, 2020). Accordingly, future studies should explore starch blends and individualised recipe adjustments to better leverage the unique functional properties of each ingredient and enhance bread quality.

Roman, Gomez and Martinez (2021) note that studies investigating the role of starchy ingredients in gluten-free bread should ideally consider evaluating bread quality attributes at constant or optimal dough consistency rather than at constant water content. This is particularly relevant given the variation in swelling and water absorption capacities among different starches. In this study, all bread samples were successfully produced using a standardised base recipe, with identical starch content and uniform processing conditions. This approach enabled direct comparison across starch types, but it may not have captured the optimal performance potential of each starch individually, which could be further investigated. This should also be combined with an improvement in the baking conditions such as internal temperature measurement of the dough during proofing, baking and cooling, to ensure a consistent result.

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9 Appendix

Appendix I

Appearance of Gluten-Free Bread
Whiteness: The brightness or lightness of the crumb color.

Crumb uniformity: Even distribution of air holes and consistency in texture throughout the bread. Does the crumb look irregular? (low uniformity) or even? (high uniformity).

Crumbliness: How easily the crumb breaks apart when touched and taken off the plate.

You are provided with the following samples of gluten-free bread labelled as:

125	859	214	754

Please visually inspect them initially in this order until you can place them in front of you from left to right in order of increasing **Whiteness** of the crumb. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of whiteness of crumb →		Highest

Comments:

____
____
____

Please visually Inspect them initially in the original order until you can place them in front of you from left to right in order of increasing **Crumb uniformity**. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of crumb uniformity →		Highest

Comments:

____
____
____

Please visually Inspect them initially in the original order until you can place them in front of you from left to right in order of increasing **Crumbliness** of crumb. You may grab the bread to see the crumbs leftover on the plate. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of crumbliness of crumb →		Highest

Smell & Taste of Gluten-Free Bread
Off-taste: chemical-like taste, for example chlorine-like or metallic.

Bread-like smell: How much does the smell resemble traditional wheat bread?

Return the order of the samples to:

125	859	214	754

Please smell them initially in this order until you can place them in front of you from left to right in order of increasing **Bread-like smell**. You may grab the bread to smell it. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of bread-like smell →		Highest

Comments:

____
____
____
____

Please cleanse your mouth and taste them initially in the original order until you can place them in front of you from left to right in order of increasing **Off-taste** of the crumb. When you have done this, write the sample codes in the boxes below:

Lowest	Rank order of off-taste →		Highest

Comments: (which off-taste did you taste, if any?)

____
____
____

Texture of Gluten-Free Bread

Softness: How easily bread compresses and breaks down when chewed, light texture.

Chewiness: Elastic texture giving the bread gives during chewing

Dryness: Lack of moisture in the bread during chewing, resulting in a dry mouthfeel.

Return the order of the samples to:

125	859	214	754

Please visually inspect them initially in this order until you can place them in front of you from left to right in order of increasing **Softness** of the crumb. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of softness of crumb →		Highest

Comments:

____

____

____

Please visually Inspect them initially in the original order until you can place them in front of you from left to right in order of increasing **Chewiness**. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of chewiness →		Highest

Comments:

____

____

____

Please visually Inspect them initially in the original order until you can place them in front of you from left to right in order of increasing **Dryness** of crumb. You may grab the bread to see the crumbs leftover on the plate. When you have decided, write the sample codes in the boxes below:

Lowest	Rank order of dryness →		Highest

Overall impression

Please give each bread an overall impression rating between 1-9, comment on why you rated the highest and lowest bread their respective scores. You may taste the bread again if you would like.

You are presented with a hedonic scale of 1-9 and their meaning shown below.

Grade	**Score**
Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like Nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1

Your samples were given in the following order, Which **Score** would you give:

125	859	214	754

Comments: Why did you choose the lowest and highest scoring breads?
____
____
____
____

Appendix II

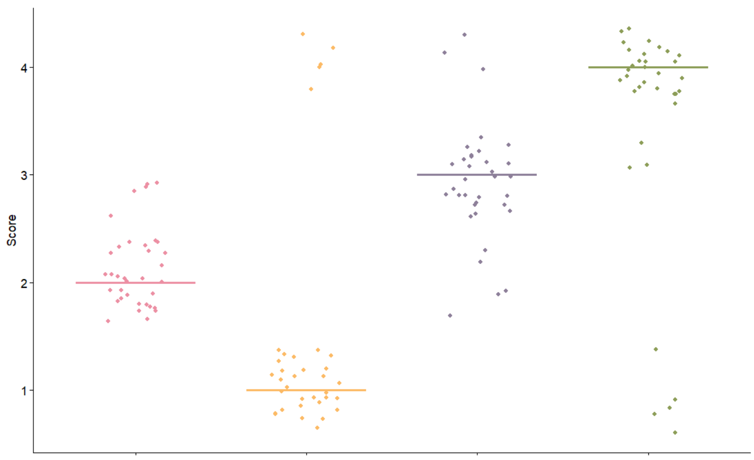

Figure A. Boxplot with jittered points, Sensory data Whiteness, left to right: Wheat, Tapioca, FPS, Amylacetate.

The boxplot for sensory results of whiteness shows the agreement amongst judges, reflected by the lack of upper and lower quartile.

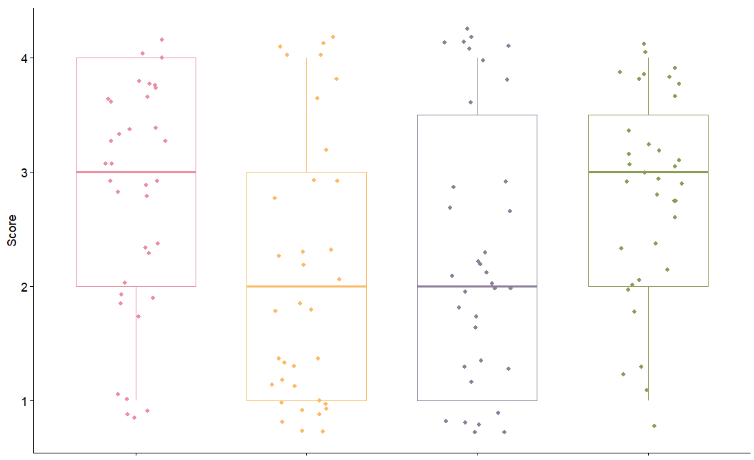

Figure B. Boxplot with jittered points, Sensory data Crumb uniformity, left to right: Wheat, Tapioca, FPS, Amylacetate.

The boxplot for sensory results of crumb uniformity shows the lack of agreement amongst judges, reflected by the points being very spread in score, receiving both highest and lowest ratings, as well as a lack of any tendency.