In vitro starch digestion rate of breads baked with added polar lipids

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Popular Science summary

Heading
Can lecithin make bread release sugar more slowly?

Introduction
White bread often causes a rapid rise in blood sugar because the starch is digested quickly. This project investigated whether lecithins from eggs, soybeans, and sunflower seeds could slow starch digestion and make bread metabolically healthier.

Main text
Bread is a staple food for millions of people. However, white bread often produces a rapid increase in blood glucose after eating. Frequent sharp rises in blood sugar are linked to diseases such as type 2 diabetes and cardiovascular disease. Researchers therefore want to develop foods that release glucose more slowly.

Starch is the main carbohydrate in bread. The rate of starch digestion strongly influences how quickly glucose enters the bloodstream. Some studies have suggested that polar lipids may slow this process. Polar lipids are special fat molecules found in foods such as egg yolk and sunflower seeds. Lecithin is one common type of polar lipid.

This project investigated whether lecithins from different sources influence starch digestion in bread. Five breads were prepared: white bread, bread with sunflower oil, bread with sunflower lecithin, bread with soy lecithin, and bread with egg lecithin.

The study used a laboratory method that simulated chewing and enzymatic digestion in the human body. The experiments measured how much starch was broken down over time. The study then estimated how quickly glucose would likely be released after eating the bread.

The results showed clear differences between the breads. All breads with added lipids showed slower starch digestion than white bread. Breads with lecithins generally showed a greater reduction than bread with sunflower oil alone. Egg lecithin and sunflower lecithin produced the strongest effects.

The results suggest that lecithins interact with starch and make it more difficult for digestive enzymes to break it down. Lecithins may also change the internal structure of bread and reduce enzyme access to starch.

Interestingly, the source of the lecithin appeared to matter. Egg lecithin produced strong effects even though it contained less polar lipid than the plant lecithins. This finding suggests that lipid composition may be important, not only the amount of fat added.

The study shows that small changes in bread ingredients can influence starch digestion. In the future, this knowledge may help researchers and food companies develop bread products with a lower glycaemic impact and a slower release of glucose after meals.

Abstract

This study investigated the effect of polar lipids from different sources on the in vitro starch digestion rate of bread. Postprandial glycaemic response is strongly influenced by the rate of starch digestion, and polar lipids have been suggested to modulate this process, although the underlying mechanisms remain unclear. Bread was used as a model food matrix, and five types of bread were prepared: white bread, bread with sunflower oil, and breads with sunflower lecithin, soy lecithin, and egg lecithin.

Starch digestion was evaluated using an in vitro method based on simulated human intestinal digestion with the addition of an initial chewing step. The degree of starch hydrolysis over time was measured, and hydrolysis index (HI) and predicted glycaemic index (pGI) were calculated.

The results showed that all lipid-enriched breads exhibited lower starch hydrolysis compared to white bread. Breads with egg lecithin and sunflower lecithin produced significantly lower HI values compared to white bread. In addition, the bread with egg lecithin also differed statistically significantly from the bread with sunflower oil. The predicted glycaemic indices followed a similar trend.

These findings suggest that starch digestion behaviour of bread may be influenced by the presence and type of added lipids. The results further indicate that breads with polar lipids, particularly those with egg or sunflower lecithin, may have potential for reducing starch digestion rate and influencing glycaemic response. Therefore, selected polar lipids may represent a promising formulation strategy for developing bread products with lower glycaemic impact.

Acknowledgement

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Bao Liu (刘保)

Table of Contents

1 Introduction

Postprandial blood glucose response is an important factor in human health. Excessive high postprandial glycemia is associated with an increased risk of metabolic disorders such as type 2 diabetes and cardiovascular disease. The glycaemic index (GI) classifies foods containing carbohydrate according to their effect on postprandial blood glucose levels and is widely used as a measure of carbohydrate quality [1].

The rate of starch digestion is a major determinant of the glycaemic response. Rapid digestion leads to a fast release of glucose and a high GI, whereas slower digestion results in a lower and more gradual glycaemic response. Therefore, understanding the factors that influence starch digestion is essential for improving the metabolic quality of foods rich in carbohydrates [2].

Food composition and structure strongly influence starch digestion. Components such as proteins, lipids, and dietary fiber can modify enzyme accessibility and alter digestion kinetics [2]. Lipids can also interact directly with starch molecules and form amylose-lipid inclusion complexes, which may reduce starch accessibility to digestive enzymes and alter starch digestibility [3,4].

Lipids, particularly polar lipids, have been shown to modulate postprandial metabolic responses. Previous in vivo studies conducted by the Lund University researchers have demonstrated that oat polar lipids and sunflower lecithin can beneficially influence postprandial metabolism in healthy adults. The inclusion of polar lipids in a solid breakfast reduced acute postprandial blood glucose responses and induced a pronounced second-meal effect, indicating a sustained metabolic impact beyond the initial meal [5-7].

Despite these findings, the mechanisms underlying the metabolic effects of polar lipids are not fully understood. In particular, it remains unclear whether polar lipids from different sources exert similar effects on starch digestion. This question is especially relevant in commonly consumed food matrices such as bread, where structure and composition strongly influence digestion behaviour.

Measurement of GI requires human intervention, making it relatively resource-intensive. Therefore, in vitro methods have been developed to estimate starch digestion and predict glycaemic response. An in vitro procedure based on simulated chewing and enzymatic digestion has been shown to correlate well with in vivo metabolic responses in cereal products [8]. Such methods provide a practical and convenient approach for identifying factors that influence starch digestibility.

Bread is a widely consumed staple food and represents a relevant starch-based matrix for studying digestion. Its structure, formed during baking through starch gelatinization and gluten network development, plays a key role in controlling enzyme accessibility. This makes bread a suitable model system for investigating the effects of added components on starch digestion.

Therefore, this study aims to investigate the effect of bread with added polar lipids from different sources on in vitro starch digestion rate. White bread without added lipids is used as a control product, while bread with sunflower oil serves as a reference representing a non-polar lipid source. Breads with sunflower lecithin, soy lecithin, and egg lecithin are compared with these two products. The study evaluates whether polar lipids influence starch digestion, examines the

nature of this effect, and compares their impact with that of a non-polar lipid source. This approach provides a mechanistic basis for understanding how polar lipids may modulate glycaemic response in complex food systems.

2 Aim

The aim of the thesis is to investigate the effect of bread with polar lipids from different sources on the in vitro starch digestion, using white bread as a control and bread with sunflower oil as a reference.

Specifically, the objectives are:

- to determine whether polar lipids influence the rate of starch digestion;
- to characterize the direction and magnitude of this effect;
- to compare the effects of different polar lipids sources (sunflower lecithin, soy lecithin, egg lecithin); and
- to evaluate whether and how breads with polar lipids differ from those with an equivalent amount of conventional sunflower oil.

3 Theoretical background

3.1 Breadmaking

Bread is a staple food made by baking a dough consisting mainly of wheat flour, water, yeast, and salt. During the baking process, a series of physical and biochemical changes transform the dough into a structured and aerated product. These changes determine key quality attributes of bread such as volume, texture and digestibility [9].

The breadmaking process consists of several key stages, including mixing, fermentation, baking, and cooling. In the straight dough method, all ingredients are mixed at once, followed by bulk fermentation before baking [9]. Mixing hydrates the flour and develops the gluten network. Fermentation enables the yeast to produce carbon dioxide, which causes the dough to rise. Finally, baking stabilizes and fixes the bread's structure through thermal transformations.

The functional properties of wheat flour, water, yeast, salt, and lipids collectively determine the structure, texture, and behaviour of bread dough. Wheat flour is one of the main ingredients that gives bread its structure. It contains starch and gluten-forming proteins, primarily gliadin and glutenin. During mixing, these proteins form a gluten network that gives the dough elasticity and allows it to retain gas. Water enables the formation of dough by hydrating flour components. It supports gluten development and allows starch granules to swell during heating. Water also affects dough consistency and processing behaviour. Yeast is essential for the fermentation process in breadmaking. It converts sugars into carbon dioxide and ethanol. The carbon dioxide becomes trapped in the gluten network and causes the dough to rise. Salt improves dough properties and controls fermentation. It strengthens the gluten network and makes the dough easier to handle. It also slows down yeast activity and contributes to flavour [9]. Lipids and emulsifiers modify the structure and stability of bread. They improve gas retention and contribute to a softer crumb. Lecithin is a widely used emulsifier. It is a polar lipid that consists mainly of phospholipids. These molecules have both hydrophilic and hydrophobic regions, which allows them to interact with water, lipids, and proteins. As a result, lecithin can stabilize gas cells and influence dough rheology [9,10].

Baking conditions strongly influence the final structure of bread. Heat causes starch gelatinization and protein denaturation, which fix the bread structure. Baking also leads to moisture loss and crust formation. These changes affect both texture and digestibility [9].

Bread structure plays an important role in starch digestion. Factors such as gluten network formation, starch gelatinization, and the presence of lipids influence the accessibility of starch to digestive enzymes [2]. These structural factors are therefore directly relevant when evaluating the effect of added polar lipids on starch digestion.

3.2 Glycaemic index

The glycaemic index (GI) classifies carbohydrate-containing foods based on their effect on postprandial blood glucose levels. It compares the blood glucose response after consuming a test food to the response after consuming a reference food, which is usually glucose or white bread. And it provides a physiological measure of carbohydrate quality rather than quantity [1].

GI is determined by measuring the incremental area under the blood glucose response curve (iAUC) over a specific time period (usually two hours) after consuming 50 grams of available

carbohydrates from a test food. The iAUC for a test food is expressed as a percentage of the iAUC for the reference food containing the same amount of available carbohydrate taken by the same subject. This method standardizes comparisons across foods and individuals [1,11].

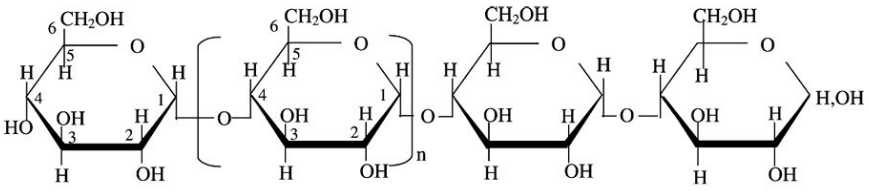
Foods are commonly classified into low, medium, and high GI categories. Low-GI foods produce a slower and smaller increase in blood glucose, while high-GI foods produce a rapid and large increase. This classification reflects differences in digestion and absorption rates of carbohydrates [1].

The GI of a food depends on multiple factors related to starch structure and food processing. The amylose-to-amylopectin ratio, degree of gelatinization, and extent of retrogradation all influence digestion rate. Processing methods such as milling, heating, and baking can further modify starch accessibility to digestive enzymes [1,2].

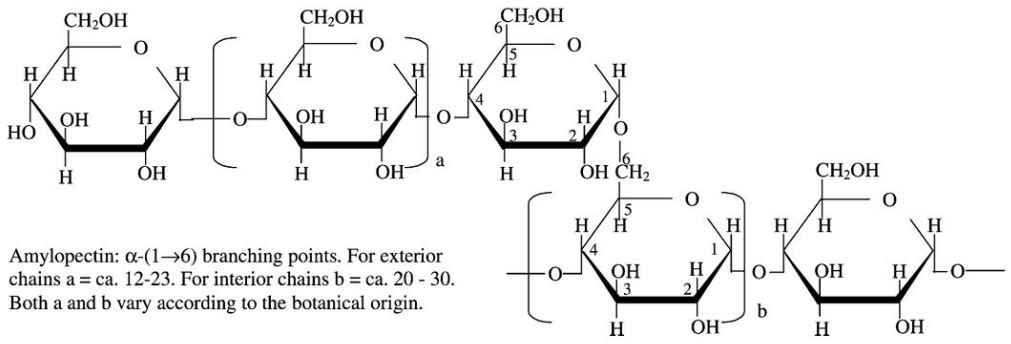
Food matrix and composition strongly affect GI. The presence of proteins, lipids, and dietary fiber can slow gastric emptying and reduce enzyme accessibility to starch. These factors can lower the rate of glucose release and decrease the GI of a food [1].

3.3 Starch

Starch is the main carbohydrate reserve in plants and a major energy source in the human diet. It is widely present in cereal grains such as wheat, which is the primary raw material in breadmaking. Starch consists of amylose and amylopectin. Amylose is mostly a linear polymer of α-(1-4)-linked glucose units, while amylopectin is a highly branched polymer with α-(1-4) backbones and α-(1-6) branch points. See *Figure 3.3-1* for the structure of amylose and amylopectin. The ratio of these two components affects the physicochemical and functional properties of starch [10].



Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).



Amylopectin: α-(1→6) branching points. For exterior chains a = ca. 12-23. For interior chains b = ca. 20 - 30. Both a and b vary according to the botanical origin.

Figure 3.3-1 Structure of amylose and amylopectin. Reproduced from Ref. [12].

Starch is organized in the form of granules with a semi-crystalline structure. These granules contain alternating crystalline and amorphous regions that arise from the ordered arrangement

of amylopectin chains. See *Figure 3.3-2* for the scanning electron microscope (SEM) and polarized light microscope (PLM) images of wheat starch granules, as well as an illustration of the semi-crystalline structure. The crystalline structure limits water penetration and enzyme access under native conditions [13].

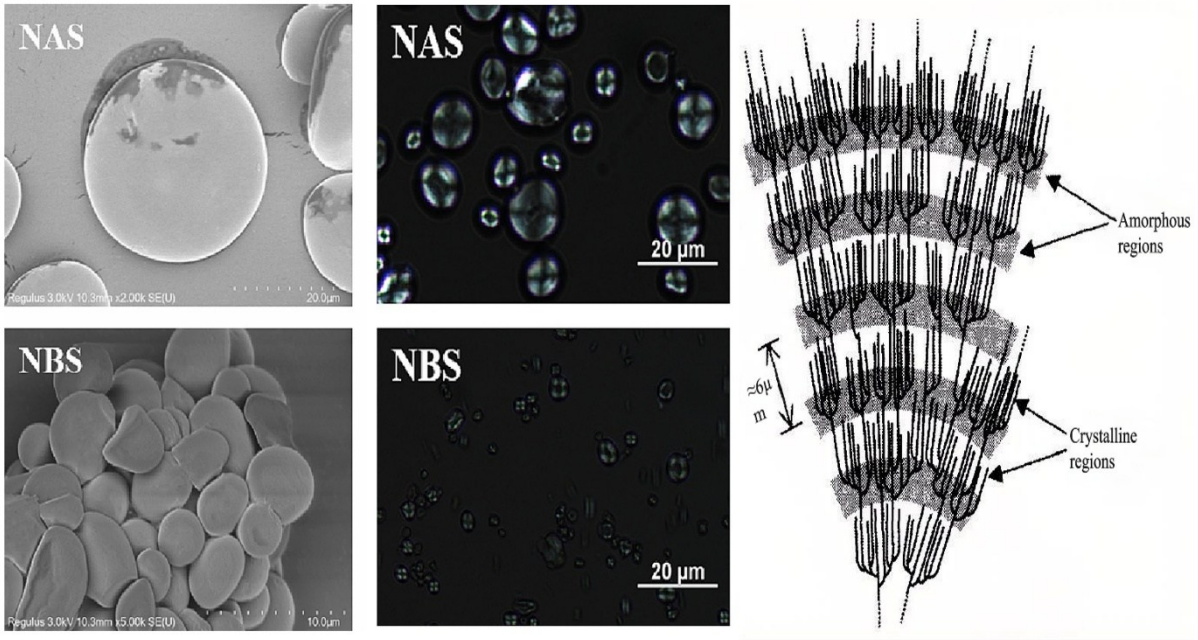


Figure 3.3-2. SEM and PLM micrographs of wheat starch granules and semi-crystalline structure of amylopectin. (A: SEM micrographs showing granule morphology; B: PLM micrographs showing birefringence patterns. NAS: native A-type wheat starch; NBS: native B-type wheat starch. C: An illustration showing the organization of the amorphous and crystalline regions) A and B adapted from Ref. [14]. C adapted from Ref. [10].

Starch undergoes gelatinization when it is heated in the presence of water. During this process, the granules absorb water, swell, and lose their crystalline structure. Gelatinization increases the accessibility of starch to enzymes and plays a key role during baking [13].

Starch can also undergo retrogradation after gelatinization. In this process, amylose and amylopectin chains reassociate and form more ordered structures during cooling and storage. Retrogradation affects the texture of bread and can reduce starch digestibility by limiting enzyme access [13].

3.3.1 Starch hydrolysis

Starch can be hydrolyzed by chemical, physical, and enzymatic methods. Enzymatic hydrolysis of starch involves the breakdown of glycosidic bonds by specific enzymes. These enzymes cleave the α-(1-4) and α-(1-6) linkages that connect glucose units in amylose and amylopectin. The process converts large starch molecules into smaller sugars such as maltose, maltotriose, and glucose [10].

α -Amylase plays a central role in starch hydrolysis. It is an endo-acting enzyme that randomly cleaves internal α-(1-4) glycosidic bonds within the starch chain. This action rapidly reduces

molecular size and produces dextrins and oligosaccharides. α-Amylase does not hydrolyze α-(1-6) branch points, which limits its ability to fully degrade amylopectin [10].

Glucoamylase further hydrolyzes starch into glucose units. It is an exo-acting enzyme that removes glucose residues from the non-reducing ends of starch chains. Unlike α-amylase, glucoamylase can hydrolyze both α-(1-4) and, more slowly, α-(1-6) linkages. This allows more complete conversion of starch into glucose [10].

Enzymatic hydrolysis depends strongly on starch structure and accessibility. Native starch granules resist enzymatic attack due to their semi-crystalline organization. Gelatinization increases enzyme access by disrupting this structure. Other factors, such as particle size, moisture content, and interactions with proteins or lipids, also influence hydrolysis rate [2,10].

3.3.2 Starch digestion

Starch digestion is a multi-step process that converts polysaccharides into absorbable monosaccharides. This process occurs mainly in the small intestine and involves coordinated enzymatic activity. The final product of starch digestion is glucose, which is absorbed into the bloodstream [15].

Digestion of starch begins in the mouth with salivary α-amylase. This enzyme initiates the hydrolysis of α-(1-4) glycosidic bonds in starch and produces smaller dextrins and maltose. However, oral digestion is limited in duration and extent due to the short residence time of food in the mouth [15].

Gastric conditions reduce the activity of salivary α-amylase. The low pH in the stomach inactivates the enzyme, and little further starch hydrolysis occurs during this phase. As a result, starch digestion is temporarily slowed in the stomach [15].

Starch digestion proceeds mainly in the small intestine through pancreatic and brush border enzymes. Pancreatic α-amylase continues the breakdown of starch into maltose, maltotriose, and α-limit dextrins. These products are then hydrolyzed by enzymes located on the intestinal brush border, including maltase and isomaltase, which produce free glucose [15].

The rate of starch digestion depends on both enzyme activity and starch structure. Gelatinized starch is digested more rapidly because enzymes can access the polymer chains more easily. In contrast, intact granules and retrograded starch are more resistant to enzymatic hydrolysis due to their compact and ordered structures, resulting in reduced starch digestion in the small intestine [2,16].

Starch can be classified based on its digestibility into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS is digested quickly and causes a rapid increase in blood glucose. SDS is digested more slowly and provides a more gradual glucose release. RS escapes digestion in the small intestine and can be fermented in the large intestine [15].

3.4 Lipids

Lipids are a broad group of compounds that include several distinct classes with different structures and functions. Major lipid classes include triglycerides, phospholipids, and sterols. These compounds differ in polarity, chemical structure, and biological role. Triglycerides are the main

storage form of energy, while phospholipids and sterols have structural and functional roles in biological systems [10,15].

Fatty acids are key building blocks of many lipids. They consist of hydrocarbon chains with a terminal carboxyl group. Fatty acids vary in chain length and degree of saturation, which strongly affects their physical and nutritional properties. Saturated fatty acids contain no double bonds, while unsaturated fatty acids contain one or more double bonds [10].

Triglycerides are the predominant form of lipids in foods and in the human diet. They consist of three fatty acids esterified to a glycerol backbone. The fatty acid composition determines the physical behaviour of triglycerides, such as melting point and fluidity. These properties influence the texture and processing characteristics of food systems [10].

3.4.1 Lipid digestion

Lipids undergo digestion through a coordinated process that involves enzymatic hydrolysis and absorption. Digestion begins in the mouth and stomach but occurs mainly in the small intestine. Enzymes hydrolyze triglycerides into free fatty acids and monoacylglycerols, which can then be absorbed by intestinal cells [15].

Pancreatic lipase plays a central role in lipid digestion in the small intestine. This enzyme hydrolyzes triglycerides at the sn-1 and sn-3 positions, producing two free fatty acids and one 2-monoacylglycerol [15]. Efficient lipid digestion also requires bile salts, which emulsify lipid droplets and increase the oil–water interfacial area available for enzyme action. Lipase activity mainly occurs at the lipid–water interface, making emulsification an essential step in the digestion process [17].

In addition to neutral lipids, polar lipids such as phospholipids also undergo enzymatic digestion in the small intestine. Phospholipids are hydrolyzed primarily by phospholipase A2, producing lysophospholipids and free fatty acids [18]. Due to their amphiphilic structure, phospholipids can act as surface-active agents and influence the physicochemical properties of lipid emulsions during digestion.

Lipid digestion products are absorbed and transported in the body through specific mechanisms. Free fatty acids, monoacylglycerols, cholesterol and lysophospholipids form mixed micelles with bile salts. These mixed micelles facilitate the transport of lipids through the aqueous environment of the intestinal lumen to the epithelial surface for absorption. After absorption, lipids are re-esterified within the small intestinal epithelial cells and packaged into chylomicrons for transport via the lymphatic system and subsequently the bloodstream [15].

3.4.2 Sunflower oil

Sunflower oil is a widely used vegetable oil obtained from the seeds of the sunflower plant (*Helianthus annuus*). It is commonly used in food processing due to its availability, mild flavour, and functional properties. The oil is extracted from sunflower seeds and is used in products such as baked goods, spreads, and cooking oils [19,20].

Sunflower oil consists mainly of triglycerides. The composition of fatty acids determines the nutritional and physical properties of the oil [20]. It is rich in unsaturated fatty acids, especially linoleic acid. Linoleic acid, also known as omega-6, is a polyunsaturated fatty acid that typically represents a large proportion of the total fatty acids in conventional sunflower oil. Oleic acid is also present, and its proportion varies depending on the sunflower variety [19,21].

Different types of sunflower oil exist based on fatty acid composition. These include high-linoleic and high-oleic sunflower oils. The fatty acid profile affects oxidative stability and functional behaviour in food systems [20,21].

Sunflower oil is liquid at room temperature due to its high content of unsaturated fatty acids. Unsaturated fatty acids reduce intermolecular packing and lower the melting point of the oil. This property influences its behaviour in food processing and formulation [20].

Sunflower oil mainly contains non-polar lipids and has limited emulsifying capacity. Unlike phospholipids, it does not contain significant amphiphilic components. Therefore, its interactions in food systems are primarily physical rather than interfacial [21].

3.5 Polar lipids

Polar lipids are a class of lipids that contain both hydrophilic and hydrophobic regions. This dual structure gives them amphiphilic properties. The hydrophobic part usually consists of fatty acid chains, while the hydrophilic part contains polar functional groups such as phosphate or sugar residues [13,22].

Polar lipids include several major groups with different chemical structures. The main groups are phospholipids and glycolipids. Phospholipids contain a phosphate group linked to a glycerol backbone, while glycolipids contain carbohydrate moieties attached to lipid structures [22,23].

Phospholipids are the most common type of polar lipids in food systems. They typically consist of a glycerol backbone, two fatty acids, and a phosphate-containing head group. Common phospholipids include phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol [10,13]. *Figure 3.5-1* below shows the structure of these three main phospholipids.

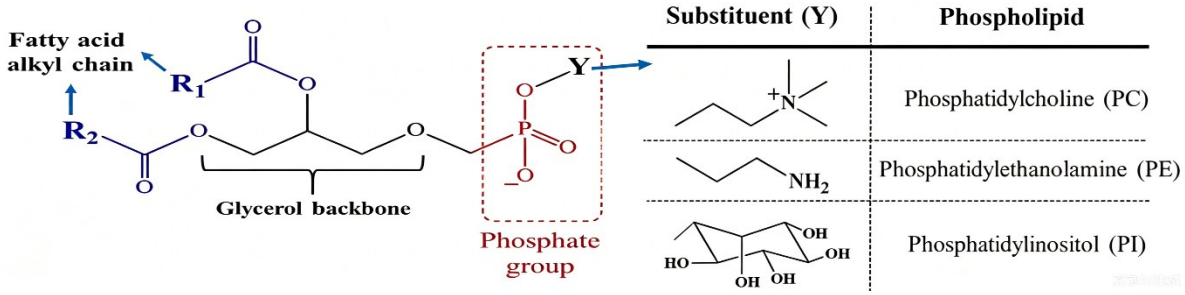


Figure 3.5-1. Structure of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Adapted from Ref. [24].

The amphiphilic nature of polar lipids allows them to form organized structures in aqueous environments. These structures include micelles, bilayers, and vesicles. The formation of these structures is driven by the tendency of hydrophobic regions to avoid water and hydrophilic regions to interact with water [22,25].

Polar lipids play a key role in biological membranes. They form the structural basis of cell membranes through bilayer organization. This structure provides both stability and selective permeability, which are essential for biological function [23,25].

Polar lipids also have important functional roles in food systems. They act as natural emulsifiers and can stabilize interfaces between water and oil phases. This property makes them important in products such as dough and emulsified foods [10,13].

Polar lipids can interact with other food components and influence their behaviour. In particular, they can interact with starch molecules through hydrophobic and molecular inclusion mechanisms. These interactions can affect starch structure and its accessibility to enzymes [13,22].

Lecithin is a mixture of polar lipids that is widely used as a natural emulsifier. It consists mainly of phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. The exact composition of lecithin depends on its biological source [10,22].

The differences in lecithin composition from various sources influence their functional behaviour. Variations in phospholipid classes and fatty acid composition affect emulsifying properties and interactions with other food components. [22].

3.5.1 Soy lecithin

Soy lecithin is one of the most commonly used commercial lecithins. It is obtained as a by-product of soybean oil refining. Soy lecithin contains a mixture of phospholipids, with phosphatidylcholine and phosphatidylethanolamine as major components. It also contains phosphatidylinositol and minor lipid components [13,22].

The composition of soy lecithin reflects the lipid profile of soybean seeds. The fatty acids in soy lecithin are mainly unsaturated, including linoleic and oleic acids. This composition influences its physical properties and functionality in food systems [26].

3.5.2 Sunflower lecithin

Sunflower lecithin is an alternative plant-based lecithin derived from sunflower seeds. It is produced during the processing of sunflower oil in a similar way to soy lecithin. Similar to soy lecithin, it contains phospholipids such as phosphatidylcholine and phosphatidylethanolamine, although the relative proportions may differ from those in soy lecithin [22].

Sunflower lecithin differs from soy lecithin in its fatty acid composition. It generally contains a high proportion of linoleic acid, reflecting the composition of sunflower oil. These differences can influence its behaviour in food systems and its interaction with other components [22].

3.5.3 Egg lecithin

Egg lecithin is derived from egg yolk and differs substantially from plant lecithins in both phospholipid composition and structure. Egg lecithin contains high proportions of phosphatidylcholine together with phosphatidylethanolamine, sphingomyelin and lysophospholipids [27]. Compared with plant lecithins, egg lecithin generally contains higher levels of saturated and monounsaturated fatty acids [28].

Previous reviews suggested that phospholipid molecular species and fatty acid composition can affect interfacial behaviour, self-assembly and interactions with other food components, including starch [26]. The relatively high phosphatidylcholine content in egg lecithin may therefore influence starch-lipid interactions differently from plant-derived lecithins. These compositional differences affect its functional properties and its interactions in biological and food systems [22,27].

3.6 Polar lipid-starch interaction

Polar lipids can interact directly with starch molecules and form molecular complexes. Early studies demonstrated that native cereal starches naturally contain endogenous lipids, especially lysophospholipids, which can associate with amylose chains [29]. These interactions mainly occur between the hydrophobic regions of lipid molecules and the helical structure of amylose. The amphiphilic structure of polar lipids facilitates this association and promotes molecular organization within the starch matrix [22].

Amylose can form inclusion complexes with lipids through a single-helical structure known as V-amylose. In this structure, the hydrophobic fatty acid chain is located inside the amylose helix, while the polar head group remains outside the helix [3]. This arrangement stabilizes the starch-lipid complex and alters the physicochemical properties of starch. Previous studies showed that complex formation depends on lipid characteristics such as fatty acid chain length, degree of saturation, and polar head group composition [30]. Fatty acid chain length may influence the stability of amylose-lipid complexes because longer hydrophobic chains can promote stronger interactions within the amylose helix. The degree of saturation may also affect complex formation, as saturated fatty acids are generally considered to pack more efficiently within the helical structure than unsaturated fatty acids. Furthermore, polar head groups can influence the orientation and molecular association of lipids with starch molecules [3,4,30].

Thermal processing promotes the formation of amylose-lipid complexes. During gelatinization, starch granules swell and partially lose their crystalline structure, which allows amylose chains to leach out and interact with surrounding lipids [31]. Baking and other heat treatments therefore create favourable conditions for starch-lipid complex formation. These complexes can remain stable during cooling and become integrated into the final food structure [3].

Amylose-lipid complex formation modifies starch functionality and digestion behaviour. Previous studies demonstrated that starch-lipid interactions can reduce starch swelling, alter gelatinization behaviour, and influence rheological properties of starch systems [30]. The formation of ordered complex structures also reduces the accessibility of starch chains to digestive enzymes. As a result, enzymatic hydrolysis becomes slower and starch digestibility decreases [2].

Polar lipids can additionally affect starch digestion through structural and interfacial mechanisms. Their emulsifying properties modify the distribution of water and lipids within the food matrix and may influence enzyme diffusion and substrate accessibility [22]. Compared with non-polar triglycerides, phospholipids often show stronger interactions with amylose because their amphiphilic structure improves molecular association and complex stability [4].

Polar lipid-starch interactions can contribute to reduced starch digestion and slower glucose release. The reduced digestion rate is commonly associated with lower enzyme accessibility and the formation of more resistant starch fractions [2]. Consequently, polar lipids may help reduce the rate of starch hydrolysis and potentially lower the glycaemic response of starch-based foods.

4 Material and Methods

4.1 Bread making

Five bread samples were baked by the Sage Bread Making Machine under the 1.0 kg Basic Light Crust program setting. Dry ingredients including wheat flour, yeast, salt, lecithin powder were mixed homogeneously and rested for 10 minutes before being added into the bread making pan containing corresponding weight of liquids. 37 ℃ water was used according to the yeast product specification. After baking, the loaves were cooled at room temperature for at least 3 hours until they had completely cooled down. Then the crust was removed and the remaining crumb was sliced into pieces. The pieces were wrapped in aluminium foil, placed in plastic freezer bags and stored in a freezer for further analysis.

The samples consisted of white bread, bread with sunflower oil, bread with sunflower lecithin, bread with soy lecithin and bread with egg lecithin. Materials used to bake them are listed below in *Table 4.1-1*. The recipe for the bread samples was developed by referencing the white bread recipe used by Johansson et al. [32]. It was then optimized based on the actual bread making machine and baking program used, as well as the characteristics of the dough throughout the baking process. The specific formulations are shown in *Table 4.1-2* below. The content of polar lipids from different sources was determined by their corresponding concentrations and the target of having 15 g of polar lipids in 50 g of available starch [5].

Table 4.1-1. Ingredients of bread samples

Ingredients	Products/Producers	Comments
Wheat flour	Vetemjöl Special 2 kg Kungsörnen, Lantmännen Cerealia AB, Stockholm, Sweden	
Yeast	Torrjäst Matbröd 14 g 2st Kronjäst, Jästbolaget AB, Sollentuna, Sweden	
Salt	Salt Finkornigt med Jod 600 g Falksalt, Salinity AB, Göteborg, Sweden	
Sunflower oil	Solrosolja 1 L ICA, ICA Sverige AB, Solna, Sweden	100% oil
Sunflower lecithin powder	Helhetshalsa AB, Borghamn, Sweden	99% phospholipids
Soy lecithin powder	AAK AB, Malmö, Sweden	97% phospholipids
Egg lecithin liquids	Akovita® ELIP 3020, AAK AB, Malmö, Sweden	34.5% phospholipids

Table 4.1-2. Formulations of bread samples

Bread samples	Formulation	Comments
White bread	Wheat flour: 530 g, water: 310 g, yeast: 4.8 g, salt: 4.8 g	Control bread
Bread with sunflower oil	Wheat flour: 530 g, water: 310 g, yeast: 4.8 g, salt: 4.8 g, sunflower oil: 102.56 g	Reference bread 15 g oil / 50 g available starch
Bread with sunflower lecithin	Wheat flour: 530 g, water: 310 g, yeast: 4.8 g, salt: 4.8 g, sunflower lecithin: 103.59 g	15 g polar lipids / 50 g available starch

Bread with soy lecithin	Wheat flour: 530 g, water: 310 g, yeast: 4.8 g, salt: 4.8 g, soy lecithin: 105.73 g	15 g polar lipids / 50 g available starch
Bread with egg lecithin	Wheat flour: 530 g, water: 310 g, yeast: 4.8 g, salt: 4.8 g, egg lecithin: 102.56 g	15 g lipids (5.175 g polar lipids + 9.825 g oil) / 50 g available starch

4.2 Determination of dry matter content of bread samples

Dry matter content of the bread samples was determined before analyzing their starch content. One slice from each bread was removed from the freezer and allowed to thaw at room temperature for 5-6 hours while kept in its aluminium foil and plastic bag. After thawing, 3-5 g of the crumb in a petri dish of known weight was placed on a tray and dried in the oven at 105°C overnight. The remaining bread was crumbled, spread onto a metal tray and placed in the fume hood to air-dry overnight. The next morning, the oven-dried bread was cooled in a desiccator for 1 hour, and then its weight was recorded. Approximately 1 g of the overnight air-dried sample was dried in the oven for another hour, then cooled and weighed following the same procedure as for the oven-dried sample. The remaining air-dried bread was milled into fine powder using a laboratory mill (FOSS CT193 Cyclotec) and the powder was collected for subsequent starch analysis.

The dry matter content of fresh thawed bread (TS1) was calculated as the oven-dried mass after overnight drying divided by the initial mass of the fresh thawed sample and expressed as a percentage. The dry matter content of air-dried bread (TS2) was calculated as the oven-dried mass after further drying divided by the initial mass of the air-dried sample and expressed as a percentage. The ratio TS1/TS2, as shown in the formula below, served as a conversion factor and was used when calculating the starch content of fresh thawed bread.

$$\frac{TS1}{TS2} = \frac{\frac{(Petri\ dish+dried\ sample)-Petri\ dish}{fresh\ thawed\ bread's\ mass}}{\frac{(Petri\ dish+dried\ sample)-Petri\ dish}{air-dried\ bread's\ mass}} = \frac{m_{dry,fresh}/m_{fresh}}{m_{dry,air}/m_{air}} \quad (1)$$

4.3 Starch analysis

Both potentially available starch content and total starch content were determined according to Holm et al. [33] and expressed in polymer weight, whereas an initial solubilization step with KOH was added for the total starch analysis [34]. Chemicals used for starch analyses are listed in *Table 4.3-1*.

Table 4.3-1. Chemicals for starch analyses

Chemicals	Total starch	Available starch	Comments
Termamyl	✓	✓	Novo A/S Copenhagen, Denmark
Amyloglucosidase	✓	✓	3500 U / 25 mL
NaAc buffer	✓	✓	0.3 M, pH 4.75
Tris buffer	✓	✓	0.5 M
Phosphate buffer	✓	✓	0.1 M, pH 6.0
KOH	✓		4.0 M
HCl	✓		5.0 M
Glucose standard	✓	✓	

Glucose oxidase-peroxidase (Glox) reagent	✓	✓

4.3.1 Available starch content

Milled air-dried bread samples were analyzed for their potentially available starch content. 500 mg of the sample powder was suspended in 20 mL phosphate buffer which provides optimum pH conditions for thermostable α-amylase (Termamyl). The breakdown of starch was initiated by incubating the suspension with Termamyl in a boiling water bath for 20 minutes. The mixture was diluted, and 1 mL of the dilution was incubated at 60℃ with amyloglucosidase for 30 minutes in NaAc buffer to continue breaking down the starch. The solution was then diluted again and incubated with Glox reagent for an hour, the absorbance at 450 nm was compared with that of a glucose standard using the glucose-Glox reaction.

4.3.2 Total starch content

Total starch content of previously ground samples was measured after solubilization in KOH. 500 mg of sample suspending in 10 mL phosphate buffer was kept for 30 minutes at room temperature and then neutralized to pH 6 after adding 10 mL of freshly prepared KOH solution. The subsequent steps were the same as those described for the available starch analysis.

4.4 Determination of hydrolysis index using the chewing method

The starch hydrolysis index of bread samples was determined using an in vitro method based on chewing, as described by Granfeldt et al. [8]. The hydrolysis index (HI) was defined as the percentage of the area under the hydrolysis curve (AUC) compared to the area for white bread chewed by the same person. The hydrolysis curve was a line graph with hydrolysis degree on the vertical axis and time (0-180 minutes) on the horizontal axis. The degree of hydrolysis was calculated by the percentage of available starch degraded into maltose. *Table 4.4-1* lists the materials required for the hydrolysis index analysis.

Table 4.4-1. Materials for hydrolysis index analyses

Materials	Comments
Dialysis tubing	Spectra/Por® 2, MWCO 12-14 kDa, 45 mm flat width (Repligen, Massachusetts, USA)
Closures	Spectra/Por®, 55 mm sealing width (Repligen, Massachusetts, USA)
Chewing buffer	0.022 M (15.15 g KH_2PO_4, 19.8 g $Na_2HPO_4 \cdot 2H_2O$, 2.0 g NaCl and deionized water), pH 6.9
Pepsin solution	2000 FIB-U/g (MERCK, Darmstadt, Germany)
α-Amylase from porcine pancreas	1100 U, A6255-100MG (Sigma, St Louis, USA)
DNS solution	10 g 2-hydroxy-3,5-dinitrobenzoic acid, 300 g $K_4C_4H_4O_6 \cdot 4H_2O$, 16 g NaOH and deionized water
Maltose standard	1 g/L
NaOH	2 M
HCl	2 M

4.4.1 Chewing session

Six healthy subjects, who had not brushed their teeth or eaten breakfast, participated in the experiment to chew the bread samples. The participants were informed of the chewing

procedure and relevant information (see Appendix **I**) beforehand. The weight of the samples to be chewed was calculated based on the previous available starch analyses, targeting an equivalent of 1 g of potentially available starch content. Bread samples with corresponding weight were provided to the chewers. The chewed samples were collected into 50 mL beakers containing 5 mL of chewing buffer, 1 mL of pepsin solution and 10 drops of HCl. The mouth was rinsed with an additional 5 mL of chewing buffer, which was then expectorated into the same beaker.

4.4.2 Analyzing session

The samples obtained after chewing were subjected to further analysis. Then, another 10 drops of HCl were added with stirring and the mixture was incubated at 37℃ for 30 minutes. After pepsin incubation, the samples were neutralized to pH 6.9 with NaOH. 30 mL of the mixture including sample, chewing buffer and α-amylase was transferred into pre-wetted dialysis tubing. The dialysis tubing (13 cm long) was sealed with clips and placed into a beaker containing 800 mL of chewing buffer in a 37℃ water bath under stirring. Duplicate 1 mL aliquots of dialysate were collected every 30 minutes up to 180 minutes. The reducing sugar content of the dialysate samples was determined using DNS solution, and the absorbance was measured at 530 nm and compared with a maltose standard curve.

4.5 Calculation and statistical methods

The starch content data were expressed as means ± standard deviation (SD).

The sample weight to be chewed for 1 g of available starch was calculated as 100 divided by the available starch content (g/100 g bread).

The degree of starch hydrolysis (%) was calculated from the maltose equivalent concentration released during digestion according to the following equation:

$$Degree\ of\ starch\ hydrolysis(\%) = \frac{C(maltose) \times 0.95 \times 800}{1000} \times 100 \qquad (2)$$

where C(maltose) is the maltose equivalent concentration measured in the in vitro starch digestion(mg/mL), 0.95 is the conversion factor used to convert maltose equivalents to starch equivalents, 800 mL is the total volume of chewing buffer surrounding the dialysis tubing, and 1000 mg represents the 1 g of available starch contained in the chewed bread sample. The result was expressed as the percentage of the initial available starch hydrolyzed.

The hydrolysis index for each test bread with each person was calculated individually for every subject as:

$$HI = \frac{AUC_{test}}{AUC_{White\ bread}} \times 100 \qquad (3)$$

where AUC is the area under the hydrolysis curve mentioned before. The AUC were calculated by the trapezoid model. Thus, the white bread has HI equals to 100. The average HI for a given sample across the six subjects was taken as the HI for that sample. All HI data are presented as mean ± SD.

Predicted glycaemic index (pGI) of the bread samples was calculated according to the empirical formula of Leeman et al. [35]:

$$pGI = 0.912 \times HI + 6.272 \quad (4)$$

Statistical analyses were performed using Minitab® (version 22.4). The significance level was set at $p < 0.05$. For the comparison of all five samples, an ANOVA using a general linear model was performed on both the HI and AUC (0-180min) results with bread type and subject as fixed factors. When the overall F-test was significant, Tukey's pairwise comparisons was performed. Additionally, Dunnett's post-hoc test was applied to compare each test bread with the white bread control. To specifically evaluate whether the three polar lipids enriched breads differed from the reference bread made with ordinary sunflower oil, Dunnett's test was again used to compare sunflower lecithin bread, soy lecithin bread, and egg lecithin bread against the bread with sunflower oil.

5 Results

5.1 Bread making

Five types of bread were made: one control white bread, one bread with sunflower oil and three breads with lecithin from sunflower, soy and egg. The four enriched breads contained 15 g of lipids per 50 g of available starch. Among them, sunflower oil bread had 15 g of sunflower oil, sunflower lecithin bread had 15 g of sunflower lecithin, soy lecithin bread had 15 g of soy lecithin and egg lecithin bread had 15 g of lipids which were 5.175 g of egg lecithin plus 9.825 g sunflower oil. The percentage of flour in the total weight of the cooled bread was calculated. This value was used to determine the theoretical available starch content of each bread. See Appendix **II** for the baking experiment notes and relevant data.

5.2 Dry matter content of the breads

Table 5.2-1 summarizes the dry matter contents and conversion factors of the bread samples. White bread showed the lowest TS1, TS2 and TS1/TS2 values. The breads containing lecithin or oil showed higher dry matter contents in both fresh and air-dried forms. All four enriched samples had very similar values of dry matter contents and conversion factors.

*Table 5.2-1. Dry matter contents (%) and conversion factor (TS1/TS2) for each bread sample**

Bread samples	TS1 (%)	TS2 (%)	TS1/TS2
White bread	56.91	91.34	0.6230
Bread with sunflower oil	61.57	93.33	0.6597
Bread with sunflower lecithin	62.14	92.94	0.6686
Bread with soy lecithin	61.60	92.76	0.6641
Bread with egg lecithin	62.03	93.34	0.6646

*(*TS1 (%) = dry matter content of fresh thawed bread, calculated as the ratio of oven-dried mass (after overnight drying at 105 °C) to the initial mass of fresh thawed bread × 100. TS2 (%) = dry matter content of air-dried bread, calculated as the ratio of oven-dried mass (after further drying of air-dried sample) to the initial mass of air-dried bread × 100. TS1/TS2 = conversion factor used to express starch content on a fresh bread basis.)*

5.3 Starch content of the breads

Table 5.3-1 summarizes the theoretical available starch content, measured total starch content and available starch content of all bread samples. The theoretical available starch contents were calculated based on the adjusted flour percentage in bread samples after cooling to room temperature, assuming that the baking loss resulted only from water evaporation. The theoretical values were used as a reference to assess the reliability of the experimentally measured available starch contents.

For all samples, the measured available starch content was lower than or similar to the measured total starch content. White bread had the highest theoretical and measured starch contents. The bread with sunflower lecithin showed the lowest theoretical starch content while the bread with soy lecithin showed the lowest total and available starch contents.

*Table 5.3-1. Starch content of the bread samples**

Bread samples	Theoretical available starch content (g / 100 g bread)	Measured total starch content (g / 100 g bread)	Measured available starch content (g / 100 g bread)
White bread	45.2	42.2 ± 0.8	42.4 ± 1.3
Bread with sunflower oil	40.3	40.5 ± 1.4	39.1 ± 0.7
Bread with sunflower lecithin	39.7	37.7 ± 0.6	37.9 ± 0.6
Bread with soy lecithin	39.9	37.4 ± 1.0	36.0 ± 1.2
Bread with egg lecithin	39.8	38.3 ± 0.6	37.7 ± 0.5

*(*Data of total starch content and available starch content are presented as means ± SD, n=6)*

Table 5.3-2 summarizes the portion size of breads for chewing. The weight of the breads to be chewed ranged from 2.36 g to 2.78 grams. White bread was the lightest while the bread with soy lecithin was the heaviest.

Table 5.3-2. Weight of the bread samples to be chewed

Bread samples	Sample weight (g, with 1 g of available starch)
White bread	2.36
Bread with sunflower oil	2.56
Bread with sunflower lecithin	2.64
Bread with soy lecithin	2.78
Bread with egg lecithin	2.65

5.4 Hydrolysis index of the breads

Table 5.4-1 summarizes the average hydrolysis degree of the bread samples from 6 subjects at pre-defined time points. As defined in Material and Methods section 3.4, the average degree of hydrolysis of the breads at different time points was obtained from the absorbance of sampling compared with the maltose standard curve. See Appendix **III** for the standard curve and the raw absorbance data of all samples from the six chewers. For more detailed data on the degree of hydrolysis of all bread samples from each subject at different time points and the area under the curve of all bread samples from each subject at different time periods, refer to Appendix **IV**.

Table 5.4-1. Average degree of hydrolysis of all bread samples at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sunflower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	12.80	11.54	10.81	9.78	9.95
60	28.03	25.77	22.84	23.89	22.30
90	39.01	37.75	34.35	34.96	32.33
120	48.35	46.33	41.52	44.24	40.07
150	57.31	53.29	48.77	51.87	46.71
180	61.13	56.66	54.55	56.17	51.80

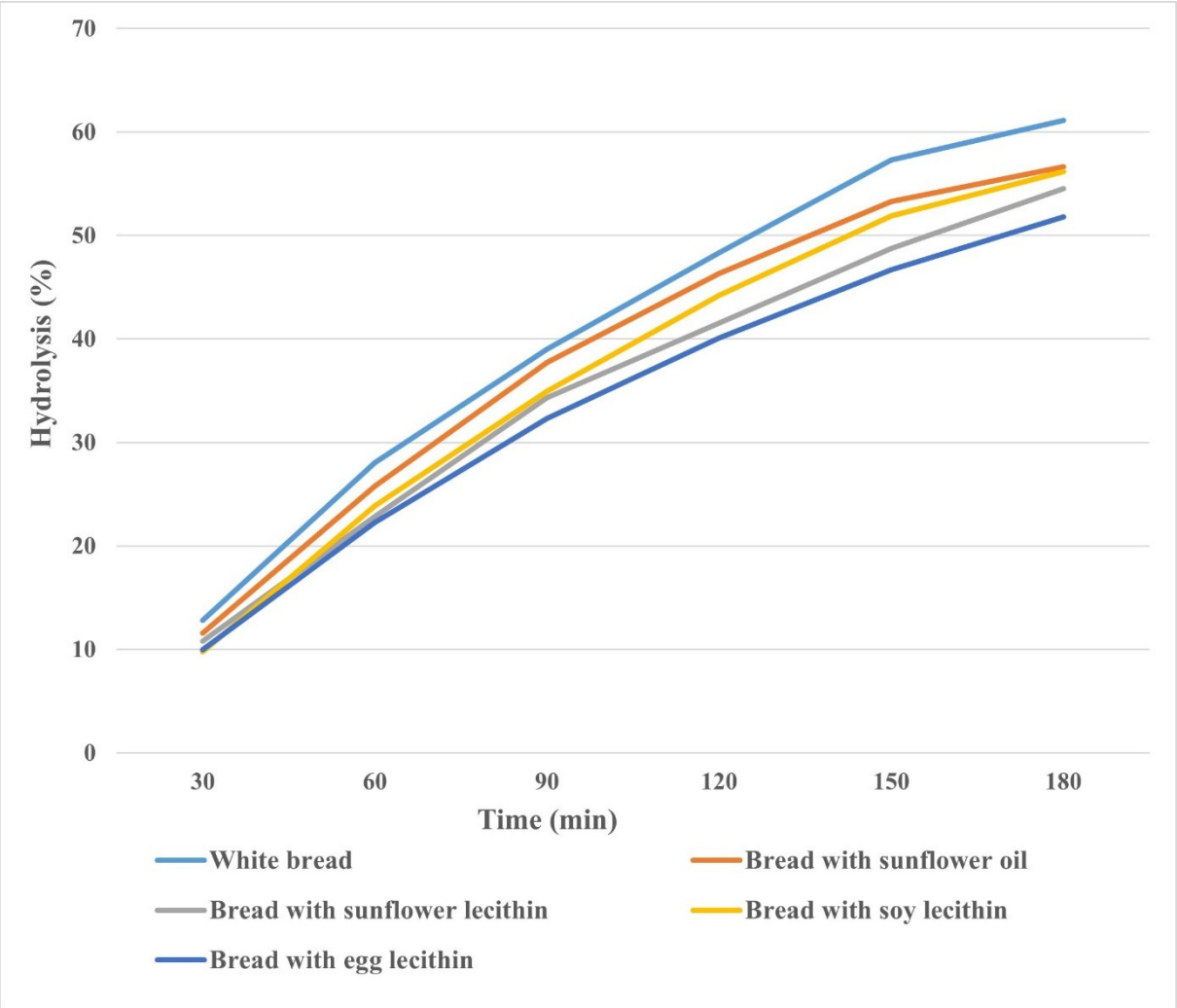


Figure 5.4-1. Hydrolysis curve of the bread samples

Figure 5.4-1 presents the hydrolysis curve of all bread samples plotted by using the data from *Table 5.4-1*, with the vertical axis representing the degree of starch hydrolysis and the horizontal axis representing time. Overall, each sample showed an increasing trend in degree of hydrolysis, with a gradual slowing of the hydrolysis rate over time and a peak at 180 minutes.

Based on visual observation of the hydrolysis curves, white bread appeared to exhibit a higher degree of hydrolysis than the other four enriched breads every sampling time point. Bread with sunflower oil generally showed the second-highest degree of hydrolysis. Bread with egg lecithin tended to show the lowest degree of hydrolysis among the five samples at all time point except 30 minutes, when its degree of hydrolysis was slightly higher than that of bread with soy lecithin, ranking second lowest. The hydrolysis curves for the three bread samples with lecithin appeared similar between 30 and 60 minutes but diverged thereafter. At the final sampling time of 180 minutes, the bread with sunflower lecithin appeared to have a lower degree of hydrolysis than the bread with soy lecithin.

Table 5.4-2 summarizes the hydrolysis index of the five breads from the six subjects. Overall, the HI of bread with sunflower oil ranged from 90.4 to 99.1, the HI of bread with sunflower lecithin ranged from 72.0 to 100.3, the HI of bread with soy lecithin ranged from 84.7 to 101.1, the HI of bread with egg lecithin ranged from 70.7 to 95.5.

Table 5.4-2. Hydrolysis Index of the bread samples from 6 subjects

Bread samples \ Subjects	1	2	3	4	5	6
White bread	100.0	100.0	100.0	100.0	100.0	100.0
Bread with sunflower oil	90.4	99.1	94.0	93.4	93.6	94.0
Bread with sunflower lecithin	72.0	88.2	100.3	80.1	93.4	83.0
Bread with soy lecithin	89.3	86.9	101.1	75.4	99.3	84.7
Bread with egg lecithin	77.1	88.8	70.7	95.5	76.9	83.1

Table 5.4-3 shows the average hydrolysis index of the four enriched bread samples chewed by six subjects. Among the four enriched breads, the HI of bread with sunflower oil was the highest, the HI of bread with egg lecithin was the lowest, the HI of bread with soy lecithin was lower than that of the bread with sunflower oil, and the HI of bread with sunflower lecithin was higher than that of bread with egg lecithin.

*Table 5.4-3. HI of the bread samples**

Bread samples	HI
White bread	100.0
Bread with sunflower oil	94.1 ± 2.8
Bread with sunflower lecithin	86.2 ± 10.0
Bread with soy lecithin	89.5 ± 9.6
Bread with egg lecithin	82.0 ± 9.0

*(*Data of the HI are presented as means ± SD, n=6. White bread was assigned an HI value of 100.)*

Table 5.4-4 summarizes the results of the ANOVA and Dunnett's post-hoc test comparing the HI and AUC (0-180min) values of all bread samples from the six subjects. For both variables, the ANOVA revealed a significant difference among the five bread samples. Dunnett's post-hoc test using white bread as the control showed that breads with egg lecithin and with sunflower lecithin differed significantly from white bread, whereas the breads with soy lecithin and with sunflower oil did not show significant differences. Tukey's pairwise comparisons showed the same result. See Appendix **V** for full data.

Table 5.4-4. Results of ANOVA and Dunnett's post-hoc test for all bread samples

	P value (HI)	P value (AUC 0-180 min)
ANOVA	0.006	0.005
Bread with sunflower oil vs White bread	0.490	0.473
Bread with sunflower lecithin vs White bread	0.019	0.015
Bread with soy lecithin vs White bread	0.087	0.076
Bread with egg lecithin vs White bread	0.002	0.002

Table 5.4-5 summarizes the results of the Dunnett post-hoc test comparing the HI and AUC (0-180min) values of four enriched bread samples from the six subjects. For both variables, Dunnett's test using sunflower oil bread as the control indicated that bread with egg lecithin differed

significantly from bread with sunflower oil, whereas the breads with sunflower lecithin and with soy lecithin did not show significant differences. See Appendix **VI** for full data.

Table 5.4-5. Results of Dunnett's post-hoc test for four enriched samples

	P value (HI)	**P value (AUC 0-180 min)**
Bread with sunflower lecithin vs Bread with sunflower oil	0.257	0.229
Bread with soy lecithin vs Bread with sunflower oil	0.691	0.660
Bread with egg lecithin vs Bread with sunflower oil	0.044	0.043

Table 5.4-6 summarizes the predicted glycaemic index of the four enriched samples predicted by the empirical equation. The bread with sunflower oil had the highest value and the bread with egg lecithin had the lowest GI.

Table 5.4-6. Predicted Glycaemic Index of the bread samples

Bread samples	**Predicted GI**
Bread with sunflower oil	92.1
Bread with sunflower lecithin	84.9
Bread with soy lecithin	87.9
Bread with egg lecithin	81.1

6 Discussion and Conclusion

6.1 Discussion

6.1.1 Bread making

In the initial experimental design, the aim was to add egg lecithin at the same polar lipid level as the other lecithin-enriched breads. However, the commercial egg lecithin product used (Akovita® ELIP 3020) was a complex lipid system containing phospholipids dispersed in sunflower oil together with minor lipid components such as cholesterol and DHA-containing phospholipids.

Based on the product specifications, the phospholipid concentration was 34.5%. This relatively low phospholipid concentration created a practical limitation for bread formulation. To achieve the theoretical target level of 15 g polar lipids per 50 g available starch, approximately 310 g of the egg lecithin product would have been required for the preparation of one loaf of bread with egg lecithin. Such a high addition level would have substantially altered the dough system and made the formulation impractical for bread making. The remaining fraction was treated as sunflower oil during formulation. Therefore, the bread with egg lecithin was formulated using a lower amount of egg lecithin combined with sunflower oil.

Future studies should consider using purified or high-phospholipid egg lecithin preparations to enable more comparable polar lipid levels among different lecithin sources without substantially altering the bread formulation. In addition, further analytical characterisation of lipid composition, such as phospholipid class distribution and fatty acid profiling, would help clarify the compositional differences between egg lecithin and plant-derived lecithins and their potential influence on starch digestion behaviour.

An additional issue was identified after completion of the experiments regarding the calculation of lipid addition levels. During formulation design, the available starch content of the wheat flour was estimated from the nutritional declaration provided by the manufacturer (Appendix **VII**). The flour contained 68 g carbohydrates and 3.5 g dietary fibre per 100 g flour. The available starch content was therefore initially estimated as 64.5 g/100 g flour by subtracting dietary fibre from total carbohydrates.

However, subsequent correspondence with the flour manufacturer clarified that the declared carbohydrate value already excluded dietary fibre according to their product developers. Therefore, the carbohydrate content relevant for formulation calculations should have been 68 g/100 g flour rather than 64.5 g/100 g flour.

As a result, the lipid additions used in the bread formulations were slightly underestimated. Based on the corrected calculation, 108.12 g of a theoretical 100% pure polar lipid preparation would have been required to achieve the intended target of 15 g polar lipids per 50 g available starch. The formulations originally used approximately 102.56 g equivalent lipid addition. Consequently, the actual formulations corresponded to approximately 14.23 g polar lipids per 50 g available starch rather than the intended 15 g/50 g. However, this difference was small and can be considered negligible. For the egg lecithin bread, the actual phospholipid content was approximately 4.91 g per 50 g available starch.

Nevertheless, all enriched breads were formulated using the same calculation principle. Therefore, the relative comparisons among treatment groups remain internally consistent. The calculation error mainly affected the absolute lipid-to-starch ratio rather than the comparative interpretation of the experimental results.

6.1.2 Dry matter content of the breads

The higher TS1 values of breads with oil or lecithin are mainly attributed to the addition of lipids, which increases the dry matter proportion of fresh bread.

The slightly higher TS2 values after air-drying may be related to changes in crumb structure and water distribution caused by lipids and emulsifiers [36]. These structural changes can influence moisture migration and water diffusion within bread systems [37], leading to differences in residual moisture after drying.

6.1.3 Starch content of the breads

The lower starch contents observed in breads with sunflower oil or lecithin compared to white bread can mainly be explained by a dilution effect. Based on the calculated flour proportions after cooling, the flour content decreased from approximately 70% in white bread to around 62% in the enriched breads. Since starch was mainly derived from wheat flour, the lower flour proportion directly reduced the theoretical starch content. This calculation assumed that baking loss resulted mainly from water evaporation, which represents a simplified assumption in bread systems.

In addition to the dilution effect, lipid type appeared to influence starch digestibility. Bread containing sunflower oil showed slightly higher available starch content than lecithin-containing breads, suggesting that polar lipids interacted more strongly with starch. Previous studies reported that phospholipids can form amylose-lipid inclusion complexes with V-type crystalline structures that are less susceptible to enzymatic hydrolysis [38,39]. These complexes may decrease enzymatic accessibility without reducing total starch content.

As discussed in the breadmaking section, the potentially available starch content of the flour was initially underestimated during formulation calculations. After correction, the theoretical starch contents of white bread, sunflower oil bread, sunflower lecithin bread, soy lecithin bread and egg lecithin bread were recalculated as 47.6, 42.5, 41.8, 42.0 and 42.0 g/100 g bread, respectively.

The corrected theoretical starch contents remained higher than the measured total starch contents by approximately 3.6-5.4 g/100 g bread, except for sunflower oil bread where the difference was approximately 2 g/100 g bread. Several factors may explain this discrepancy. The theoretical values were calculated estimates based on simplified assumptions that all starch remained fully recoverable during breadmaking and sample processing. In practice, baking, freezing, thawing, air-drying and milling may alter starch structure and reduce enzymatic accessibility during analysis. Enzymatic starch assays are also sensitive to sample processing conditions, starch retrogradation and starch-lipid interactions, all of which may influence starch recovery and quantification [33,34].

The slightly lower available starch content observed in soy lecithin bread may indicate differences in the complex-forming ability of lecithins from different sources. Lecithins differ in phospholipid composition, which may influence their interaction with amylose [31]. However,

the differences among lecithin-containing breads were relatively small and should be interpreted cautiously unless supported by statistical significance.

Lipid addition may also modify bread structure and starch-protein interactions, thereby influencing enzyme accessibility and starch hydrolysis rates [40]. These structural effects, together with starch-lipid complex formation, likely contributed to the differences in available starch content among the bread samples.

Finally, the observation that measured available starch content was similar to or slightly higher than measured total starch content in some samples may reflect methodological variability associated with enzymatic assays and KOH pretreatment. Differences in substrate accessibility, enzyme specificity and sample processing conditions may introduce variability when comparing different starch fractions.

6.1.4 Hydrolysis index of the breads

The in vitro starch hydrolysis curves showed clear differences in digestion kinetics among the bread samples. White bread showed the highest hydrolysis rate and extent throughout the digestion period, whereas all enriched breads showed lower starch hydrolysis. Bread with sunflower oil showed hydrolysis kinetics similar to white bread during the early digestion phase (0-90 min), followed by a moderately lower hydrolysis rate at later stages (90-180 min). In contrast, breads with lecithins consistently showed lower hydrolysis rates and extents throughout the digestion period, suggesting that polar lipids exerted a stronger inhibitory effect on starch digestion.

The hydrolysis index (HI) results supported these observations. All enriched breads showed HI values below 100, indicating lower starch digestibility than white bread. However, when inter-individual variability and the repeated-measures structure were considered using ANOVA followed by Tukey's and Dunnett's post-hoc tests, only sunflower lecithin bread and egg lecithin bread differed significantly from white bread. The ANOVA corrected for multiple comparisons and therefore provided an appropriate assessment of treatment effects in the present experimental design.

Both Tukey's and Dunnett's post-hoc tests identified significant differences between sunflower lecithin bread and white bread, as well as between egg lecithin bread and white bread. Tukey's test compares all possible pairwise group combinations, whereas Dunnett's test specifically compares treatment groups against a selected control group. These consistent results strengthen the reliability of the observed effects. The reduced starch hydrolysis observed for sunflower lecithin bread is also consistent with previous human studies showing that sunflower lecithin can influence postprandial metabolic responses [5].

Soy lecithin bread did not differ significantly from white bread in the ANOVA followed by Dunnett's post-hoc test, although the p-values for HI ($p = 0.087$) and AUC ($p = 0.076$) were close to the significance threshold. These results may indicate a tendency for soy lecithin to reduce starch digestion, although the effect appeared weaker than that observed for sunflower lecithin and egg lecithin under the present experimental conditions. The relatively large inter-subject variability and limited sample size may have reduced the statistical power to detect moderate effects. Future studies including larger numbers of subjects may help clarify whether soy lecithin exerts a biologically meaningful effect on starch hydrolysis.

The lower starch hydrolysis observed in lecithin-containing breads can be explained by starch-lipid interactions. Previous studies showed that amylose can form inclusion complexes with lipids, particularly polar lipids such as phospholipids, resulting in V-type crystalline structures that are less susceptible to enzymatic hydrolysis [38,39]. These complexes reduce enzyme accessibility and thereby decrease starch digestion. Compared with neutral lipids such as triglycerides in sunflower oil, polar lipids have a greater ability to interact with amylose because of their amphiphilic structure, which may facilitate their insertion into the amylose helix [39].

Lipids may also influence starch digestion through structural modifications of the bread matrix. Lipid addition can alter dough rheology, crumb porosity and starch-protein interactions, thereby affecting enzyme diffusion and substrate accessibility [40]. These structural effects may act together with starch-lipid complex formation to reduce starch hydrolysis in lipid-enriched breads.

Egg lecithin bread showed significantly lower HI and AUC values than sunflower oil bread in Dunnett's post-hoc comparisons (HI: $p = 0.044$; AUC: $p = 0.043$). This result suggests that egg lecithin influenced starch digestion differently from ordinary sunflower oil. Differences in phospholipid composition and minor lipid components may have contributed to this effect. Egg lecithin is known to contain phospholipid classes such as phosphatidylcholine and lysophosphatidylcholine, which can form amylose-lipid inclusion complexes and reduce starch susceptibility to amylase hydrolysis [41].

Interestingly, the actual phospholipid content in the egg lecithin bread (approximately 4.91 g per 50 g available starch) was substantially lower than that in the sunflower lecithin and soy lecithin breads (approximately 14.23 g per 50 g available starch). Despite this lower phospholipid level, egg lecithin bread still showed significantly lower starch hydrolysis than sunflower oil bread. This observation may indicate that phospholipid composition, rather than total phospholipid quantity alone, contributes to the modulation of starch digestibility.

However, this interpretation should be made cautiously because the commercial egg lecithin product used in this study had a complex composition dispersed in sunflower oil, and detailed phospholipid characterization was not performed. Therefore, the specific components responsible for the observed effect remain unclear and require further investigation.

The predicted glycaemic index (pGI) values followed the same trend as the HI values, with lecithin-containing breads showing lower pGI values than sunflower oil bread. This result agrees with previous studies showing that in vitro starch hydrolysis kinetics can be used to estimate glycaemic response [8,35]. However, these predictions were based on empirical equations and may not fully reflect in vivo physiological responses.

Despite the reductions observed, all breads still showed relatively high pGI values (>80), indicating that the enriched breads would still be classified as high-GI foods according to conventional GI classification. This may reflect the inherently rapid digestibility of white wheat bread matrices, where starch is highly gelatinized and readily accessible to digestive enzymes [39,40]. Nevertheless, the reductions in HI and pGI observed for lecithin-containing breads may still be nutritionally relevant, as postprandial glycaemic responses occur along a continuous scale [1].

One limitation of the present study was the design of the chewing sessions. All subjects consumed the same bread type on the same experimental day. Although this approach improved

procedural consistency, it may also have introduced confounding effects related to experimental day, chewing adaptation or experimental handling.

A more robust experimental design would use a randomized crossover approach in which each subject consumes all bread types in randomized and balanced order across different sessions. Such a design would distribute day-to-day variability more evenly across treatments and reduce systematic bias. Balanced sample order would also help minimize potential learning effects during repeated chewing sessions.

Although the overall trends were relatively consistent among subjects, the absence of a fully randomized and balanced crossover design may have reduced the ability to detect subtle differences among enriched breads. Future studies should therefore include randomized and balanced crossover designs together with larger subject numbers to improve statistical power and strengthen the reliability of the conclusions.

Overall, the results showed that the addition of certain polar lipids, particularly egg and sunflower lecithin, reduced starch digestion rate of bread. Starch-lipid complex formation and structural modifications of the bread matrix likely contributed to these effects. These findings further support the importance of lipid type in modulating starch digestion and may help guide the development of bakery products with lower glycaemic impact.

6.2 Conclusion

In conclusion, breads added with polar lipids generally showed lower in vitro starch digestion rate than white bread and bread with the same level of sunflower oil. Egg lecithin and sunflower lecithin produced the strongest effects as they yielded significantly lower hydrolysis index values than white bread. Egg lecithin bread also differed significantly from sunflower oil bread. Soy lecithin bread showed a similar tendency, although the differences were not statistically significant under the present experimental conditions. The results indicate that polar lipids may reduce starch digestibility in bread, likely through starch-lipid interactions and structural modifications of the bread matrix that may decrease enzyme accessibility to starch. The findings also suggest that the source and composition of polar lipids may influence the magnitude of this effect. Overall, the study suggests that certain polar lipid ingredients, particularly egg and sunflower lecithin, may have potential for the development of bread products with reduced starch digestion rate and potentially lower glycaemic impact.

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Appendices

I. Information for chewing participants

Thank you very much for helping with this experiment.

Please follow the steps below for the chewing procedure:

1. **Rinse your mouth with the water**. (After rinsing, you can spit the water into the empty unmarked cup.)
2. Take the provided bread sample and **chew the bread 15 times** at a normal, comfortable pace.
3. After 15 chews, **spit the sample completely into the provided beaker**.
4. **Collect saliva once** and spit it into the same beaker.
5. Take **5 mL of buffer solution** into your mouth and **rinse for 1 minute**.
6. Spit the rinse solution into the same beaker.
7. Finally, **collect saliva one more time** and spit it into the beaker.

Please make sure all material is collected in the beaker and try to follow the chewing procedure as accurately as possible.

After completing the procedure, you may rinse your mouth again using the water from the cup in step 1 and spit it into the unmarked waste cup. You are then free to leave, and I will take care of the remaining materials.

Thank you again for your help!

Ingredients:

White bread 2.36g: Wheat flour, water, yeast, salt.

Bread with sunflower lecithin 2.64g: Wheat flour, water, yeast, salt and sunflower lecithin powder.

Bread with sunflower oil 2.56g: Wheat flour, water, yeast, salt and sunflower oil.

Bread with soy lecithin 2.78g: Wheat flour, water, yeast, salt and soy lecithin powder.

Bread with egg lecithin 2.65g: Wheat flour, water, yeast, salt and egg lecithin liquids.

Chewing buffer 5mL: KH_2PO_4, $Na_2HPO_4 \cdot 2H_2O$, NaCl and water.

Figure 8.1-1. Information for chewing participants

II. Notes on bread making

*Table 8.2-1. Notes on bread making**

Bread samples	Formulation	Actual weight (g)	Ingredient percentage (%)	Total weight before baking (g)	Total weight after cooling (g)	Baking loss (%)	Adjusted ingredient percentage (%)
White bread	Flour 530g	530.03	62.38	849.69	757.18	10.89	70
	Water 310g	310.05	36.49				
	Yeast 4.8g	4.81	0.57				
	Salt 4.8g	4.80	0.57				
Bread with sunflower oil	Flour 530g	530.08	55.66	952.39	848.70	10.89	62.46
	Water 310g	310.09	32.56				
	Yeast 4.8g	4.81	0.50				
	Salt 4.8g	4.85	0.50				
	Sunflower oil 102.56g	102.56	10.78				12.08
Bread with sunflower lecithin	Flour 530g	530.17	55.60	953.47	862.35	9.56	61.48
	Water 310g	310.02	32.52				
	Yeast 4.8g	4.83	0.50				
	Salt 4.8g	4.83	0.50				
	Sunflower lecithin 103.59g	103.62	10.87				12.02
Bread with soy lecithin	Flour 530g	530.19	55.47	955.59	857.50	10.27	61.83
	Water 310g	310.02	32.45				
	Yeast 4.8g	4.81	0.50				
	Salt 4.8g	4.83	0.50				
	Soy lecithin 105.73g	105.74	11.07				12.33
Bread with egg lecithin	Flour 530g	530.00	55.66	953.71	858.27	10.01	61.75
	Water 310g	310.01	32.56				
	Yeast 4.8g	4.83	0.50				
	Salt 4.8g	4.80	0.50				
	Egg lecithin 102.56g	104.07	10.78				12.13

*(*Baking loss = ratio of mass after cooling to the total mass of all ingredients. Adjusted ingredient percentage = ratio of mass of the ingredient to the mass after cooling.)*

III. Raw data for absorbance

Table 8.3-1. Absorbance at 530 nm of the maltose standard curve

Maltose concentration (mg / mL)	Absorbance (Abs)
0	0.095
0.1	0.137
0.2	0.193
0.4	0.337
0.8	0.597
1.0	0.733

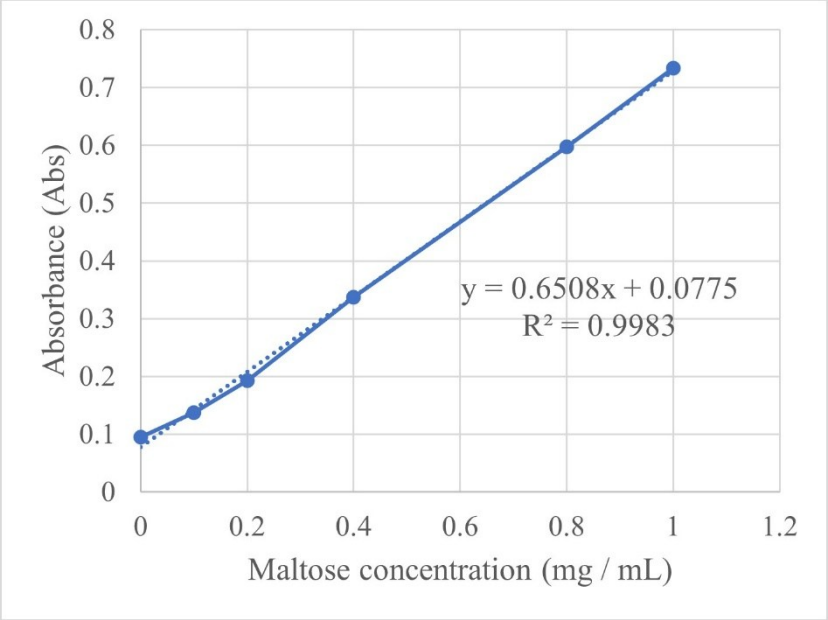


Figure 8.3-1. Maltose standard curve

Table 8.3-2. Absorbance at 530 nm of the white bread from 6 subjects at different time points

Time (min) \ Subjects	1	2	3	4	5	6
30	0.191	0.168	0.205	0.169	0.183	0.198
	0.191	0.176	0.185	0.187	0.179	0.213
60	0.33	0.277	0.334	0.32	0.268	0.356
	0.347	0.291	0.318	0.327	0.297	0.345
90	0.389	0.34	0.375	0.416	0.389	0.475
	0.403	0.403	0.385	0.43	0.403	0.468
120	0.511	0.465	0.463	0.492	0.464	0.532
	0.51	0.487	0.537	0.496	0.473	0.542
150	0.576	0.538	0.538	0.55	0.517	0.594
	0.595	0.565	0.572	0.558	0.558	0.617
180	0.616	0.579	0.592	0.593	0.578	0.637
	0.589	0.614	0.544	0.571	0.608	0.643

Table 8.3-3. Absorbance at 530 nm of the bread with sunflower oil from 6 subjects at different time points

Time (min) \ Subjects	1	2	3	4	5	6
30	0.179	0.173	0.178	0.172	0.156	0.197
	0.171	0.193	0.168	0.164	0.154	0.201
60	0.285	0.287	0.257	0.299	0.285	0.325
	0.27	0.325	0.279	0.311	0.286	0.349
90	0.385	0.406	0.368	0.381	0.381	0.435
	0.414	0.36	0.375	0.402	0.389	0.444
120	0.431	0.478	0.439	0.477	0.445	0.505
	0.47	0.47	0.455	0.481	0.451	0.51
150	0.488	0.529	0.485	0.53	0.507	0.563
	0.539	0.56	0.505	0.524	0.528	0.583
180	0.57	0.542	0.534	0.557	0.55	0.61
	0.546	0.538	0.534	0.526	0.568	0.628

Table 8.3-4. Absorbance at 530 nm of the bread with sunflower lecithin from 6 subjects at different time points

Time (min) \ Subjects	1	2	3	4	5	6
30	0.139	0.173	0.19	0.161	0.167	0.19
	0.15	0.168	0.19	0.163	0.168	0.182
60	0.233	0.268	0.306	0.246	0.276	0.299
	0.235	0.269	0.317	0.269	0.284	0.275
90	0.307	0.37	0.427	0.354	0.382	0.408
	0.326	0.359	0.401	0.347	0.382	0.397
120	0.389	0.42	0.465	0.417	0.437	0.455
	0.396	0.424	0.477	0.398	0.455	0.464
150	0.447	0.433	0.537	0.449	0.49	0.509
	0.457	0.491	0.541	0.48	0.517	0.533
180	0.499	0.509	0.546	0.52	0.431	0.556
	0.513	0.533	0.588	0.505	0.574	0.576

Table 8.3-5. Absorbance at 530 nm of the bread with soy lecithin from 6 subjects at different time points

Time (min) \ Subjects	1	2	3	4	5	6
30	0.158	0.15	0.163	0.136	0.17	0.179
	0.156	0.152	0.178	0.138	0.176	0.179
60	0.286	0.264	0.302	0.232	0.297	0.296
	0.288	0.273	0.319	0.22	0.307	0.301
90	0.386	0.334	0.396	0.323	0.394	0.396
	0.387	0.357	0.411	0.329	0.397	0.412
120	0.464	0.418	0.487	0.403	0.468	0.465
	0.474	0.438	0.49	0.407	0.483	0.479
150	0.532	0.481	0.542	0.456	0.539	0.534
	0.54	0.503	0.577	0.474	0.537	0.545

180	0.556	0.532	0.589	0.508	0.573	0.57
	0.584	0.544	0.616	0.501	0.471	0.556

Table 8.3-6. Absorbance at 530 nm of the bread with egg lecithin from 6 subjects at different time points

Subjects / **Time (min)**	**1**	**2**	**3**	**4**	**5**	**6**
30	0.158	0.156	0.152	0.195	0.141	0.175
	0.155	0.15	0.149	0.183	0.153	0.185
60	0.267	0.256	0.235	0.3	0.237	0.294
	0.264	0.264	0.24	0.32	0.249	0.295
90	0.33	0.361	0.309	0.405	0.311	0.392
	0.347	0.358	0.309	0.408	0.322	0.4
120	0.408	0.437	0.359	0.456	0.374	0.462
	0.412	0.446	0.361	0.471	0.389	0.473
150	0.463	0.494	0.409	0.511	0.435	0.52
	0.464	0.505	0.424	0.534	0.451	0.52
180	0.505	0.544	0.405	0.567	0.489	0.56
	0.511	0.552	0.466	0.588	0.497	0.569

IV. Raw data for hydrolysis degree and AUC

Table 8.4-1. Degree of starch hydrolysis in all samples from Subject 1 at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sun-flower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	13.25	11.39	7.82	9.28	9.23
60	30.48	23.36	18.28	24.47	21.95
90	37.19	37.60	27.91	36.08	30.48
120	50.57	45.84	36.79	45.72	38.83
150	59.32	53.89	43.73	53.54	45.08
180	61.31	56.11	50.04	57.51	50.27

Table 8.4-2. Area under curve in all samples from Subject 1 at different time periods

Time periods	AUC of White bread	AUC of Bread with sunflower oil	AUC of bread with sunflower lecithin	AUC of bread with soy lecithin	AUC of bread with egg lecithin
0-30	198.82	170.79	117.36	139.26	138.38
30-60	656.01	521.13	391.50	506.24	467.70
60-90	1015.10	914.38	692.79	908.25	786.51
90-120	1316.40	1251.58	970.44	1227.06	1039.63
120-150	1648.34	1495.94	1207.79	1488.94	1258.59
150-180	1809.50	1650.09	1406.61	1665.86	1430.26
0-180	6644.16	6003.92	4786.49	5935.60	5121.07

Table 8.4-3. Degree of starch hydrolysis in all samples from Subject 2 at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sun-flower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	11.04	12.32	10.86	8.58	8.82
60	24.11	26.68	22.30	22.30	21.31
90	38.01	38.36	33.52	31.30	32.93
120	46.54	46.30	40.23	40.93	42.51
150	55.35	52.73	48.29	48.41	49.28
180	60.61	54.01	51.79	53.78	54.94

Table 8.4-4. Area under curve in all samples from Subject 2 at different time periods

Time periods	AUC of White bread	AUC of Bread with sunflower oil	AUC of bread with sunflower lecithin	AUC of bread with soy lecithin	AUC of bread with egg lecithin
0-30	165.53	184.80	162.91	128.75	132.25
30-60	527.26	585.06	497.48	463.32	451.94
60-90	931.90	975.69	837.31	804.03	813.66
90-120	1268.22	1269.98	1106.19	1083.42	1131.59
120-150	1528.35	1485.43	1327.78	1340.04	1376.83
150-180	1739.43	1601.04	1501.20	1532.73	1563.38
0-180	6160.69	6102.01	5432.87	5352.29	5469.65

Table 8.4-5. Degree of starch hydrolysis in all samples from Subject 3 at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sun-flower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	13.72	11.75	13.14	10.86	8.52
60	29.02	23.44	27.33	27.21	18.68
90	35.33	36.18	39.30	38.07	27.03
120	45.02	45.47	45.95	48.00	32.99
150	55.76	51.38	53.89	56.29	39.59
180	60.08	56.17	59.62	61.31	41.81

Table 8.4-6. Area under curve in all samples from Subject 3 at different time periods

Time periods	AUC of White bread	AUC of Bread with sunflower oil	AUC of bread with sunflower lecithin	AUC of bread with soy lecithin	AUC of bread with egg lecithin
0-30	205.82	176.28	197.07	162.91	127.87
30-60	641.12	527.91	606.96	571.05	408.14
60-90	965.18	894.30	999.34	979.19	685.79
90-120	1205.16	1224.71	1278.73	1291.00	900.37
120-150	1511.71	1452.67	1497.70	1564.26	1088.68
150-180	1737.68	1613.25	1702.64	1763.95	1220.93
0-180	6266.67	5889.11	6282.44	6332.36	4431.78

Table 8.4-7. Degree of starch hydrolysis in all samples from Subject 4 at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sun-flower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	11.74	10.57	9.87	6.95	13.02
60	28.73	26.57	21.02	17.34	27.15
90	40.35	36.67	31.88	29.02	38.42
120	48.64	46.89	38.54	38.25	45.08
150	55.65	52.49	45.19	45.25	51.97
180	58.92	54.19	50.80	49.86	58.39

Table 8.4-8. Area under curve in all samples from Subject 4 at different time periods

Time periods	AUC of White bread	AUC of Bread with sunflower oil	AUC of bread with sunflower lecithin	AUC of bread with soy lecithin	AUC of bread with egg lecithin
0-30	176.04	158.53	148.02	104.23	195.31
30-60	606.96	557.04	463.32	364.35	602.58
60-90	1036.12	948.54	793.52	695.42	983.57
90-120	1334.79	1253.33	1056.27	1008.97	1252.46
120-150	1564.26	1490.69	1255.96	1252.46	1455.65
150-180	1718.41	1600.17	1439.89	1426.75	1655.35
0-180	6436.59	6008.30	5156.98	4852.18	6144.93

Table 8.4-9. Degree of starch hydrolysis in all samples from Subject 5 at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sun-flower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	12.09	9.05	10.51	11.15	8.12
60	23.94	24.29	23.65	26.22	19.33
90	37.19	35.91	35.56	37.14	27.91

120	45.66	43.27	43.03	46.48	35.50
150	56.11	51.38	49.75	53.78	42.68
180	60.20	56.23	57.98	57.86	48.52

Table 8.4-10. Area under curve in all samples from Subject 5 at different time periods

Time periods	AUC of White bread	AUC of Bread with sunflower oil	AUC of bread with sunflower lecithin	AUC of bread with soy lecithin	AUC of bread with egg lecithin
0-30	181.30	135.76	157.65	167.29	121.74
30-60	540.40	500.11	512.37	560.54	411.65
60-90	917.01	903.00	888.11	950.29	708.56
90-120	1242.82	1187.65	1178.89	1254.21	951.17
120-150	1526.60	1419.74	1391.72	1503.83	1172.76
150-180	1744.68	1614.18	1615.93	1674.62	1368.07
0-180	6152.81	5760.43	5744.67	6110.77	4733.94

Table 8.4-11. Degree of starch hydrolysis in all samples from Subject 6 at different time points

Time (min)	White bread	Bread with sunflower oil	Bread with sun-flower lecithin	Bread with soy lecithin	Bread with egg lecithin
30	14.95	14.19	12.67	11.85	11.97
60	31.88	30.30	24.47	25.81	25.34
90	46.01	42.27	37.95	38.13	37.19
120	53.66	50.22	44.61	46.07	45.54
150	61.66	57.86	51.79	53.95	51.67
180	65.69	63.24	57.05	56.70	56.87

Table 8.4-12. Area under curve in all samples from Subject 6 at different time periods

Time periods	AUC of White bread	AUC of Bread with sunflower oil	AUC of bread with sunflower lecithin	AUC of bread with soy lecithin	AUC of bread with egg lecithin
0-30	224.22	212.83	190.06	177.80	179.55
30-60	702.43	667.39	557.04	564.92	559.67
60-90	1168.38	1088.68	936.28	959.05	938.03
90-120	1495.07	1387.34	1238.44	1262.97	1241.07
120-150	1729.79	1621.19	1446.02	1500.32	1458.28
150-180	1910.22	1816.50	1632.58	1659.73	1628.20
0-180	7230.10	6793.93	6000.41	6124.78	6004.79

V. Results of ANOVA and post-hoc tests (Tukey and Dunnett) for all samples.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bread	4	1169.0	292.25	4.93	0.006
Subjectx	5	217.9	43.59	0.73	0.606
Error	20	1186.2	59.31		
Total	29	2573.1			

Regression Equation

HI = 90.33 - 8.33 Bread_Egg + 3.72 Bread_Oil - 0.89 Bread_Soy - 4.17 Bread_Sunflower
+ 9.67 Bread_White - 4.57 Subjectx_1x + 2.25 Subjectx_2x + 2.87 Subjectx_3x
- 1.47 Subjectx_4x + 2.32 Subjectx_5x - 1.39 Subjectx_6x

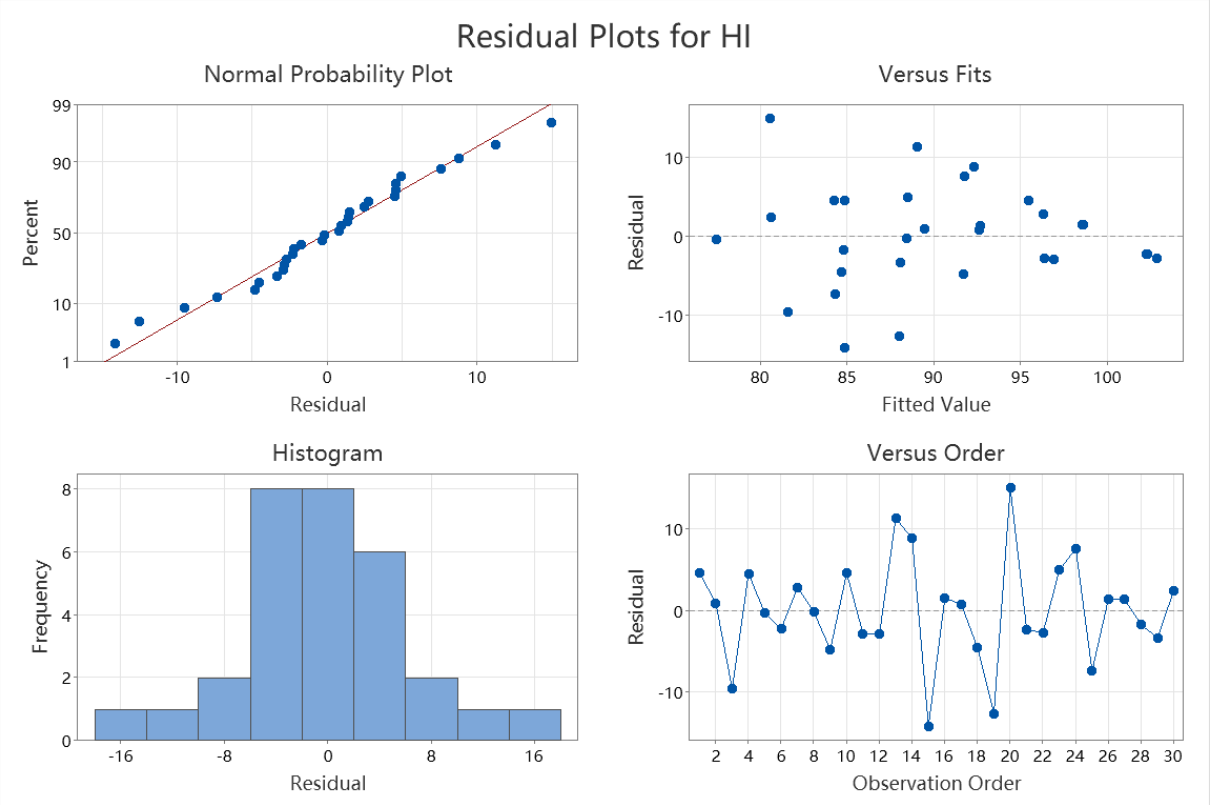


Figure 8.5-1. Results of ANOVA comparing for HI of all bread samples

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Bread	4	4954623	1238656	5.09	0.005
Subjectx	5	2106020	421204	1.73	0.174
Error	20	4870240	243512		
Total	29	11930884			

Regression Equation

AUC(0-180) = 5848.9 - 531 Bread_Egg + 244 Bread_Oil - 64 Bread_Soy - 282 Bread_Sunflower
+ 633 Bread_White - 151 Subjectx_1x - 145 Subjectx_2x - 8 Subjectx_3x
- 129 Subjectx_4x - 148 Subjectx_5x + 582 Subjectx_6x

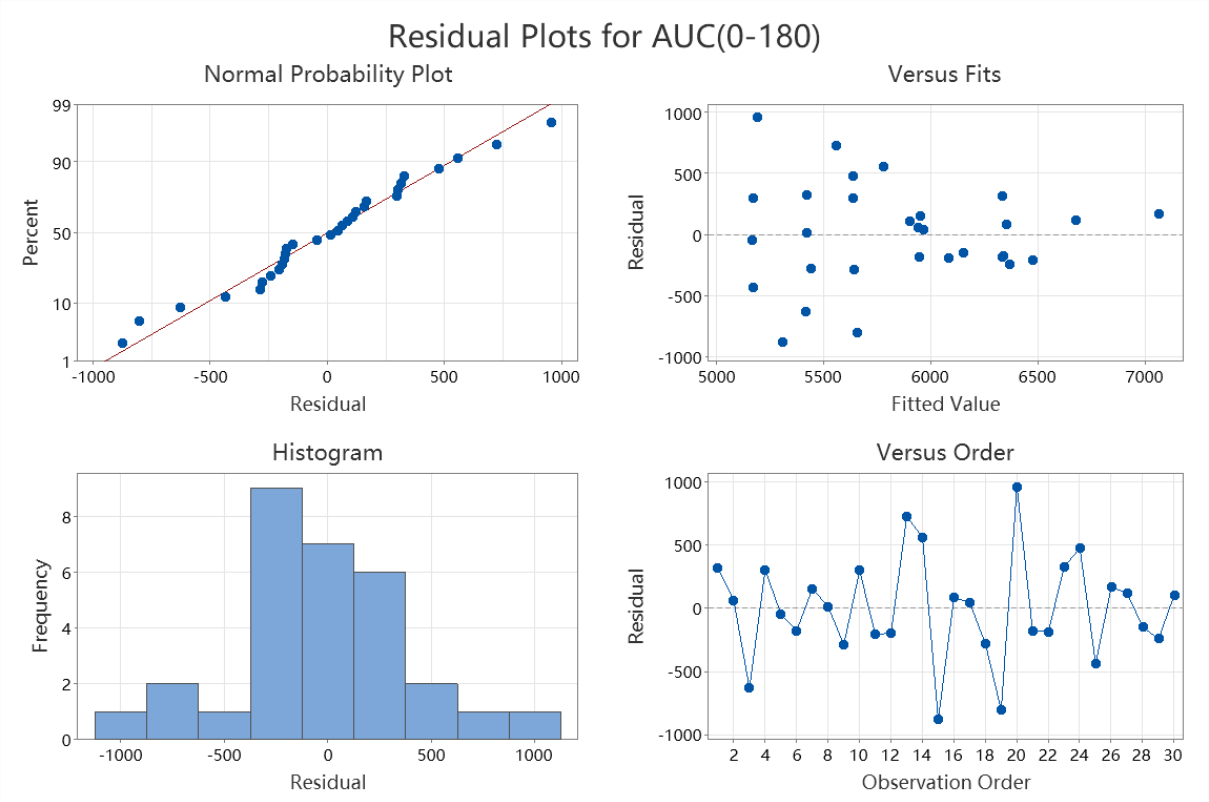


Figure 8.5-2. Results of ANOVA comparing for AUC (0-180min) of all bread samples

Tukey Pairwise Comparisons: Bread

Grouping Information Using the Tukey Method and 95% Confidence

Bread	N	Mean	Grouping	
White	6	100.000	A	
Oil	6	94.054	A	B
Soy	6	89.446	A	B
Sunflower	6	86.159		B
Egg	6	82.007		B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Bread Levels	Difference of Means	SE of Simultaneous Difference	95% CI	T-Value	Adjusted P-Value
Oil - Egg	12.05	4.45	(-1.25, 25.35)	2.71	0.088
Soy - Egg	7.44	4.45	(-5.86, 20.74)	1.67	0.472
Sunflower - Egg	4.15	4.45	(-9.15, 17.45)	0.93	0.880
White - Egg	17.99	4.45	(4.69, 31.29)	4.05	0.005
Soy - Oil	-4.61	4.45	(-17.91, 8.69)	-1.04	0.836
Sunflower - Oil	-7.89	4.45	(-21.19, 5.40)	-1.78	0.414
White - Oil	5.95	4.45	(-7.35, 19.25)	1.34	0.672
Sunflower - Soy	-3.29	4.45	(-16.59, 10.01)	-0.74	0.945
White - Soy	10.55	4.45	(-2.75, 23.85)	2.37	0.164
White - Sunflower	13.84	4.45	(0.54, 27.14)	3.11	0.039

Individual confidence level = 99.28%

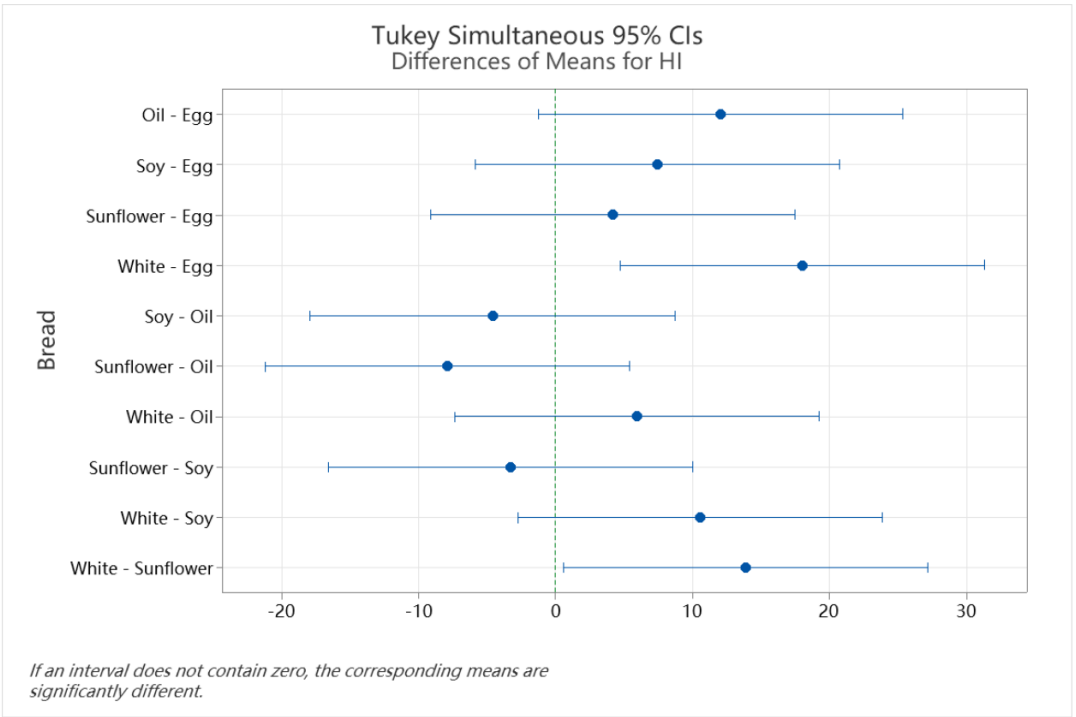


Figure 8.5-3. Tukey's pairwise comparison of HI results

Tukey Pairwise Comparisons: Bread

Grouping Information Using the Tukey Method and 95% Confidence

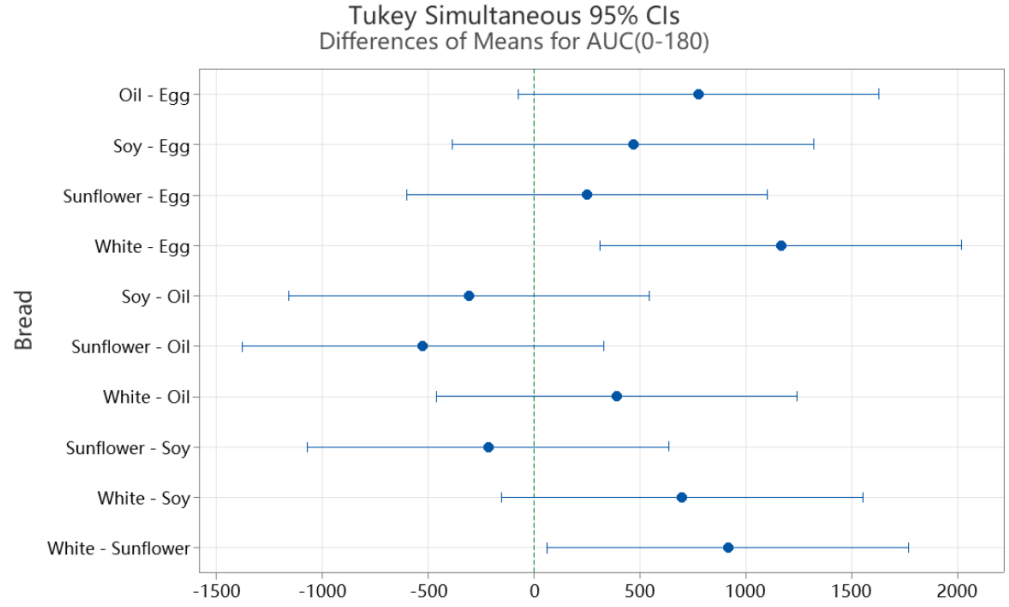
Bread	N	Mean	Grouping	
White	6	6481.84	A	
Oil	6	6092.95	A	B
Soy	6	5784.67	A	B
Sunflower	6	5567.31		B
Egg	6	5317.69		B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Bread Levels	Difference of Means	SE of Simultaneous Difference	95% CI	T-Value	Adjusted P-Value
Oil - Egg	775	285	(-77, 1627)	2.72	0.086
Soy - Egg	467	285	(-385, 1319)	1.64	0.491
Sunflower - Egg	250	285	(-603, 1102)	0.88	0.902
White - Egg	1164	285	(312, 2016)	4.09	0.005
Soy - Oil	-308	285	(-1160, 544)	-1.08	0.814
Sunflower - Oil	-526	285	(-1378, 327)	-1.84	0.377
White - Oil	389	285	(-463, 1241)	1.36	0.656
Sunflower - Soy	-217	285	(-1070, 635)	-0.76	0.938
White - Soy	697	285	(-155, 1549)	2.45	0.144
White - Sunflower	915	285	(62, 1767)	3.21	0.032

Individual confidence level = 99.28%



If an interval does not contain zero, the corresponding means are significantly different.

Figure 8.5-4. Tukey's pairwise comparison of AUC (0-180min) results

Dunnett Multiple Comparisons with a Control: Bread

Grouping Information Using the Dunnett Method and 95% Confidence

Bread	N	Mean	Grouping
White (Control)	6	100.000	A
Oil	6	94.054	A
Soy	6	89.446	A
Sunflower	6	86.159	
Egg	6	82.007	

Means not labeled with the letter A are significantly different from the control level mean.

Dunnett Simultaneous Tests for Level Mean - Control Mean

Difference of Bread Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Egg - White	-17.99	4.45	(-29.78, -6.21)	-4.05	0.002
Oil - White	-5.95	4.45	(-17.73, 5.84)	-1.34	0.490
Soy - White	-10.55	4.45	(-22.34, 1.23)	-2.37	0.087
Sunflower - White	-13.84	4.45	(-25.63, -2.05)	-3.11	0.019

Individual confidence level = 98.47%

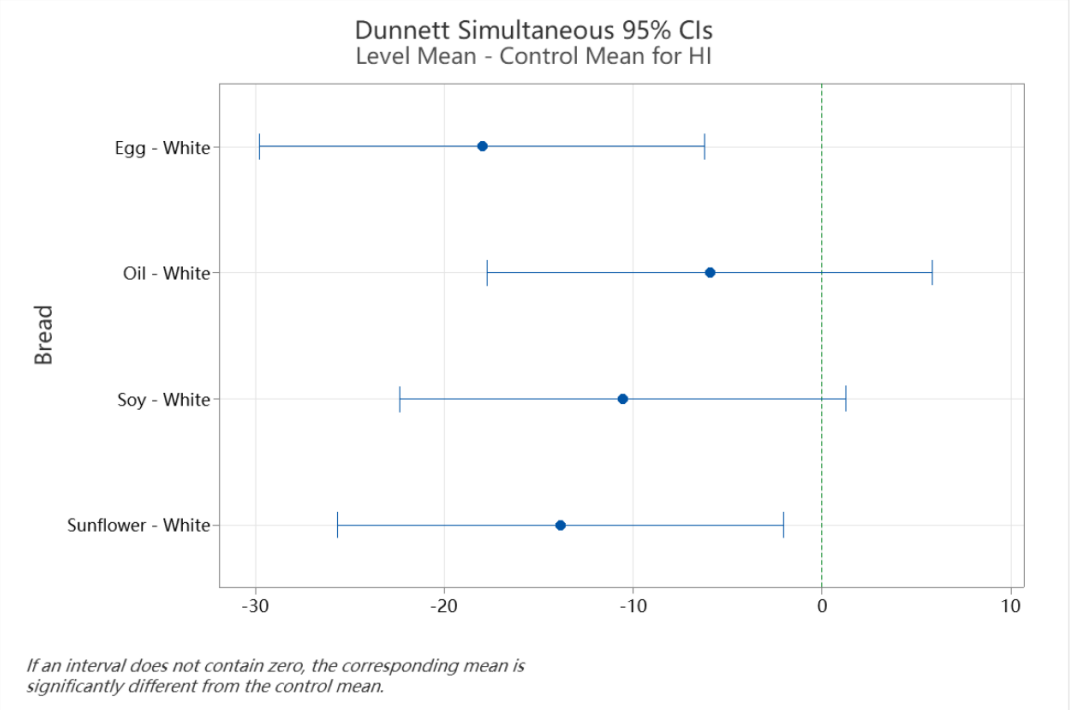


Figure 8.5-5. Results of Dunnett's post-hoc test comparing for HI using white bread as the control group

Dunnett Multiple Comparisons with a Control: Bread

Grouping Information Using the Dunnett Method and 95% Confidence

Bread	N	Mean	Grouping
White (Control)	6	6481.84	A
Oil	6	6092.95	A
Soy	6	5784.67	A
Sunflower	6	5567.31	
Egg	6	5317.69	

Means not labeled with the letter A are significantly different from the control level mean.

Dunnett Simultaneous Tests for Level Mean - Control Mean

Difference of Bread Levels	Difference of Means	SE of Simultaneous Difference	95% CI	T-Value	Adjusted P-Value
Egg - White	-1164	285	(-1919, -409)	-4.09	0.002
Oil - White	-389	285	(-1144, 366)	-1.36	0.473
Soy - White	-697	285	(-1452, 58)	-2.45	0.076
Sunflower - White	-915	285	(-1670, -159)	-3.21	0.015

Individual confidence level = 98.47%

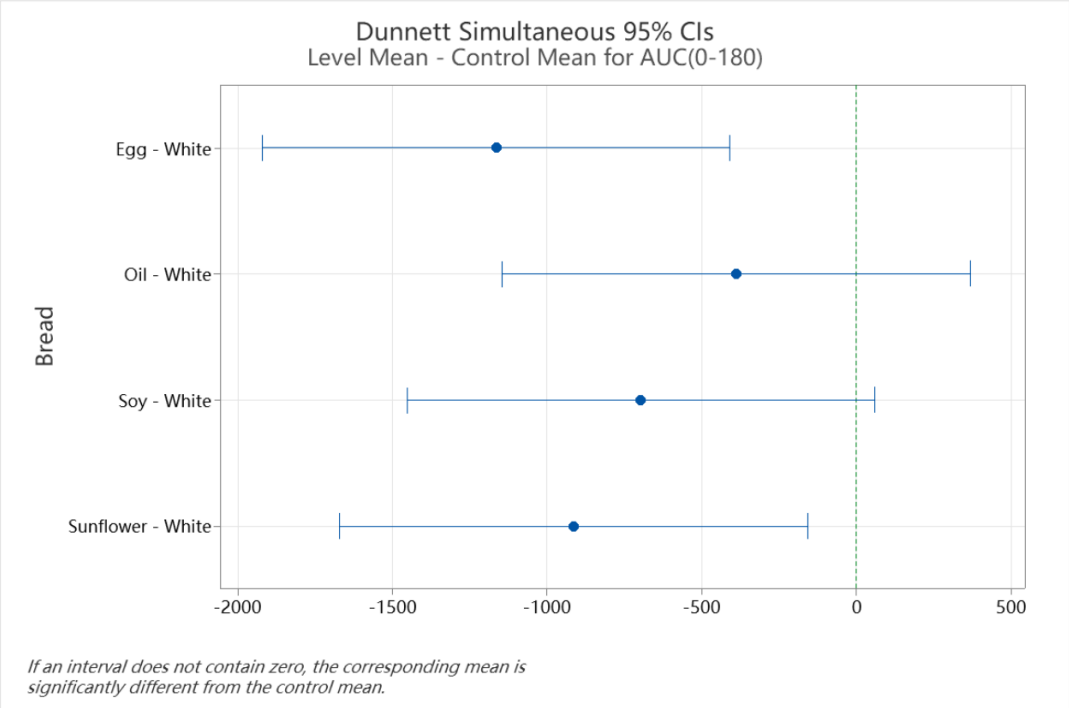


Figure 8.5-6. Results of Dunnett's post-hoc test comparing for AUC (0-180min) *using white bread as the control group*

VI. Results of the Dunnett's post-hoc test with sunflower oil bread as the control group

Dunnett Multiple Comparisons with a Control: Bread

Grouping Information Using the Dunnett Method and 95% Confidence

Bread	N	Mean	Grouping
Oil (Control)	6	94.054	A
White	6	100.000	A
Soy	6	89.446	A
Sunflower	6	86.159	A
Egg	6	82.007	

Means not labeled with the letter A are significantly different from the control level mean.

Dunnett Simultaneous Tests for Level Mean - Control Mean

Difference of Bread Levels	Difference of Means	SE of Simultaneous 95% Difference	CI	T-Value	Adjusted P-Value
Egg - Oil	-12.05	4.45	(-23.83, -0.26)	-2.71	0.044
Soy - Oil	-4.61	4.45	(-16.40, 7.18)	-1.04	0.691
Sunflower - Oil	-7.89	4.45	(-19.68, 3.89)	-1.78	0.257
White - Oil	5.95	4.45	(-5.84, 17.73)	1.34	0.490

Individual confidence level = 98.47%

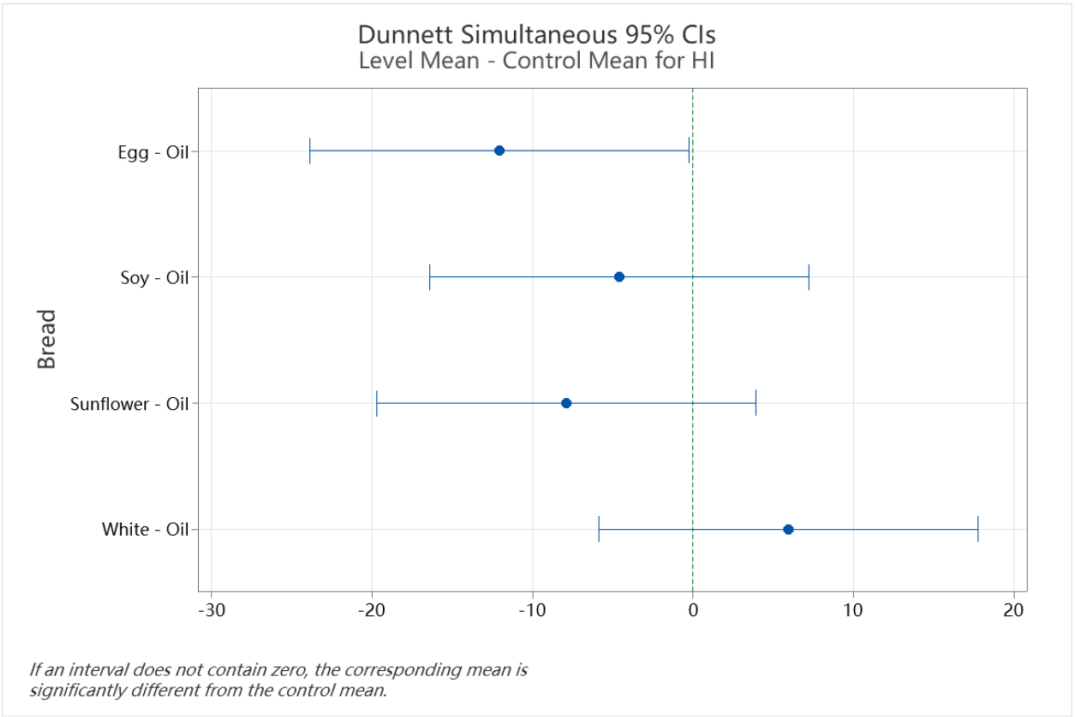


Figure 8.6-1. Results of Dunnett's post-hoc test comparing for HI using bread with sunflower oil as the control group

Dunnett Multiple Comparisons with a Control: Bread

Grouping Information Using the Dunnett Method and 95% Confidence

Bread	N	Mean	Grouping
Oil (Control)	6	6092.95	A
White	6	6481.84	A
Soy	6	5784.67	A
Sunflower	6	5567.31	A
Egg	6	5317.69	

Means not labeled with the letter A are significantly different from the control level mean.

Dunnett Simultaneous Tests for Level Mean - Control Mean

Difference of Bread Levels	Difference of Means	SE of Simultaneous Difference	95% CI	T-Value	Adjusted P-Value
Egg - Oil	-775	285	(-1531, -20)	-2.72	0.043
Soy - Oil	-308	285	(-1064, 447)	-1.08	0.660
Sunflower - Oil	-526	285	(-1281, 230)	-1.84	0.229
White - Oil	389	285	(-366, 1144)	1.36	0.473

Individual confidence level = 98.47%

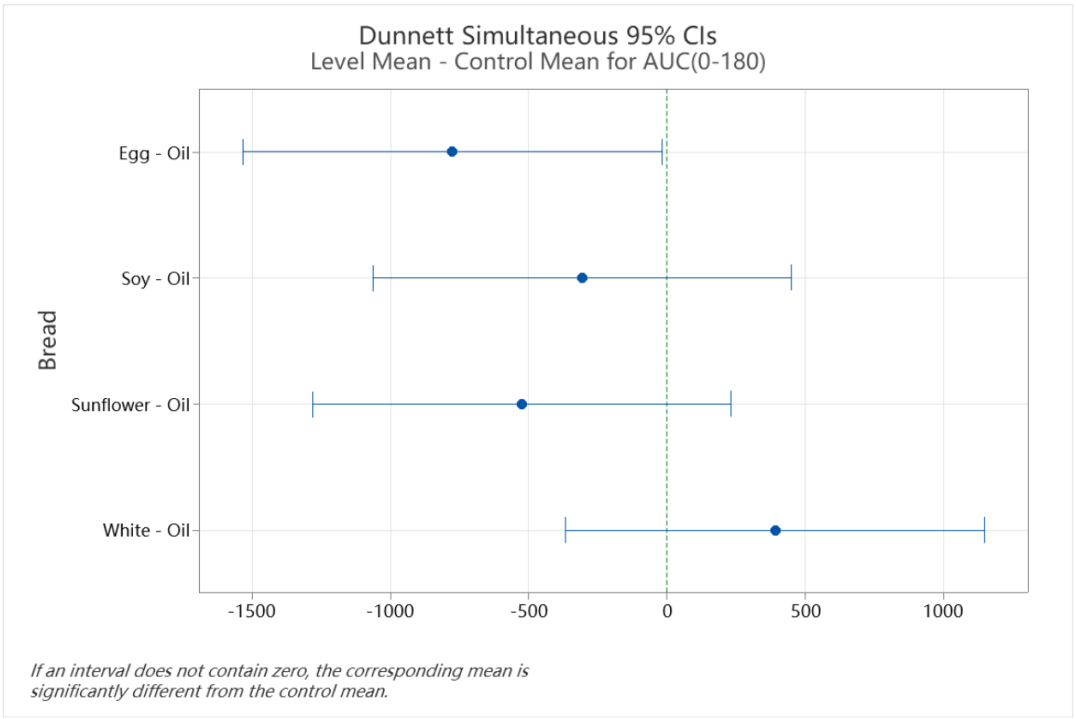


Figure 8.6-2. Results of Dunnett's post-hoc test comparing for AUC (0-180min) using bread with sunflower oil as the control group

VII. Nutritional information of wheat flour

Table 8.7-1. Nutritional declaration of Vetemjöl Special 2kg Kungsörnen

Näringsvärde	100 Gram
Energi (kcal)	346 kcal
Energi (kJ)	1447 kJ
Fett	1.6 g
Varav mättat fett	0.4 g
Kolhydrat	68 g
Varav sockerarter	0.3 g
Fiber	3.5 g
Protein	12 g
Salt	0 g