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Effects of Apple Pomace on Starch Digestibility of White Bread

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1. INTRODUCTION AND OBJECTIVES

In recent years, the role of dietary food components has increasingly been recognized for modulating metabolic diseases. One such nutritional food gaining popularity is apple (*Malus domestica*) pomace.

Apple and apple products are widely consumed in Europe and the United States. Last year, the World Apple and Pear Association (WAPA) forecasted that 11.4 million metric tons (MT) of fresh apple crops would be produced in the European Union (European Union, 2023). Forty percent of this will be processed into apple juice concentrate. Out of this processed portion, 25-30% will be left as waste or the apple pomace. Apple pomace, encompassing pulp, peel, and seeds, represents a high-nutrient food processing by-product, with its fiber content reportedly surpassing cereal (Bhushan et al., 2008). However, it has a high moisture content of 70-75% (Bhushan et al., 2008), due to which apple pomace is susceptible to degrade very quickly. Having huge piles of apple pomace outside or near the processing facility for an extended time can contribute to microbiological and environmental hazards, ultimately resulting in increased risk in the facility and the surrounding community.

Transporting the high load of apple pomace can also be costly, and while they have tried to dry the apple pomace under the sun before, doing so leads to the oxidation and enzymatic reaction of apple pomace, making it unfit for further processing. In the US, it was recorded that they spent $10 million annually to dispose of apple pomace (Skinner et al., 2018). Producers also tried to use them as animal fodder and fertilizer. However, the latter can cause more damage than help to the environment due to its high acidity, anti-germinant activity, and the inherent organic compounds in such by-products (Grigoras et al., 2013).

Given the high nutritional value of apple pomace and the problems mentioned above, we can deduce the importance of finding a way for processors to directly process the apple pomace to upscale it into high-value products like dietary supplements, functional food, and/or food additives for human consumption. Finding solutions for repurposing apple pomace can help solve the economic and environmental hazards posed to the processors.

The World Health Organization (WHO) (2023) recorded that the number of people in the world with diabetes rose from 1980 to 422 million in 2014, especially in middle to low-income countries. The abrupt spike in glucose levels in the blood, also known as hyperglycemia, is caused by the rapid absorption of simple sugars through the portal vein circulation. When this happens extensively, the individual is exposed to a high risk of Type II diabetes. De Oliveira Raphaelli et al. (2019) explained in their study that even though people

cut down on the consumption of simple sugars when starch is consumed, it can still be further hydrolyzed by the amylase enzyme in our mouth, followed by the enzymes in the stomach and/or intestinal tract.

Recent studies suggest that the inherent bioactive components in apple pomace can inhibit critical enzymes responsible for carbohydrate digestion. In one of the studies conducted, different fractions of apple phenolic extracts were studied for their α-Glucosidase activity to inhibit carbohydrate digestion. Results showed that the purified apple extract and fractions with high total phenolic content exhibited the most substantial inhibition, 12 to 46 times stronger than acarbose, a known drug for type II diabetes (de Oliveira Raphaelli et al., 2019). Moreover, phenolic substances were also accounted to benefit other physiological processes. One notable substance from this group was phlorizin, which is predominant in many apple cultivars and is said to be antihyperglycemic in human digestion (Ehrenkranz et al., 2005).

Hence, this study wants to focus on observing the effects of apple pomace on carbohydrate digestion. Specifically, we aim to:

1. Identify a simple and effective valorization technique to process apple pomace to influence carbohydrate digestion.
2. Investigate the presence of bioactive compounds that affect the observed effects.
3. Study the impact of apple pomace when co-consumed with bread on postprandial glucose levels using a modified *in vitro* Infogest digestion protocol with a semi-dynamic gastric phase.

2. LITERATURE REVIEW

2.1. Food Loss and Food Waste

This study mainly focuses on the 12th Sustainable Development Goals (SDGs) of the United Nations, responsible production and consumption. The 2030 Agenda for Sustainable Development, specifically SDG 12.3, calls for reducing the per capita food waste by half at the retail and consumer levels, as well as reducing food losses along production, supply chains, and post-harvest (Food and Agriculture Organization (FAO), 2022).

According to FAO's (2019) State of Food and Agriculture report, 14% of the world's food is lost after harvest before reaching consumers. Meanwhile, the United Nations Environment Programme's (UNEP) (2021) Food Waste Index Report shows that 17% is wasted in retail stores and households.

Efforts from all levels of society are critical to tackling food loss and food waste, which can help solve various issues and other SDGs. Some are zero hunger (SDG 2), climate change (SDG 13), food security, nutrient deficiency, and sustainability in our agrifood systems.

Governments must spearhead and implement regulations and campaigns to keep all sectors and individuals aligned with the goal. Primary producers, manufacturers, and the research community must work hand-in-hand to develop and implement new technologies and innovations to avoid food loss during harvest and production. The general public must be informed and engaged so that they may adopt policies and good practices regarding food waste at home.

2.2. Apple and Apple Pomace

2.2.1. Background

According to a report from the United States Department of Agriculture (USDA) (2023), there is an expected increase in world apple production in the years 2023 to 2024 from 175,000 metric tons (tons) to 83.1 million. Table 1 shows the forecasted world fresh apple production of top producing countries. Hence, it is unsurprising that there is an increased interest in the research and development efforts on valorization, effective

utilization, and value addition of nutritionally-rich agro-industrial residues such as apple pomace.

Table 1: World production of fresh apples (Source: USDA, 2023)

Country	2022/23 (1,000 MT)	2023/24 (1,000 MT)
China	44500	45000
European Union	12683	12208
Turkey	4968	4850
United States	4300	4356
India	2400	2410
Iran	2241	2241
Russia	1742	1844
Brazil	1297	1297
Ukraine	1279	1279
South Africa	1150	1230
Other	6374	6394
Total	82934	83109

*MT - metric tons

2.2.2. Apple Juice Production

Forty percent of the apple produce will be processed into juice or concentrate. Out of this processed portion, 25-30% will be left as residue or apple pomace (Bhushan et al., 2008). Apple pomace consists of leftover flesh and peels (95%), seeds (4%), and stems (1%). Furthermore, Bushan et al. (2008) also mentioned that apple juice concentrate (AJC) is the primary processed product worldwide, comprising 64% of all processed apple products.

Figure 1 represents a typical apple juice concentrate processing flow. During the clarification process, there was a total recovery of 70% to 75% concentrate, 25% to 30% apple pomace, and some 5% to 11% liquid waste.

After harvest, apple fruits undergo sorting and washing before they are pressed to extract the juice. After the extraction, the apple pomace can be obtained. The raw juice extract will further undergo a series of treatments and clarifications to finally produce the apple juice concentrate at the end of the process.

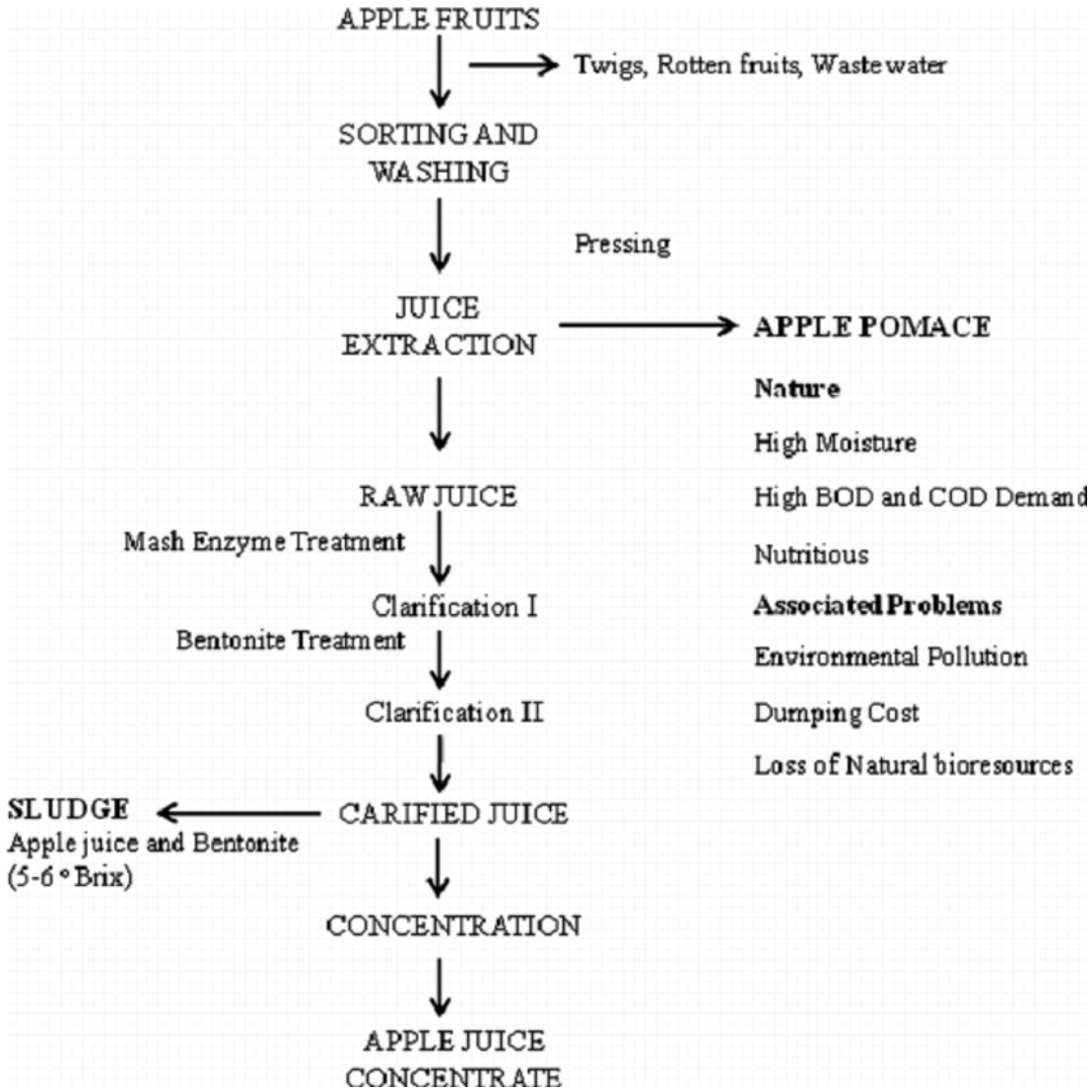


Figure 1: Industrial processing of apple juice concentrate (AJC)

(Source: Bushan et al., 2008)

2.2.3. Nutritional Composition

Skinner et al. (2018) tabulated a comprehensive comparison of the nutritional composition of apple and apple pomace, as shown in Table 2.

Table 2: Comparison of the nutrient composition of whole apples versus apple pomace (Source: Skinner et al., 2018)

Constituents (fresh weight)	Whole apple*	Apple Pomace**
Macronutrients, %		
Fat	0.16–0.18	1.1–3.6
Protein	0.24–0.28	2.7–5.3
Total carbohydrate	13.81	44.5–57.4
Simple carbohydrates, %		
Fructose	5.8–6.0	44.7
Glucose	2.4–2.5	18.1–18.3
Complex carbohydrates, %		
Total fiber	2.1–2.6	4.4–47.3
Insoluble fiber	1.54	33.8–60.0
Soluble fiber	0.67	13.5–14.6
Pectin	0.71–0.93	3.2–13.3

Table 2 shows that apple and apple pomace have very low protein and fat composition but high total carbohydrates. Apple pomace has significantly higher total carbohydrates than whole apples due to its high fructose and glucose content. Skinner et al. (2018) explained that this is due to the inclusion of sugar-containing seeds in apple pomace. Apple pomace also contains high amounts of fiber.

These values agree with Waldbauer et al.'s (2017) representation of the major composition of dried apple pomace, as seen in Figure 2. The major components are 36.89% total dietary fibers, 15.96% fructose, 13.96% starch, 7.51% glucose, and 7.33% moisture content.

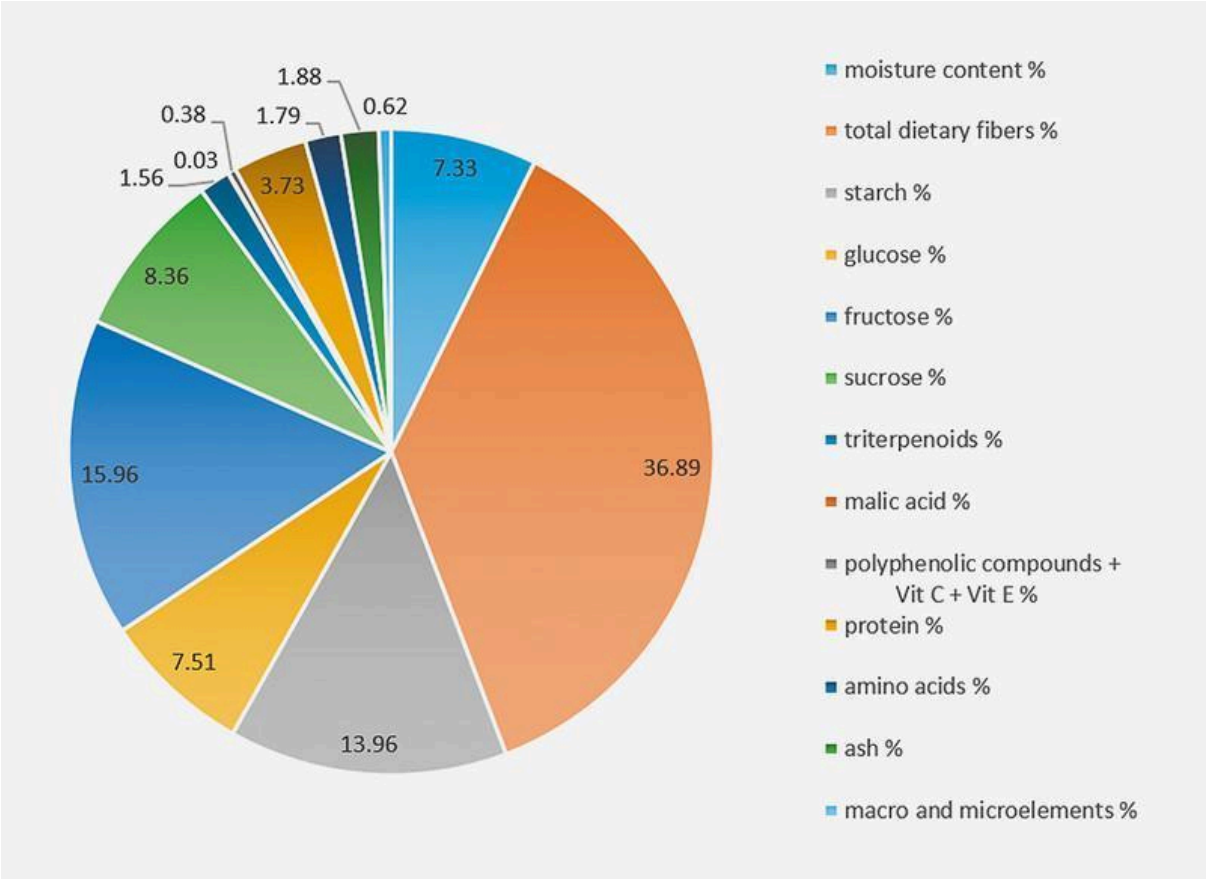


Figure 2: Approximate composition of dried apple pomace. Data provided by studies of different pomace sources were partly averaged and normalized to 100%.
(Source: Waldbauer et al., 2017)

2.2.4. Preparation and Effects on Bioactive Components

Rana et al. (2015) employed different drying techniques to observe their influence on apple pomace's cell wall properties and phenolic profile. Their results have shown that freeze-drying is one of the best methods to remove moisture in apple pomace as it gives the best dietary fiber yield (74%). An array of functional properties such as density, water and oil holding capacity, swelling capacity, and glucose dialysis retardation index (36.91%) was found better in freeze-dried fraction. The higher total phenolic content was also recorded in freeze-dried samples. We have learned from this study the importance of moisture removal to harness its nutritional and economic potential.

Since apple pomace consists of different parts and sizes from apple processing, grinding is essential for homogenization and enhancing extraction efficiency. The smaller the particle size, the greater the surface area and the smaller the diffusion path for solvents.

A study by Pinelo et al. (2008) compared the extraction efficiency of phenolic compounds using different particle sizes (300, 900, and 1500 μm). Results showed that the smallest particle size extracted the most concentrated total phenolics.

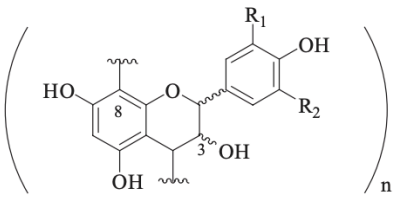
We aim to extract as many phenolic components as possible that can positively affect the postprandial glucose release. Perussello et al. (2017) have cited that the three most common solvents for extracting polyphenols are ethanol, methanol, and acetone. Hence, we have prioritized testing the acetone extract together with the simple water suspension. Previous studies have shown positive results for acetone-extracted apple pomace.

Rana et al. (2015) have shown that apple pomace powder extracted with acetone exhibited the highest antioxidant activity, total phenolic content (TPC), and total flavonoids content (TFC) in comparison to methanol and ethanol. Similarly, Reis et al. (2012) reported that when water was used to extract phenolic contents, it gave the highest TPC, TFC, and proanthocyanidins content (PAC). Furthermore, the extracted amount is even higher when the pomace undergoes subsequent extraction with aqueous methanol and acetone. Fernández‐Suárez et al.'s (2010) study also showed that higher polyphenols were extracted using acetone.

2.3. Procyanidins

2.3.1. Background

Condensed tannins, or proanthocyanidins (PAs), are polymers of dimers, oligomers, and catechin polymers bound together by links between C4, C8, or C6 (Buelga-Santos and Scalbert, 2000, as cited in Seeram, 2006). Figure 3 shows the chemical structure of proanthocyanidins.



$R_1 = R_2 = H$, Propelargonidins
$R_1 = H, R_2 = OH$, Procyanidins
$R_1, R_2 = OH$, Prodelphinidins

Figure 3: Chemical structure of proanthocyanidins

(Source: Seeram, 2006)

Procyanidins are referred to by Seeram (2006) as the most ubiquitous type of PA, which consists of individual (epi)catechin units. They can be found in abundance in different dietary fruits, vegetables, nuts, legumes, and grains (Rue et al., 2017). Moreover, consumption of PA-containing food is linked to various health benefits like cardiovascular diseases, certain cancers, diabetes, inflammation, neurodegeneration, allergies, and geriatric disorders.

Figure 4 shows the chemical structure of the different types of procyanidin. Rue et al. (2017) further explained that procyanidins are characterized by their ability to form a polymer and their degree of polymerization (DP). Monomers form linkages, forming oligomers and further forming polymers. The most common monomeric unit is (−)-epicatechin, with B-type being the most prominent. Depending on the stereo configuration and linkage between monomers, there are two types of procyanidin: A-type and B-type. The most abundant are the B-type procyanidins, with procyanidins B1, B2, B3, and B4 occurring most frequently. They have one interflavan bond between carbon-4 of the B-ring and carbon-8 or carbon-6 of the C-ring (Figure 4). On the other hand, the A-type has a second ether linkage between the A-ring hydroxyl group and carbon-2 of the A-ring in addition to the interflavan bond that B-type has. A1 and A1 are the most common A-type procyanidins.

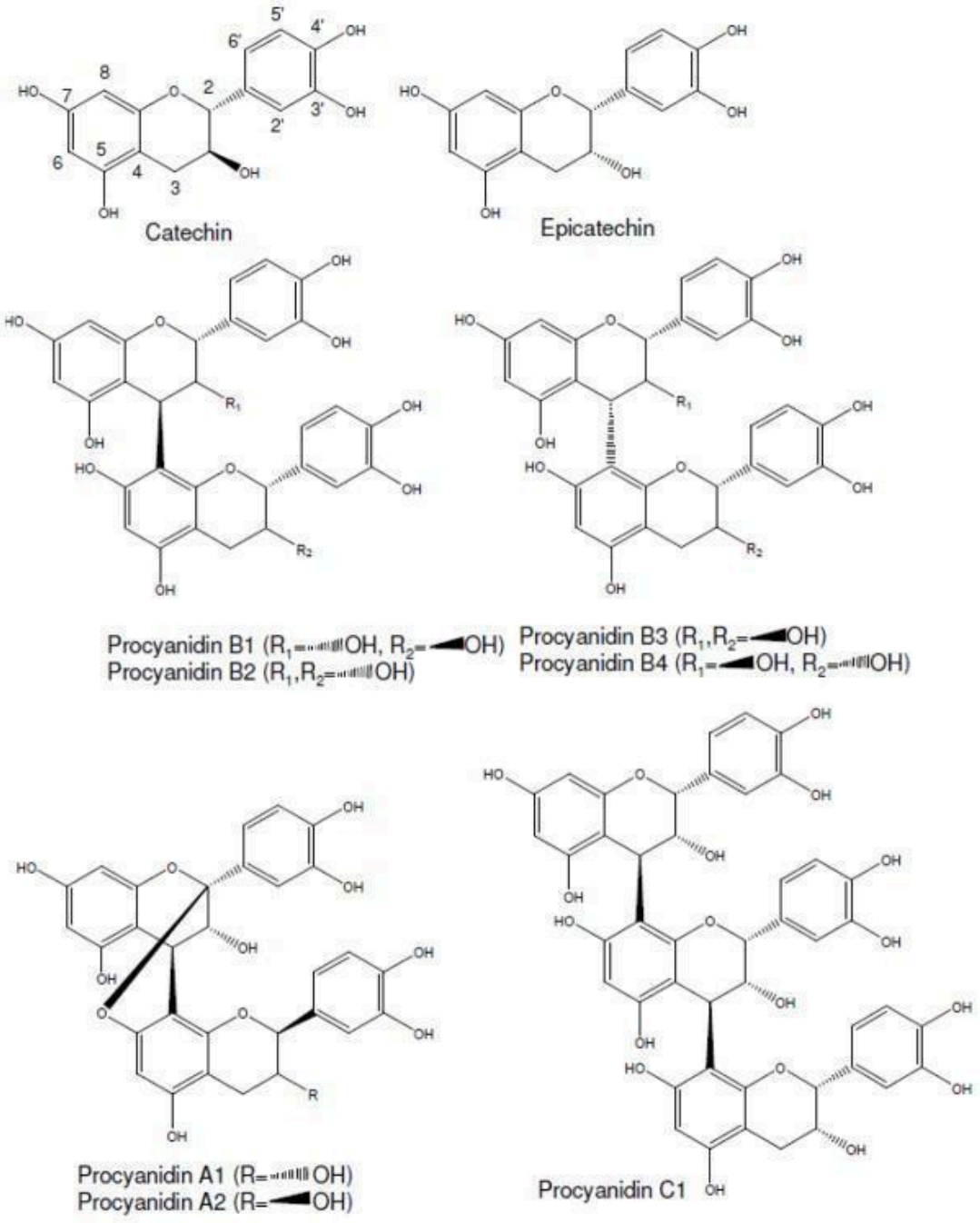


Figure 4: Chemical structures of monomeric and polymeric procyanidins
(Source: Xie and Dixon, 2005; Tsao, 2010 as cited in Rue et al., 2017)

Plant carbohydrates, fats, and proteins can form complexes with procyanidins that interfere with their extraction and isolation (Jakobek, 2015, as cited in Rue et al., 2017). Hence, different solvents like methanol, ethanol, water, dimethyl sulfoxide, and acetone have been used to improve their bioaccessibility.

2.3.2. Procyanidins in Apple Pomace

The apple fruit contains abundant amounts of polyphenols, including PA which Mendoza-Wilson et al. (2016) found to be mostly concentrated on the skin or peel. The

Antonic et al. (2020) reported that procyanidin B2 is one of the main polyphenol compounds present in apple pomace, with content ranging from 48.8 to 590.2 mg/kg of the dry weight of apple pomace.

Similarly, Arraibi et al. (2021) identified phenolic compounds in freeze-dried apple pomace from Spain, which include procyanidins B-type (epi)catechin dimer, B-type (epi)catechin trimer, and B-type (epi)catechin tetramer.

In a study conducted by Krasnova & Segliņa (2019), they determined the tannin content of 12 different cultivars of apple and its pomace. Results from their experiment showed that the tannin content in apple pomace ranges from 0.2911 mg tannic acid equivalents (TAE)/g dry weight to 0.7340 mg TAE/g dry weight, while water extracts of apple pomace contained 0.1356 mg TAE/g dry weight to 0.2602 mg TAE/g dry weight.

The apple cultivar used for this experiment was the Idared apple. Wolfe et al. (2003) found Idared cultivars to have one of the highest total phenolic content in their study containing 588.9 ± 83.2 gallic acid equivalents/100 g of peels. Lončarić et al. (2019) quantified the procyanidins present in Idared apple peels as follows: 0.134 mg/g dry weight (DW) procyanidin A2, 0.352 mg/g DW procyanidin B1, and 0.292 mg/g DW procyanidin B2.

2.4. Diabetes

Diabetes is a chronic metabolic disease that negatively affects millions of lives, especially in middle to low-income countries. According to the World Health Organization (WHO) (2023), from 1980 to 2014, the number of people with diabetes rose from 108 to 422 million. It can cause a lot of other major complications if left unregulated. Some of them are cardiovascular complications, kidney failure, blindness, and nerve damage resulting in limb amputation. Hyperglycemia, an increase in blood glucose level, is one of the effects of diabetes, and it can cause damage to the nerves and blood vessels.

There are two types of diabetes: Type I and II. Type I refers to the type of diabetes that happens when the body can’t produce the needed amount of insulin. Hence, regular

insulin administration is needed for individuals who have this disease. In 2017, nine million people had this type of diabetes (WHO, 2023). The cause and treatment for this are currently unknown.

Ninety percent of people with diabetes have Type II diabetes (T2D), which happens when the body cannot synthesize insulin well and is not able to normalize the blood sugar level (WHO, 2023). When our cells do not respond normally to insulin, the pancreas produces more insulin to try to get the cells to respond (*Type 2 Diabetes*, 2023). Eventually, this will lead to the pancreas malfunctioning and blood sugar levels rise. Type II diabetes can go undetected for several years and can often be prevented through lifestyle and diet changes.

Beigrezaei et al. (2019) investigated the relationship between Type II diabetes and diet. They found two dietary patterns among their participants: the healthy and Western types of diet. The healthy pattern has an inverse relationship with the occurrence of T2D, while the Western type significantly increases the risk of getting the disease. They discussed that the risk stems from the Western diet's higher intake of red meat, processed meat, and refined grains.

2.5. Hypoglycaemic Effects of Procyanidins

Another critical factor that should be considered in this study is understanding how procyanidins are metabolized and how they interact with glucose or starch in white bread. In the study conducted by Deshpande & Salunkhe (1982), they investigated how tannic acid and catechin bind with five different legume starches and how the binding affects the *in vitro* digestibility of these starches. This knowledge can help explain procyanidins' effect on the glucose release of white bread.

In their experiment, they found out that tannic acid binds to starch more than catechin. Moreover, when starch binds to tannic acid and catechin, the *in vitro* digestibility using porcine pancreatic α-amylase (PPA) is lowered by 8.8 - 17% and 6.7 -15.5%, respectively.

Coe et al. (2013) also observed this type of inhibition when they used polyphenol-rich baobab fruits (*Adansonia digitata* L.). They found that when 1.88% or higher concentrations of baobab were added to bread, sugar release was significantly reduced at 20 and 60 minutes into the duodenal phase of digestion compared with the control (white bread only). They further explained that since polymeric polyphenols cannot be absorbed significantly, they have the potential to bind starch molecules in food, resulting in a decrease in the rate of starch

breakdown. The polyphenols present may be inhibiting digestive enzymes such as α-amylase and α-glucosidase.

Gonthier et al. (2003) studied how procyanidins affect metabolism in rats. They fed rats with different polymers of procyanidins. They discovered that the higher the degree of polymerization of procyanidin, the more they are poorly absorbed in the gut. Due to the polymeric nature of procyanidin, they contribute to inhibiting extracellular microbial enzymes in the gut by forming complexes with the substrates required for microbial growth. The same could be happening to the digestive enzymes during starch degradation inhibition.

Moreover, Sun et al. (2018) observed that polyphenols found in tea have an inhibitory effect on α-amylase even though it is already bonded to starch. Hence, starch digestion is also inhibited. They further discussed that tea polyphenols could inhibit PPA activity by binding to the enzyme's active site, resulting in a competitive inhibition (Hara & Honda, 1990; Sun et al., 2017; Sun et al., 2016 as cited by Sun et al., 2018). When the inhibitor binds to α-amylase, the enzyme may have changes in structure that affect its binding capacity with starch. It was also previously reported that polyphenols can directly interact with starch through hydrophobic forces and hydrogen bonding, reducing starch digestibility.

2.6. Testing Glycemic Response

2.6.1. Glycemic Index / Load

Sami et al. (2017) defined glycemic index (GI) as an indicator of the post-prandial glucose response to food per gram carbohydrate compared to a reference test food like white bread or glucose. Kim (2020) described GI as an observed effect in the serum glucose after consuming carbohydrate-containing food.

The GI value is affected by the type of carbohydrate. Low-GI foods are digested and absorbed more slowly, resulting in a gradual and slow increase in blood glucose. High-GI foods are the opposite and cause a rapid increase.

Glycemic load (GL) accounts for the product of the food's GI value and the amount of available carbohydrates per serving. Foods with a higher GL can raise serum glucose and insulin per serving size than those with a lower GL. Table 3 gives a summary of how GI and GL values can be interpreted.

Table 3: Guide to Interpreting GI and GL (Source: Kim, 2020)

Glycemic index	**Glycemic load**
The smaller the number, the less impact the food has on your blood sugar	The smaller the number, the less impact the food has on your blood sugar per serving size
55 or less = low	10 or less = low
56 - 69 = moderate	11 - 19 = moderate
70 or higher = high	20 or more = high

Brand-Miller et al. (2008) studied the effect of consuming low, medium, and high GI food in eight food groups. They found that GI plays a crucial role in predicting glycemic response and is an important factor in reducing or managing postprandial glycemia. The results of the glucose response are illustrated in Figure 5. The higher GI food typically gets a higher plasma glucose release, and the overall shape of postprandial glycemia is similar regardless of whether it is low, medium, or high GI.

Vlachos et al. (2020) regarded postprandial hyperglycemia as a major factor contributing to the development and progression of micro- and macrovascular complications in T2D. Their study supported previous studies in concluding that low-GI/GL meals positively affect postprandial hyperglycemia in patients with T2D. Moreover, they noted that the quality and quantity of carbohydrates primarily affect the postprandial glycemic response; hence, they should be regulated.

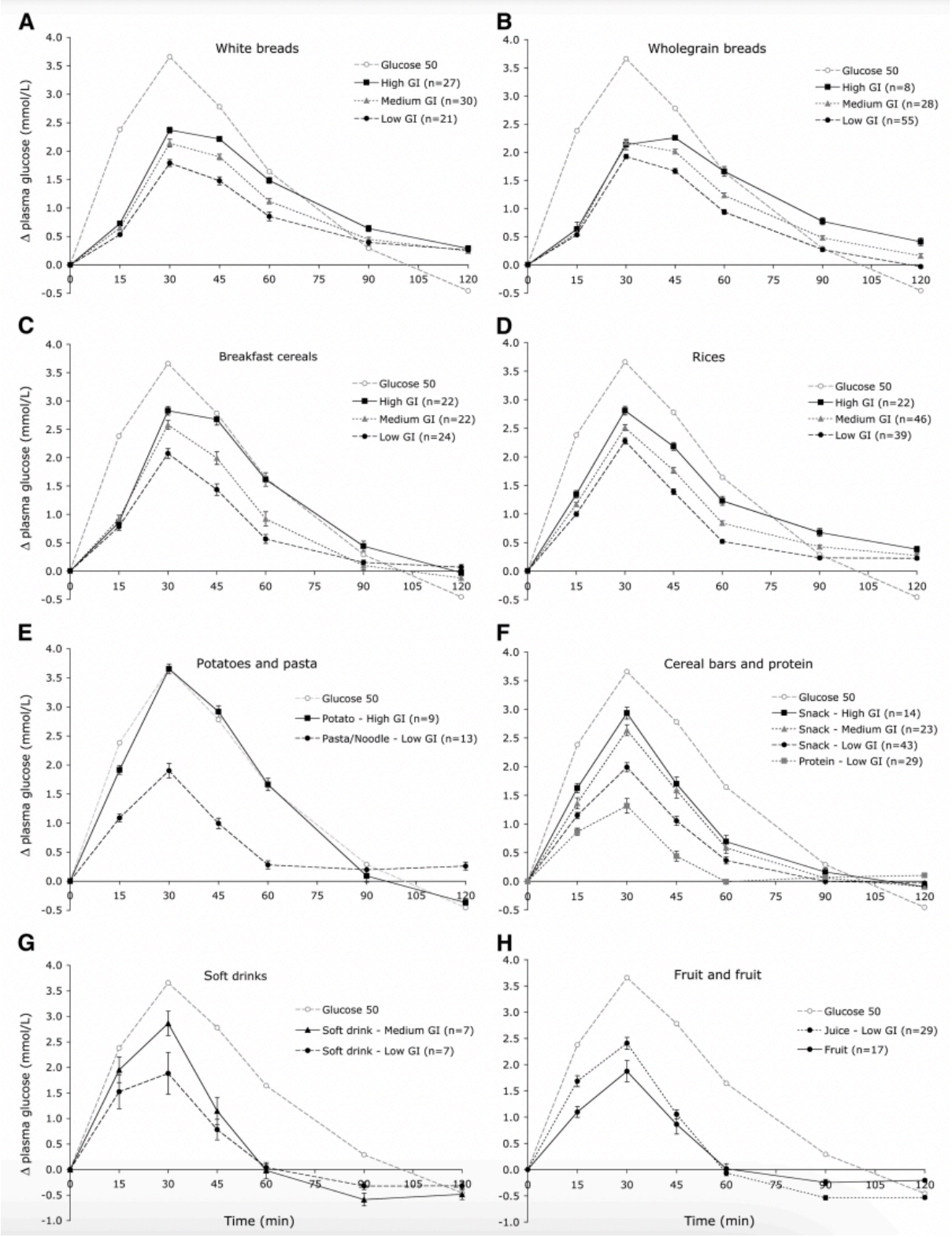


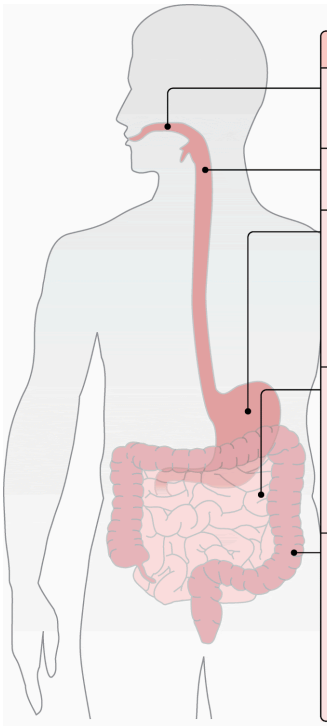
Figure 5: Incremental blood glucose profiles for high, medium, and low glycemic index (GI) foods within eight categories.

(Brand-Miller et. al, 2008)

2.6.2. Test Methods

2.6.2.1. *In vivo*

The process of digestion is a series of mechanical and enzymatic processes starting from the mouth going through the esophagus, stomach, and small and large intestines, and ending at the anus. Figure 6 summarizes the entire digestive process in the gastrointestinal tract (GIT).



Organ	Physical process	Chemical process
Oral cavity (mouth)	**Mastication** Goal: Bolus formation	**Enzymatic hydrolysis** Goal: α-amylase: Starch breakdown Lingual lipase: Lipid breakdown
Esophagus	**Peristalsis** Goal: Bolus transport	
Stomach	**Peristalsis** Goal: Continued food breakdown	**Enzymatic hydrolysis** Goal: Pepsin: Protein breakdown Lipase: Lipid breakdown **Acid hydrolysis** Goal: Gastric acid (pH ~2): Soften food texture and break down structure
Small intestine	**Peristalsis** Goal: Digesta transport **Segmentation** Goal: Mixing to facilitate absorption **Diffusion (active/passive)** Goal: Absorption of nutrients	**Enzymatic hydrolysis** Goal: Lipase, phospholipase A: Lipid breakdown Amylase, amyloglucosidase: Starch breakdown Trypsin, chymotrypsin, carboxypeptidase, elastase: Protein breakdown
Large intestine (colon)	**Peristalsis** Goal: Digesta transport **Segmentation** Goal: Mixing to facilitate absorption **Diffusion (active/passive)** Goal: Absorption of fermentation by-products and water	**Fermentation** Goal: Produces short-chain fatty acids and other by-products

Figure 6: Summary of critical physical and chemical processes that occur in the gastrointestinal tract

(Source: Heuman et al., 1997 and Seidel & Long, 2006, as cited in Bornhorst & Singh, 2014)

Digestion test methods allow researchers in the food industry to understand how food is broken down in the body. This is essential in designing and optimizing novel food products with health benefits such as increased satiety, more extensive nutrient availability, or decreased blood glucose response (Bornhorst & Singh, 2014). By understanding how food is digested, we also know how it can affect human health.

In vivo digestion models use human or animal subjects to observe how food is digested. This process tends to be more complicated than *in vitro* as it requires more technical and financial capabilities and ethical considerations.

Blood glucose centration and glycemic index can be measured *in vivo* by drawing blood samples from the finger with a capillary tube (Wolever et al., 1991, Bornhorst & Singh, 2014). Monitoring blood glucose levels for two to three hours after meal consumption helps determine the glycemic response. The values measured will be compared to baseline levels.

The GI is determined by measuring the area under the curve (AUC) of the glucose response exhibited by a test food, which is then compared with the AUC of a control, typically a glucose solution or white bread. Test meals contain 50 grams of available carbohydrates (Jenkins et al., 1981, as cited in Bornhorst & Singh, 2014). GL, on the other hand, is calculated similarly to GI. Instead of using 50 grams of carbohydrates for test meals, a recommended serving portion is used depending on the test subject's cultural, geographical, or nutritional requirements.

Animal test subjects are also often used when using a human model is impossible. Animals that are commonly used for digestion studies are dogs, rats, chickens, and pigs. Ingestible markers such as chromium oxide (Cr_2O_3) or titanium dioxide (TiO_2) are added to the meals, samples of chyme are then collected from the GIT and can be done either by cannulation or slaughter (Bornhorst & Singh, 2014).

2.6.2.2. *In vitro*

The COST INFOGEST network created an international consensus to standardize the *in vitro* digestion protocol based on data collected from *in vivo* studies (Brodkorb et al., 2019). Researchers can efficiently perform digestion experiments and compare data with other studies using a simplified and standardized protocol. The use of *in vitro* digestion protocol gives reproducibility, allowing a large number of samples to be measured in parallel in a controlled condition for mechanistic studies and hypothesis building (Minekus et al., 2014).

Brodkorb et al. (2019) further explained that there are two most commonly used types of *in vitro* digestion methods: static and dynamic. A dynamic setup is suitable for testing food and pharmaceutical products in different population groups and for different purposes. However, it is more complex and expensive to set up and maintain. The static model, on the

other hand, is more commonly used and is more straightforward because it uses a constant ratio of food to enzymes and electrolytes and a constant pH for each digestive phase.

One limitation that should be considered when using this digestion model is that it cannot completely mimic the dynamic complex of the digestion process. For example, in the static mode, the gastric pH is kept constant. In actuality, there should be a gradual addition of gastric fluid and gastric emptying during the gastric phase of digestion.

As shown in Figure 7, the digestion process consists of three main steps: oral phase, gastric phase, and intestinal phase. During the oral phase, the test food is diluted in a 1:1 ratio with the SSF for two minutes at pH 7. The resulting oral bolus will be diluted again in a 1:1 ratio with SGF with pepsin and lipase and incubated under agitation at pH 3 for two hours. The intestinal phase will be simulated by diluting the gastric chyme with SIF, bile salts, and pancreatic enzyme at a 1:1 ratio and incubated at pH 7 for another two hours.

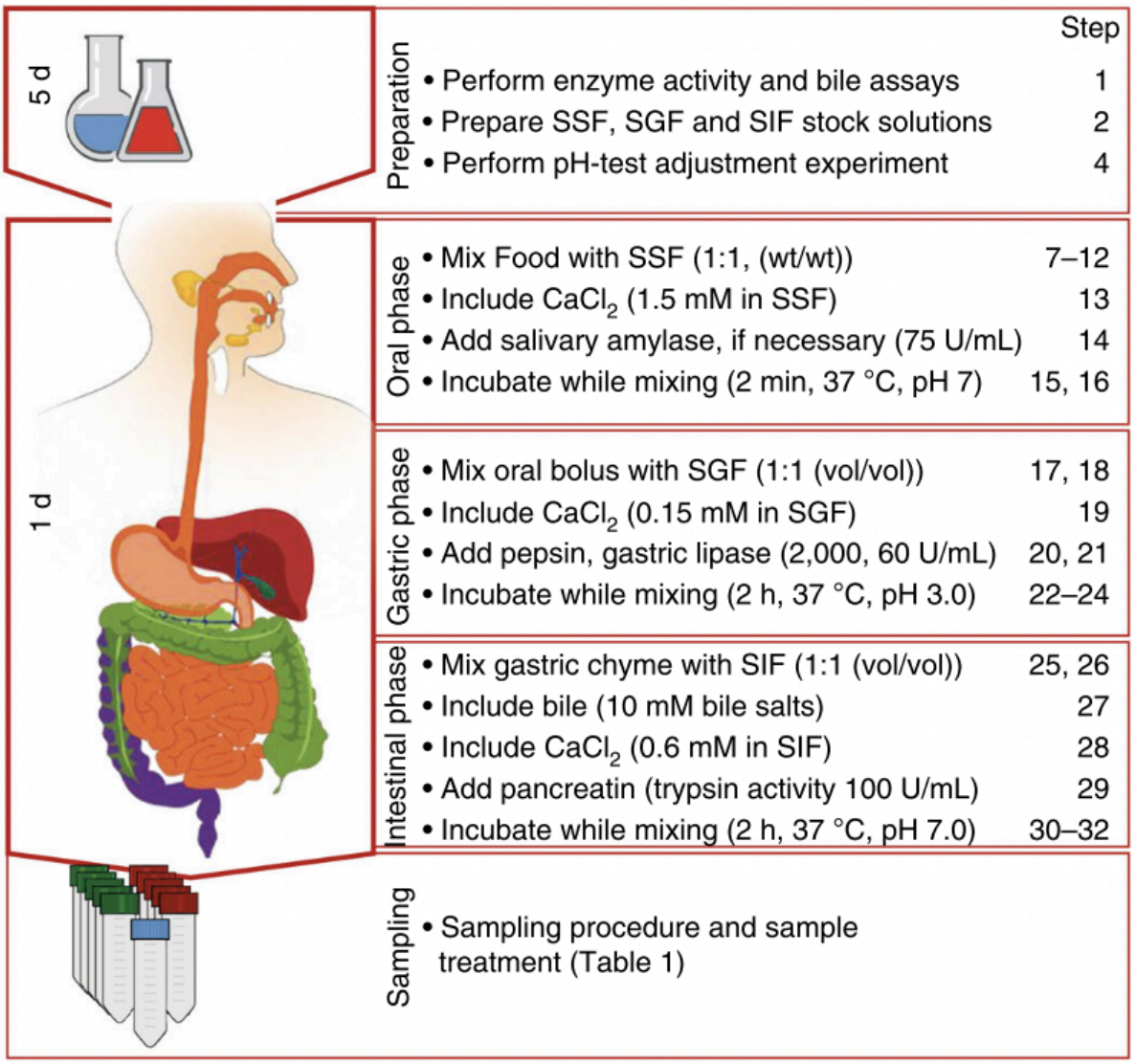


Figure 7: Flow diagram of the INFOGEST 2.0 digestion method. Timing and flow diagram of the INFOGEST 2.0 in vitro digestion method for food. The expected time frame (left) and stages and corresponding step numbers in the Procedure (right) are given. SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SSF, simulated salivary fluid.
(Source: Brodkorb et al., 2019)

Minekus et al. (2014) recommended exposing the food sample to a half-gastric emptying time of two hours to cover a wide range of meals. Therefore, the enzyme-substrate ratio during digestion is set to mimic this condition. Moreover, gastric lipase was not included in the protocol because it has a lower activity in the gastric tract compared to that of the duodenal tract.

2.6.3. Semi-dynamic *In vitro* Digestion For Testing Starch Hydrolysis

The modification done for the *in vivo* oral phase was standardized by Tormási et al. (2023) using two parameters: chewing time and chewing number. They got the average chewing time and chewing number of three volunteers from the lab. The oral phase happens for around two to five minutes, and then digestion moves to the gastric phase. Despite recent suggestions that salivary α-amylase (HSA) is not an integral component for *in vitro* digestion because of inactivation just after the oral phase, Freitas et al. (2018) study proves that HSA is just as important as pancreatic α-amylase. They found that the amylolytic activity of saliva plays a preponderant role in hydrolyzing up to 80 % of bread starch in the first 30 min of gastric digestion. Furthermore, they discussed that HSA can continue hydrolyzing the starch in the stomach until the pH lowers below 4 in which the enzyme will be inactivated. Their study imposes the importance of HSA in both *in vitro* and *in vivo* digestion setups.

The semi-dynamic gastric phase was employed to simulate the gastric phase pH decrease and emptying (Xavier & Mariutti, 2021). For a Western-type solid meal, gastric emptying is usually completed between three and four hours, and the pH usually increases to 5 and above because of the food's buffering capacity (Kalantzi et al., 2006, as cited in Minekus et al., 2014). Later in the digestion, the pH will return to a fasted pH of 2 or below (Carrière et al., 1991 as cited in Minekus et al., 2014).

In the intestinal phase, the sample's pH was raised to 7 by adding 1 M NaOH to stop the pepsin activity (Brodkorb et al., 2019).

3. MATERIALS AND METHODOLOGY

3.1. Materials

- Scanvac Coolsafe 110-4 freeze dryer
- Kitchen scale food grinder
- Ultra-turrax
- Ultrasonic bath - Realsonic Cleaner
- Thermo Scientific Evolution 300 UV-Vis spectrophotometer
- 70% acetone
- Methanol
- Scanvac centrifuge for vacuum concentrator
- Megazyme D-glucose standard 10mg/ml in 0.2% benzoic acid
- Sigma-Aldrich bile extract porcine (20 mM)
- Sigma-Aldrich pancreatin from porcine pancreas (100 U/ml)
- Sigma-Aldrich pepsin from porcine gastric mucosa 500 units/mg protein (2000 U/ml)
- Silvateam Welltan product line quebracho tree extract (*Schinopsis lorentzii*) with 95% condensed tannin
- 2 w/v% solution of $NH_4Fe(SO_4)_2$ x 12 H_20
- 6M HCl
- BuOH-HCl solution
- Simulated gastric fluid (SGF) pH 7
- 0.3M $CaCl_2$
- 3M HCl
- 1M NaOH
- Amyloglucosidase
- GOPOD (Glucose Oxidase/Peroxidase) reagent
- Hettich Mikro 22R centrifuge (for pancreatin preparation)
- MPW - 260R centrifuge (for sample preparation)

3.2. Samples

3.2.1. White Bread

White bread was used as a reference food during the experiments. White bread was made according to the recipe from the ISO 6820:1985 standard. In a Bosch MUM4830 type kitchen mixer equipped with the dough hook, 500 g of wheat flour (0.55% (d.w) ash), 7 g of instant yeast, 4 g of sugar, 7 g of salt, and approximately 340 ml water (dependent on the quality of the flour) was mixed until smooth texture was reached. The dough was left to rest for 30 min at 50 °C to rise and was baked for 50 min at 180 °C in a Memmert UF110 drying cabinet. The baked and cooled bread was sliced to 1 cm thickness, and each slice was packed in plastic bags and stored at – 20 °C. A slice of white bread was thawed before digestion experiments.

3.2.2. Apple pomace

The apple pomace used in the experiment is an Idared apple variety, it was pressed under normal industrial conditions with no enzyme treatment. It was harvested during the beginning of Autumn 2022. It was procured from Eszat Kft. Address: 4700 Mátészalka, Jármi út, 57.

3.3. Drying and Pulverization of Apple Pomace

The apple pomace sample was obtained directly from an apple processing facility. It was stored in the freezer and pre-processed before testing for glucose release and bioactive components. The pre-processing procedures were freeze-drying and grinding the apple pomace into powder to stabilize and homogenize it for further processing. Figure 8 shows the fresh apple pomace samples, Figure 9 shows the samples after freeze-drying, and Figure 10 represents the powdered apple pomace after grinding.

Figure 8: Petri dishes with fresh apple pomace

(Source: own work)

Figure 9: Freeze-dried apple pomace

(Source: own work)

Figure 10: Powdered apple pomace

(Source: own work)

Drying was carried out using Scanvac Coolsafe 110-4 freeze dryer. Frozen apple pomace was measured onto Petri dishes with a known weight. The plates are then covered with parafilm with small holes in it. All of the plates containing samples were freeze-dried for six days. The moisture content was calculated using the initial and final weight of the sample. The freeze-dried apple pomace samples were pulverized using a kitchen-scale electric grinder.

3.4. Extraction of Apple Pomace

Two extraction procedures were implemented: water suspension and acetone extraction. For the water suspension, one gram of apple pomace was weighed in a 50 ml centrifuge tube. Twenty milliliters of water at 30 ℃ were added, and the mixture was homogenized using an ultraturrax for two minutes. Three replicates were made.

The acetone extraction was done using 70% acetone. Four grams of apple pomace were weighed in a 50 ml centrifuge tube. Forty milliliters of 70% acetone were added, and the mixture was homogenized using an ultraturrax for two minutes. Three replicates were done and centrifuged at 4 ℃ for 10 minutes at 13000 rpm. The supernatant was dried in a Scanvac centrifuge for a vacuum concentrator to remove acetone up to the 10 ml mark to have a known concentration.

The water suspension and acetone extract were co-digested with white bread. The procyanidin content was also determined for both samples.

The first sample prepared and tested was the water suspension, as seen in Figure 11, and then the acetone extract, as seen in Figure 12.

Figure 11: Apple Pomace water suspension

(Source: own work)

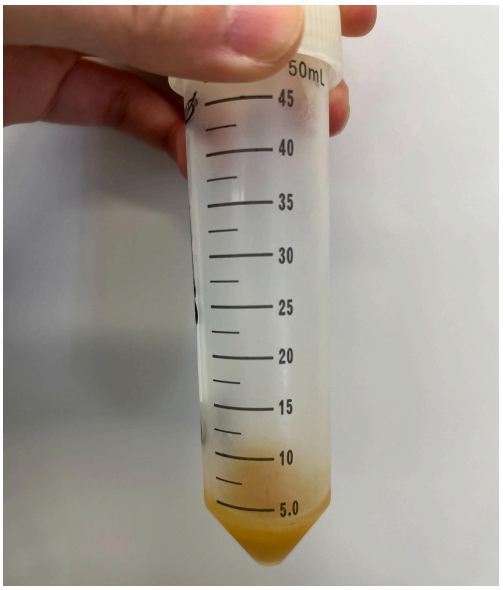


Figure 12: Apple Pomace acetone extract

(Source: own work)

After drying the acetone extract to 30% of the original volume, we tried to freeze-dry some samples. However, the resulting sample became very hygroscopic. We inferred that the sample still has considerable amounts of sugar that need to be removed.

3.5. Proanthocyanidin (Condensed Tannin) Determination

For the procyanidin measurement, we adopted Porter et al.'s (1985) methodology, which is still being used in studies, such as Nemes et al.'s (2018) determination of the proanthocyanidin profile of Hungarian sour cherry.

Water suspension and acetone-extracted samples were prepared according to the original protocol. The condensed tannins are extracted using methanol to create a 0.02 w/v% stock solution. First, 600 µl of the apple pomace acetone extract was transferred into a 15 mL centrifuge tube and mixed with 10 mL of methanol. Methanolic extracts then were analyzed as is and after dilutions to 1:2, 2:5, 3:10, and 1:5. The water suspension did not dissolve in methanol fully, therefore it was subjected to an ultrasonic bath at 37 ℃ for five minutes and then centrifuged at 4 ℃ 6000 rpm for 10 minutes. The supernatant was used for the analysis.

For the calibration, we used the Silvateam Welltan product line quebracho tree extract (*Schinopsis lorentzii*) with 95% condensed tannin content. We did five dilutions with concentrations of 0.200, 0.067, 0.057, 0.046, 0.027 mg/ml.

Two reagents were made for this measurement: 2 w/v% solution of $NH_4Fe(SO_4)_2$ x 12 H_20 and BuOH-HCI solution with a BuOH:HCl ratio of 95:5.

The 2 w/v% solution of $NH_4Fe(SO_4)_2$ x 12 H_20 was prepared by measuring 0.2 g of the salt into a 15 mL centrifuge tube and adding 3.33 mL of 6 M HCl and 6.66 mL distilled water. It was mixed until the salt was dissolved.

To make the BuOH-HCI solution, 237.5 mL of butanol was transferred into a 250 mL screw-top jar and mixed with 12.5 mL of 37% HCl under the fume hood.

For the reaction, 1 mL of the methanolic extract was transferred into an 8 mL screw cap vial, added with 6 mL of the BuOH-HCl solution and 0.2 mL of the iron reagent. Mixed and placed into a 95 °C water bath for 40 mins. The absorbance of the solutions was then measured at 550 mm using a

Based on the calibration curve, results were calculated in mg condensed tannin equivalent/mL extract.

3.6. Modified *In vitro* Digestion Based on Infogest Protocol

The digestion method used was *in vitro* digestion simulation, specifically a modified Infogest protocol using a semi-dynamic gastric phase. The same modifications were adopted from the work of Tormási et al. (2023). Figure 13 presents a schematic of the actual process used in the experiment, while Figure 14 shows the actual set-up. The digestion procedure was performed for each sample in four to six parallels for each treatment.

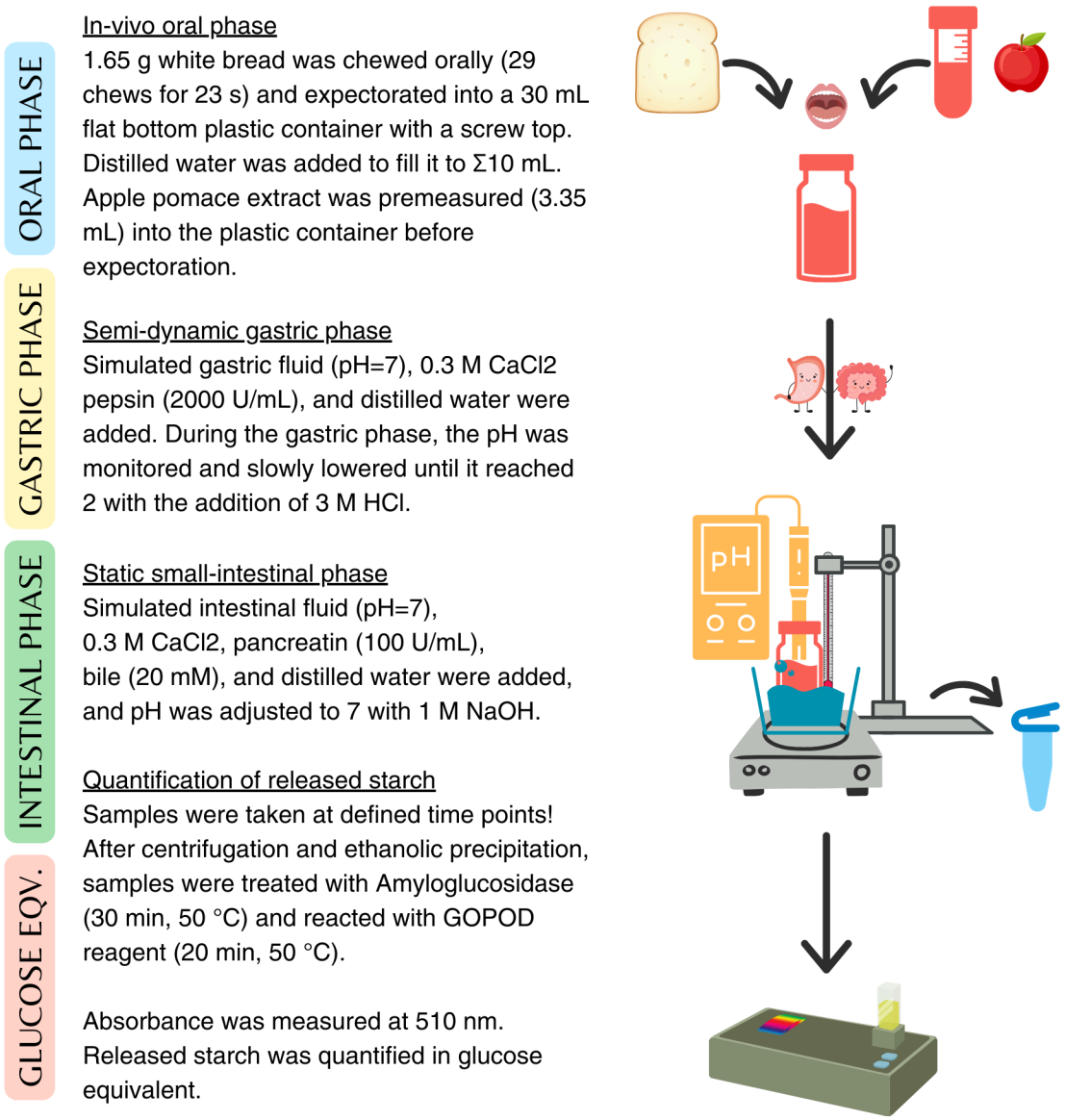


Figure 13: Schematic flow diagram for the modified *in vitro* digestion (source: own work)

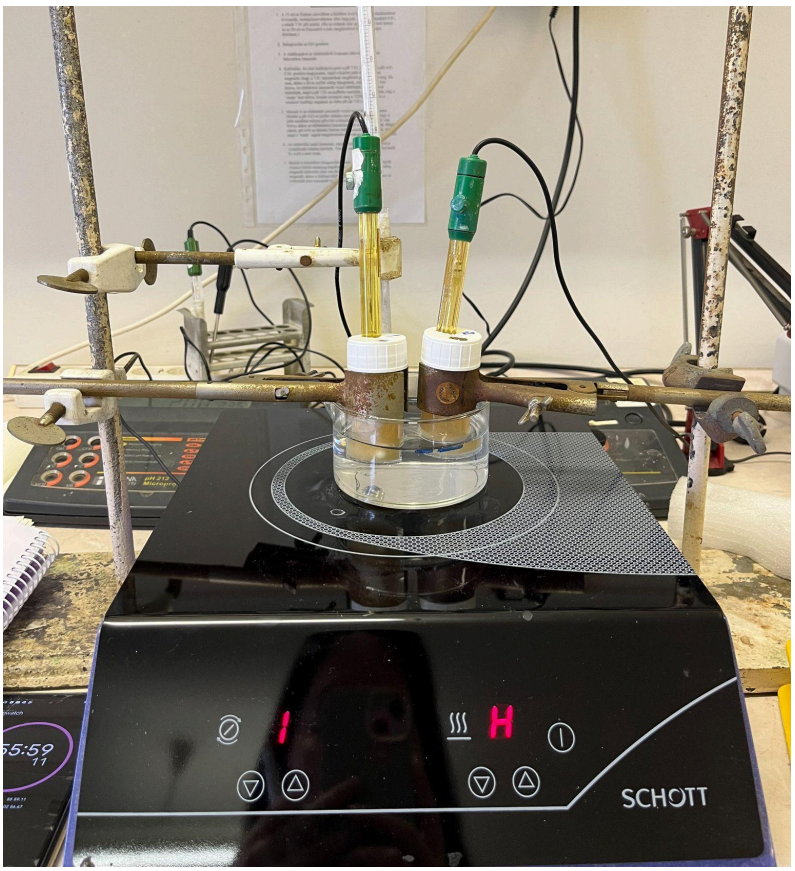


Figure 14: Actual digestion set-up used in the experiment (source: own work)

Before digestion, the following reagents must be prepared for gastric and intestinal digestion: 46.2 mg Sigma-Aldrich pepsin from porcine gastric mucosa 500 units/mg protein (2000 U/ml) dissolved in 3.8 ml SGF, 265.1 mg Sigma-Aldrich pancreatin from porcine pancreas (100 U/ml) dissolved in 5.5 ml SIF, and 4.2 mg Sigma-Aldrich bile extract porcine (20 mM) dissolved in 2.6 ml SIF.

The pancreatic fluid was prepared by dissolving 265.1 mg to 5.5 ml SIF, vortexed for 10 seconds, put in a Realsonic Cleaner ultrasonic bath at 35 ℃ for five minutes, and then centrifuged (Hettich Mikro 22R centrifuge) at 4 ℃ 6000 rpm for five minutes. The pancreatic supernatant was transferred to another tube. Bile and SIF were incubated at 37 ℃ while the pancreatic fluid was kept in the refrigerator before use.

The digestion vial was pre-weighed before adding 3.35 ml of apple pomace extract. The total weight of the extract and vial was also recorded. 1.65 g of white bread was weighed onto a Petri dish. The start of the digestion simulation in the mouth was improvised by chewing the bread in the mouth for 29 chewing motions for 23 seconds. The macerated bread

was put in the vial, and its weight was recorded. A known amount of distilled water was added by calculating:

$$Amount\ of\ water\ = 10\ -\ (weight\ after\ putting\ bread\ -\ tare\ weight\ of\ vial).$$

After the oral phase, the vial was loaded into the digestion setup, which consists of the water bath maintained at 37 ℃, a magnetic stirrer inside the vial to mix the digesta continuously, and a pH probe to monitor the pH of the vial contents.

When the vial was set up, the gastric fluids were added in the following manner: 6.4 ml SGF, 5 µl 0.3 M $CaCl_2$, 1.6 ml pepsin, and 1.545 ml distilled water. The pH was recorded, and then 10 µl to 20 µl of 3 M HCl was added. HCl was gradually added every five minutes until pH 2 was attained, and the pH was also recorded every five minutes.

At 120 minutes, the digestion shifted to small intestinal digestion, and the intestinal fluids were added in the following manner: 4.26 ml SIF, 20 µl 0.3 M $CaCl_2$, 1.25 ml bile, and 2.5 ml pancreatic fluid. After putting all the fluids, 1 M NaOH was added gradually until the sample reached pH 7, adding 10 µl to 20 µl per addition until the desired pH was reached. Distilled water was added, depending on the amount of NaOH added. The total volume of NaOH and water should be 1.980 ml. From this point, the pH was measured, and samples were collected at the time mentioned above.

During the digestion simulation, sampling was made at set time points to measure the glucose release later. So two-milliliter tubes were pre-weighed, and after 15 mins, 30 mins, 45 mins, 60 mins, 90 mins, 120 mins, 150 mins, 180 mins, 210 mins, and 240 mins, 2 ml of digesta from the vial were placed in the tubes. The weight of the tubes after putting the sample was also recorded.

3.7. Evaluation of glucose release

After digestion, glucose release was measured. The collected samples were extracted using ethanol and then diluted. The starch in the diluted samples was hydrolyzed into D-glucose using amyloglucosidase. The hydrolyzed glucose will be treated with a GOPOD reagent to produce quinoneimine, which can be detected in the UV-Vis spectrophotometer.

The tubes were first centrifuged at 4 ℃ 13000 rpm for 10 mins. Then, 400 µl of the supernatant was extracted with 1600 µl ethanol in a second tube and centrifuged at the same setting. After the second centrifugation, 50 µl of the supernatant was diluted with 950 µl water.

The diluted samples were used for the spectrophotometric analysis. Aliquots of 25 µl AMG were mixed with 25 µl samples in Eppendorf tubes. Two standards were prepared: one contains AMG, and the other contains only distilled water. The tubes with AMG and samples were subjected to a water bath at 50 ℃ for 30 mins. After 30 mins, 750 µl GOPOD reagent was added to each tube and then returned to the water bath for 20 mins.

After the water bath, the absorbance of samples was determined at 510 nm in the UV-Vis spectrophotometer.

The amount of glucose released was plotted in a graph based on the data collected and calculated during the whole process.

3.8. Inherent Glucose Measurement

The inherent glucose composition was determined with liquid chromatographic separation (HPLC) and refractive index detection (RID). Agilent 1200 HPLC (Agilent Technologies, Santa Carla, California) was used for the measurement of fructose, glucose, and sorbitol, and ChemStation software (B.04.02, Agilent Technologies, Santa Carla, California) for the evaluation of the data. A mixture of acetonitrile and water in a ratio of 87:13 (v/v%) was used as eluent. The separation of compounds was performed on a Phenomenex Luna NH_2 (250 mmx4.6, 5um) HPLC column. The flow rate was set to 1.5 ml/min and the temperature to 30°C.

Typically, adjusting for glucose content suffices to derive accurate glucose release results. As seen, when analyzing sour cherry juice, as done by Tormási et al. (2023), only the directly measured glucose content was used for adjustment. Yet, the outcomes were debatable when we applied this method to apple pomace. We inferred that apple pomace likely contains some oligo-, polysaccharides which provide additional glucose released during digestion, which was not initially available but was generated through hydrolyzation during the experiment. Therefore, we measured the glucose release from apple pomace acetone extract, and water suspension (without the bread) during digestion.

3.9. Statistical Analysis

The digestion for bread was replicated three times, for water suspension and bread four times, and six times for acetone extract and bread. Results were expressed as the means

± SEM. To determine the extent of the effect of the apple pomace samples in bread, t-test analysis was employed to know at which time points there are significant differences in glucose release between the control (bread only) and the experimental (bread co-digested with apple pomace samples). Significance was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

4. RESULTS AND DISCUSSION

The study aims to determine the effect of apple pomace during the digestion of white bread. Two sample preparation procedures were used to extract possible bioactive components present in the pomace that can affect digestion: water suspension and acetone extract. The proanthocyanidin content of each apple pomace sample was measured. Subsequently, the bread sample was co-digested with the apple pomace samples using a semi-dynamic Infogest *in vitro* digestion protocol.

4.1. Results of Moisture Content Determination

Freeze drying was done to remove moisture from apple pomace. As discussed in the literature review, freeze-drying was found to be one of the best drying methods to remove moisture from apple pomace while retaining most of its bioactive components. Moreover, the dried apple pomace was ground and homogenized for greater the surface area and the smaller the diffusion path for solvents resulting in greater extraction efficiency. The freeze-drying data and calculated moisture content of apple pomace are represented in Table 4.

Table 4: Freeze drying data and calculated moisture content of apple pomace (source: own work)

Samples	Weight of petri dish, g	Weight of sample + petri dish before drying, g	Weight of sample before drying, g	Weight of sample + petri dish after drying, g	Weight of sample after drying, g	Moisture content, %
1	79.40	118.56	39.16	90.47	11.07	71.73
2	72.85	117.39	44.55	85.42	12.57	71.78
3	70.88	121.60	50.72	85.13	14.25	71.90
4	70.06	115.44	45.37	82.79	12.72	71.96
5	66.45	122.63	56.18	82.01	15.55	72.32
6	69.35	124.36	55.00	84.68	15.33	72.13
7	86.70	174.42	87.71	111.12	24.42	72.16
8	70.16	132.25	62.09	86.95	16.79	72.96
9	114.35	204.02	89.67	138.26	23.90	73.34
10	116.06	193.92	77.87	137.14	21.09	72.92
					Average	72.32±0.56

The moisture content of apple pomace was calculated using the below formula:

$$\%\ Moisture\ = \frac{Weight\ of\ sample\ before\ drying\ (g) - Weight\ of\ sample\ after\ drying\ (g)}{Weight\ of\ sample\ before\ drying\ (g)} \text{ x } 100$$

Fernandes et al. (2019) reported that apple pomace contained 81% moisture, resulting in its high perishability. Based on Table 4, the average moisture content calculated after freeze-drying was 72%, which is also considerably high.

4.2. Proanthocyanidin Measurement of Water Suspension and Acetone Extract Samples

A 95% condensed tannin from a Quebracho tree sample was used to create a calibration curve. Figure 15 shows the absorbance measured for the five different dilutions made. It indicates that the higher the tannin concentration, the higher the absorbance measured.

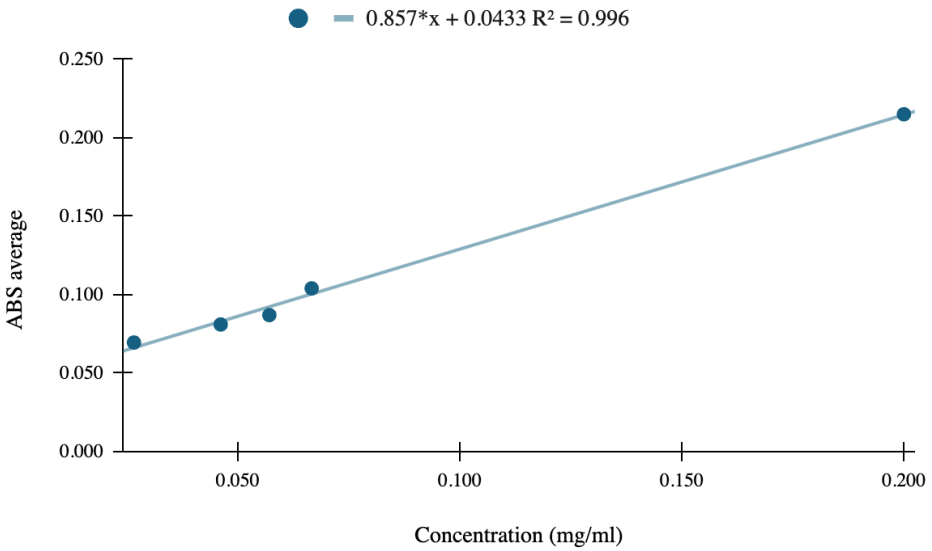


Figure 15: Calibration curve for proanthocyanidin measurement (source: lab reference)

Using the calibration curve from Figure 15, the proanthocyanidin content presented as the tannin equivalent of the apple pomace samples was calculated in mg/g. Four dilutions

were done for the acetone-extracted apple pomace, from these dilutions an average of 0.127 mg/g tannin equivalent was calculated as shown in Table 5.

Table 5: Proanthocyanidin concentration of apple pomace acetone extract (source: own work)

Conc. (mg/ml)	ABS average	Diluted tannin eqv. (mg/ml)	Dilution Factor	Tannin eqv. (mg/ml)	Tannin eqv. (mg/g)
2.000	0.118	0.087	10.000	0.872	0.131
0.057	0.118	0.087	11.667	1.010	0.152
0.046	0.104	0.070	14.333	1.007	0.151
0.027	0.085	0.049	10.000	0.487	0.073
				AVG	0.127
				STDEV	0.037
				RSD	29%

The concentration of proanthocyanidin in the water suspension samples was also measured, no dilution was done but two parallel sets of samples were used. The results calculated are shown in Table 6, the average tannin equivalent is 0.028 mg/g was obtained.

Table 6: Proanthocyanidin concentration of apple pomace water suspension (source: own work)

Conc. (mg/ml)	ABS average	Diluted tannin eqv. (mg/ml)	Dilution Factor	Tannin eqv. (mg/ml)	Tannin eqv. (mg/g)
0.200	0.056	0.015	2.667	0.041	0.024
0.200	0.061	0.020	2.667	0.054	0.032
				AVG	0.028
				STDEV	0.006
				RSD	20.159

The proanthocyanidin concentrations of the apple pomace water suspension and acetone-extracted apple pomace were calculated from the measured absorbance and the use of the calibration curve. The tannin equivalent for the water-suspended sample is 0.028 mg/g and 0.127 mg/g for the acetone-extracted one. In a study conducted by Krasnova & Segliņa (2019), they determined the tannin content of 12 different cultivars of apple and its pomace. Results from their experiment showed that tannin content in apple pomace ranges from

0.2911 mg TAE/g dry weight to 0.7340 mg TAE/g dry weight, while water extracts of apple pomace contained 0.1356 mg TAE/g dry weight to 0.2602 mg TAE/g dry weight. Based on these values, we can say that the measured tannin contents were lower than the literature values. Acetone-extracted apple pomace gave higher tannin equivalent values, while the water suspension sample produced a lower amount of tannin.

4.3. Observed pH Change During Digestion

Figure 16 shows the pH change throughout the digestion process, which explicitly represents the pH change of acetone-extracted apple pomace co-digested with bread. The same patterns were observed in the other digestions made during the study.

The initial pH is 5 and will gradually decrease to a fasted pH of 2 at the end of the gastric phase. The pH was controlled in the experiment by adding 3 M HCl every five minutes and then recording it. The amount of HCl added was based on Tormási et al.'s (2023) study and was adjusted accordingly to achieve a gradual decrease in pH. The amount ranges from 10 µl to 20 µl per addition.

In the intestinal phase, the sample's pH was raised to 7 by adding 1 M NaOH to stop the pepsin activity (Brodkorb et al., 2019).

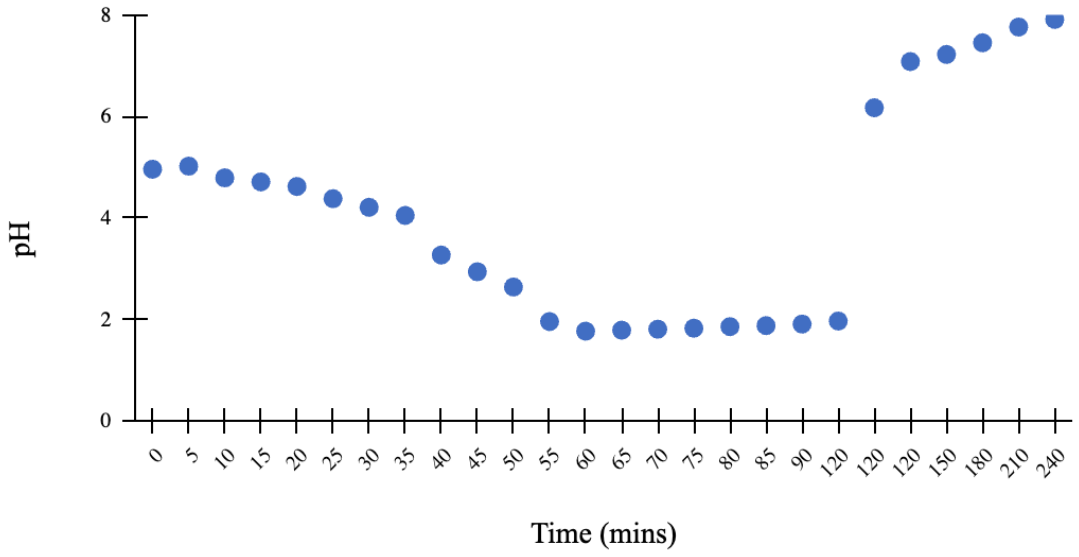


Figure 16: pH change during digestion of acetone-extracted apple pomace (source: own work)

4.4. *In vitro* Digestion of Reference Bread

Throughout the study, samples of white bread from our laboratory, with a standardized recipe, were utilized. The bread's glucose release was measured as a control; the calculated data are shown in Table 7.

Table 7: Calculated glucose release data of bread (source: lab reference)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	Standard Dev. (SD)	Relative Standard Dev. (RSD)	Standard Error of the Mean (SEM)
15	22.70	3.50	15.30	2.00
30	23.60	4.10	17.20	2.35
45	29.40	3.90	13.30	2.26
60	26.70	2.20	8.20	1.26
90	31.40	13.00	41.50	7.53
120	35.90	0.90	2.50	0.51
150	39.60	4.00	10.00	2.30
180	38.30	3.90	10.20	2.26
210	40.70	2.20	5.40	1.27
240	41.10	2.80	6.90	1.63

The values from Table 7 were plotted on a graph. In Figure 17, we can see the increasing trend of glucose release through time.

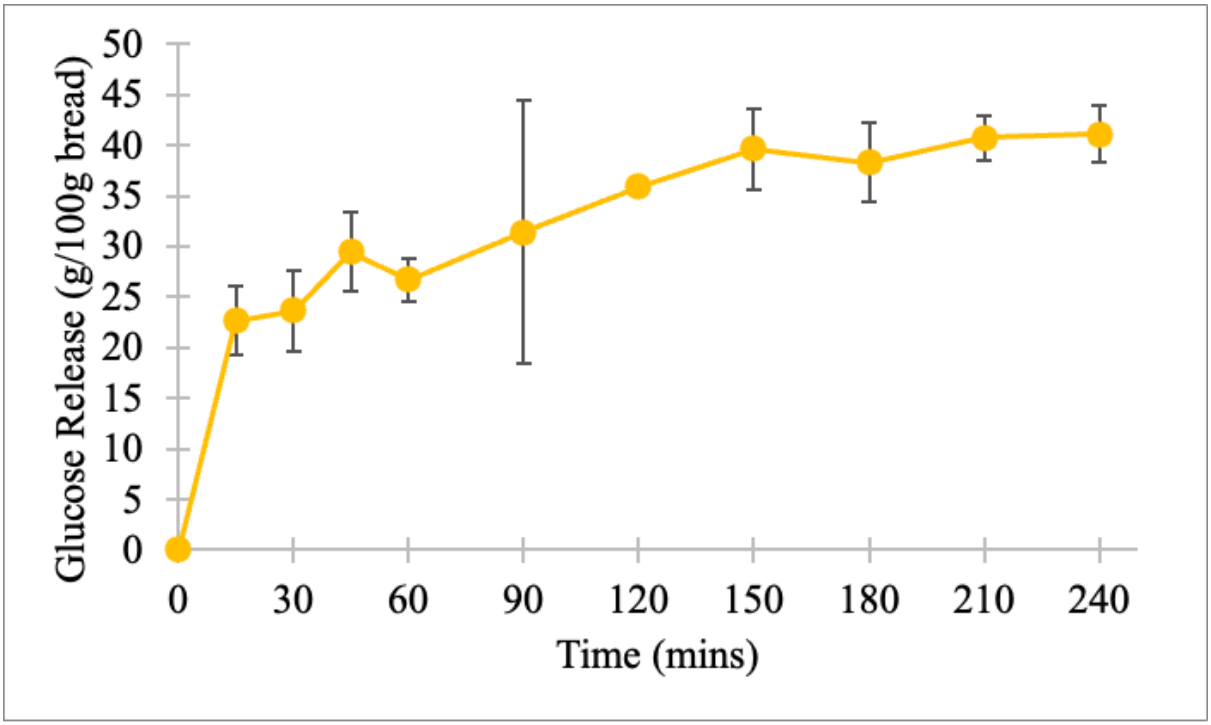


Figure 17: Glucose release during bread digestion

(source: lab reference)

Figure 18 presents the amount of reducing sugars released (mg/g starch) during in vitro digestion for the control bread and bread treated with codfish powder and semolina flour. The pattern of release in this graph suggests that there is an increase in sugar release during the first 20 minutes, after that, continuous there is an increase without decline until the endpoint at 140 minutes. The pattern in our reference bread is similar which exhibited a continuous increase without decline until the endpoint at 240 minutes.

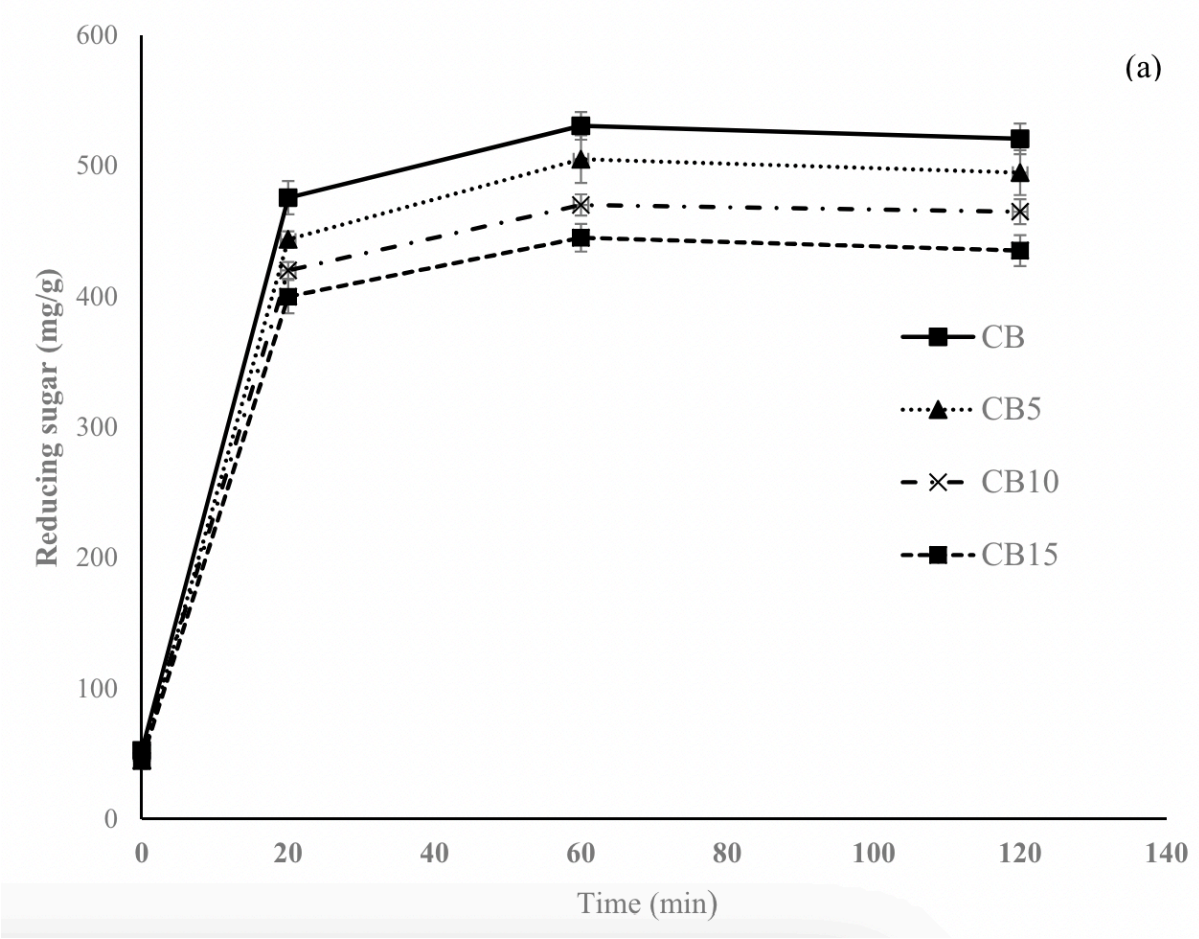


Figure 18: Amount of reducing sugars released (mg/g starch) during in vitro digestion. Control bread (CB); CB5, CB10, and CB15: bread produced with 5, 10, and 15 g codfish powder, 100 g semolina four (Desai et. al, 2021)

4.5. *In vitro* Co Digestion of Apple Pomace Water Suspension With Bread

After measuring the glucose release in white bread, the two treatments: water suspension and acetone extract were co-digested with white bread to determine their effect on the post-prandial glucose release.

Table 8 shows the glucose equivalent data when the apple pomace water suspension sample was co-digested with bread. Results are visually represented in Figure 19 compared to our bread data.

Table 8: Calculated glucose release data of apple pomace water suspension and bread (source: own work)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	**Standard Dev. (SD)**	**Relative Standard Dev. (RSD)**	**Standard Error of the Mean (SEM)**
15	21.30	7.00	32.90	3.50
30	27.20	8.20	30.20	3.36
45	28.60	5.50	19.30	2.25
60	28.60	10.40	36.20	4.23
90	30.20	6.00	19.80	2.44
120	53.90	12.90	23.90	5.25
150	52.80	12.50	23.70	5.10
180	55.50	15.40	27.80	6.29
210	55.10	10.60	19.10	4.31
240	53.20	10.80	20.30	4.41

As seen in Figure 19, during the first 90 minutes, the amount of glucose released between the two data sets is almost the same. During the rest of the digestion process, the apple pomace water suspension exhibited higher glucose releases than the control. To gather information on the effect of added extracts, raw data should be corrected with the inherent glucose content of the added treatment. Therefore, the glucose content of apple pomace water suspension was measured with liquid chromatographic separation (HPLC) and refractive index detection (RID). The measured glucose was 18.23 mg/g. However, when subtracted from the above data the same pattern was observed and no difference from the initial result was noted.

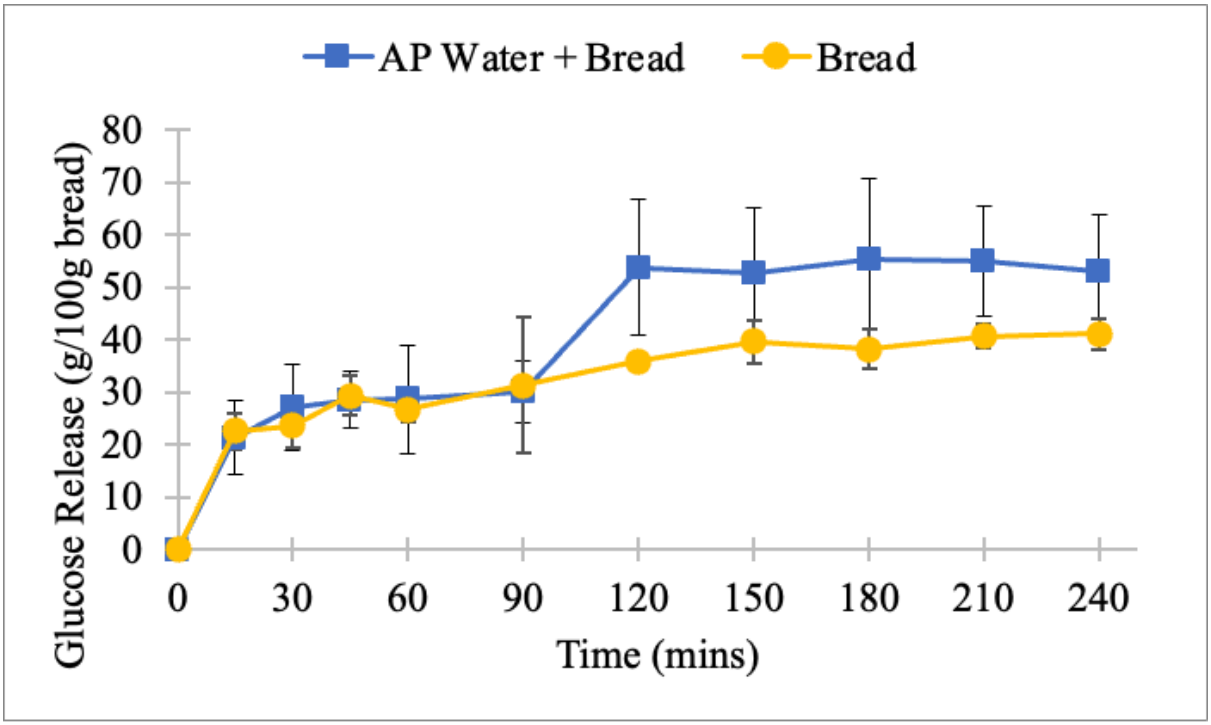


Figure 19: Glucose release during digestion of apple pomace water suspension with bread vs. bread only (source: own work)

Typically, adjusting for glucose content suffices to derive accurate glucose release results. As seen, when analyzing sour cherry juice, as done by Tormási et al. (2023), only the directly measured glucose content was used for adjustment. Yet, the outcomes were debatable when we applied this method to apple pomace. We inferred that apple pomace likely contains some oligo-, polysaccharides which provide additional glucose released during digestion, which was not initially available but was generated through hydrolyzation during the experiment. Therefore, we proceeded to measure the release of glucose from apple pomace during digestion, which will be elaborated upon in the subsequent section.

4.6. Measurement of Inherent Sugar in Apple Pomace Water Suspension

Table 9 shows the glucose release data of apple pomace water suspension digested alone. The results show that there is a substantial amount of glucose that could be released during the digestion of apple pomace water suspension, sugar which does not originate from the white bread when co-digested.

Table 9: Calculated glucose release data of apple pomace water suspension (source: own work)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	Standard Dev. (SD)	Relative Standard Dev. (RSD)	Standard Error of the Mean (SEM)
15	6.60	0.10	2.20	0.10
30	6.80	0.20	3.20	0.15
45	6.70	0.10	1.10	0.05
60	6.70	0.10	1.80	0.08
90	6.60	0.00	0.20	0.01
120	16.60	0.00	0.30	0.04
150	16.70	0.20	1.40	0.17
180	16.50	1.10	6.60	0.77
210	16.70	0.80	4.60	0.54
240	16.80	0.70	4.30	0.51

We used the data from Table 9 to account for the effect of the apple pomace's inherent glucose in the co-digestion data. Table 10 below shows the glucose equivalent data of the apple pomace water suspension and bread co-digestion when the inherent glucose was subtracted.

Table 10: Calculated glucose release data of apple pomace water suspension and bread after subtracting inherent sugar in apple pomace (source: own work)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	Standard Dev. (SD)	Relative Standard Dev. (RSD)	Standard Error of the Mean (SEM)
15	14.70	7.00	47.70	3.50
30	20.50	8.20	40.20	3.36
45	21.80	5.50	25.20	2.25
60	21.90	10.40	47.20	4.23
90	23.60	6.00	25.30	2.44
120	37.30	12.90	34.50	5.25
150	36.10	12.50	34.60	5.10
180	39.00	15.40	39.50	6.29

210	38.40	10.60	27.50	4.31
240	36.40	10.80	29.70	4.41

Data from Table 10 plotted in Figure 20 shows that when the inherent glucose equivalent of apple pomace was subtracted from the apple pomace water suspension and bread co-digestion result, the glucose release was lower during the gastric phase or the first 90 minutes compared to bread. The release for the rest of the digestion, the intestinal phase, will be almost similar to bread alone.

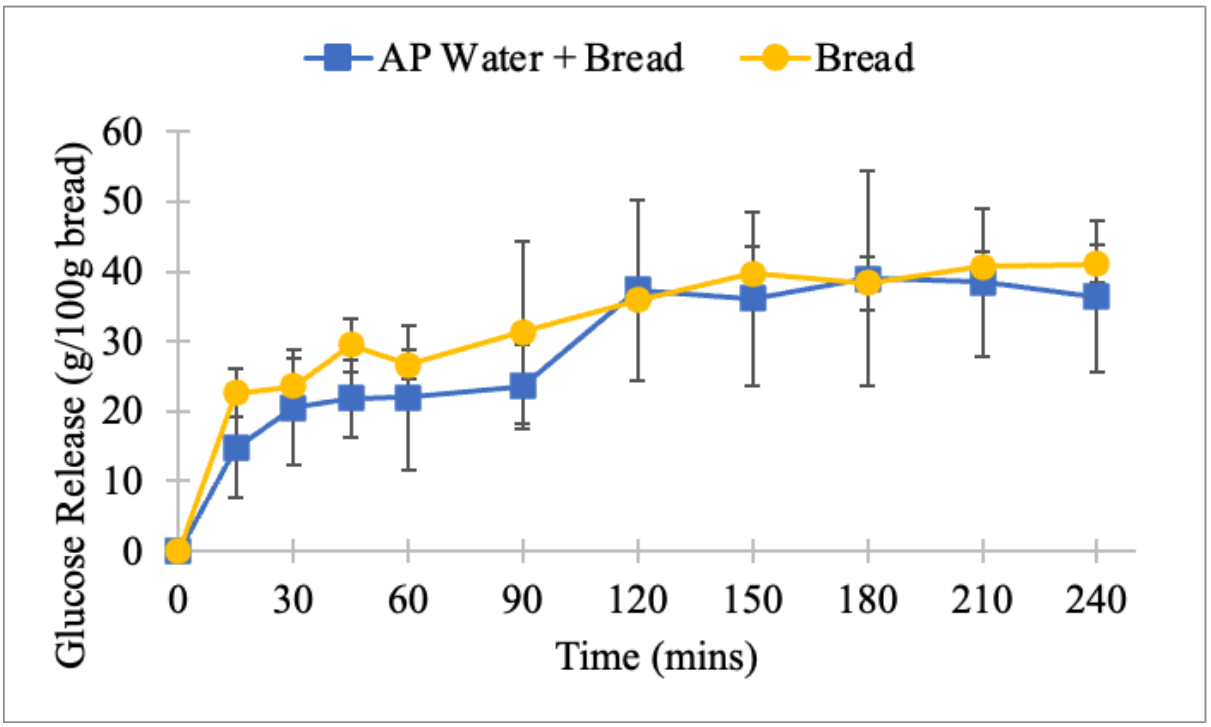


Figure 20: Glucose release during digestion of apple pomace water suspension with bread after subtracting inherent sugar in apple pomace vs. bread only. *p≤0.05, **p≤0.01, ***p≤0.001 (source: own work)

From this result, although the glucose release was lowered after subtracting the inherent glucose, the effect may still not be sufficient to say that apple pomace water suspension effectively lowered the glucose release when co-consumed with bread. T-test at p-values 0.05, 0.01, and 0.001 was done to account for differences between the control (bread) and the experimental (bread and apple pomace). The values calculated range from 0.065 to 0.873, therefore no significant differences were accounted for any time points.

Hence, minimal processing is not suitable for achieving the objective of lowering the glucose release when apple pomace is co-consumed with bread. Pre-processing should be employed to remove the inherent glucose that impacts digestion.

4.7. *In vitro* Co Digestion of Apple Pomace Acetone Extract with Bread

Table 11 shows the glucose equivalent data when the apple pomace acetone extract sample was co-digested with bread. Results are visually represented in Figure 21 compared to our bread data.

Table 11: Calculated glucose release data of apple pomace acetone extract and bread (source: own work)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	Standard Dev. (SD)	Relative Standard Dev. (RSD)	Standard Error of the Mean (SEM)
15	16.60	5.00	50.30	2.05
30	14.80	3.30	40.30	1.33
45	13.90	3.90	54.60	1.61
60	13.60	2.00	29.80	0.84
90	14.10	4.10	54.80	1.67
120	32.80	8.70	53.40	3.54
150	36.20	7.50	38.60	3.06
180	36.90	6.10	29.90	2.49
210	34.70	6.50	36.30	2.67
240	37.80	4.70	22.30	1.91

As seen in Figure 21, the glucose release is lower when acetone-extracted apple pomace is co-digested with bread. Significant differences are particularly observed between time points 30 to 90 minutes, or during the gastric phase of the digestion. This was supported by the t-test done using p-values 0.05, 0.01, and 0.001. The calculated values obtained from the t-test are 0.009, 0.001, 4.6×10^{5}, and 0.016 for 30, 45, 60, and 90 minutes respectively.

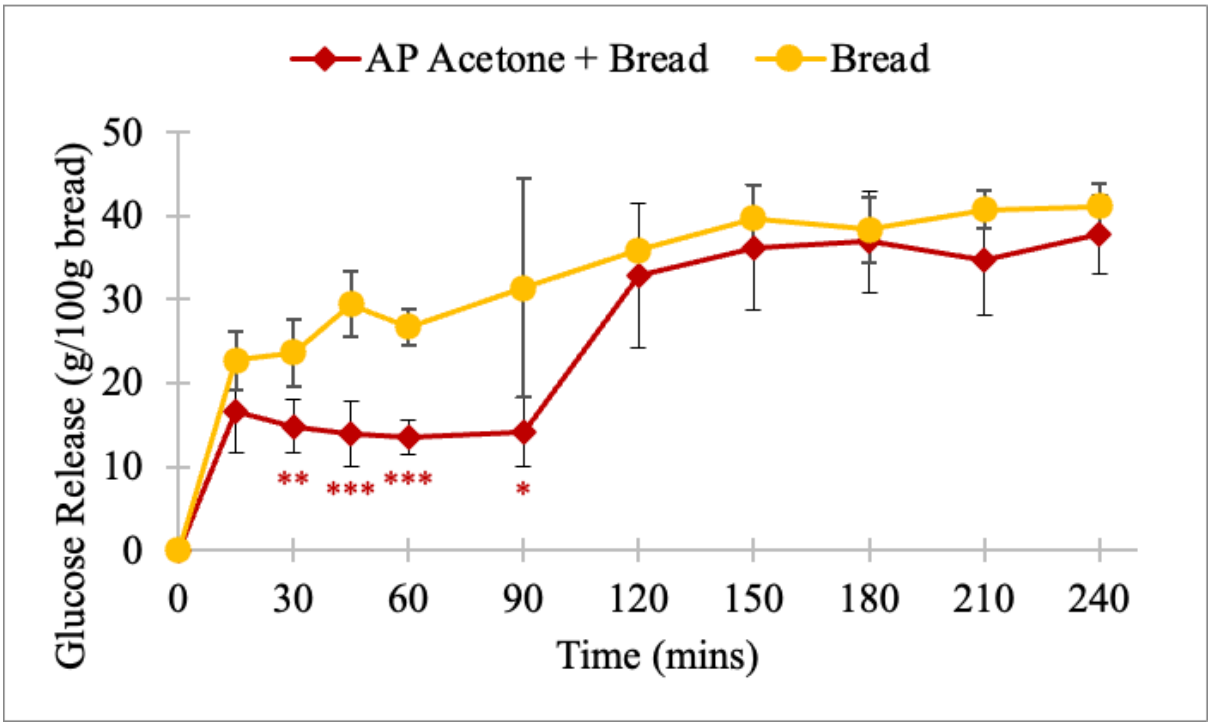


Figure 21: Glucose release during digestion of apple pomace water suspension with bread vs. bread only. *p≤0.05, **p≤0.01, ***p≤0.001 (source: own work)

4.8. Measurement of Inherent Sugar in Apple Pomace Acetone Extract

Table 12 shows the glucose release data of apple pomace acetone extract digested alone. The results show that there is a substantial amount of glucose that could be released during the digestion of apple pomace acetone extract, the sugar that does not originate from the white bread when co-digested.

Table 12: Calculated glucose release data of apple pomace acetone extract (source: own work)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	**Standard Dev. (SD)**	**Relative Standard Dev. (RSD)**	**Standard Error of the Mean (SEM)**
15	5.70	5.00	50.30	4.18
30	7.60	3.30	40.30	0.03
45	6.30	3.90	54.60	0.48
60	6.40	2.00	29.80	0.01
90	6.70	4.10	54.80	0.21
120	16.00	8.70	53.40	0.44
150	20.30	7.50	38.60	0.26
180	21.40	6.10	29.90	0.66
210	19.10	6.50	36.30	0.42
240	22.20	4.70	22.30	0.55

Figure 22 represents the glucose release from the water suspension and acetone extract from Table 9 and Table 12. It can be noted that the release from both treatments is almost similar, with a peak release during the intestinal phase of digestion from 120 to 240 minutes. This implies that apple pomace contains substantial glucose, and possible oligo-, polysaccharide that contributes to the release and should be removed before it can be utilized as a nutritional additive for food. The Idared apple cultivar has the highest total sugars among the cultivars studied by Akagić et al. (2019), containing 105.45 ± 2.42 g/kg fresh weight.

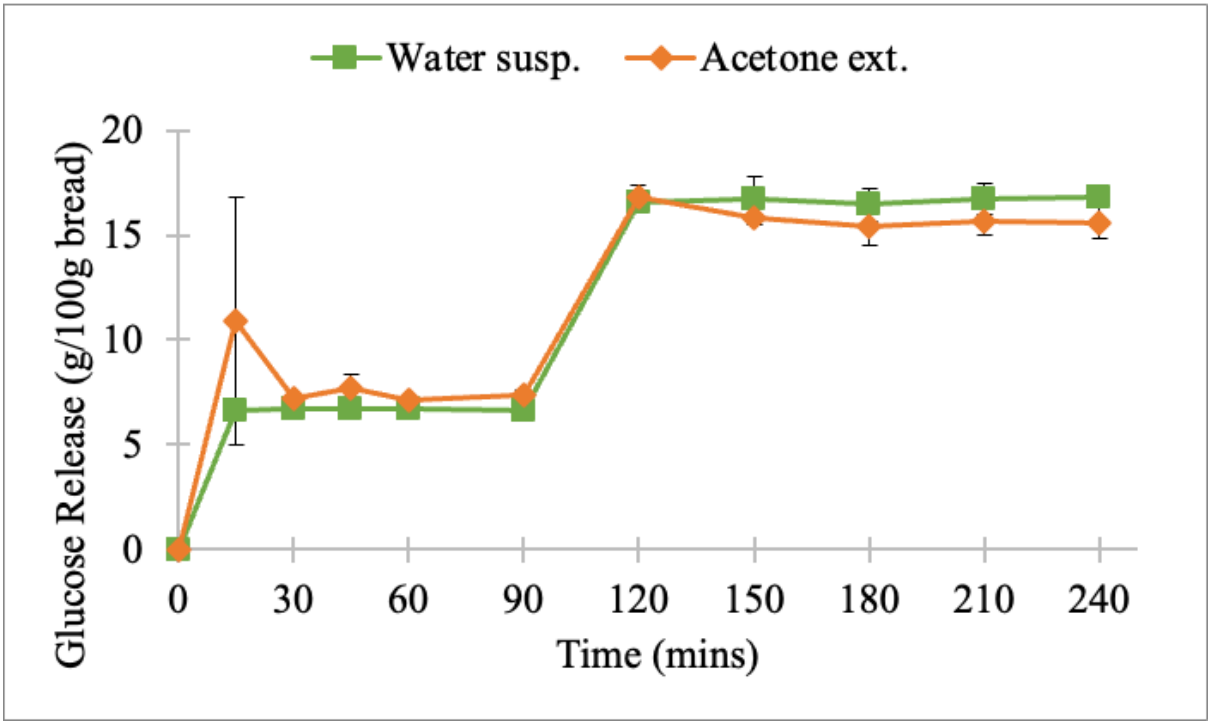


Figure 22: Glucose release during digestion of apple pomace water suspension and apple pomace acetone extract (source: own work)

We used the data from Table 12 to account for the effect of the apple pomace's inherent glucose in the co-digestion data. Table 13 below shows the glucose equivalent data of the apple pomace acetone extract and bread co-digestion when the inherent glucose was subtracted.

Table 13: Calculated glucose release data of apple pomace acetone extract and bread after subtracting inherent sugar in apple pomace (source: own work)

Time (min)	Glucose Equivalent (g/100g bread)			
	Average	Standard Dev. (SD)	Relative Standard Dev. (RSD)	Standard Error of the Mean (SEM)
15	5.70	5.00	50.30	2.05
30	7.60	3.30	40.30	1.33
45	6.30	3.90	54.60	1.61
60	6.40	2.00	29.80	0.84
90	6.70	4.10	54.80	1.67
120	16.00	8.70	53.40	3.54

150	20.30	7.50	38.60	3.06
180	21.40	6.10	29.90	2.49
210	19.10	6.50	36.30	2.67
240	22.20	4.70	22.30	1.91

Figure 23 below shows that when extracted with acetone and dried down (acetone removal), apple pomace is effective in lowering glucose release when co-digested with bread, especially during 15 to 60 minutes, and 210 to 240 minutes.

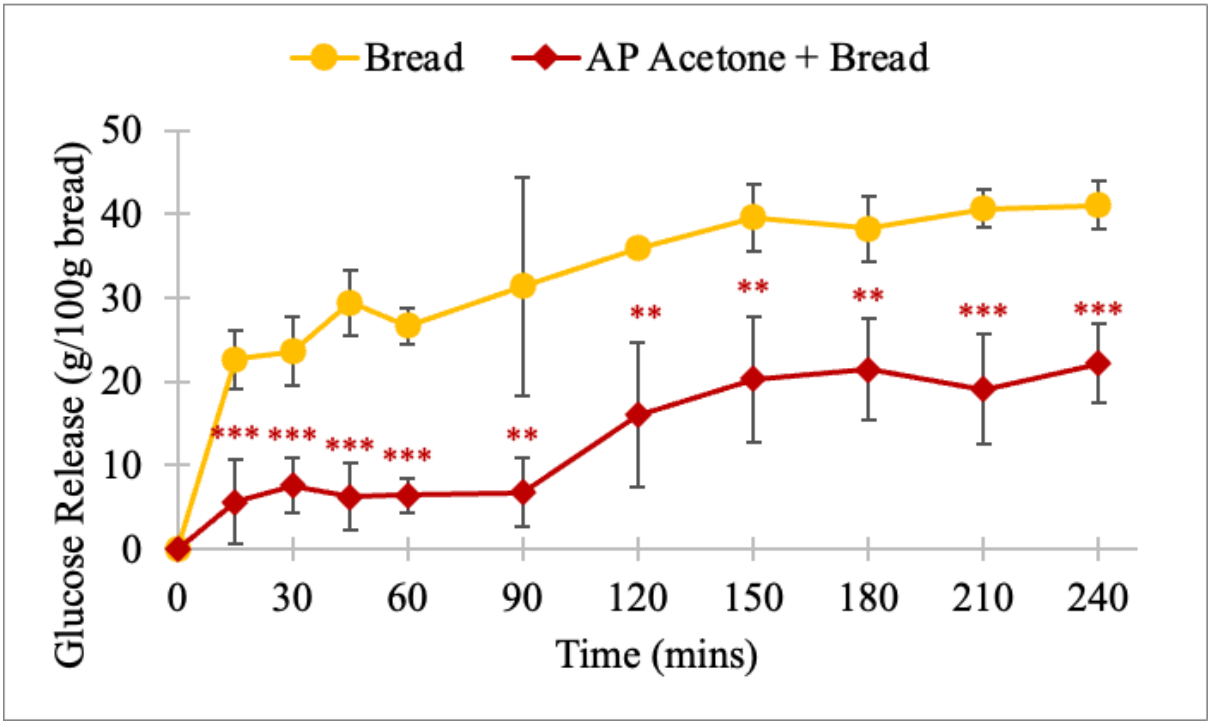


Figure 23: Glucose release during digestion of apple pomace acetone extract with bread after subtracting inherent sugar in apple pomace vs. bread only. *p≤0.05, **p≤0.01, ***p≤0.001 (source: own work)

Overall, the acetone extract is successful in lowering the glucose release at all time points of digestion compared to bread alone. This can be supported by the t-test conducted which resulted in the following values seen in Table 14.

Table 14: T-test results for apple pomace acetone extract co-digestion vs. bread only (source: own work)

Time (min)	T-statistic
15	0.001
30	3,42E-04
45	6,98E-05
60	2,55E-06
90	0.003
120	0.007
150	0.005
180	0.004
210	0.001
240	3,95E-04

Returning to the tannin equivalent for the water-suspended sample, which is 0.028 mg/g and 0.127 mg/g for the acetone-extracted one. Therefore, we can conclude that one of the possible reasons behind the changes in glucose release could be the higher amount of condensed tannin lowers the postprandial glucose of bread, particularly during the gastric phase.

Figure 24 summarizes the glucose release for all samples tested during the experiment. As discussed above, the acetone-extracted apple pomace showed the most significant effect during the digestion of bread. This suggests that apple pomace can decrease salivary α-amylase activity between 15 to 60 minutes, and possibly the enzymatic activities in the intestinal phase as well because of the significant lowering at 120 minutes onwards.

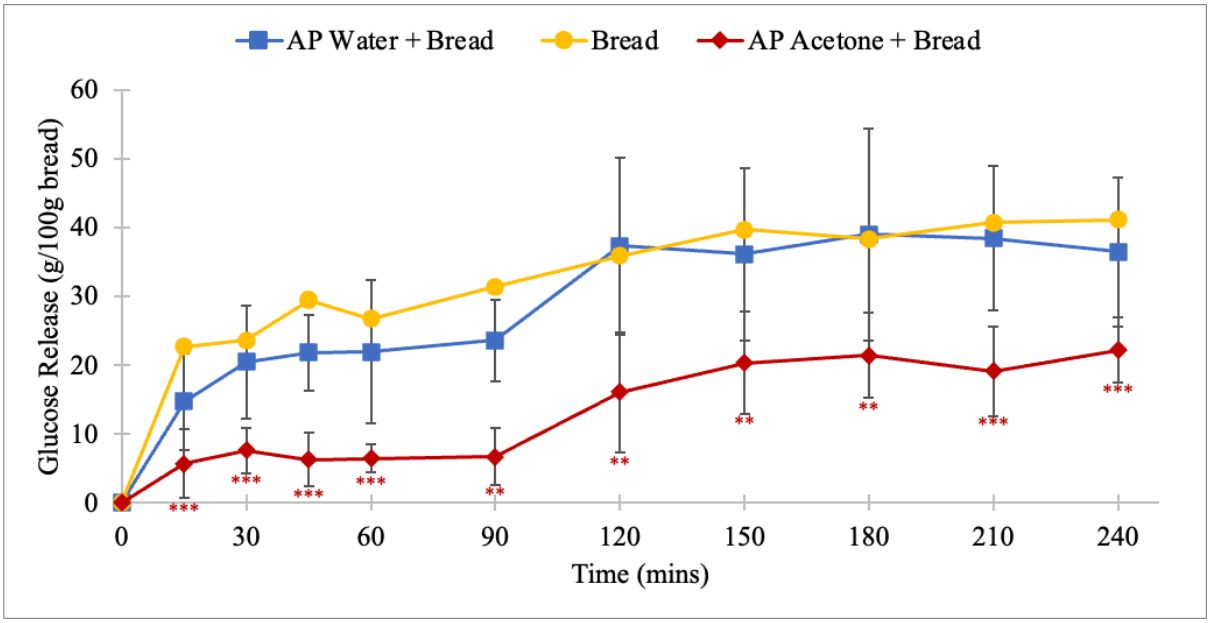


Figure 24: Glucose release comparison of bread vs. water suspension vs. acetone extract. *p≤0.05, **p≤0.01, ***p≤0.001 (source: own work)

Similar results were observed in Tormási et al.'s (2023) study of the effect of sour cherry (*Prunus cerasus*) on the bioaccessibility of white bread. They concluded that sour cherry decreases salivary amylase activity between 15 to 90 minutes of digestion.

Another study by Coe et al. (2013) was conducted using polyphenol-rich baobab fruits (*Adansonia digitata* L.) where they found that when 1.88% or higher concentrations of baobab are added to bread, there was a significant reduction in sugar release at 20 and 60 minutes into the duodenal phase of digestion compared with the control (white bread only). They further explained that since polymeric polyphenols cannot be absorbed significantly, they have the potential to bind starch molecules in food, resulting in a decrease in starch breakdown. These polyphenols may be inhibiting digestive enzymes such as α-amylase and α-glucosidase.

5. SUMMARY AND CONCLUSION

Apple pomace contains high nutritional value due to its high polyphenol content, which studies have found to be concentrated in the apple peel. However, apple pomace becomes an environmental hazard if left near the processing facility due to its high moisture content, leaving it susceptible to microbial growth and decay. Transporting bulk apple pomace can also be costly. Because of this, we want to determine how to utilize apple pomace as a nutritional component using a simple valorization technique that can be implemented in industrial settings.

This study aims to identify a simple and effective valorization technique to process apple pomace to influence carbohydrate digestion. To determine the effect of apple pomace the *in vitro* Infogest digestion protocol with a semi-dynamic gastric phase was used when consumed with white bread. We also wanted to account for the effect by determining which bioactive component can affect the release of glucose during digestion.

The apple pomace sample was dried and homogenized before extraction. Freeze-drying was done to remove moisture from the apple pomace. The dried apple pomace was ground and homogenized to increase the surface area and reduce the diffusion path for solvents, resulting in greater extraction efficiency.

After obtaining the apple pomace powder, two sample preparation procedures were used to extract possible bioactive components present in the pomace that can affect digestion: water suspension and acetone extract. The proanthocyanidin content of each apple pomace sample was measured. Lastly, the bread sample was co-digested with the apple pomace samples using a semi-dynamic Infogest *in vitro* digestion protocol.

Various researches have revealed that proanthocyanidin is one of the major phenolic components present in apple pomace. The proanthocyanidin concentrations of the apple pomace water suspension and acetone-extracted apple pomace were calculated from the measured absorbance and the use of the calibration curve. The tannin equivalent for the water-suspended sample is 0.028 mg/g and 0.127 mg/g for the acetone-extracted one. The measured tannin contents were lower than the literature values. Acetone-extracted apple pomace gave higher tannin equivalent values, while the water suspension sample produced significantly lower tannin.

Co-digestion of the water suspension sample with bread shows that apple pomace has high inherent glucose that should be adjusted or removed first to see the effect on the glucose release of bread. Therefore, the glucose content of the apple pomace water suspension was

measured by HPLC-RID. The measured glucose was 18.23 mg/g. However, when subtracted from the above data, the same pattern was observed and no difference from the initial result was noted. We inferred that apple pomace likely contains some oligo-, polysaccharides which provide additional glucose released during digestion, which was not initially available but was generated through hydrolyzation during the experiment. Therefore, we proceeded to measure the release of glucose from apple pomace during digestion. Although the glucose release was lowered after subtracting the inherent glucose, the effect may still not be sufficient to say that apple pomace water suspension effectively lowered the glucose release when co-consumed with bread.

The acetone-extracted apple pomace was used and co-digested with bread after the acetone was removed. Compared to the water suspension, this trial exhibited a more promising result with a significantly different and lower glucose release during all time points in the digestion compared to the digestion of bread alone. Overall, the acetone extract is successful in lowering the glucose release at all time points of digestion compared to bread alone, also after accounting for the inherent glucose.

Returning to the tannin equivalent for the water-suspended sample, which is 0.028 mg/g and 0.127 mg/g for the acetone-extracted one. Therefore, we can conclude that one of the possible reasons behind the changes in glucose release could be that the higher amount of condensed tannin lowers the postprandial glucose of bread, particularly during the gastric phase.

As discussed above, the acetone-extracted apple pomace showed the most significant effect during the digestion of bread. This suggests that apple pomace can decrease salivary α-amylase activity in the gastric phase, and possibly the enzymatic activities in the intestinal phase as well, because of the significant lowering throughout the digestion process.

Finally, we can say that acetone extraction of apple pomace is effective in valorizing bioactive components like procyanidin, which lowers the post-prandial glucose release in white bread.

For further studies, preliminary sugar removal is suggested to explore for further studies to have a more accurate observation of the effect. Some other factors that can be accounted for in future studies are the effect of fiber content in the digestion, other valorization solvents, the effect of time and temperature during extraction, and comparison with different drying methods for apple pomace.

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7. LIST OF FIGURES AND TABLES

8. DECLARATIONS

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Department: Inst. of Food Science & Technology
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