# Development of innovative ultrasound-treated emulsion gels for its application in dysphasia food

By

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## Abstract

Dysphagia is an oral disability characterized by swallowing difficulties, aspiration, and choking. The bulk of most prepared foods present a risk for people with dysphagia; hence their diet consists of modified-texture foods. In this study, two emulsion gels formulated with Alkylresorcinols-saponin, and Inulin-Psyllium husk combinations were assessed in the improvement of texture, stability, and enzyme inhibition of food purees. Ultrasound enhanced encapsulation and reduced droplet size to improve emulsion stability. The addition of the emulsion gels improved the consistency and Total Expressible Fluids (TEF) of the puree samples. Enzyme tests showed inhibition of α-amylase, α-glucosidase, and xanthine oxidase by AR-Saponin and Inulin-Husk emulsion gels which transferred to purees, with higher inclusions showing better inhibition. The delay in the activity of these enzymes helps to improve the physicochemical properties of foods during oral processing for people with dysphagia. Therefore, these developed emulsion gels could be beneficial in formulating modified-texture foods.

## Acknowledgements

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## List of abbreviations

| | |
|---|---|
| ARs | Alkylresorcinols |
| US | Ultrasound |
| ANOVA | Analysis of variance |
| PLM | Polarized light microscopy |
| SEM | Scanning Electron Microscope |
| HPLC | High performance liquid chromatography |
| HLB | Hydrophilic-lipophilic Balance |
| TEF | Total expressible fluid |
| W-Sa | Saponin-stabilized whey permeate emulsion gel |
| W-Sa-AR | Saponin & alkylresorcinols stabilized whey permeate emulsion gel |
| B-Sa | Saponin-stabilized buttermilk emulsion gel |
| B-Sa-AR | Saponin & alkylresorcinols stabilized buttermilk emulsion gel |

# Chapter 1: Introduction and Literature review

## 1.1 Dysphagia

Dysphagia is characterized as difficulty swallowing (an oral disorder), which may range from total lack of swallowing ability to safely ingest food, fluids, or saliva (NIDCD, 2014). In Canada, about 35% of the elder population is affected by dysphagia. Among hospitalized elders, approximately 50% have dysphagia which not only impacts their nutrition and hydration status but also medication intake and overall quality-of-life (Werstuck & Steel, 2021). Dysphagia can be caused by weak tongue, cheek, or throat muscles, making it difficult to move food around in the mouth for chewing and transferring meals to the stomach (NIDCD,2014 & Hadde et, al., 2021). Therefore, because patients with dysphagia usually require a longer oral transit time (> 3 s), they often have poorly timed swallowing and breathing which could cause food materials to incorrectly enter the airway (Hadde et, al., 2021). Aspiration, aspiration pneumonia, dehydration, malnutrition, morbidity, and death are among risks associated with dysphagia (Tagliaferri et al., 2019).

Dysphagia is clinically managed through the provision of thickened liquids and texture-modified foods. These modified foods and liquids reduce the risk of aspiration and increase hydration and nutrition. (Seshadri et al., 2018). Texture modified foods include pureed foods. Pureed foods are naturally or mechanically altered so that the original

food becomes moist, smooth and of one texture. The modification reduces muscle activity needed for chewing and swallowing (Keller et al., 2012). In the preparation of pureed foods, thickeners are an integral ingredient which improve consistency and cohesiveness and decrease syneresis (e.g., released liquid) of food products (Nishinari et. al., 2019; Alvarez et al., 2012). The use of thickener in liquids for dysphagia patients increases the viscosity of the liquid which eases swallowing. Thickened fluids tend to flow more slowly than liquids (e.g., water), providing an appropriate reflex reaction time when swallowing (Hadde et. al., 2021).

### 1.11 Hydrocolloids as thickening components

Food ingredients used as thickening agents in purees are typically hydrocolloids. Hydrocolloids are a heterogeneous group of long chain polymers (such as polysaccharides and proteins) that have an ability to form viscous dispersions and/or gels when dispersed in water (Saha & Bhattacharya, 2010). Due to the presence of many hydroxyl (-OH) groups, which bind to water molecules, hydrocolloids have a thickening effect. The formation of gels is a characteristic of some hydrocolloids and is useful in food applications. In gel formation, an aqueous solution is converted to solid through cross-linking of the polymer chains to form a three-dimensional matrix that trap the water inside it to create a structure that is resistant to flow (Saha & Bhattacharya, 2010). Therefore, hydrocolloids are usually incorporated in pureed foods to provide a

desired consistency and to reduce syneresis.

Examples of hydrocolloids used in foods include starch, xanthan gum, gum arabic, carboxymethylcellulose, carrageenan and alginate. Of these, modified starch granules are traditionally used as thickening agents in pureed food for the management of dysphagia (Newman et al., 2016).

### 1.111 Starch

Starch is a naturally occurring polysaccharide in plants consisting of glucose units connected in α-(1,4) and α-(1, 6) bonds. Starch is made up of two main structural components, amylose and amylopectin (Figure 1.1). Amylose is a linear chain molecule of α-1,4-glycosidic bonds between two glucose units, while amylopectin is a highly branched molecule with α-1,4-glycosidic bonds between two glucose units in the straight chain and α-1,6-glycosidic bonds at the branch (Figure 1.1) (Ismail et al.,2013). Starch is the most used thickener in texture modified foods because it is relatively cheap and abundant, and it does not impart any foreign taste (Saha & Bhattacharya, 2010). When starch is heated with water, it absorbs water and swells, resulting in an acute increase in liquid viscosity (Newman et al., 2016 & Egharevba, 2019). Therefore, starch-based thickeners are traditionally used in the management of dysphagia

(Newman et al., 2016).

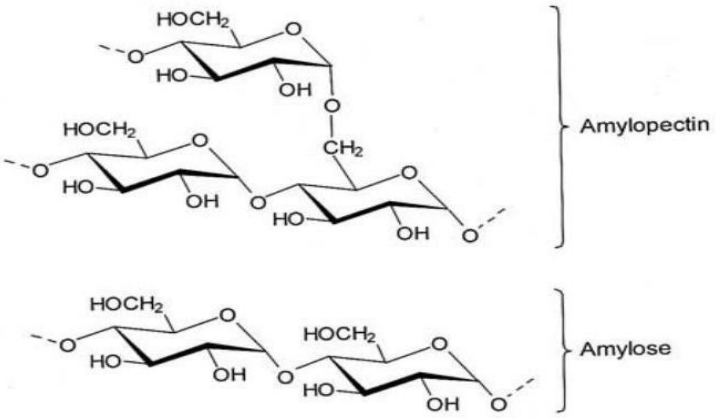


**Figure 1.1 Structure of amylopectin and amylose in starch (Ismail et. al., 2013).**

Despite the advantages of starch mentioned above, research has shown that starch-based thickeners have numerous limitations in clinical applications. For example, its viscosity is decreased during oral processing (Payne et al., 2012) or it might lead to an unwanted grainy texture in food (Matta et al., 2006). Previous study has reported that the viscosity of starch-thickened liquid was reduced by 90% in 10 seconds of oral processing (Hanson et al., 2011). The sudden decrease of viscosity in starch-thickened food during oral processing is due to the action of α-amylase, an enzyme presented in saliva. The amylase catalyzes the hydrolysis of α-1,4 glycosidic linkages between glucose units in starch, resulting in the breakdown of amylose and amylopectin. (Sukkar et al., 2018 & Souza, 2010). Hence, the stability of thickener to carbohydrate-hydrolyzing enzymes, should be taken into consideration in food preparation for dysphagia patients (Sukkar et al., 2018).

## 1.112 Carbohydrate-hydrolyzing enzymes

The main carbohydrate-hydrolyzing enzymes are α-amylase and α-glucosidases. α-amylases are hydrolytic enzymes that act upon the α-(1,4)- and/or α-(1,6)-linkages of starch polymers (Goesaert et al., 2009). When food is ingested, α-amylase in saliva randomly hydrolyses α-(1–4) glycosidic bonds of starch components to produce oligosaccharides of various lengths and a different α-limit dextrins with have α-(1–6) bonds (Figure 1.2). As a result, the digestion process also breaks down the mechanical structure of food, which is created by starch granules. It could also lower the viscosity of fluids that are thickened by starch-based thickeners (Hanson et al., 2011). The oligosaccharides and dextrins released from amylase action on starch can be further hydrolyzed into monosaccharides by α-glucosidases which. catalyze the hydrolysis of α-1,4 and α-(1,6) bonds (Tomasik & Horton, 2012).

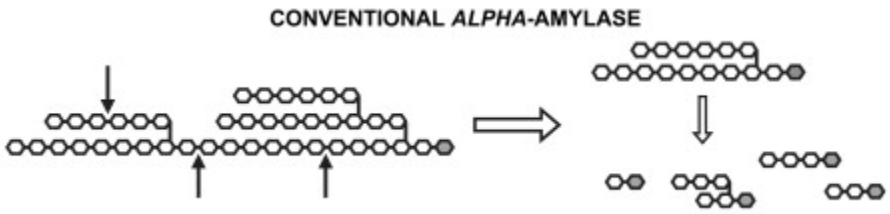


**Figure1.2 Schematic representation of the action of α-amylase on starch (amylopectin) polymers, yielding branched and linear dextrins. The gray ring structure represents a reducing glucose residue (Goesaert et al., 2009).**

## 1.12 Salivary antioxidant activity and xanthine oxidase analysis

Effective improvement of the swallowing function of patients with dysphagia can actively reduce the risks associated with dysphagia (such as aspiration, pneumonia and malnutrition) and lead to an improvement in the prognosis and overall quality-of-life (Liang et al., 2021). It has been reported that dysphagia could be caused by mucositis (inflammation of the mucosa) which is associated with decrease of salivary antioxidant activity (Rosenthal et al., 2006 & Avivi et al., 2009). If the salivary antioxidant molecules fail to overcome the oxidative damage, it might promote mucosal injury and mucositis-related symptoms (such as dysphagia).

The detection of salivary antioxidant activity can be achieved by using the assay involving the enzyme xanthine oxidase (Avivi et al., 2009). Xanthine oxidase is the only enzyme which is linked with the production of substantial levels of superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$) (Özyürek et al., 2009). Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid, and molecular oxygen ($O_2$) is reduced to $O_2^-$ and $H_2O_2$ (Figure 1.3) This process increases the oxidative stress in the body and high oxidative stress further lead to pathological conditions and diseases such as vascular damage, inflammatory disorders, and chronic heart failure (Özyürek et al., 2009).

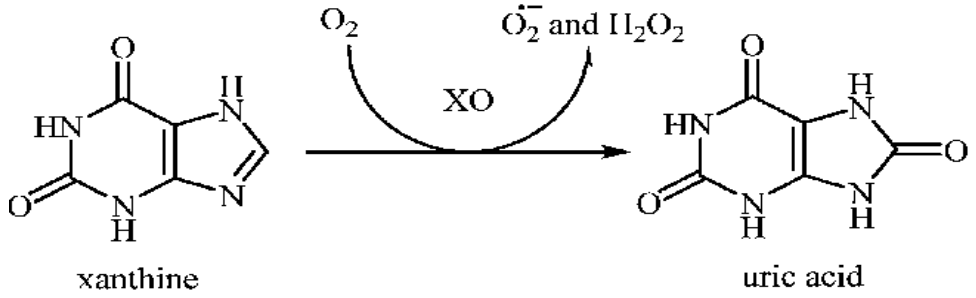


**Figure 1.3 The conversion of xanthine to uric acid by xanthine oxidase (Özyürek et al., 2009)**

## 1.2 The use of emulsions in modified-texture foods

Although hydrocolloids can be used as thickener in modified-texture foods, they are not all capable of maintaining the stability of the modified food. Reports of limitations in the use of hydrocolloid as thickener in modified-texture foods include an undesirable viscosity declination with starch-based hydrocolloids. Non-starch gum thickeners would have higher stickiness, which negatively affects swallowing (Raheem et al., 2021). In addition, some hydrocolloids, such as psyllium husk gum, have extremely strong gel-forming ability and water-absorption property, and so forms a solid gel rapidly when it binds with water, making it difficult to incorporate into food homogeneously (Yu et. al., 2003).

To overcome above mentioned limitations, development of an emulsion for use in modified-texture foods to enhance consistency and cohesiveness, and to reduce food syneresis are considered. Besides, applications of emulsions is expected to continue to grow in the food industry because emulsions in food have multiple functional attributes,

such as lowering calories, improving digestion behavior, and enhancing bioavailability of bioactive compounds (Tan & McClements, 2021).

## 1.21 Emulsions

An emulsion is a dispersion of one liquid in another immiscible liquid, most commonly, oil and water. The spatial distribution of the different phases relative to each other in the system is used to classify emulsions. For example, a system in which oil droplets are dispersed in a watery phase is known as an oil-in-water (O/W) emulsion, and a system in which water droplets are dispersed in an oily phase is a water-in-oil (W/O) emulsion (McClements, 2010). The main types of emulsions are oil-in-water (O/W) and water-in-oil (W/O) emulsion, oil-in-water-in-oil (O/W/O) emulsion and water-in-oil-in-water (W/O) emulsion (Figure 1.4).

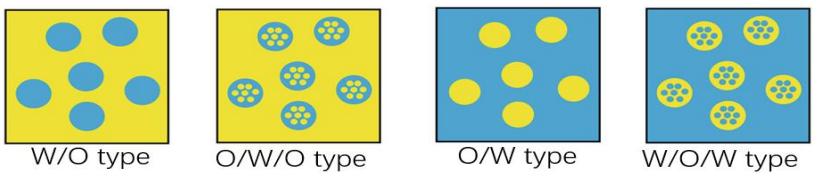


**Figure 1.4 Main types of emulsions (Lu et.al., 2019).**

Although emulsions are used to alter the texture and increase the palatability of food, as a sole component, they do not offer the viscosity stability that is needed for modified-texture foods. However, emulsions can be created with hydrocolloids to improve modified-texture foods suitable for people with dysphagia.

## 1.22 Emulsion gels

Emulsion gels are formed by gelling the continuous phase of emulsions or by aggregating the emulsion droplets through the addition of hydrophilic polymers (Figure 1.5). With the gelling of the continuous phase, the resulting medium is a soft solid that can entrap emulsified lipid droplets inside the gel matrix. Common food emulsion gels include cheese, yogurt, several dairy sweets, and reformulated meat products are examples of common food emulsion gels (Lu et.al., 2019). The nature of emulsion gels means they possess the properties of emulsions in terms of delivering lipid-soluble components (Lamprecht et. al., 2001), and the physical stability and mechanical properties of gels (Torres et. al., 2016). As a result, functional compounds incorporated into emulsion gels often have better storage stability compared with when incorporated into emulsion. The storage stability is due to limited oil movement within the emulsion gel systems (Cofrades et.al., 2017). Emulsion gels with desirable textural and characteristics can be achieved by adjusting the oil/water phase fractions, droplet distribution, interfacial composition, and gelation triggers (e.g., salt, heat, enzyme) (Mao et. al., 2014). The adjustable textural properties have made emulsion gels widely used in fat-reduced foods.

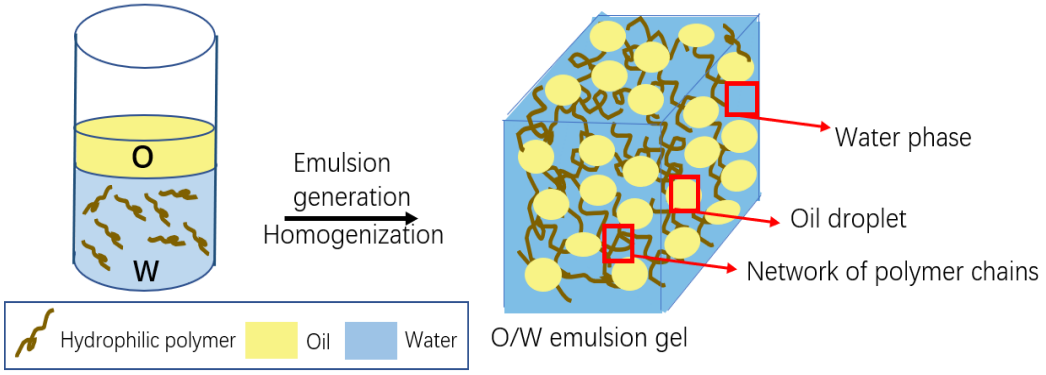


**Figure 1.5 The formation of an emulsion gel (O/W), adapted from Lu et.al. (2019).**

## 1.23 Emulsion stability and Emulsifiers

The capacity of an emulsion to resist change in appearance over time is characterized as emulsion stability. Storage instability of emulsions is evident by processes such as sedimentation, flocculation, Ostwald ripening, coalescence, and phase inversion, (Figure 1.8). (Yvonne and Victoria, 2018).

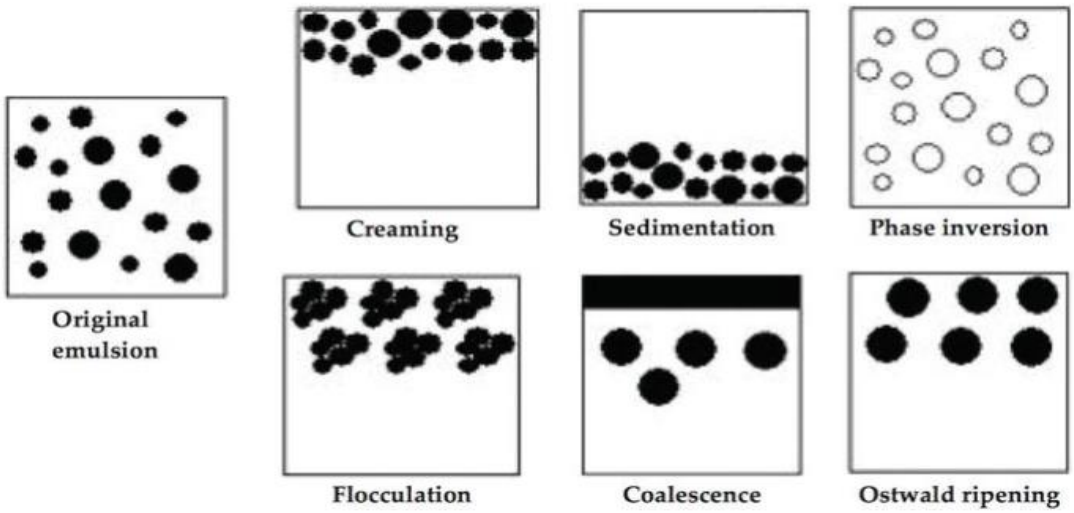


**Figure 1.6 Mechanisms of emulsion destabilization, adapted from (Yvonne and Victoria, 2018).**

Factors that affect emulsion stability include droplet size, droplet dispersion, density between the dispersed and continuous phases, and the chemical integrity of the dispersed phase (Huan & Vardhanabhuti, 2013).

Emulsion stability is a critical factor in determining the shelf-life of food products (Juttulapa et.al., 2017). To assess the stability of an emulsion, centrifugation is used to accelerate phase separation. During this test, droplets with a high density move to the bottom of the centrifuge tube and then form a layer after centrifugation. In the need to improve on storage stability, compounds known as stabilizers are often added to emulsion preparations. The stabilizer is included to improve the kinetic stability of the emulsion. Based on the mode of action, stabilizers can be classified as emulsifiers, texture modifiers, weighting agents, or ripening inhibitors (David, 2015). Emulsifiers are surface-active substances having amphiphilic qualities that can interact with the oil–water interface to stabilize droplets and prevent droplet aggregation (Jacobsen, 2016). Proteins, polysaccharides, and phospholipids are common emulsifiers used in food products. A texture modifier (such as hydrocolloids) improves emulsion stability by thickening or gelling the continuous phase to slow or prevent droplet movement (David, 2015). Weighting agents are hydrophobic components that included into the dispersed (oil) phase of an emulsion to decrease the density disparity between the phases and thus avoiding gravitational separation of oil droplets and surrounding liquid (Piorkowski & McClements, 2014).

In the food industry, a combination of proteins and polysaccharides are often employed as emulsifiers to their benefits such as rapid adsorption, steric repulsion, and viscosity increase (Juttulapa et.al., 2017). When protein and polysaccharide are mixed, protein-polysaccharide complexes are produced by electrostatic contact, resulting in higher emulsion stability and longer shelf life (Juttulapa et.al., 2017).

## 1.24 Ingredients used in the formulation of emulsion gels

### 1.241 Whey permeate

Whey permeate is a by-product of cheese manufacturing. Whey permeate is utilized as an emulsifier because it has amphiphilic proteins, which include -lactoglobulin, -lactalbumin, and bovine serum albumin (Kiokiaset.al., 2007). Along the polypeptide chain of these proteins are a uniform distribution of hydrophilic and hydrophobic acidic and basic amino acids (Minj & Anand, 2020). The amphiphilic properties of these proteins enable them to orientate themselves at the oil-water or air-water interface and to reduce the surface tension. Whey proteins are denatured, and exhibit unfolded molecular structures at temperatures over 70°C, resulting in an increase in the number of non-polar and sulfhydryl groups and an increase in the hydrophobic contacts operating between the droplets and aggregation of the protein fractions (Chanasattruet.al., 2009). Whey proteins partly unfold at lower temperatures, which

improves their adsorption at the interface and forms a thicker protein coating, increasing their emulsifying capacity and emulsion stability (Kiokiaset.al., 2007).

### 1.242 Alginate polymer

Alginates are indigestible polysaccharides that are typically obtained from brown marine algae. The structure is an unbranched polymer consisting of D-mannosyluronic acid (M) and L-gulusyluronic acid (G) residues linked together by 1,4-glycosidic linkages (Figure 1.7). Alginate is a cold-water soluble polymer; therefore, it can form gels without heat treatment, and the generated gel is heat stable (Takarini et.al., 2017). Alginate is also a polyuronide, which is a natural ion exchanger. The presence of bivalent cations (such as $Ba^{2+}$ and $Ca^{2+}$) trigger conformational changes in alginate. Changes like an alignment of the G-blocks due to ion exchange between two chains and the development of divalent salt bridges, which consequently lead to the formation of a gel (Senturk et.al., 2018).

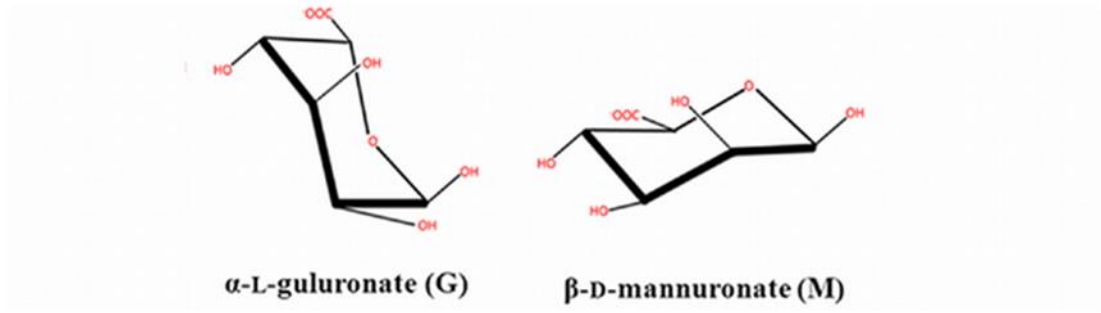


**Figure 0.7 Chemical structure of alginate, adapted from (Paredes et.al., 2014).**

### 1.243 Buttermilk

Buttermilk is a by-product of butter manufacturing. It contains skim milk proteins as well as material, made up of proteins and phospholipids, derived from the original milk fat globule membrane (MFGM) (Corredig, & Dalgleish, 1997). MFGM is important in fat globule stability in the milk, for example keeping the milk fat in suspension (Corredig, & Dalgleish, 1997 & Jiménez-Flores, 2022). Milk fat globules are coated by MFGM to maintain the structural integrity and to reduce surface tension. The coating ability of MFGM is due to amphiphilic properties imparted by its components (Malik et. al., 2015).

### 1.244 Inulin

Inulin is a dietary fiber (polysaccharide) in which D-fructose subgroups are bonded in β (2→1) linkage and attached to an end glucose residue with a β (1→ 2)) bond (Figure 1.8). The number of fructose units in inulin can vary from 2 to 60 (Shoaib et.al., 2016). Inulin is soluble in water and would bind with water to form a gel at 20°C at high concentrations (>15% (w/w)) for a degree of polymerization of 9 or higher (Mensink et.al., 2015). Inulin gels have a white creamy appearance with a spreadable texture and properties similar to those of a fat crystal network in oil. As such inulin has been used as fat replacer in spreads, butter-like products, cream cheeses to provide a spreadable texture as well as in milk drinks and yogurts where it imparts a creamy mouthfeel

(Chiavaro et. al., 2007).

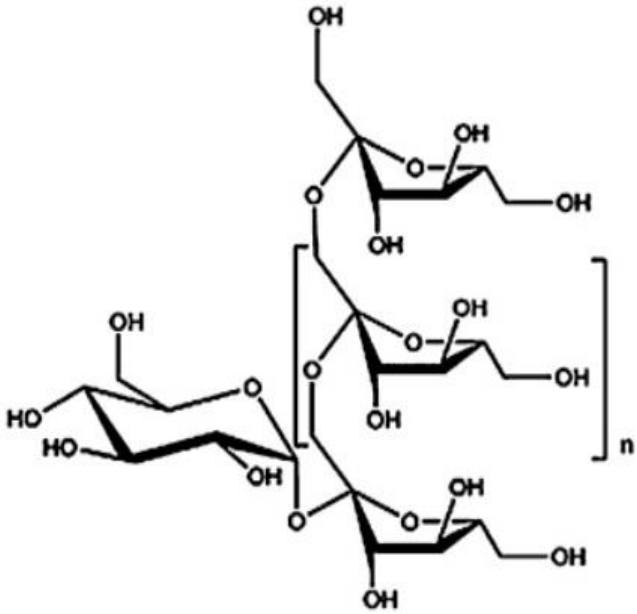


**Figure 1.8 Chemical structure of inulin (Shoaib et.al., 2016)**

## 1.245 Psyllium husk

Psyllium husk is obtained after milling of the psyllium seeds and is an excellent source of both soluble and insoluble fiber (Raymundo et al., 2014). It contains a high proportion of hemicellulose, composed of a xylan backbone linked with arabinose, rhamnose, and galacturonic acid units (arabinoxylans) by β (1→3) and β (1→4) glycosidic linkage (Figure 1.9). About 80% of the fiber in the husk is soluble and classified as a mucilaginous fiber due to its powerful ability to form a gel in water (Raymundo et al., 2014). It is reported that weaker psyllium husk gel will be formed when psyllium husk is hydrated at temperatures lower than 60°C due to the formation

of longer sidechains or (1→3) linkages which enhance microstructural heterogeneity and increase distribution of molecular arrangement in the psyllium gel, and hence weaken the gel (Ren, et al., 2020). Hence, many studies suggest hydrating the psyllium husk at temperatures around 80 °C (Guo et al., 2009 & Ren, et al., 2020). Psyllium husk is used in the food and pharmaceutical industries as a thickening agent, colloidal stabilizer, and gelling agent because psyllium mucilage characteristics make them unique for utilization as a matrix for transport and/or for trapping of various drug types, proteins and cells (Agrawal, 2021).

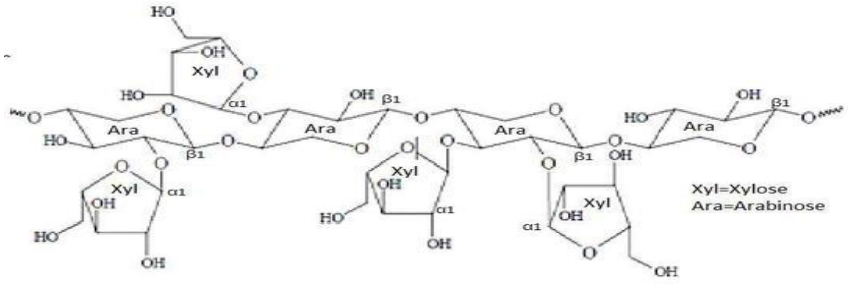


**Figure 1.9. Backbone structure of psyllium fiber (Agrawal, 2021).**

Saponins

Saponins are natural surface-active agents which are found mainly in plants, especially *Quillaja saponaria* Molina tree (Wojciechowski et. al., 2014). The saponin from the extract of *Quillaja saponaria* Molina's bark is mainly triterpene aglycones, which is composed of two polar sugar chains and one non-polar aglycone linked by a glycosidic bond (Figure 1.10). The amphiphilic properties of *Quillaja* saponin enables its application in the food, pharmaceutical and cosmetic industries as a foaming agent, emulsifier, nutraceutical, and cholesterol lowering agent (Banerjee et. al., 2021 & Wojciechowski et. al., 2014). *Quillaja* saponins are able to produce emulsion with nano-sized droplets (d < 200 nm) at low emulsifier-to-oil ratios (0.1:1). it can generate strong electrostatic repulsive forces between emulsified droplets and hence provide broad-

ranging stability to emulsions against pH, ionic strength, and temperatures (Dahlawi et al., 2020).

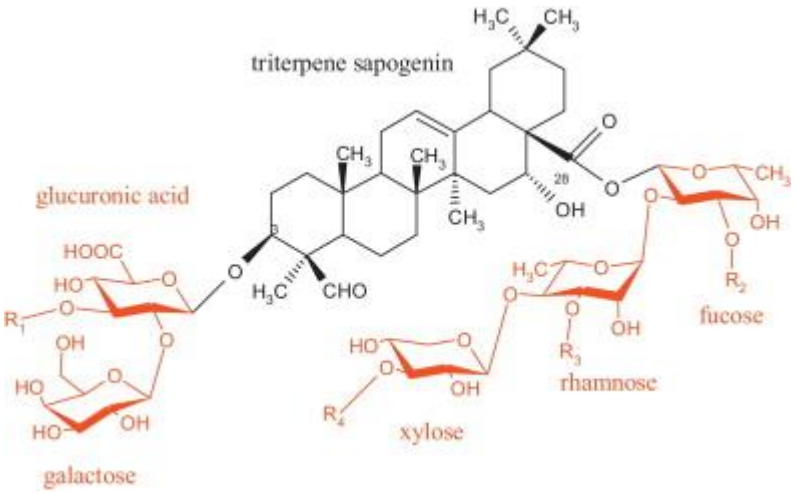


**Figure 1.10 Basic structure of Quillaja bark saponin. R1–R4 indicate H atoms or sugar groups. The hydrophilic parts of Quillaja bark saponin are highlighted in red (Wojciechowski et. al., 2014).**

## 1.246 Wheat Alkylresorcinols

Alkylresorcinols (ARs) are phenolic lipids which are mostly found in bran fraction of cereal such as wheat, whereas no, or little AR are presented in starchy endosperm and germ (Ross et.al. 2001). ARs are 1,3-dihydroxy-5-alkylbenzene homologues with odd-numbered alkyl side chains connected to the benzene ring at position 5 (Agil et. al., 2012). The presence of a polar "head" is soluble in water (dihydroxybenzene group) while the non-polar alkyl "tail" is insoluble in water which gives the ARs their amphiphilic nature (Figure 1.11). The longer the aliphatic chain, the more hydrophobic the AR. The alkyl tail of alkylresorcinols may vary in degrees of unsaturation, chain

length, ring, or have a keto or hydroxyl group replaced on the alkyl chain depending on the source (Ross et.al., 2004). Primary homologues of ARs found in wheat are C17:0, C19:0, C21:0, C23:0, and C25:0 a (Ross et.al., 2004).

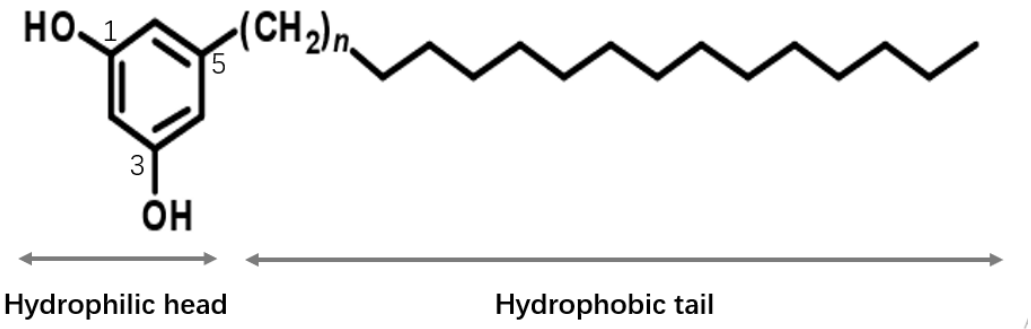


**Figure 1.11 Basic structure of Alkylresorcinols**

### 1.3 Classification of emulsifiers

With a variety of food emulsion systems and compounds that could act as emulsifiers, it becomes difficult to choose the best emulsifier for a food product. As such, classification based on hydrophilic-lipophilic balance (HLB), ionic charge and crystal stability can serve as a performance indicator of emulsifiers in a particular medium (Msagati, 2012)

#### 1.3.1 Hydrophilic-lipophilic Balance (HLB)

The HLB value denotes the activity of the emulsifier and the type of emulsion in which the emulsifier would be suitable for (Hasenhuettl and Hartel, 2008). Based on the

chemical structure of the emulsifier, the HLB value is calculated using the equation HLB = 7 + Σ (hydrophilic group numbers) + Σ (lipophilic group numbers) (Guo et. al., 2006 & Khoshdast et. al., 2012). Table 1.1 provides the values for the reactive groups of chemical compounds used in HLB calculation.

**Table 1.1 the value of hydrophilic and lipophilic groups for calculating the HLB**

| hydrophilic groups | value | lipophilic groups | value |
|---|---|---|---|
| -O- | 1.3 | =CH- | − 0.475 |
| Free -OH | 1.9 | $-CH_2-$ | − 0.475 |
| $-CH_2CH_2O-$ | 0.33 | $-CH_3$ | − 0.475 |
| -COOH | 2.1 | $-CH_2-CH_2-CH_2-O-$ | − 0.15 |
| -OH (sorbitan ring) | 0.5 | -CH- | -0.475 |

Hydrophilic emulsifiers with higher HLB values (10-18) (Figure 1.12) are used to stabilize and emulsify oil-in-water (O/W) emulsions. On the other hand, lipophilic emulsifiers with lower HLB (3-6) (Figure 1.12) were shown to be beneficial in stabilizing water-in-oil (W/O) emulsions (Pant, Jha & Singh, 2019)

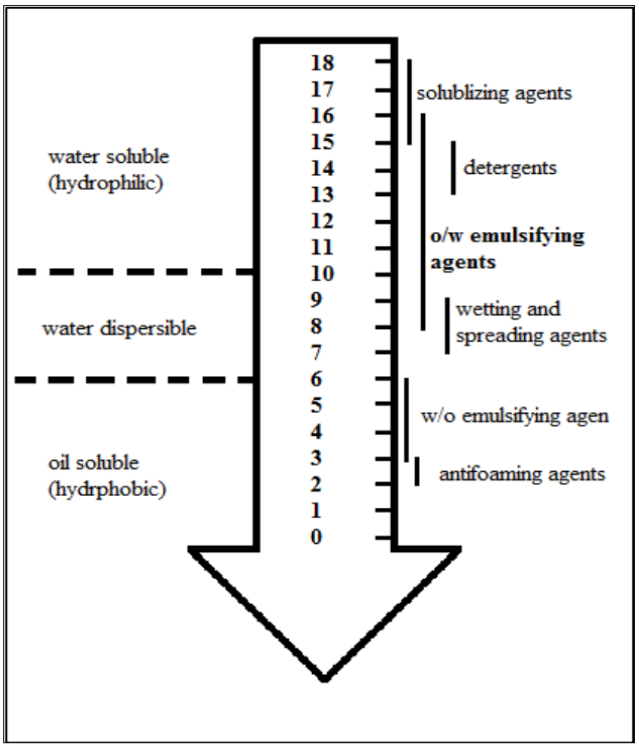


**Figure 1.12 HLB Scale for emulsifiers (Pant, Jha & Singh, 2019).**

The HLB value of the emulsifier is also used to determine which oils would be best for creating the emulsion. This is because different types of oil have different HLB requirements (Pasquali, Taurozzi, & Bregni, 2008). The selected emulsifiers should have HLB values that are equivalent to the corresponding oils to achieve maximal emulsion stability. If two or more emulsifiers are used in an emulsion, their ratio required to reach HLB value of certain oil phase is calculated by:

$$\%(A)=\frac{100(X-HLB_{(B)})}{HLB_{(A)}-HLB_{(B)}}$$

$$\%(B)=\%100-\%(A)\ ,$$

Where A and B are two kinds of emulsifiers. X is the blended HLB (Griffin, 1954).

## 1.4 Ultrasonic homogenization

The formation of emulsions requires energy to agitate the two immiscible phases together. Ultrasonic homogenization is a high-energy emulsification process that is also a cost-effective, energy-efficient, easy, and environmentally friendly approach to break up aggregates and generate tiny droplets with a narrow size distribution (Leong et.al., 2017). Smaller droplet size can improve emulsion stability by avoiding gravity separation; hence, ultrasound-treated emulsion has higher stability than that of untreated emulsion. Ultrasonic wave at the range from 20 to 100 kHz has extensively been used for controlling and improving physicochemical properties of food components and products (Ashokkumar, 2015; Jayasooriya et.al. 2004). Two mechanisms have been proposed for ultrasonic emulsification. Firstly, when ultrasonic waves flow through a liquid medium, they cause molecular mobility, which induces the oil phase to disperse into the water phase in the form of mid- to large-sized droplets. Secondly, the ultrasonic field will create acoustic cavitation (Figure 1.12), which could further induce shear force by rapidly collapsing cavitation bubbles, resulting in the breaking up of the originally formed droplets of dispersed oil into sub-micron sizzed droplets. (Abbas et.al., 2013 & Leong et.al., 2017)

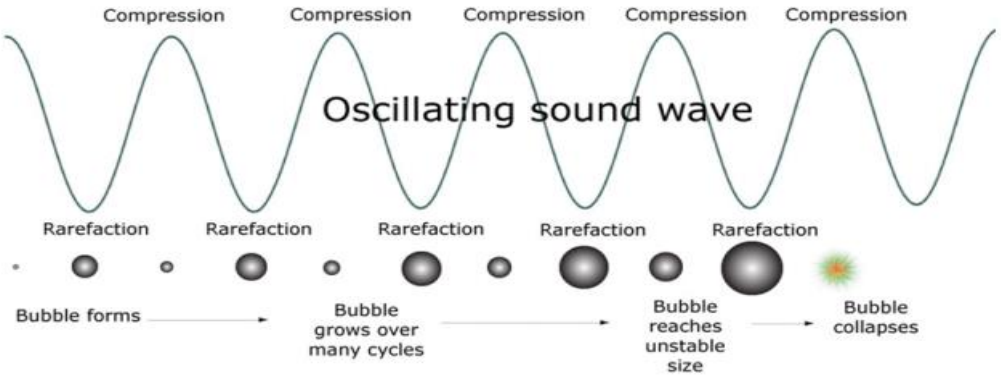


Figure 1.12 Growth and collapse of bubble in acoustic cavitation process (Leong et.al. 2011).

## 1.5 Industrial challenges

Industrial production of food for people with dysphagia is mostly pureed food (Raheem et.al., 2021). To promote ease of swallowing, these pureed foods should be moist, smooth and of one texture. Therefore, hydrocolloids are frequently used in pureed foods because they provide a desired texture, consistency, reduce syneresis and improve appearance. However, the food modified by the hydrocolloids are not usually stable throughout storage and oral processing. For these reasons, studies are looking into the application of emulsion gels to modify pureed food for patients with dysphagia.

## 1.5 Hypothesis and objectives

During storage, syneresis occurs in pureed foods which makes them less palatable. In addition, the purees without any modification do not afford good levels of inhibition to carbohydrate hydrolysis enzymes, α-amylase, and β-glucosidase.

To this effect, HFS Apetito, a company that produces pureed foods for hospitals, provided food samples for this research.

### 1.51 Hypothesis

This research is aimed at creating novel emulsions gels for application in modified-textured foods for people with dysphagia.

that:

(1) Utilization of ultrasonic homogenization for emulsion gel preparation could improve emulsion gel stability.

(2) Incorporation of saponin-wheat alkylresorcinols (ARs) emulsion gel or allergy-free inulin-husk emulsion gel could improve the physicochemical properties and decrease the activity of alpha amylase, alpha-glucosidase, and xanthine oxidase in puree samples.

## 1.52 Objectives

The objectives of this study were to:

(1) To study the effects of combination of wheat ARs and saponin, and ultrasonic homogenization on stability of emulsion gels and food purees containing the gels.

(2) To develop and optimize food-grade psyllium husk emulsion gel by using inulin as delivery ingredient for incorporation into food purees.

(3) To investigate the inhibitory effects of wheat ARs emulsion gel and psyllium husk emulsion gel on the activity of alpha-amylase, alpha-glucosidase, and xanthine oxidase in puree samples.

# Chapter 2: Formulation of a stable wheat bran-phenolic lipid emulsion gel using ultrasound and the enzyme inhibitory effects of the formulated gel alone and incorporated into purees.

## 2.1 Introduction

Alkylresorcinols (ARs) are amphiphilic phenolic lipids mostly found in bran fraction of cereals such as wheat, rye, and barley (Ross et.al. 2001; Landberg e al., 2014). The amphiphilic nature of ARs make a case for their use as potential emulsifier to develop innovative emulsion gels. Whole cereals are known to have better effect on blood glucose levels compared with refined cereals. The mechanism for this effect is most likely due to the presence of compounds that act as α-amylase and α-glucosidase inhibitors. Extracts prepared from parts of cereals such as bran, hulls, milled grains, germinated grains etc. from wheat, millet, corn, exert different inhibitory levels based on the components of the extract (Gong et al., 2020). ARs are some of the major components found in wheat bran extracts that have been studied to have antioxidant, anti-mutagenic, anti-cancer (Landberg et al., 2014) and α-glucosidase inhibition (Tu et al., 2013).

Therefore, the formulation of an emulsion gel with wheat AR could impart the benefits of enzyme inhibition and improved emulsion stability to food products prepared for people with dysphagia.

As much as the ingredients are important in emulsion stability, so is the mode of preparation. Ultrasonic homogenization is a high-energy emulsification process that is also cost-effective, energy-efficient, easy, and an environmentally friendly approach to break up aggregates and generate tiny droplets with a narrow size distribution (Leong et al., 2017). Ultrasound use and its effect on emulsion gels formulated with wheat AR are also assessed in this study.

## 2.2 Materials and Methods

### 2.21 Material

AC Carberry wheat (cultivar Ultima) bran was received from Alberta Agriculture, Food and Rural Development (Edmonton, Alberta, Canada).. Ethanol was purchased from Global greenfield (Brampton, ON, Canada). Acetic acid was purchased from Aldrich Chemical Company Inc. Methanol and Tween 80 (polyoxyethylene-20-sorbitan monooleate) were purchased from Fisher Scientific Company (Ottawa, ON, Canada). Canola oil from Saporito foods (Montreal, Quebec, Canada). Whey permeate (Darisweet 200) was purchased from Parmalat (Toronto, ON, Canada). Sodium alginate (Landor Trading Company) was purchased from Amazon. ARs extracts was obtained from previous section. Sodium carbonate was from VWR International Co. (Mississauga, ON, CA). α-glucosidase (Cat. no. G5003), α–amylase (Cat. no. A3403),

P-NPG (p-Nitrophenyl-α-D-glucopyranoside, Cat. no. 487506), xanthine (Cat. no. X7375), xanthine oxidase from bovine milk (Cat. no. X1875), 3,5-Dinitrosalicylic acid (Cat. no. 128848), potassium sodium tartrate tetrahydrate (Cat. no. S2377), Quillaja bark saponin (Cat. no. 84510, sapogenin 8–25%) and AR standards (C15:0, C17:0, C19:0, C21:0, C23:0, and C25:0) with 95% purity were from Sigma-Aldrich (St Louis, MO, USA). Salmon puree, chicken puree, chicken stew puree and beef puree were kindly provided by HFS-Apetito.

**2.22 Solvent extraction of ARs**

Solvent extraction of ARs was conducted following the method of Gliwa et.al. (2011). Wheat bran was mixed ethanol in a ratio of 1 :40 (w/v, g/ml) for 24 h with continuous stirring on a stirring plate (CorningPC 420D, Corning Inc., NY, US) at room temperature. The solution was then filtered using Whatman paper P5 and evaporated to dryness in a Rotavapor R-245 (Buchi-Brinkman, R100 Switzerland). The dried extract was collected, weighted, and stored at 4°C before analysis.

**2.23 HPLC analysis**

Alkylresorcinols (ARs) analysis were performed using a Waters Alliance® HPLC system e2695 Separation Module equipped with Empower 3 software and a 2998 Photodiode Array UV Detector (Milford, Massachusetts Waters Corporation, Milford MA, USA) and an Atlantis C18 column (4.6 x150mm, 5 μm inner diameter),(Waters

Corporation, Milford MA, USA). The wheat bran extract was mixed with methanol to a concentration of 50 mg/ml. The solution was filtered through a 0.45 µm PTFE filter (Whatman) prior to analysis with slight modification (Gunenc et. al., 2015). The mobile phase consisted of 4% acetic acid in ddH2O (eluent **A**) and 1% acetic acid in methanol (eluent **B**), in a gradient program (Table 2.1) and a column temperature of 30 °C. Total run time was 45 min and the UV-detector measured absorbance at 280nm. Alkylresorcinols were quantified by using calibration curves of the AR standards (C15:0, C17:0, C19:0, C21:0, C23:0, and C25:0) mixture dissolved in methanol to concentrations of 0.1; 0.2; 0.4; 0.8; 1 mg / ml.

**Table 2.1 Gradient program for HPLC**

| Time (min) | A (%) | B (%) | Flow rate (ml/min) |
|---|---|---|---|
| 0-10 | 10 | 90 | 0.5 |
| 10-35 | 0 | 100 | |
| 35-40 | 10 | 90 | |

## 2.24 Preparation of emulsion

### 2.241 Different formulations of emulsion

The combination of a high HLB emulsifier with a low HLB emulsifier improves emulsion stability, the HLB value of selected oil or biosurfactants used throughout the emulsion formulation is shown in table 2.2. In present work, wheat ARs and saponin were selected as surfactants for emulsion gel formulation, and span 80 was selected as

a substitute surfactants of wheat ARs due to their similar HLB value. The required HLB value of sunflower oil is 7 (SaffireBlue, 2018). Based on the equation in Figure 2.1. The ratio of wheat ARs to saponin was calculated to be 7:2.

Emulsions with different percentage of ingredients were prepared as listed in table 2.3. Sodium alginate was included in the formulation because it can from a stronger gel with the calcium ion presented in the whey permeate and buttermilk. In the preparation of whey permeate-control emulsion, first, whey permeate was dissolved completely at water (68 ℃), then sodium alginate and sunflower oil were added to the water phase, followed with mechanical stirring using a Tissuemiser (Fisher Scientific) until a desired consistency was achieved. To investigate the effect of saponin, wheat ARs, span 80, the combination of saponin and wheat ARs or the combination of saponin and span 80 on the control emulsion, saponin was added to the water phase, while the wheat ARs or span 80 was added to the oil phase. The formulated emulsions were cooled to room temperature (23℃) for further analysis. Buttermilk emulsion was prepared in the same manner as whey permeate emulsion.

**Table 2.2 HLB value of oil or biosurfactants used throughout the emulsion formulation**

| Oil or biosurfactant | Sunflower oil | Saponin | ARs | Span 80 |
|---|---|---|---|---|
| HLB value | 7 | 14.5 | 4~5 | 4.3 |

$$\%(A)=\frac{100(X-HLB_{(B)})}{HLB_{(A)}-HLB_{(B)}}$$

$$\%(B)=\%100-\%(A)$$

**Figure 2.1 Equation of blended HLB calculation, where A and B are two kinds of emulsifiers. X is the blended HLB.**

**Table 2.3 Different formulations of whey permeate or buttermilk emulsion**

| Formulation | Saponin (%) | Whey permeate or buttermilk (%) | Water (%) | Sunflower oil (%) | Sodium Alginate (%) | ARs (%) |
|---|---|---|---|---|---|---|
| Control | 0 | 3.6 | 77.8 | 17.6 | 1 | 0 |
| Saponin | 1 | 3.6 | 76.8 | 17.6 | 1 | 0 |
| Span 80 | 0 | 3.6 | 77.8 | 14.1 | 1 | 0 |
| ARs | 0 | 3.6 | 77.8 | 14.1 | 1 | 3.5 |
| Span 80 & Saponin | 1 | 3.6 | 76.8 | 14.1 | 1 | 0 |
| ARs & saponin | 1 | 3.6 | 76.8 | 14.1 | 1 | 3.5 |

## 2.242 Ultrasound-treated emulsion gel

To investigate the effect of ultrasound on the emulsions (Table 2.3), the preparations were based on the method by Leong et.al. (2017). For the oil phase, 7.05 g of sunflower oil was first mixed with 1.75 g of wheat ARs using ultrasound at 2 W calorimetric power for 10 seconds. Then 1.8 g of whey permeate and 0.5 g of saponin was mixed with 13.7g of water, before being mixed with the oil phase using ultrasound at 10 W calorimetric power for 60 seconds. The obtained mixture was then mixed with 25 g of alginate solution (0.5 g of sodium alginate in 24.5 g of water) at 10 W until the emulsion

formed was homogenous in appearance. The formulated emulsions were cooled to room temperature (23℃) for further analysis.

**2.243 Emulsion stability**

Both ultrasound-treated and untreated emulsion gels were stored at one month for 30 ℃ based on similar procedure by Tipvarakarnkoon, Einhorn-Stoll & Senge (2010), to check for phase separation during storage.

**2.25 Microscopic observation of emulsion gel**

Ultrasound treated and untreated emulsion gel were observed under PLM (polarized light microscope) (Axioplan 2 imaging and Zeiss Axiophot 2 universal microscope, Carl Zeiss Inc., Jena, Germany) at the end of the homogenization and at 7 days after storage at 4°C. The images were taken with a Retiga 1300 camera linked to the Northern eclipse software on the computer.

**2.26 Modification of meat puree samples**

Puree samples (salmon, chicken, chicken stew and beef) were stored at -20 ºC until needed and thawed at room temperature before use. Emulsion gel formulations of whey permeate and buttermilk containing saponin, AR, or saponin + AR were used for preparing the puree samples for analysis. 5% to 10% of 20 g puree samples was replaced with the formulated emulsion gel. Each treatment was mixed homogeneously using a Tissuemiser (Fisher Scientific, Ontario, CA). After each mixing, the resultant puree

samples were stored at ~-20°C before analysis.

### 2.261 Polarized light microscopic (PLM) observation of puree samples

Pure puree samples (salmon, chicken, chicken stew and beef) and puree mixed with emulsion gel samples were observed under PLM with the same equipment used for the emulsion gels.

### 2.262 Total expressible fluid (TEF) determination

The total expressible fluid (TEF) was determined by following the procedures described by Ismail et. al. (2021) and Colmenero et.al. (1995) with minor modification. In the water bath heating method, three replicates of puree (~10 g) at room temperature were centrifuged (1 min, 3250 g), heated in a water bath (30 min, 70°C) and immediately recentrifuged. After centrifugation, the supernatant was removed, and the residue was weighed. The total expressible fluid (TEF) was calculated according to the following equation (Ismail et. al., 2021):

$$\text{TEF (\%)} = \frac{\text{Weight of the sample before heating} - \text{Weight of the sample after heating}}{\text{Weight of the sample before heating}} \times 100$$

### 2.27 Inhibition assay for alpha-amylase activity

The inhibitory activity of wheat ARs, emulsion gels and meat sample to α-amylase were carried out according to the standard method with minor modification (Telagari & Hullatti, 2015 & Bhutkar & Bhise, 2012). In a 96-well plate, reaction mixture

containing 20 µl α–amylase (1 U/ml) and 60 µl of varying diluted sample solution was preincubated at room temperature for 30 min. Then, 80 µl of 1% soluble starch in buffer (20 mM phosphate buffer pH 6.9) was added as a substrate and incubated further at 37°C for 10 min; 80 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (BioTek Cytation 5, Ottawa, Canada).

Wheat ARs were diluted in buffer to final concentrations of 0.01, 0.1, 1 and 10 mg/ml. A positive control contained buffer and enzyme. A negative control contained buffer and either wheat ARs, pure puree or emulsion gel. The test sample contained buffer, enzyme and either AR or emulsion gel or puree. Emulsion samples were diluted in buffer to give a final concentration of 5 mg/ml, and then centrifuged at 10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was used for the test. In case of meat sample, a blank was prepared without emulsion sample and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium phosphate buffer, PH = 6.9). Meat samples were diluted in buffer to give a final concentration of 50 mg/ml and then were centrifuged at10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was used for the test. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity.

Each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula:

Inhibitory activity (%) = $[(Abs_{bank} - bs_{sample})/ Abs_{bank}] \times 100$

Where $Abs_{bank}$ is the absorbance of the bank and $Abs_{sample}$ is the absorbance of the sample.

**2.271 Kinetics of alpha-amylase inhibition**

The mode of inhibition of wheat ARs against alpha-amylase activity was measured with increasing concentrations of starch (0. 25 – 4%) as substrate in the absence or presence of the wheat ARs concentrations of 0.1 and 1 mg/ml. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the wheat ARs on alpha-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot.

**2.28 Inhibition assay for alpha-glucosidase activity**

The inhibitory activity of wheat ARs, emulsion gels and meat sample on α-glucosidase were carried out according to the standard method with minor modification (Telagari & Hullatti, 2015 & Picot et.al., 2014). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (0.1 mM, pH = 6. 9), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying diluted sample solution was preincubated at 37°C for 15 min. Then, 20 µl P-NPG (1 mM) was added as a substrate and incubated further at 37°C for 30 min. The reaction was stopped by adding 50 µl $Na_2CO_3$ (0.1 M). The absorbance of the released

p-nitrophenol was measured at 405 nm using Multiplate Reader (BioTek Cytation 5, Ottawa, Canada). Wheat ARs were diluted in buffer to give a final concentration of 0.1, 1 and 10 mg/ml. Emulsion samples were diluted in buffer to give a final concentration of 5 mg/ml, and then were centrifuged at 10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was used for the test. Meat samples were diluted in buffer to give a final concentration of 50 mg/ml and then were centrifuged at10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was used for the test. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. A blank was prepared without sample and another without the glucosidase enzyme, replaced by equal quantities of buffer (0.1 mM phosphate buffer, PH = 6.9).

Each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula:

Inhibitory activity (%) = $[(Abs_{bank} - Abs_{sample})/ Abs_{bank}]\times 100$

Where $Abs_{bank}$ is the absorbance of the bank and $Abs_{sample}$ is the absorbance of the sample.

**2.281 Kinetics of alpha-glucosidase inhibition**

The mode of inhibition of wheat ARs against alpha-glucosidase activity was measured with increasing concentrations of PNPG (0.125, 0.25, 0.5, 1 and 2 mM) as substrate in the absence or presence of the wheat ARs concentrations of 0.05 and 0.1 mg/ml. A

double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the wheat ARs on alpha-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot.

**2.29 Inhibition assay for xanthine oxidase activity**

The assay carrying out based on the procedure reported by Ferraz et. al. (2006), with modification. 1% (v/v) ethanol and 0.1% (w/v) Tween 80 in water was prepared to dilute wheat ARs, emulsion gels and meat puree samples. In a 96-well plate, reaction mixture containing 36 µl of diluted sample solution, 55 µl of 1/15M phosphate buffer (pH 7.5) and 14 µl enzyme solution (0.2 units/mL in phosphate buffer). A blank was prepared with sample replaced by equal quantities of water, and another blank with the enzyme replaced by equal quantities of buffer (1/15 M phosphate buffer, PH = 7.5). After preincubation of the mixture at 25 ◦C for 1 min, the absorbance at 295 nm was measured spectrophotometrically every 12s for 2 min. Then, the reaction was initiated by adding 107 µl of substrate solution (0.6mM in water). The assay mixture was incubated at 25◦C with the absorbance (295 nm) measured spectrophotometrically every 6 s, using a Multiplate Reader (BioTek Cytation 5, Ottawa, Canada).

Wheat ARs were diluted in buffer to give a final concentration of 0.1, 1 and 10 mg/ml.

Emulsion samples were diluted in buffer to give a final concentration of 5 mg/ml, and then were centrifuged at 10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was used for the test. Meat samples were diluted in buffer to give a final concentration of 50 mg/ml and then were centrifuged at 10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was used for the test. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity.

Each experiment was performed in triplicates. The results were expressed as percentage inhibition, calculated using the formula:

Inhibitory activity (%) = $[(Abs_{bank} - Abs_{sample})/ Abs_{bank}]\times 100$

Where $Abs_{bank}$ is the absorbance of the bank and $Abs_{sample}$ is the absorbance of the sample.

**2.291 Kinetics of xanthine oxidase inhibition**

The mode of inhibition of wheat ARs against xanthine oxidase activity was measured with increasing concentrations of xanthine (0. 15 – 1.2 mM) as substrate in the absence or presence of the wheat ARs concentrations of 0.05 and 0.1 mg/ml. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the wheat ARs on xanthine oxidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot.

## 2.30 Statistical analysis

Statistical analyses were performed with SAS Software (SAS Institute Inc, Cary, NC). One-way analysis of variance (ANOVA) by Duncan's Multiple Range test was used to compare the mean values and to find significances between results ( $\alpha$ =0.05). Differences were significant at $p < 0.05$.

## 2.3 Results and Discussion

## 2.31 Solvent extraction and Characterization of ARs

HPLC chromatograms for ARs extracted with ethanol from wheat bran are shown in Figure 2.2, and the quantity of each AR homologue are shown in table 2.4.

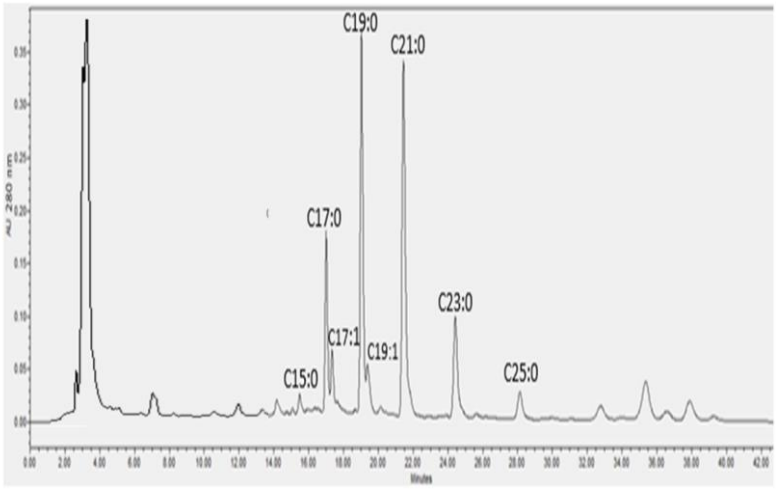


**Figure 2.2 HPLC chromatograms (at 280 nm) for ARs extracted from wheat bran**

**Table 2.4 Quantity of each AR homologue extracted with ethanol from wheat bran.**

| AR homologue | C17:0 | C17:1 | C19:0 | C19:1 | C21:0 | C23:0 | C25:0 | total ARs |
|---|---|---|---|---|---|---|---|---|
| mg ARs/100g dry matter | 10.0 [c] | 8.5 [c] | 31.9 [a] | 4.7 [d] | 35.4 [a] | 7.3 [c] | 22.2 [b] | 120.0 |

**Different letters represent significant different at $p<0.05$.**

According to the HPLC chromatograms, the alkylresorcinols homologue (C17:0, C17:1, C19:0, C19:1, C21:0, C23:0 and C25:0) were detected in wheat bran, and the dominant AR homologues in wheat bran are C21:0 and C19:0, which is in agreement with previous studies (Chen et al. 2004 & Agil et al. 2012). Total ARs were found to be 119.0 mg per 100g dry matter.

Chloroform, hexane, ethanol, diethylether, dichloromethane and acetone are solvents commonly used for the extraction of ARs from cereals include (Mullin et.al., 1992). In this study, ethanol was selected as the extracting solvent because ethanol is the only food-graded solvent, so the wheat ARs from ethanol extraction will be qualified to be incorporated in food for a question of food safety.

## 2.32 Emulsion stability

Visual observation of the untreated and ultrasonic treated emulsion gels prepared by buttermilk is shown in Figure 2.3 and Figure 2.4, respectively. The untreated and ultrasonic treated emulsion gels prepared by whey permeate is shown in Figure 2.5 and

Figure 2.6, respectively.

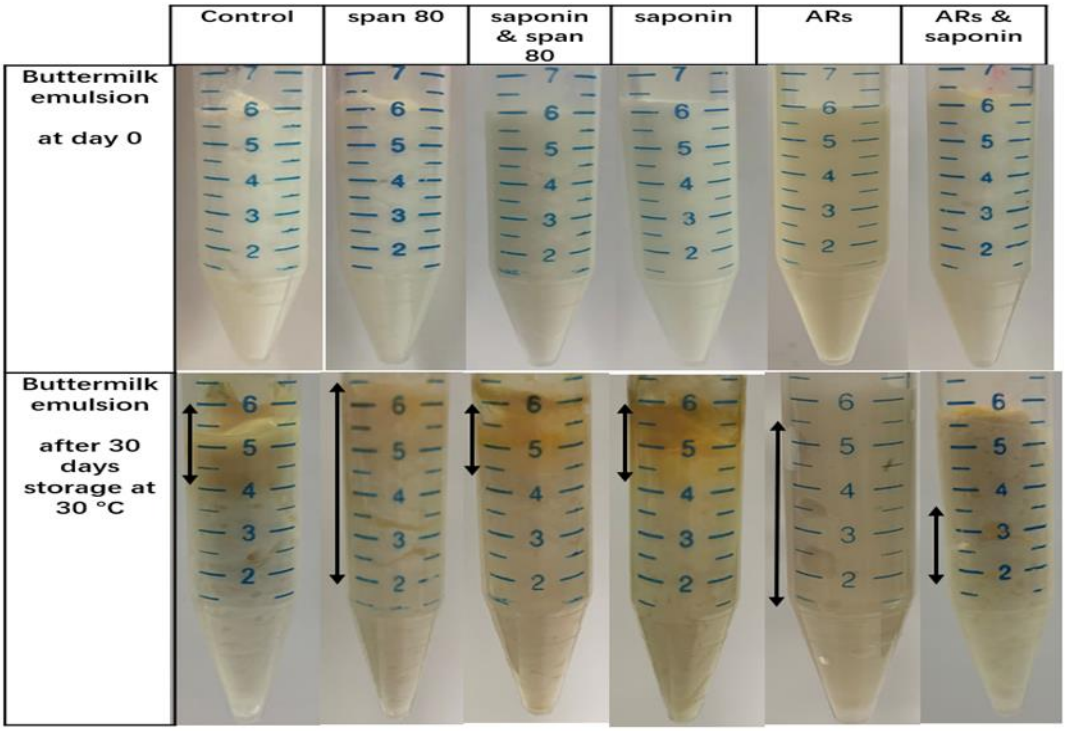


**Figure 2.3 Photographs of untreated buttermilk emulsion gels before and after 30 days storage at 30 °C. Dark arrow indicates apparent oil aggregation or degradation of emulsion gel**

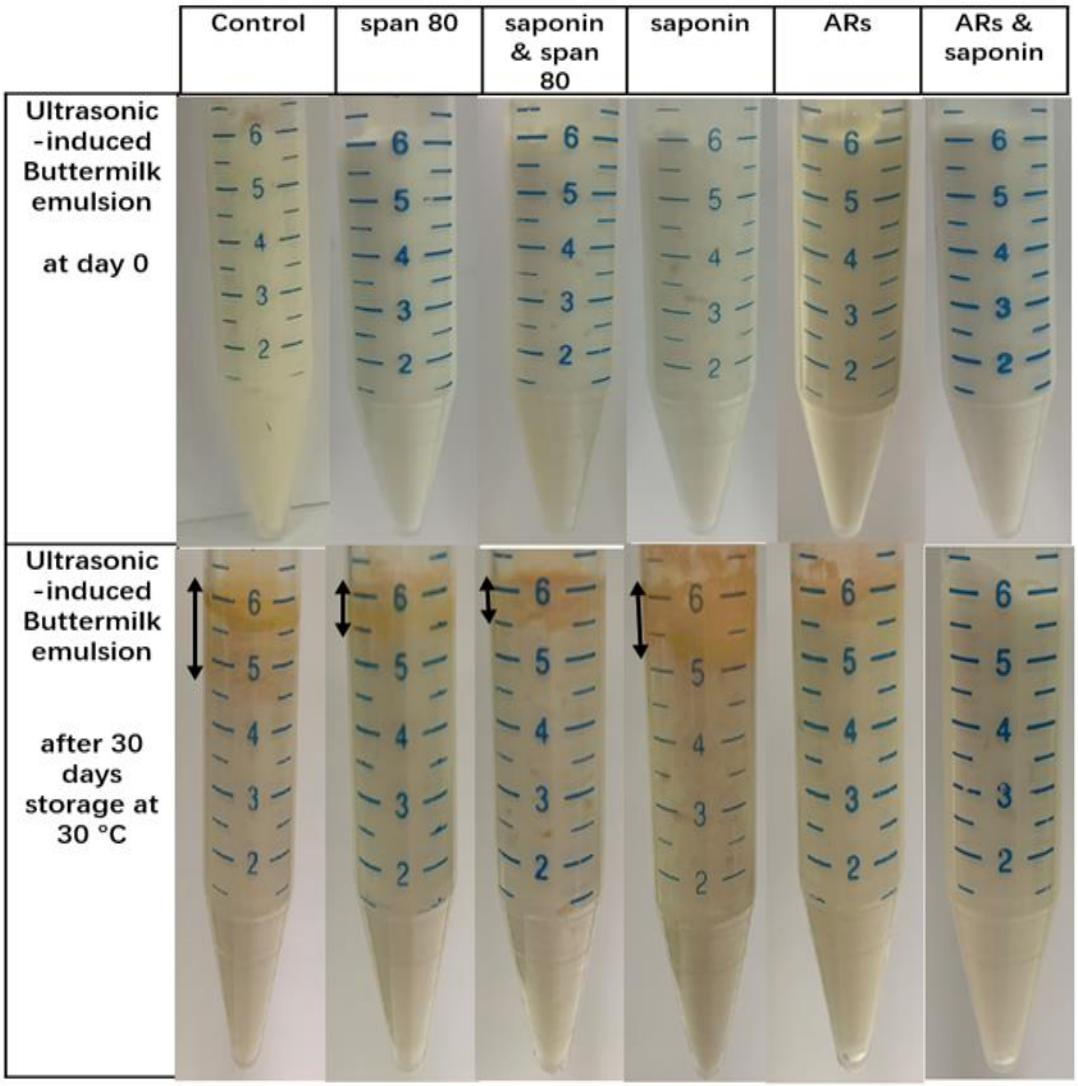


**Figure. 2.4 Photographs of ultrasonic-induced buttermilk emulsion gels before and after 30 days storage at 30 °C. Dark arrow indicates apparent oil aggregation or degradation of emulsion gel**

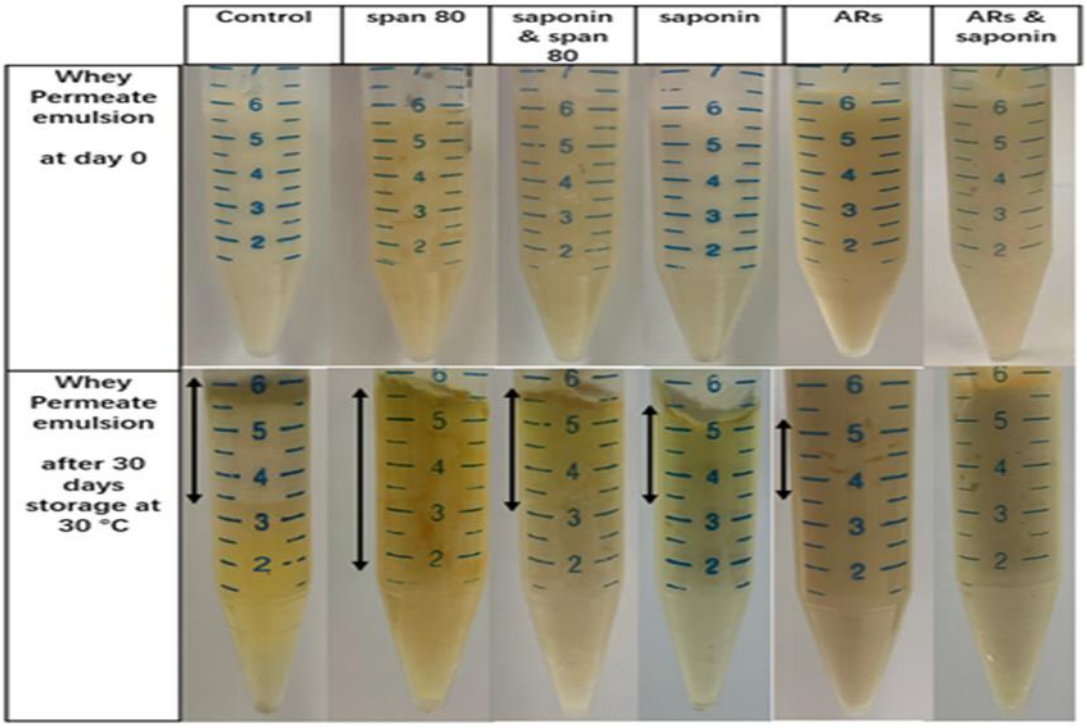


**Figure. 2.5 Photographs of untreated whey permeate emulsion gels before and after 30 days storage at 30 °C. Dark arrow indicates apparent oil aggregation or degradation of emulsion gel**

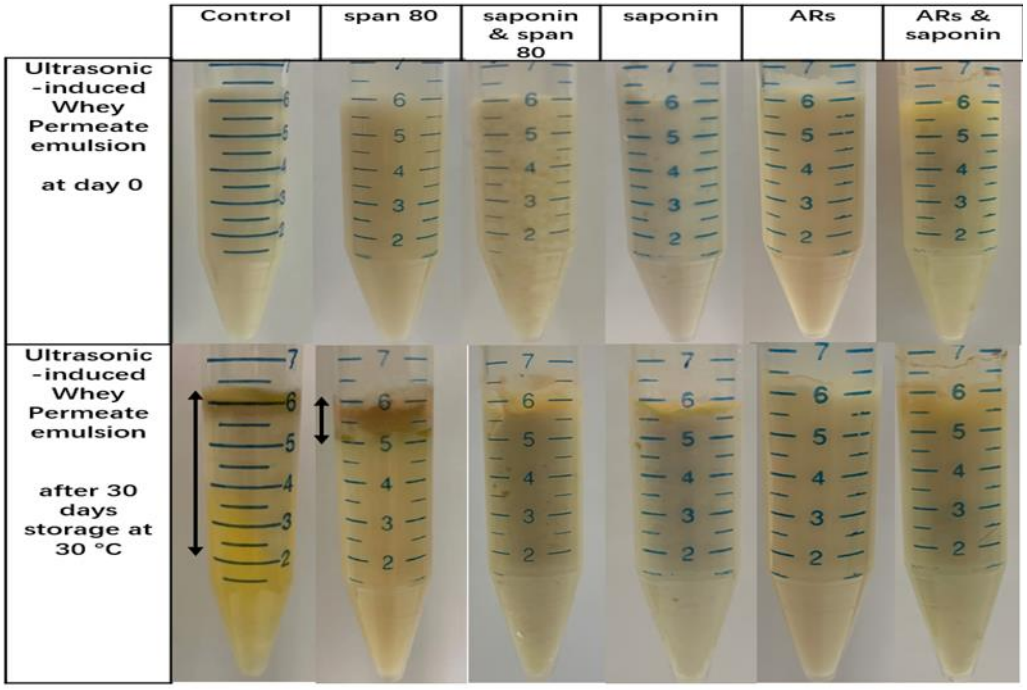


**Figure. 2.6 Photographs of ultrasonic-induced whey permeate emulsion gels before and after 30**

**days storage at 30 °C. Dark arrow indicates apparent oil aggregation or degradation of emulsion gel**

Most emulsions tend to break down during storage, and the stability of emulsion can be defined as its ability to resist phase separation or change in appearance over time (Maphosa and Jideani, 2018).

After preparation, both ultrasonic-treated and untreated buttermilk emulsion gels (Figure 2.3 and Figure 2.4) did not show phase separation at day 0. In untreated butter milk emulsion gels (Figure 2.3), the control emulsion gel, span 80-stabilized emulsion gel, span 80 & saponin-stabilized emulsion gel, and saponin- stabilized emulsion gel showed obvious oil separation (the yellow part in the image of emulsion gel), while the ARs-stabilized emulsion gel and the emulsion gel with ARs & saponin showed obvious degradation during 30 days of storage at 30 °C. This storage condition was chosen based on similar test conducted by Tipvarakarnkoon, Einhorn-Stoll & Senge (2010), in their study, emulsion with modified acacia gum did not show any phase separation at either room temperature (20 ℃) or 30 ℃.

No degradation was observed in ultrasonic-treated butter milk emulsion gel (Figure 2.4). In addition, the ultrasonic-treated control emulsion gel, span 80-stabilized emulsion gel, span 80 & saponin-stabilized emulsion gel, saponin-stabilized emulsion gel and the ARs-stabilized emulsion gel also showed oil aggregation, but its apparent oil

aggregation was less compared with that of emulsion gel without ultrasonic treatment, showing that ultrasonic homogenization greatly improved the emulsion stability by preventing its phase separation and degradation. No phase separation or degradation was observed in the ultrasonic-treated ARs & saponin-stabilized emulsion gel, indicating the ultrasonic- treated emulsion gel stabilized by saponin & ARs exhibited the best stability among all emulsion gel during 30 days of storage at 30 °C.

Similar results can be seen in whey permeate emulsion. Both ultrasonic treated (Figure 2.6) and untreated whey permeate emulsion gel (Figure 2.5) did not show phase separation at day 0. The untreated whey permeate emulsion gel stabilized by the combination of ARs and saponin exhibited the best stability against phase separation and degradation among all untreated emulsion gel during 30 days of storage at 30 °C. In the ultrasonic-treated emulsion gel, only the control emulsion gel and the span 80-stabilized emulsion gel showed apparent oil aggregation among all emulsion gel during 30 days of storage at 30 °C.

A stabilizer, such as Span or Tween, is typically employed to improve the kinetic stability of the emulsion and avoid phase separation in the emulsion system. However, because of the demands for natural, nontoxic, biocompatible, and high ecological acceptability, the use of these surfactants is becoming more limited (Fu et al., 2022). In this study, the results indicated that the ultrasonic homogenization greatly promoted

emulsion stability, and the emulsion with the combination of ARs and saponin had obviously higher stability during storage, compared to emulsions with span 80 alone or with the combination of span 80 and saponin. So, it can be concluded that the use of combination of ARs and saponin could successfully replace the use of span 80 for emulsion stability.

As a result, ultrasonic-treated buttermilk and whey permeate emulsion gel with saponin & ARs were selected to be incorporated into the puree samples as the positive control and compared with puree samples incorporated with saponin-stabilized emulsion gels to investigate the effect of ARs in puree sample.

## 2.33 Microstructure

The images of the microscopic observation are shown in Figure 2.7 and Figure 2.8.

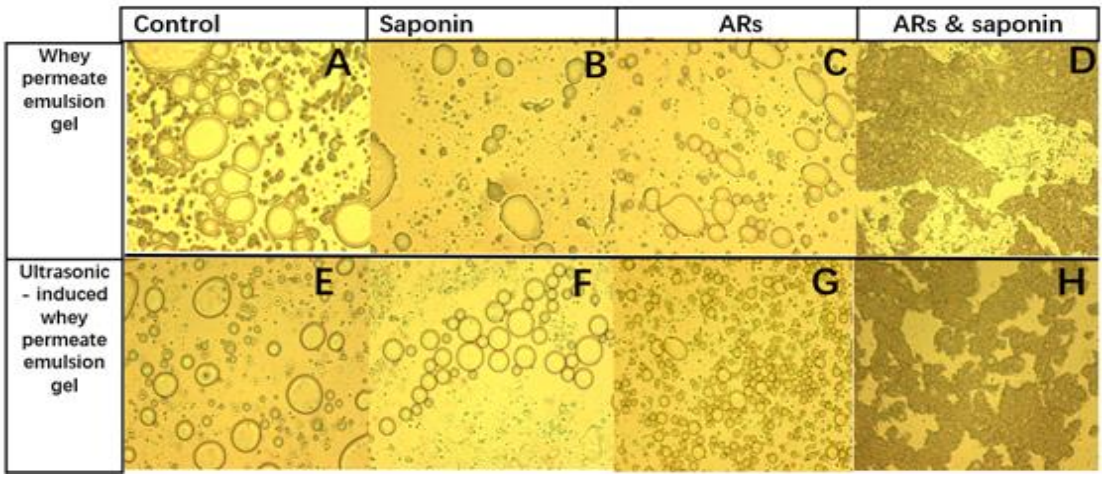


**Figure 2.7 Visual and microscopic observation (50X) of whey permeate emulsion gels. Dark**

**arrow indicates length of 20 µm.**

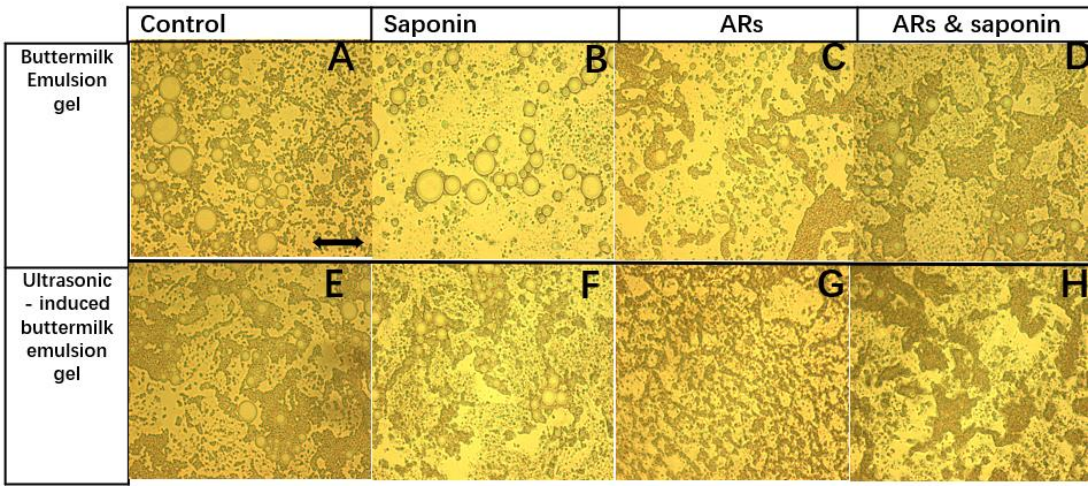


**Figure 2.8 Visual and microscopic observation (50X) of buttermilk emulsion gels. Dark arrow indicates length of 20 µm.**

In the untreated whey permeate emulsion gel, the oil droplets were oval in the control emulsion gel (Figure 2.7 A), the saponin-stabilized emulsion gel (Figure 2.7 B) and the ARs- stabilized emulsion gel (Figure 2.7 C), while were changed to spherical in the saponin & ARs-stabilized emulsion gel (Figure 2.7 D). The oil droplets in the untreated whey permeate emulsion gel became smaller and uniform with the combination of saponin and ARs suggesting that the combination of saponin and ARs had synergistic effect on emulsion stability.

Similar results were also observed in the untreated buttermilk emulsion gel, the saponin & ARs-stabilized emulsion (Figure 2.8 D) showed smaller droplet diameters than the control emulsion (Figure 2.8 A) and the saponin-stabilized emulsion (Figure 2.8 B). In

the untreated emulsion gels, aggregation of droplets was observed in the ARs-stabilized emulsion gels (Figure 2.7 C and Figure 2.8 C) and saponin & ARs-stabilized emulsion gels (Figure 2.7 D and Figure 2.8 D). This might be because the presence of ARs promote interactions with the hydrophobic domains in buttermilk and whey permeate. According to Phan et. al (2014), the formation of aggregates in protein emulsion is due to the reduction of electrostatic repulsions between the protein molecules by increasing interactions between hydrophobic domains.

When emulsion droplets near each other are large and polydispersed, it would increase the flocculation rate and reduce stability of the emulsion system (Yvonne and Victoria, 2018). It probably could explain why the untreated emulsion gel with saponin & ARs had highest stability among untreated emulsion gels during storage. A similar result was reported by Hajimohammadi & Johari-Ahar (2018). Their research found that rhamnolipid positioned them at the interface and saponin protruded into the aqueous phase through hydroxyl groups, allowing them to form hydrogen bonds with water molecules. Thus, increasing the favorable interaction between hydrocarbon chains and water molecules. As such, their combination could successfully increase the stability.

In addition, compared to the untreated emulsion, the ultrasonic-treated emulsions showed higher stability with smaller particle size. Reduction of droplet size by ultrasound can be attributed to the shear force generated by the cavitation of the

ultrasonic waves (Guo et al. (2014). The results indicate that ultrasonic homogenization, and combination of saponin and ARs help to make stronger and more stable emulsion gel.

## 2.34 Total expressible fluid (TEF) determination

Figure 2.9 shows the results of TEF form different meat puree samples.

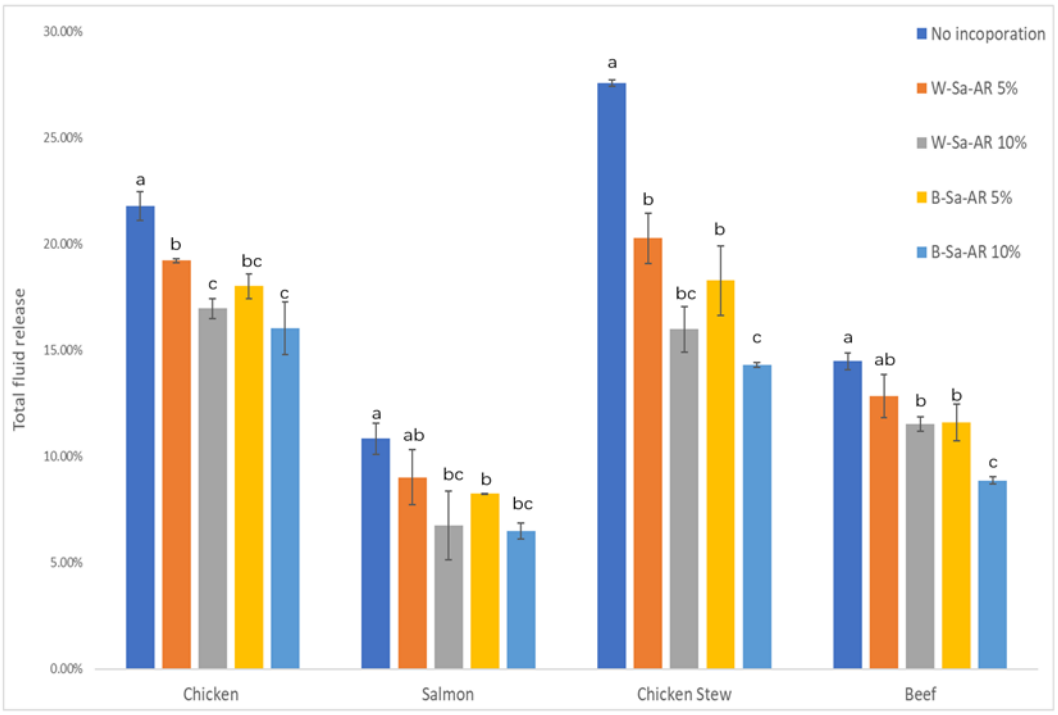


**Figure 2. 9 Effect of incorporation of 5% or 10% buttermilk-saponin-ARs or whey permeate-saponin-ARs emulsion gel in puree samples on total fluid releases test. Different letters represent significant different at $p<0.05$.**

The mean TEF of water bath-heated pure puree (chicken, salmon, chicken stew and beef without emulsion gel incorporation) was higher than that of puree with 5% w/w

W-SA-AR or 5% w/w B-SA-AR emulsion gel incorporation. With increased amount of W-SA-AR or B-SA-AR emulsion gel (from 0% to 10%) incorporated in meat pure, the TEF of puree was further reduced.

In Figure 2.9, the results show pure chicken puree (no emulsion gel incorporation) had the highest TEF among all chicken puree, with a mean value of 21.79%. There were no significant differences ($P<0.05$) in TEF value of the chicken puree with 10% w/w replacement by W-SA-AR and B-SA-AR emulsion gel, with mean values of 16.96% and 16.03%, respectively. Similar result was also observed in the other puree groups, the puree without emulsion gel incorporation had the highest TEF and the puree with 10% w/w emulsion gel replacement had the lowest TEF within the same group.

The reduction of TEF by emulsion gel incorporation may be due to ingredients in the emulsion gel forming a crosslink with the polar groups of the matrix protein to give a stronger three-dimensional network structure, leading to stronger binding properties and less fluid release during heating (Colmenero et al., 2005 & Kumar, 2021).

### 2.35 Microstructural properties of meat

Figure 2.10 shows micrographs of the of meat puree by PLM.

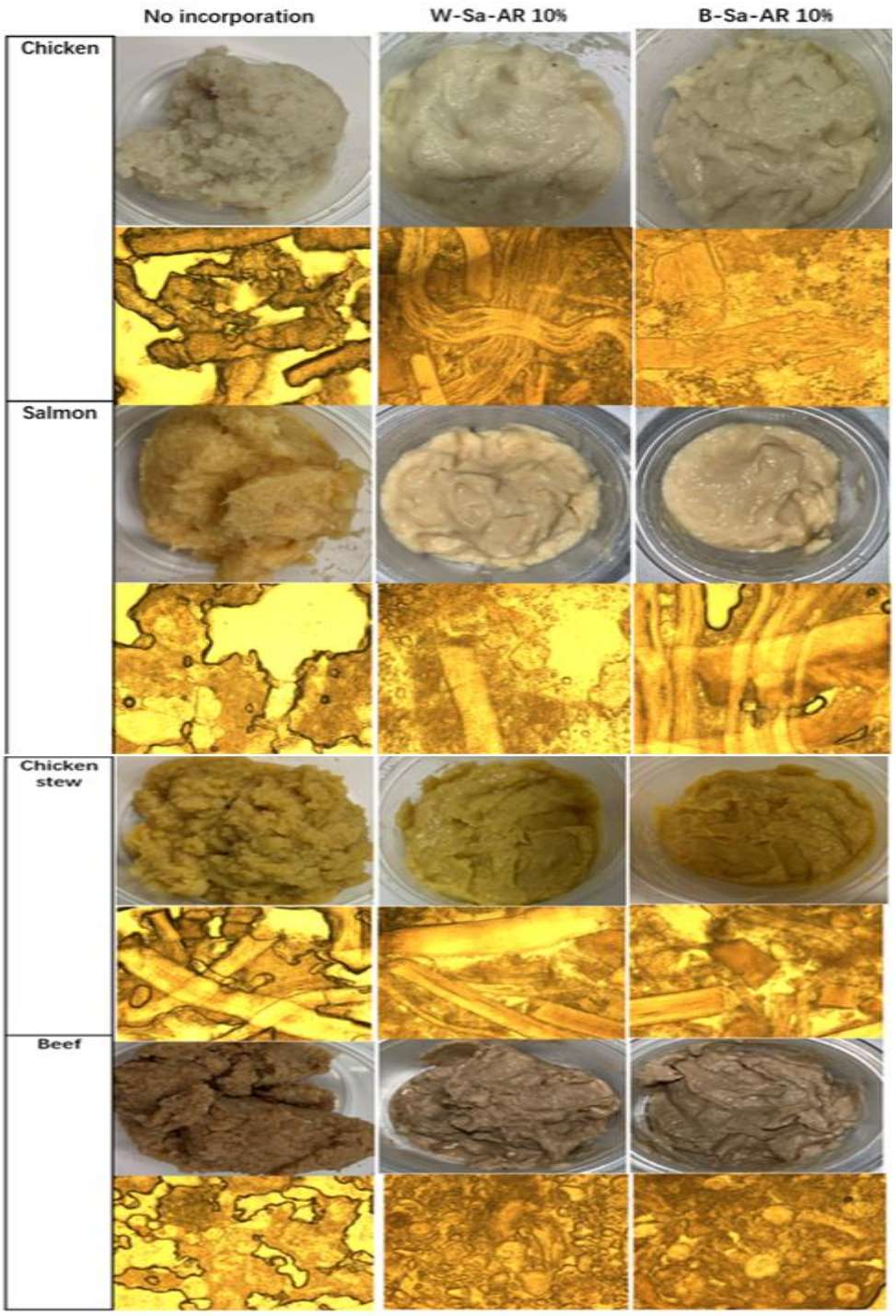


**Figure. 2.10 visual and microscopic observation (20X) of puree samples before and after incorporation with 10% buttermilk-saponin-ARs or 10% whey permeate-saponin-ARs emulsion gel**

In PLM images, the morphology of the control purees showed larger voids. When 10%

W-SA-AR or B-SA-AR emulsion gel was added in the puree, less or no void was observed compared to the puree without addition of emulsion gel. The images indicate the complete incorporation of emulsion in the meat matrix and so improve homogeneity of the puree structure, in agreement with Mejia et al. (2018). The high proportion of voids can be interpreted as the expansion of water and air in the protein network (Paglarini et.al. 2022). The change in the structure could also be explained that the formation of a consistent distribution of oil droplets in the meat emulsion is enabled when animal fat (high melting point) is substituted with vegetable oil (low melting point), resulting in a more stable and homogenous protein–water–fat matrix (Kumar et al. (2017).

According to Colmenero et al. (1995), meat matrices with homogeneous and more compact (less and smaller voids) structures, could be classified as strong meat gels with good water and fat binding properties. On the other hand, meat matrices with irregular and less compact (more and larger voids), and aggregate structures, could be classified as poor meat gels with weak binding properties. These results were also confirmed by the result of total expressible fluid (TEF) determination (Figure 2.9), which showed emulsion gel incorporation reduce the released fluid from purees after water thermal treatment and centrifugation.

## 2.36 Alpha-amylase inhibition test

Alpha-amylase is a key enzyme in the digestion of dietary starch, producing oligosaccharides that can be broken down further into glucose. Results of the α-amylase inhibition activity by wheat ARs are shown in Figure 2.11.

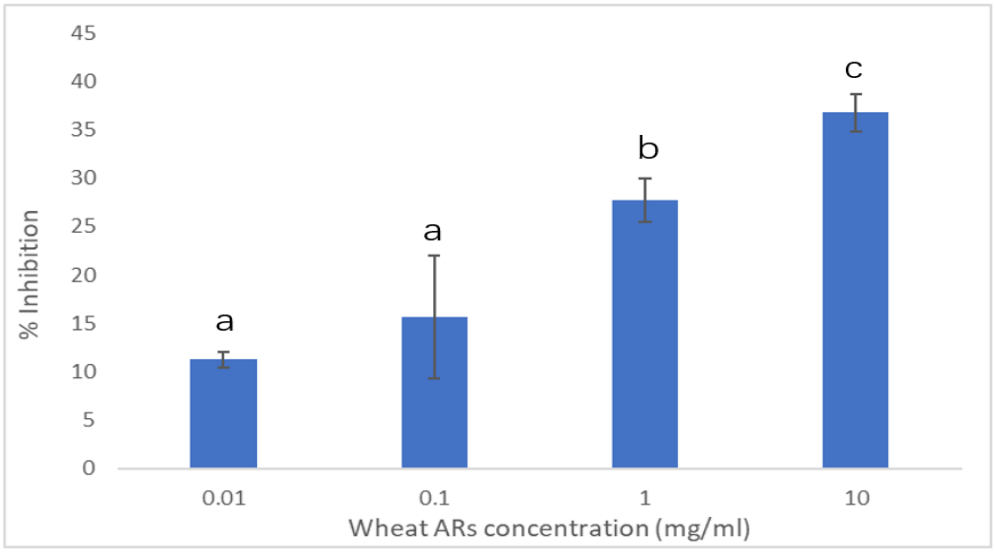


**Figure 2.11 Inhibitory potency of wheat alkylresorcinols against α-amylase (1 U/mL) activity at 1% starch concentration. Different letters represent significant different at $p<0.05$.**

The inhibitory activity of wheat ARs were conducted at concentrations of 0.01, 0.1, 1 and 10 mg/ml with the highest inhibitory activity at 10 mg/ml. Results show that inhibitory activity increased with increasing wheat ARs concentration, indicating a dose-dependent effect. The inhibitory activity of wheat ARs at 10 mg/ml on α-amylase was 36.49 ± 1.75%.

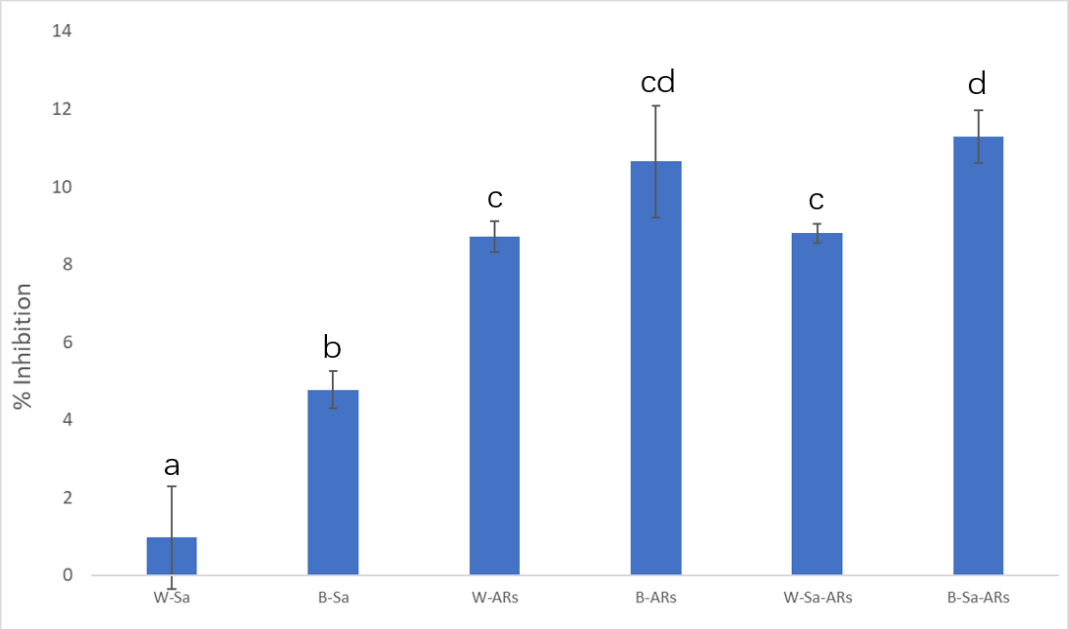


**Figure 2.12 Inhibitory potency of 0.5% whey permeate emulsion gel and buttermilk emulsion gel in the absence or presence of wheat alkylresorcinols against *α*-amylase (1 U/mL) activity at 1% starch concentration. Different letters represent significant different at p<0.05.**

The inhibitory effects of emulsion stabilized by W-Sa, B-Sa, W-ARs, W-ARs, W-Sa-ARs and B-Sa-ARs at 5 mg/ml on the activity of α-amylase are shown in Figure 2.12. W-ARs stabilized emulsion gel had the same inhibitory activity ($p>0.05$) with W-Sa-ARs stabilized emulsion gel, with the value of 8.72 ± 0.39% and 8.80 ± 0.25%, respectively, and these values were significantly higher than that of W-Sa stabilized emulsion gel. The results indicate that ARs contributed majorly to amylase inhibition ability in the samples. Similar result was observed in the buttermilk emulsion gels, B-ARs stabilized emulsion gel had the same inhibitory activity ($p>0.05$) with B-Sa-ARs stabilized emulsion gel, with the values of 10.65 ± 1.44% and 11.28 ± 0.68%,

respectively, which were higher than that of B-SA emulsion gel.

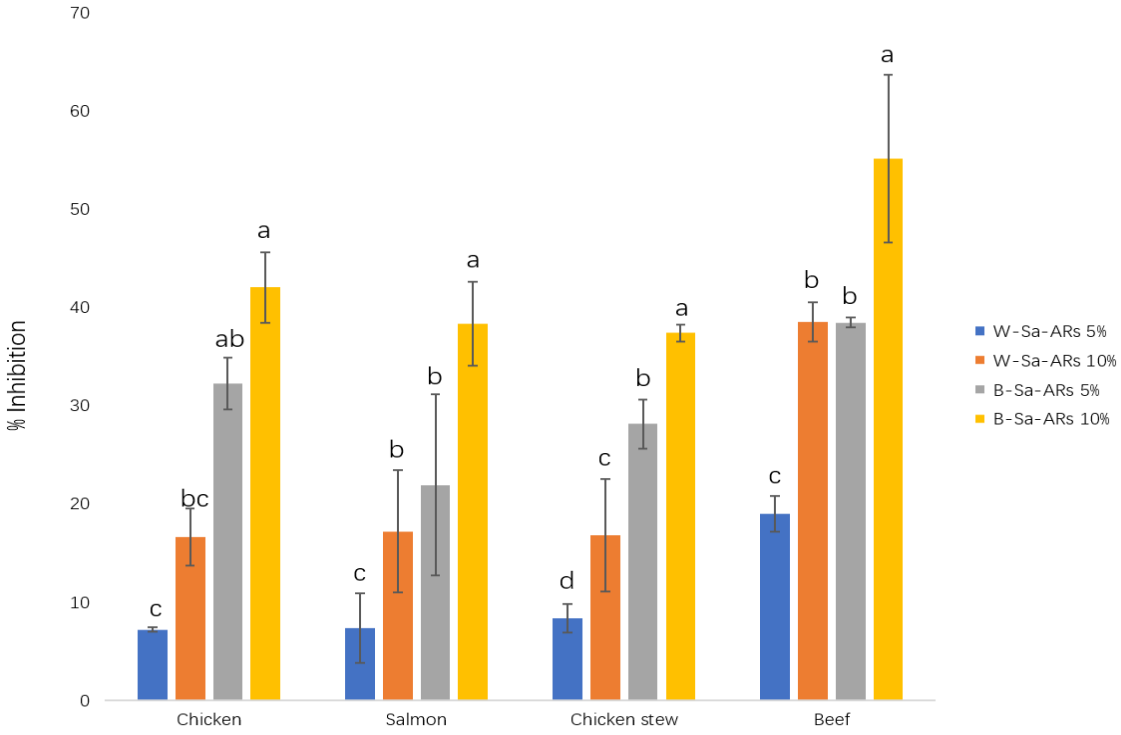


**Figure 2.13 Inhibitory potency of puree samples with incorporated 5% or 10% whey permeate-saponin-ARs or buttermilk-saponin-ARs emulsion gel against *α*-amylase (1 U/mL) activity at 1% starch concentration. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. Different letters represent significant different at p<0.05.**

The inhibitory effects of puree samples (salmon, chicken, chicken stew or beef) with incorporation of 5% w/w and 10% w/w W-Sa-ARs or B-Sa-ARs stabilized emulsion gel on the activity of α-amylase are shown in Figure 2.13. Puree samples without emulsion incorporation was representative of the 100% enzyme activity. It showed all puree samples have the ability to delay α-amylase activity after incorporation with

buttermilk or whey permeate emulsion gel. However, the inhibitory activity of puree samples with 10% w/w replacement by W-Sa-ARs or B-Sa-ARs emulsion gel was higher than that of puree with 5% w/w replacement. The results indicate that incorporation of emulsion gel improved the α- amylase inhibitory activity of the puree samples. The increase of inhibitory activity after incorporation of emulsion gel was confirmed in the result of amylase inhibitory test by whey ARs (Figure 2.12) and emulsion gel (Figure 2.13), which showed the emulsion gel containing ARs have ability to delay α-amylase activity. Thus, the presence of higher amount of W-Sa-ARs or B-Sa-ARs emulsion gel in the puree would result in higher inhibitory activity.

Alpha-amylase inhibitory activity of ARs from wheat bran has not been reported until now. However, a study was reported by Martirosova et al (2014). on the effect of two alkylresorcinols, methylresorcinol ($C_7H_8O_2$) and hexylresorcinol ($C_{12}H_{18}O_2$) on the activity of β-amylase. The study found only the hexylresorcinol at high concentration had inhibitory effect on β-amylase, so they reported that difference in alkyl radical length of alkylresorcinols leads to differences in their influence on enzyme activity, and long-chain ARs showed an inhibitory effect but not short-chain ARs (Martirosova et al., 2014). Notably, all five major ARs homologues from wheat bran (C17:0, C19:0, C21:0, C23:0, and C25:0), have longer alkyl chain than hexylresorcinol.

## 2.361 Alpha-Amylase Kinetics of wheat ARs

In this study, the initial velocity '*v*' of the hydrolysis reactions catalyzed by α-amylase was measured at 0.25-4 % starch concentrations [*S*] in the absence and presence of 0.1 mg/mL and 1 mg/mL ARs, as shown in Figure 2.14. The kinetic properties of wheat ARs concentration on alpha-amylase are shown in table 2.5.

**Table 2.5 Kinetic properties of wheat ARs concentration on alpha-amylase**

| Wheat ARs concentration | Vmax (mol maltose /min) | KM (%) |
|---|---|---|
| 0 mg/ml | 1.75 | 2.26 |
| 0.1 mg/ml | 1.29 | 2.26 |
| 1 mg/ml | 1.09 | 2.26 |

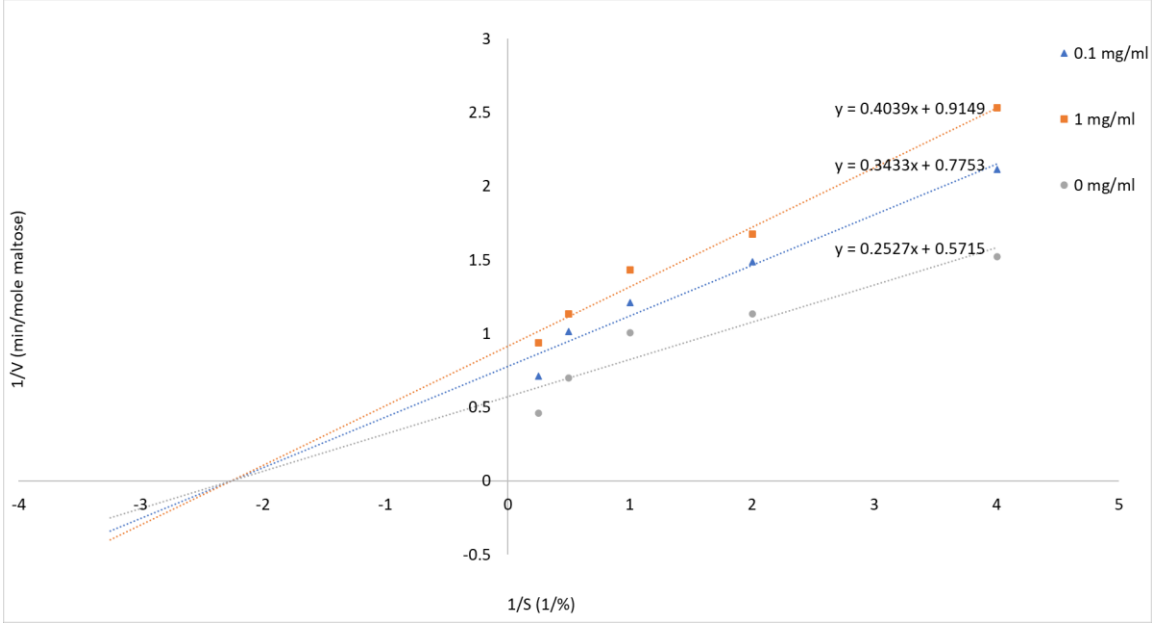


**Figure 2.14 Lineweaver–Burk plot of alpha-amylase (1 U/mL) reaction with variable starch concentrations in the absence or presence of alkylresorcinols. Starch concentrations change between 0.25% - 4%**

With increasing ARs concentration, both the slope and vertical axis intercept increased, but the lines intersect at the x-axis at the same point. These results indicated that ARs affected the velocity of the α-amylase-catalyzed reaction proportional to the concentration of ARs in the reaction mixture, without affecting the $K_m$. These results suggest that the inhibition of ARs on α-amylase was non-competitive. The Vmax values of ARs at 0.1 mg/mL and 1 mg/mL were found to be 129 and 109 mol/min, respectively, while their Km values remained unchanged (2.26 %).

Non-competitive inhibitors are known to block allosterically and change the structure of the enzyme. They have the same affinity for free enzymes as they do for enzyme-substrate complexes. As a result, in non-competitive inhibition, the inhibitors have no effect on the Km since the change in conformation has no effect on its affinity for the substrate (Delaune et al., 2021). Alpha-amylase inhibitory activity of ARs from wheat bran had not been reported until now.

The inhibition mode established in this study was similar to phenolic compounds extracted from millet seed coat (Shobana et al., 2009), which were all non-competitive inhibitors.

## 2.37 Alpha-glucosidase inhibition test

Results of the α-glucosidase inhibition activity by wheat ARs are shown in Figure 2.15.

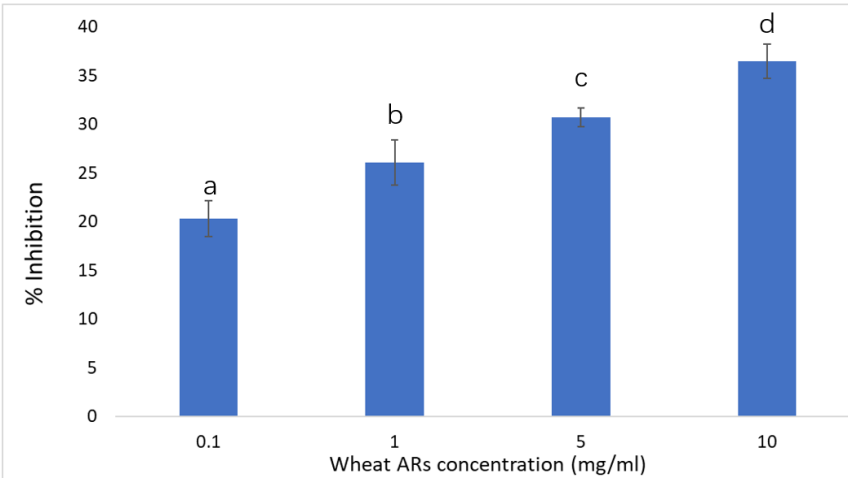


**Figure 2.15 Inhibitory potency of wheat alkylresorcinols against α-glucosidase (1 U/mL) activity at 1mM PNPG concentration. Different letters represent significant different at p<0.05.**

The inhibitory activity of the wheat ARs were conducted at concentrations of 0.1, 1, 5 and 10 mg/ml with 10 mg/ml exhibiting the highest inhibition. The results showed increased inhibitory activity with increasing wheat ARs concentration, indicating a dose-dependent effect. The inhibitory activity of wheat ARs at 50 mg/ml on α-glucosidase was 36.78 ± 1.94%.

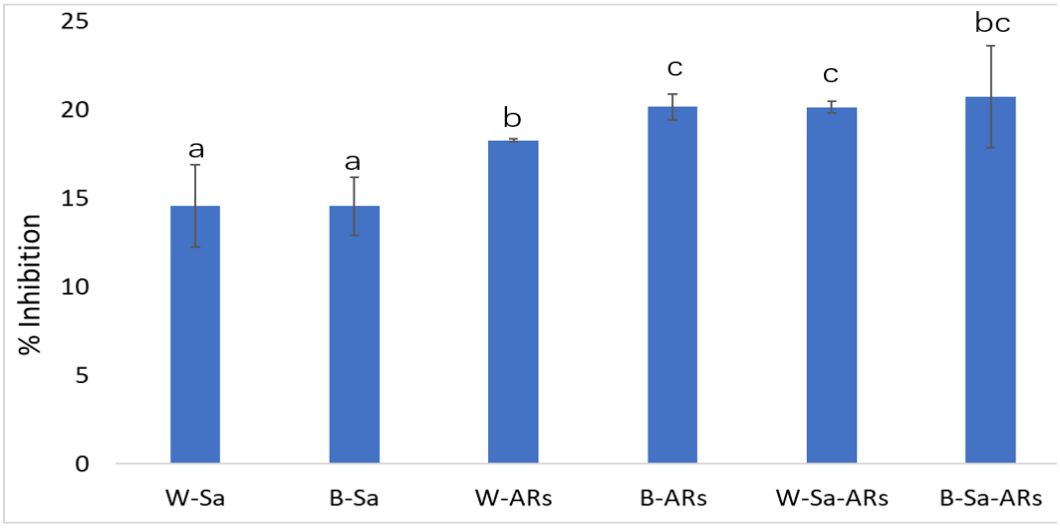


**Figure 2.16 Inhibitory potency of 0.5% whey permeate emulsion gel and buttermilk emulsion gel in the absence or presence of wheat alkylresorcinols against alpha-glucosidase (1 U/mL) activity at 1mM PNPG concentration. Different letters represent significant different at $p<0.05$.**

Within whey-permeate emulsions (Figure 2.16), W-Sa-ARs stabilized emulsion gel had higher inhibitory activity ($p<0.05$) compared to W-ARs stabilized emulsion gel, with values of 20.15 ± 0.73% and 18.28 ± 0.07%, respectively. These values were also significantly ($p<0.05$) higher than that of W-Sa stabilized emulsion gel (14.55 ± 2.31%). The results indicate that ARs and saponin contribute to glucosidase inhibition of the samples. In the buttermilk emulsion gels, B-ARs stabilized emulsion gel had the same inhibitory activity ($p>0.05$) with B-Sa-ARs stabilized emulsion gel, with the value of 20.16 ± 0.73% and 20.74 ± 2.87%, respectively, which was also higher than that of B-SA emulsion gel (14.55 ± 1.64%).

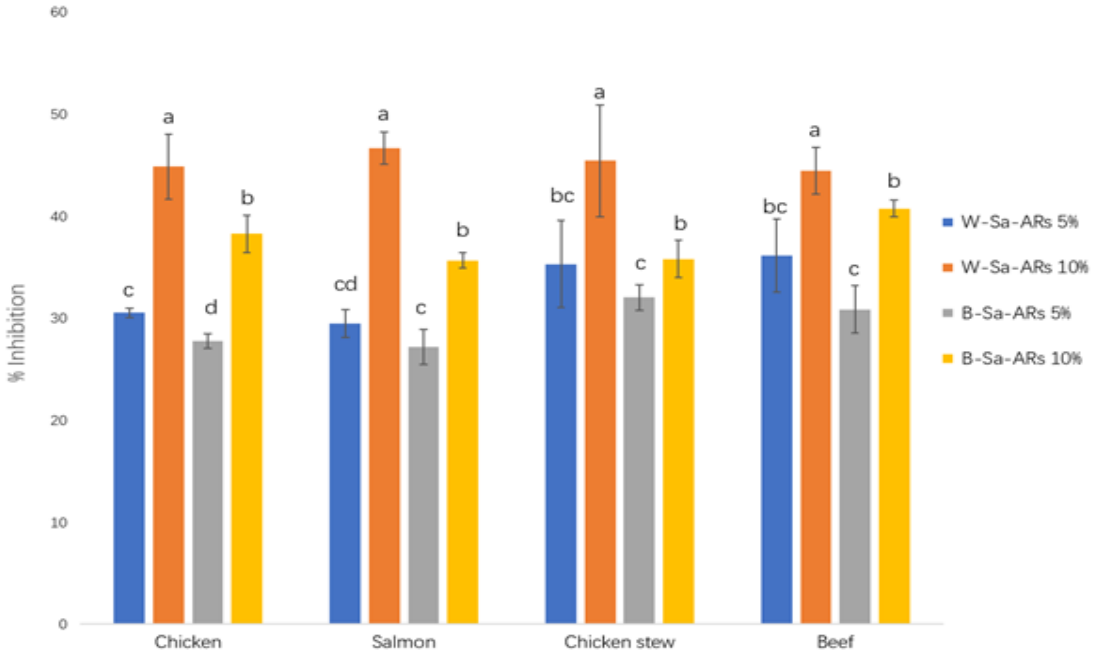


**Figure 2.17 Inhibitory potency of puree samples with incorporated 5% or 10% % whey permeate-saponin-ARs or buttermilk-saponin-ARs emulsion gel against alpha-glucosidase (1 U/mL) activity at 1mM PNPG concentration. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. Different letters represent significant different at $p<0.05$.**

The inhibitory effects of puree samples (salmon, chicken, chicken stew or beef) with incorporation of 5% w/w and 10% w/w W-Sa-ARs or B-Sa-ARs stabilized emulsion gel emulsion gel on the activity of α- glucosidase are shown in Figure 2.17. Puree samples without emulsion incorporation was representative of 100% enzyme activity. It showed all puree samples have ability to delay α- glucosidase activity after incorporation with buttermilk or whey permeate emulsion gel. The inhibitory activity

of puree samples with 10% w/w replacement by W-Sa-ARs or B-Sa-ARs emulsion gel was higher than that of puree with 5% w/w replacement. The increase of inhibitory activity after incorporation of emulsion gel was confirmed by the result of glucosidase inhibitory test by whey ARs (Figure 2.15) and emulsion gel (Figure 2.16). These results showed that emulsion gels containing ARs have ability to delay α-glucosidase activity, and so the presence of higher amount of W-Sa-ARs or B-Sa-ARs emulsion gel in the puree would result in higher inhibitory activity.

### 2.371 Alpha- glucosidase Kinetics of wheat ARs

In this study, the initial velocity '*v*' of the hydrolysis reactions catalyzed by α-glucosidase was measured at 0.125-4mM PNPG concentrations [*S*] in the absence and presence of 0.05 mg/mL and 0.1 mg/mL ARs, as shown in Figure 2.18. The kinetic properties of wheat ARs concentration on α-glucosidase is shown in table 2.6.

**Table 2.6 Kinetic properties of wheat ARs concentration on alpha-glucosidase**

| Wheat ARs concentration | Vmax (mol maltose /min) | KM (%) |
|---|---|---|
| 0 mg/ml | 0.09 | 7.32 |
| 0.05 mg/ml | 0.07 | 7.62 |
| 0.1 mg/ml | 0.06 | 7.82 |

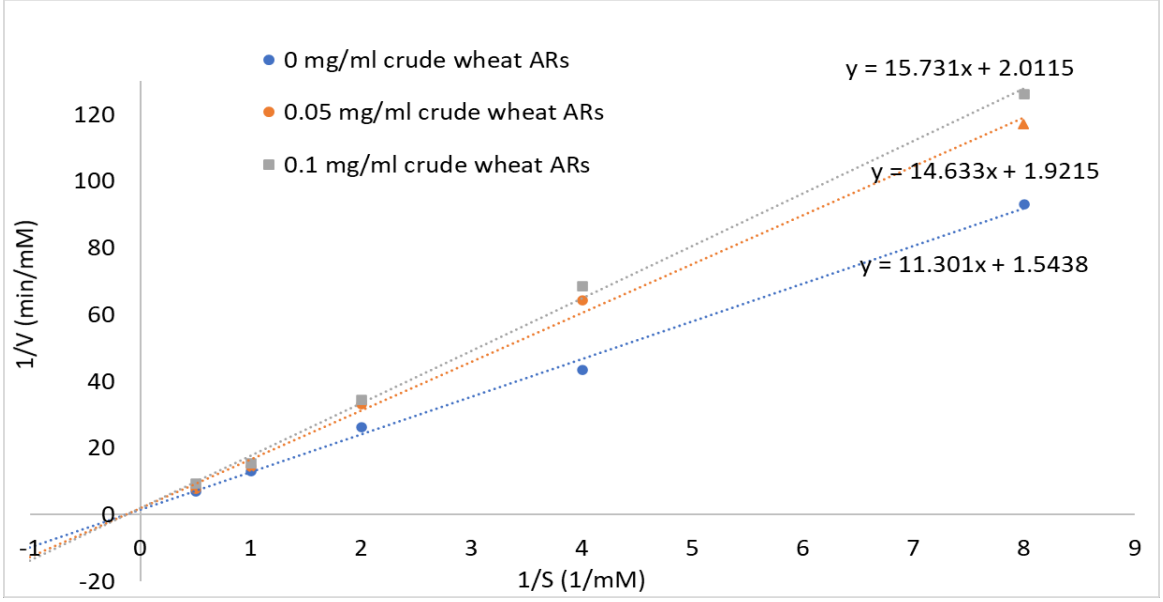


**Figure 2.18 Lineweaver–Burk plot of alpha-glucosidase (1 U/mL) reaction with variable PNPG concentrations in the absence or presence of wheat alkylresorcinols. PNPG concentrations change between 0.125 – 2 mM**

With increasing ARs concentration, the velocity of the reaction catalyzed by α-glucosidase decreased, and the Km increased. These results suggest that the mode of ARs on α- glucosidase was mixed non-competitive inhibition. The kinetic properties of wheat ARs concentration on alpha- glucosidase (table 2.6) showed the Vmax values of ARs at 0. 05 mg/mL and 0.1 mg/mL were found to be 0.07 and 0.06 mmol/min, respectively, while their Km values were 7.62 and 7.82, respectively. The mode of mixed non-competitive inhibition indicates that the active components of the inhibitors do not compete with the substrate for binding to the active site, but instead bind to a separate site on the enzyme to delay disaccharide conversion to monosaccharides (Jung et/ al., 2016). A study from Tu et al. (2013) reported that the inhibition mode of ARs on

α-glucosidase was non-competitive. The inconsistency with literature about the inhibition mode of ARs from wheat bran on α-glucosidase might have been due to differences in source of wheat bran, cultivar factors and extraction method.

## 2.38 Xanthine oxidase inhibition test

Results of the xanthine oxidase inhibition activity by wheat ARs are shown in Figure 2.19.

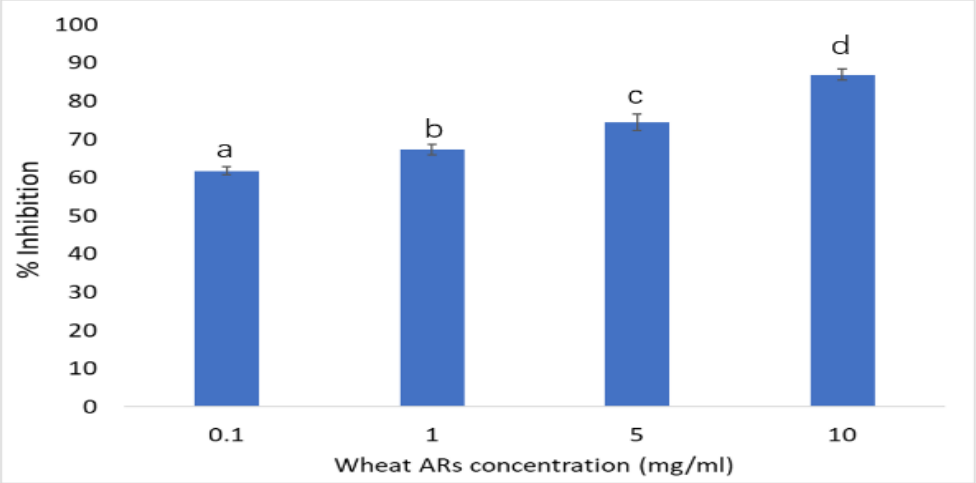


**Figure 2.19 Inhibitory potency of wheat alkylresorcinols against xanthine oxidase (0.2 U/mL) activity at 0.6mM xanthine concentration. Different letters represent significant different at $p<0.05$.**

The inhibitory activity of wheat ARs were conducted at concentrations of 0.01, 0.1, 1 and 10 mg/ml and the concentration at which the samples had the highest inhibitory

activity was 10 mg/ml. It showed inhibitory activity increased with increasing wheat ARs concentration, indicating a dose-dependent effect. The inhibitory activity of wheat ARs at 10 mg/ml on xanthine oxidase was 86.94 ± 1.43%.

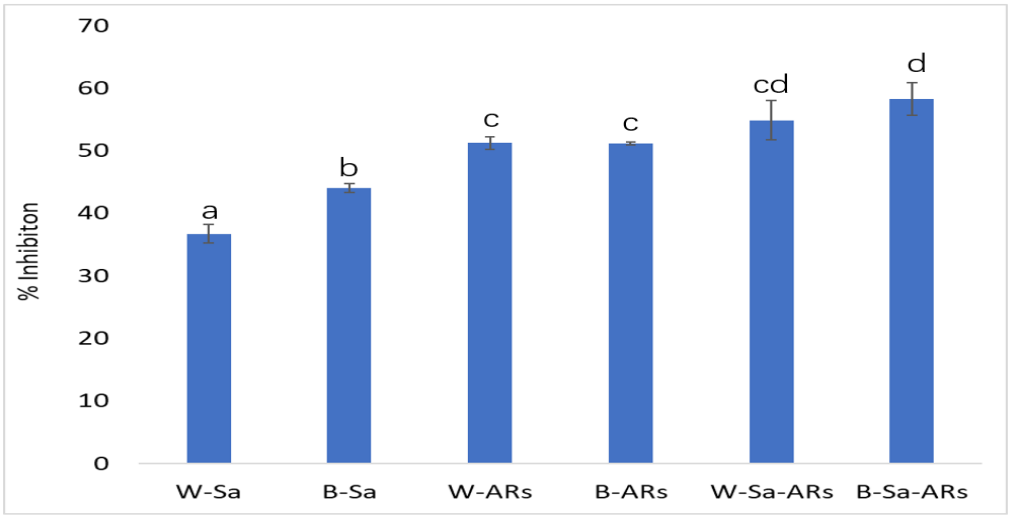


**Figure 2.20 Inhibitory potency of 0.5% whey permeate and buttermilk emulsion gel in the absence or presence of wheat alkylresorcinols against xanthine oxidase (0.2 U/mL) activity at 0.6mM xanthine concentration. Different letters represent significant different at p<0.05.**

The inhibitory effects of emulsion stabilized by W-Sa, B-Sa, W-ARs, W-ARs, W-Sa-ARs and B-Sa-ARs at 5 mg/ml on the activity of xanthine oxidase are shown in Figure 2.20. W-ARs stabilized emulsion gel had the same inhibitory activity (p>0.05) with W-Sa-ARs stabilized emulsion gel, with the value of 51.23 ± 0.99% and 54.86 ± 3.12%, respectively. These values were significantly higher than that of W-Sa stabilized emulsion gel (36.7 ± 1.44%), indicating ARs contributed to xanthine oxidase inhibition in the samples. Similar result was observed in the buttermilk emulsion gels, B-Sa-ARs stabilized emulsion gel had the highest inhibitory activity, with the value of 58.25 ±

2.64%, followed by B-ARs and B-Sa stabilized emulsion gel, with the value of 51.17 ± 0.98% and 44.00 ± 0.72%, respectively.

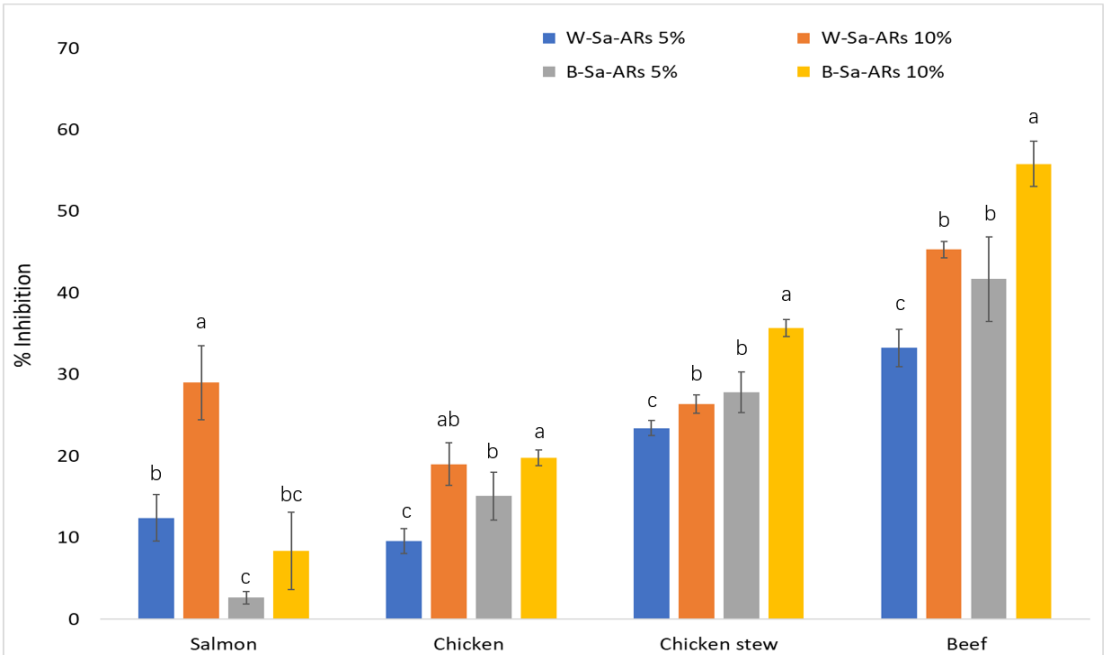


**Figure 2.21 Inhibitory potency of puree samples with incorporated 5% or 10% whey permeate-ARs or buttermilk-ARs emulsion gel against xanthine oxidase (0.2 U/mL) activity at 0.6mM xanthine concentration. Puree sample (salmon, chicken, chicken stew or beef) without emulsion gel incorporation was representative of the 100% enzyme activity. Different letters represent significant different at $p<0.05$.**

Figure 2.21 shows that all puree samples had ability to delay xanthine oxidase activity after incorporation with buttermilk or whey permeate emulsion gel. The inhibitory activity of puree samples with 10% w/w replacement by W-Sa-ARs or B-Sa-ARs emulsion gel was higher than that of puree with 5% w/w replacement. The results indicate that incorporation of emulsion gel improved the xanthine oxidase inhibitory

activity of the puree samples. The increase of inhibitory activity after incorporation of emulsion gel was confirmed by the result of xanthine oxidase inhibitory test of whey ARs (Figure 2.19) and emulsion gel (Figure 2.20), which showed the emulsion gel containing ARs have ability to delay xanthine oxidase activity. Hence, the presence of higher amount of W-Sa-ARs or B-Sa-ARs emulsion gel in the puree would result in higher inhibitory activity.

### 2.381 Xanthine oxidase Kinetics of wheat ARs

In this study, the initial velocity '*v*' of the hydrolysis reactions catalyzed by xanthine oxidase was measured at 0.15-1.2mM xanthine concentrations [*S*] in the absence and presence of 0.1 mg/mL and 1 mg/mL ARs was showed in Figure 2.22, and the kinetic properties of wheat ARs concentration on xanthine oxidase was showed in table 2.7.

**Table 2.7 Kinetic properties of wheat ARs concentration on xanthine oxidase**

| Wheat ARs concentration | Vmax (mol maltose /min) | KM (%) |
|---|---|---|
| 0 mg/ml | 1.14 | 0.046 |
| 0.1 mg/ml | 0.33 | 0.069 |
| 1 mg/ml | 0.31 | 0.070 |

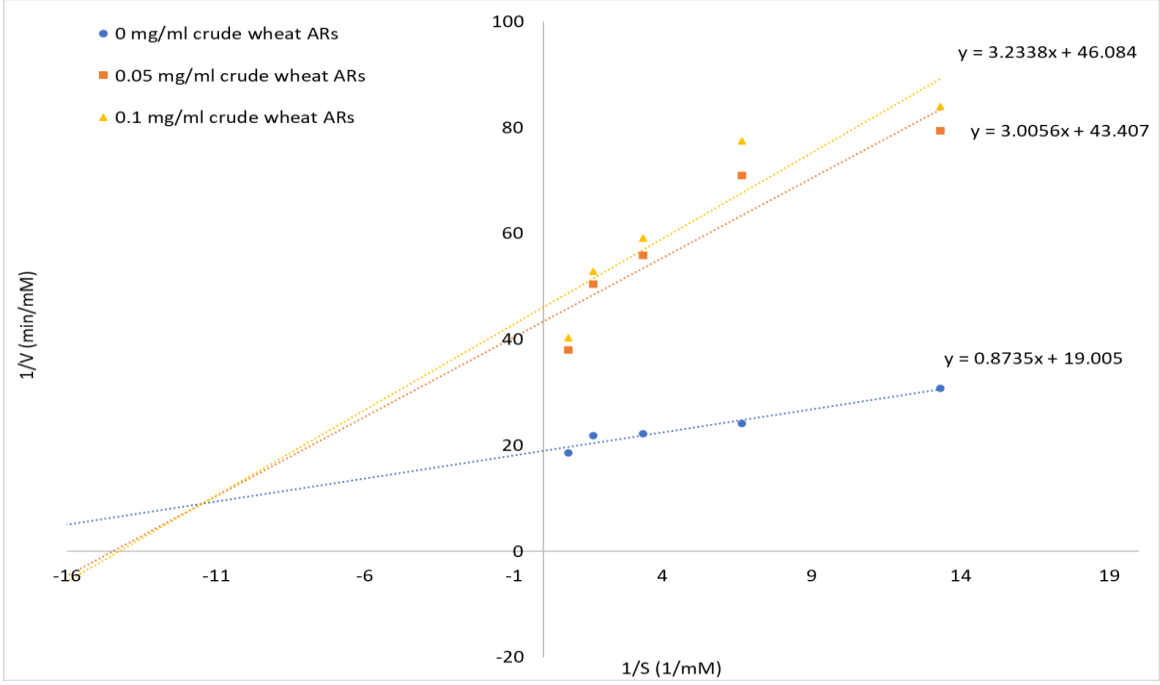


**Figure 2.22 Lineweaver–Burk plot of xanthine oxidase (0.2 U/mL) reaction with variable xanthine concentrations in the absence or presence of alkylresorcinols. Xanthine concentrations change between 0.15 – 1.2 mM**

With increasing ARs concentration, the velocity of the reaction catalyzed by xanthine oxidase decreased, and the Km increased. These results suggest that the mode of ARs on xanthine oxidase was mixed non-competitive inhibition. The kinetic properties of wheat ARs concentration on xanthine oxidase (table 2) show the Vmax values of ARs at 0. 1 mg/mL and 1 mg/mL were e 0.33 and 0.31 mmol/min, respectively, while their Km values were 0.69 and 0.70, respectively. Although the inhibitory activity and mechanism of ARs against xanthine oxidase to date has not been described in the literature, the finding of mixed-type inhibition mechanism suggests that some active component may interact with the amino acid residue of xanthine oxidase domains distal

to the substrate binding site, and then weaken the activity of xanthine oxidase (Hu et al., 2021).

## 2.4 Conclusion

ARs extracted from wheat bran were quantified to be 120.0 mg ARs/100g dry matter. A combination of saponin and ARs in the emulsion gel exhibited the best stability against phase separation and degradation among emulsion gels without ultrasound during 30 days of storage at 30 °C. Ultrasonic homogenization further reduced the size of emulsion droplet to improve the emulsion stability. The liquid holding capacity of purees was improved with the addition of emulsion gels. Purees with 10% w/w emulsion gel had less TEF values compared to purees without the emulsion gel. The positive effect of AR and Saponin whey permeate and buttermilk emulsion gels in purees was evident in the almost void-less images taken with the PLM.

The stability of the emulsion gel and modified puree (with emulsion gel) against the activity of α-amylase, α-glucosidase and xanthine oxidase was positive. ARs alone, and in emulsion gel exhibited significant inhibition on α-amylase compared to emulsions gels with only saponin. Thus, ARs in either buttermilk or whey permeate emulsions were the enzyme inhibiting compound. The inhibitory effect was transferred when emulsions gels containing ARs were incorporated into purees. At 10% w/w emulsion gel incorporation, purees showed better enzyme inhibition than at 5%w/w incorporation.

These results were not unique to α-amylase but were replicated in α-glucosidase and xanthine oxidase tests.

The oral breakdown of starch-based thickeners by carbohydrate-hydrolyzing enzymes is a major safety concern in pureed foods for people with swallowing difficulties. The results obtained in this study imply that development of emulsion gels with wheat ARs for use in puree foods, not only improve the texture of the puree but also enhance the stability of food to oral hydrolysis.

This study presents a different outlook on the applications of emulsion gels in the production of modified texture foods for people with dysphagia. Hitherto, starch and gums were the modifiers of choice but results from this study show that emulsion gels could replace starch or gums and potentially provide better texture-modification. In addition, the emulsion gels were formulated with by-products of the milk and wheat industries which provides another avenue for creating value-added products. The presence of the wheat alkylresorcinols (AR) will also impart the health benefits associated with ARs in any food matrix that is incorporated with the emulsion gel.

# Chapter 3 Optimized psyllium husk emulsion gel stabilized by ultrasound as a functional ingredient in pureed foods to inhibit alpha-amylase, alpha-glucosidase, and xanthine oxidase.

## 3.1 Introduction

Psyllium husk is a hydrophilic substance that is obtained after milling of psyllium seeds and is well-known for its water absorption properties, gelling abilities, and soluble and insoluble fiber content. Psyllium husk can absorb water more than 50 times its initial weight, and it expands and creates a smooth bulky gel (Thari, et.al., 2018). Psyllium has been reported for the treatment of constipation, diarrhea, diabetes, and hypercholesterolemia (Madgulkar, et.al., 2015). The Canadian government authorized the use of health claims on food products containing soluble fiber from psyllium to state that they are associated with a decreased risk of heart disease (Health Canada 2011).

Inulin is a natural plant polysaccharide. In food manufacturing, inulin is commonly exploited as a fat or sugar substitute or to add desired properties to processed foods (Zeng et.al., 2020). When completely combined with water, inulin forms a gel-like structure with a white creamy appearance, a spreadable texture, and characteristics that are similar to those of a fat crystal network in oil, making inulin an interesting ingredient to be incorporated into foods to replace fats in food production (Chiavaro, et.al., 2007). It has been established that inulin delivers only 25–35 percent energy in

comparison with carbohydrates and may be an ideal component to make reduced calorie diets for diabetics to regulate blood sugar levels (Zeng et.al., 2020). Inulin also plays a key function in the pharmaceutical business, where it may be employed as an excipient and stabilizer in drug delivery system, or as a wall material on encapsulation of bioactive compounds (Zabot, et.al., 2016).

Food allergy is an immune system response that is aberrant or exacerbated because of exposure to relatively innocuous particular food proteins or dietary antigens (Ahmed, et.al., 2018). Food allergy is a serious public health issue that affects around 5–8% of young children and 2–4% of adults; however, it is dependent on hereditary variables, early exposure to allergenic materials, and food choices (Luo, et.al., 2020). There are over 170 different foods have been documented to provoke allergic reactions, of these, wheat is one of the nine most common food allergies, together with peanuts, milk, eggs, wheat, nuts, soybeans, fish, crustaceans, and shellfish; these nine most common foods responsible for 90% of reported allergic reactions (Boye, 2012). Wheat bran as the major food sources used for extracting ARs from previous chapter, it might be a concern for its use in patients with wheat allergy because ARs may be contaminated with residual wheat allergen during extraction. Prevention of food allergies continues to be an important objective for persons who suffer from food sensitivities, which frequently demands full avoidance of problematic foods. Hence, the main goal of this chapter is to use psyllium husk and inulin to develop an allergy-free emulsion formulation for

elders who have dysphagia and food allergy

## 3.2 Materials and methods

### 3.21 Chemicals and regents

Sunflower oil from Saporito foods (Montreal, Quebec, Canada). Inulin was extracted from Jerusalem artichoke by Nutia Food Ingredients (Kentwood, Michigan, USA). Sodium alginate (Landor Trading Company) was purchased from Amazon. Psyllium husk was from a local grocery store (Bulkbarn.ca.). Sodium carbonate was from VWR International Co. (Mississauga, ON, CA). Tween 80 (polyoxyethylene-20-sorbitan monooleate) was from Fisher Scientific (CA). α-glucosidase (Cat. no. G5003), α–amylase (Cat. no. A3403), P-NPG (p-Nitrophenyl-α-D-glucopyranoside, Cat. no. 487506), xanthine (Cat. no. X7375), xanthine oxidase from bovine milk (Cat. no. X1875), 3,5-Dinitrosalicylic acid (Cat. no. 128848) and potassium sodium tartrate tetrahydrate (Cat. no. S2377) were from Sigma-Aldrich (St Louis, MO, USA). Salmon puree, chicken puree, chicken stew puree and beef puree were kindly provided by HFS-Apetito.

### 3.22 Psyllium husk emulsion gel formation and storage stability

In present work, to build on the emulsion gel formulated in chapter two, all ingredients were maintained with the exception of wheat ARs, saponin, buttermilk and whey permeate. Emulsions with different percentage of psyllium husk (10 formulations) were

prepared as listed in table 3.1. The ingredients consist of sunflower oil, distilled water, psyllium husk and sodium alginate. First psyllium husk was dissolved completely at water (80 ℃) then cooled to room temperature (23℃). Sodium alginate and sunflower oil were added to the water phase, followed with mechanical stirring using a Tissuemiser (Fisher Scientific, Ontario, CA). The formulated emulsions were stored under refrigeration (4℃) for two weeks to check for phase separation during storage.

**Table 3.1 Different formulations of emulsion properties for screening the most stable emulsions**

| Control formulation | psyllium husk (%) | Water (%) | Sunflower oil (%) | Sodium Alginate (%) |
|---|---|---|---|---|
| 1 | 0 | 81.4 | 17.6 | 1 |
| 2 | 0.5 | 80.9 | 17.6 | 1 |
| 3 | 0.8 | 80.6 | 17.6 | 1 |
| 4 | 1 | 80.4 | 17.6 | 1 |
| 5 | 1.2 | 80.2 | 17.6 | 1 |
| 6 | 1.5 | 79.9 | 17.6 | 1 |
| 7 | 2 | 79.4 | 17.6 | 1 |
| 8 | 2.5 | 78.9 | 17.6 | 1 |
| 9 | 3 | 78.4 | 17.6 | 1 |
| 10 | 4 | 77.4 | 17.6 | 1 |

### 3.221 Optimization of emulsion formulations

Based on results from Psyllium husk emulsion gel formation, formulation 9 (3% w/w) was chosen for emulsion optimization. Emulsions with 0%, 5%, 10%, 15% and 20% (w/w) inulin were prepared to investigate suitable inulin concentration. The percentages of other ingredients were prepared as shown in table 3.1 based on formulation 9. In preparation, inulin was dissolved in water phase to replace the same amount of water

in the formulation, following the same method as described in Section 3.22.

**Table 3.2 different concentration of Jerusalem artichoke inulin in 3% psyllium husk emulsion gel**

| Formulation | psyllium husk (%) | Water (%) | Sunflower oil (%) | Sodium Alginate (%) | Inulin (%) |
|---|---|---|---|---|---|
| A | 3 | 78.4 | 17.6 | 1 | 0 |
| B | 3 | 73.4 | 17.6 | 1 | 5 |
| C | 3 | 68.4 | 17.6 | 1 | 10 |
| D | 3 | 63.4 | 17.6 | 1 | 15 |
| E | 3 | 58.4 | 17.6 | 1 | 20 |

### 3.222 Ultrasound-treated emulsion gel

Emulsions were prepared with formulation shown in table 3.2 and with ultrasound to investigate its effect. The method of Leong et.al. (2017) was followed with modification. 1.5 g of husk was first dissolved completely in 13.7g of water (80 ℃) then cooled to room temperature (23℃). 0, 2.5, 5, 7.5 or 10 g of inulin was mixed in the husk solution by ultrasound at 2 W calorimetric power for 10~20 seconds. 8.8 g of sunflower oil phase was then mixed with the aqueous phase by ultrasound at 10 W calorimetric power for 60~120 seconds. The obtained mixture was then mixed with 25 g of alginate solution (0.5 g of sodium alginate in 24.5 g of water) at 10 W until the emulsion formed was homogenous in appearance. The formulated emulsions were stored at 4℃ for further analysis.

### 3.23 Polarized light microscopic (PLM) observation of emulsion gel

Ultrasound treated and untreated formulation A, C and E emulsion gels were observed

under PLM. The images were taken with a Retiga 1300 camera linked to the Northern eclipse software.

### 3.24 Cryo-Scanning Electron microscopic observation of emulsion gel

Ultrasound treated and untreated formulation A, C and E emulsion gels were observed under cryo-SEM (cryo-scanning electron microscope, Nano Imaging Facility Laboratory of Carleton University, Ottawa, ON). The method of Liu & Lanier (2015) was employed with slight modification. The internal portion of the fresh emulsion gels were cut into blocks of 0.5 cm, then the blocks were frozen on a metal plate surrounding by liquid nitrogen for 20 s. The block was placed on a copper specimen holding and observed under a Cryo-SEM. The microscope was operated at 20 kV in low vacuum mode (40 Pa), with the temperature at <−50 °C.

### 3.25 Droplet size measurement

Droplet size of A, B, C, D and E emulsions from table 3.2 were determined by polarized light microscope (Axioplan 2 imaging and Zeiss Axiophot 2 universal microscope, Carl Zeiss Inc., Jena, Germany). The images were taken with a Retiga 1300 camera linked to Northern eclipse software. The dispersed droplet size from the images was analyzed via Image J software.

### 3.26 Meat sample preparation

Puree samples (salmon, chicken, chicken stew and beef) were stored at least -20 ºC

until needed and thawed until room temperature for the experiment. Different percentages of puree sample (5% and 10% w/w) were replaced with 3% psyllium husk -0%, 10%, or 20% inulin emulsion gels. Each treatment was mixed homogeneously using a Tissuemiser (Fisher Scientific, Ontario, CA). After each mixing, the resultant puree samples were stored at -20 ºC until analysed.

### 3.261 Polarized light microscopic (PLM) observation of puree samples

Pure puree samples (salmon, chicken, chicken stew and beef) and puree samples with 5% and 10% w/w replacement by 3% psyllium husk -20% inulin emulsion gels were observed under PLM. The images were taken with a Retiga 1300 camera linked to the Northern eclipse software.

### 3.262 Cryo-Scanning Electron microscopic observation of puree samples

Pure puree samples (salmon, chicken, chicken stew and beef) and puree samples with 10% w/w replacement by 3% psyllium husk -20% inulin emulsion gels were observed under cryo-SEM (cryo-scanning electron microscope, Nano Imaging Facility Laboratory of Carleton University, Ottawa, ON) to see differences between their morphological characteristics, and the effect of emulsion gels on microstructure of puree samples. Specific method was the same as described in “Section 3.24”.

### 3.263 Total expressible fluid (TEF) determination

The total expressible fluid (TEF) was determined by following the procedures described by Ismail et. al. (2021) and Colmenero et.al. (1995) with minor modification. Three replicates of puree (~10 g) at room temperature were centrifuged (1 min, 3250 g), heated in a water bath (30 min, 70°C) and immediately recentrifuged. In case of microwave oven heating method, three replicates of puree (~10 g) at room temperature were centrifuged (1 min, 3250 g), heated by the household microwave oven (Model DMW799BL, Danby, CA, ON) for 1 min at 700W and immediately recentrifuged. After centrifugation, the supernatant is removed, and the residue was weighed. The total expressible fluid (TEF) was calculated according to the following equation (Ismail et. al., 2021):

$$\text{TEF (\%)} = \frac{\text{Weight of the sample before heating} - \text{Weight of the sample after heating}}{\text{Weight of the sample before heating}} \times 100$$

### 3.264 Texture analysis

Hardness of puree samples were analysed using CT3 Texture Analyzer (Brookfield Engineering Labs Inc., Middleboro, Massachusetts, USA). The trigger force of the analysis was set to 5g, a cylindrical piston 120 mm diameter was used to penetrate the sample at a depth of 10mm at 2mm /s speed. The result is presented as shear force (g). The measurements were performed in triplicate.

### 3.27 Alpha-amylase inhibitory activity

The inhibitory activity of 3% husk emulsion gels and meat sample to α-amylase were carried out according to the standard method with minor modification (Telagari & Hullatti, 2015 & Bhutkar & Bhise, 2012). In a 96-well plate, reaction mixture containing 20 µl α–amylase (1 U/ml) and 60 µl of varying diluted sample solution was preincubated at room temperature for 30 min. Then, 80 µl of 1% soluble starch in buffer (20 mM phosphate buffer pH 6.9) was added as a substrate and incubated further at 37°C for 10 min; 80 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (BioTek Cytation 5, Ottawa, Canada).

3% psyllium husk emulsion gel were diluted in buffer to give a final concentration of 6.25, 12.5, 25, and 50 mg/ml. Additionally, 3% psyllium husk -0%, 5%, 10%, 15%, and 20% inulin emulsion gels were diluted in buffer to give a final concentration of 5 mg/ml. Diluted emulsion samples were centrifuged at 10,000 rpm for 2 mins at room temperature, then the clear supernatant was applied to the test. In case of meat puree sample, a blank was prepared without sample and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium phosphate buffer, PH = 6.9). Meat puree samples were diluted in buffer to give a final concentration of 50 mg/ml and then were centrifuged at10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was applied to the test. Puree sample (salmon,

chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity.

Each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula:

Inhibitory activity (%) = $[(Abs_{bank} - Abs_{sample})/ Abs_{bank}]\times 100$

Where $Abs_{bank}$ is the absorbance of the bank and $Abs_{sample}$ is the absorbance of the sample.

### 3.28 Alpha -glucosidase inhibitory activity

The inhibitory activity of emulsion gels and meat sample on α-glucosidase were carried out according to the standard method with minor modification (Telagari & Hullatti, 2015 & Picot et.al., 2014). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (0.1 mM, pH = 6. 9), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying diluted emulsion gels was preincubated at 37°C for 15 min. Then, 20 µl P-NPG (1 mM) was added as a substrate and incubated further at 37°C for 30 min. The reaction was stopped by adding 50 µl $Na_2CO_3$ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader (BioTek Cytation 5, Ottawa, Canada). A blank was prepared without diluted emulsion gels and another without the glucosidase enzyme, replaced by equal quantities of buffer (0.1 mM phosphate buffer, PH = 6.9). 3% psyllium husk -0% inulin emulsion gel were diluted

in buffer to give a final concentration of 5, 6.25, 12.5, 25, and 50 mg/ml. Additionally, 3% psyllium husk -0%, 5%, 10%, 15%, and 20% inulin emulsion gels were diluted in buffer to give a final concentration of 5 mg/ml. In case of meat sample, a blank was prepared without emulsion sample and another without the amylase enzyme, replaced by equal quantities of buffer (0.1 mM phosphate buffer, PH = 6.9). Meat samples were diluted in buffer to give a final concentration of 50 mg/ml and then were centrifuged at 10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was applied to the test. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. A blank was prepared without sample and another without the glucosidase enzyme, replaced by equal quantities of buffer (0.1 mM phosphate buffer, PH = 6.9).

Each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula:

Inhibitory activity (%) = $[(Abs_{bank} - Abs_{sample})/ Abs_{bank}]\times 100$

Where $Abs_{bank}$ is the absorbance of the bank and $Abs_{sample}$ is the absorbance of the sample.

### 3.29 Xanthine oxidase inhibitory activity

The assay carrying out based on the procedure reported by Ferraz et. al. (2006), with modification. The diluted emulsion gels were dissolved in ethanol and Tween 80 and

diluted with water to give final concentration of 1% (v/v), ethanol and 0.1% (w/v) Tween 80 in the diluted emulsion gels solution. The diluted emulsion gels from 3% psyllium husk -0% inulin emulsion gel were diluted to give a final concentration of 6.25, 12.5, 25, and 50 mg/ml. Additionally, 3% psyllium husk -0%, 5%, 10%, 15%, and 20% inulin emulsion gels were diluted to give a final concentration of 5 mg/ml. In a 96-well plate, reaction mixture containing 36 µl of diluted emulsion gels solution, 55 µl of 1/15M phosphate buffer (pH 7.5) and 14 µl enzyme solution (0.2 units/mL in phosphate buffer). To ensure that there was no absorbance change due to the plant material, after preincubation of the mixture at 25 ◦C for 1 min, the absorbance (295 nm) was measured spectrophotometrically every 12 s for 2 min. Then, the reaction was initiated by adding 107 µl of substrate solution (0.6mM in water). The assay mixture was incubated at 25◦C with the absorbance (295 nm) measured spectrophotometrically every 6 s, using a Multiplate Reader (BioTek Cytation 5, Ottawa, Canada). Meat samples were diluted in buffer to give a final concentration of 50 mg/ml and then were centrifuged at 10,000 rpm for 2 mins at room temperature. After centrifugation, the clear supernatant was applied to the test. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity.

A blank was prepared without sample was replaced by equal quantities of water, and another blank without the enzyme was replaced by equal quantities of buffer (1/15 M

phosphate buffer, PH = 7.5). Each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula:

Inhibitory activity (%) = $[(Abs_{bank} - Abs_{sample})/ Abs_{bank}]\times 100$

Where $Abs_{bank}$ is the absorbance of the bank and $Abs_{sample}$ is the absorbance of the sample.

### 3.30 Statistical analysis

Statistical analyses were performed with SAS Software (SAS Institute Inc, Cary, NC). One-way analysis of variance (ANOVA) by Duncan's Multiple Rangetest was used to compare the mean values and to find significances between results ($\alpha$=0.05). Differences were significant at $p < 0.05$.

## 3.3 Results and discussion

### 3.31 Psyllium husk emulsion gels formation and stability

The condition of the Figure ten formulations of psyllium husk emulsion gel after two weeks of storage at 4℃ is presented in Figure 3.1. A 4℃ temperature is representative of household refrigerated storage. Formulations were mostly all one phase and stable, except the emulsions with 0% husk and with 4% husk. Phase separation was observed in the emulsion with 0% husk while with the 4% husk, a bulk gel was produced which could not disperse homogeneously in emulsion. Incorporation of 0.5% husk in emulsion gel greatly improved its stability compared to emulsion with 0% husk. As husk concentration increased, texture of emulsion became coarse, and its color became darker. Emulsions with 0% ~ 2% husk were liquid and creamy. Emulsions with 2.5% ~ 4% were gel-like solid and did not flow, indicating gels were formed (Li et al., 2021). Therefore, emulsion gel with 3% husk was chosen for optimization.

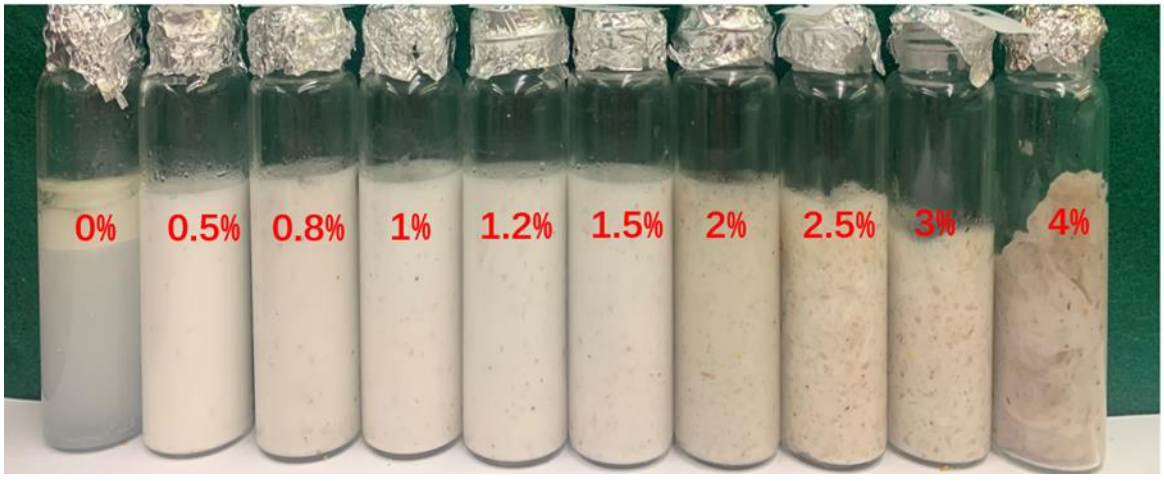


**Figure 3.1 Control emulsion with different concentration of psyllium husk after two weeks**

**storage at 4℃.**

### 3.311 Effect of inulin on emulsion gels

Figure 3.2 shows different concentrations of inulin in 3% husk emulsion gels, with all emulsion gels homogenized and stable. As inulin concentration increased, texture of emulsion gel became creamier, and became easier to spread. Similar results were observed by Bot et.al (2004), who demonstrated that inulin is able to form a gel-like structure with a white creamy appearance, a spreadable texture, and characteristics that are similar to those of a fat crystal network in oil. These characteristics make inulin an interesting ingredient to be incorporated into foods to replace fats (Chiavaro, et.al., 2007).

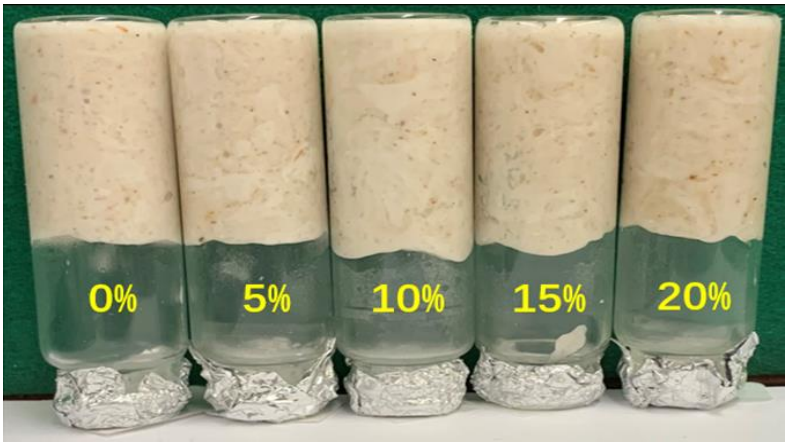


**Figure 3.2 different concentration of Jerusalem artichoke inulin in 3% psyllium husk emulsion gel**

### 3.32 Polarized light microscopy observation of emulsion gels

Polarized light microscopy observation, as shown in Figure 3.3, indicates that inulin concentration had a significant influence on the morphologies of emulsion gels prepared with 3% psyllium husk.

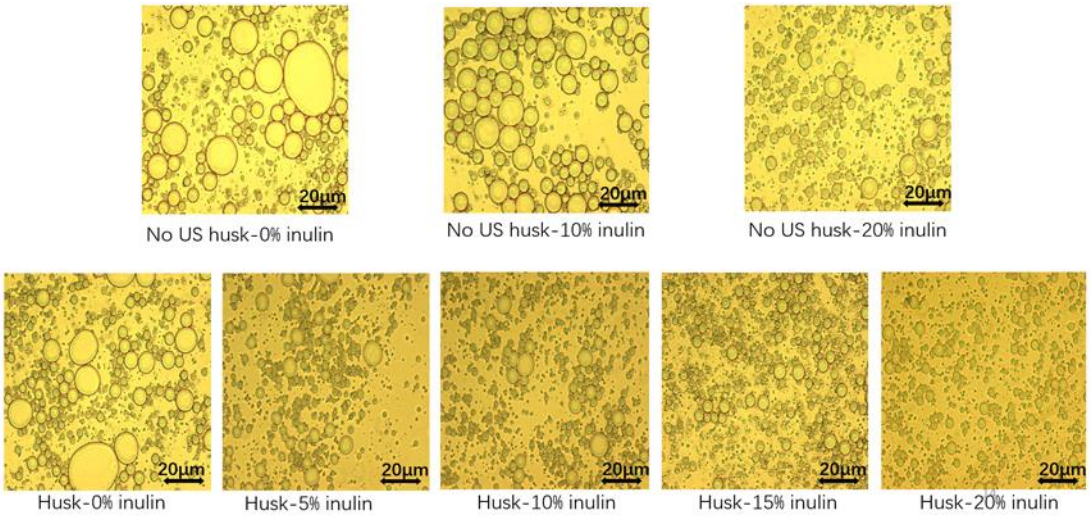


**Figure 3.3 microscopic observation (50X) of ultrasonic-treated and untreated 3% psyllium husk emulsion gel with different concentration of inulin. Dark arrow indicates length of 20 µm.**

At 0% inulin concentration, emulsion droplets in close proximity to each other were large and polydispersed, which increased the flocculation rate. A higher degree of flocculation of emulsion droplets indicates the emulsion system has less stability (Yvonne and Victoria, 2018). The oil droplets in the emulsion gels became smaller and uniform as the inulin concentration increased suggesting that the husk-emulsions were stable at higher inulin concentration. A similar result was reported by Xu et. al. (2020), who reported that the particle size of oil-in-water emulsion was reduced with the increasing concentration of inulin. The decrease in the droplet might be because high

concentration of inulin could form a secondary interfacial layer which enhance its coating on the surface of oil droplets to prevent against droplet aggregation and coalescence, and so result in smaller oil droplets and more stable emulsion (Sarkar et al., 2018). These results were further confirmed by the particle analysis and SEM image.

In comparison to untreated emulsion, the ultrasonic-treated emulsions showed higher stability with smaller particle size. Reduction of droplet size by ultrasound could be attributed to shear force generated by the cavitation of the ultrasonic wave breaking droplets into smaller sizes (Guo et al. (2014).

### 3.321 Particle size analysis

The droplet size distribution of husk emulsion gel system was determined using ImageJ software and results shown in Figure 3.4, with average particle size collated in table 3.3.

**Table 3.3 Average particle size of ultrasonic treated 3% psyllium husk emulsion gel with different concentration of inulin.**

| Ultrasonic-treated emulsion | Average particle size (μm) |
|---|---|
| Husk-0% inulin | $14.98 \pm 9.84^{a}$ |
| Husk-5% inulin | $5.86 \pm 5.28^{b}$ |
| Husk-10% inulin | $2.20 \pm 1.50^{c}$ |

| | |
|---|---|
| Husk-15% inulin | 2.60 ±2.18$^{c}$ |
| Husk-20% inulin | 1.58 ±1.46$^{d}$ |

Values are the mean±SD.

Different letter superscripts in the same row are significantly different ($p<0.05$).

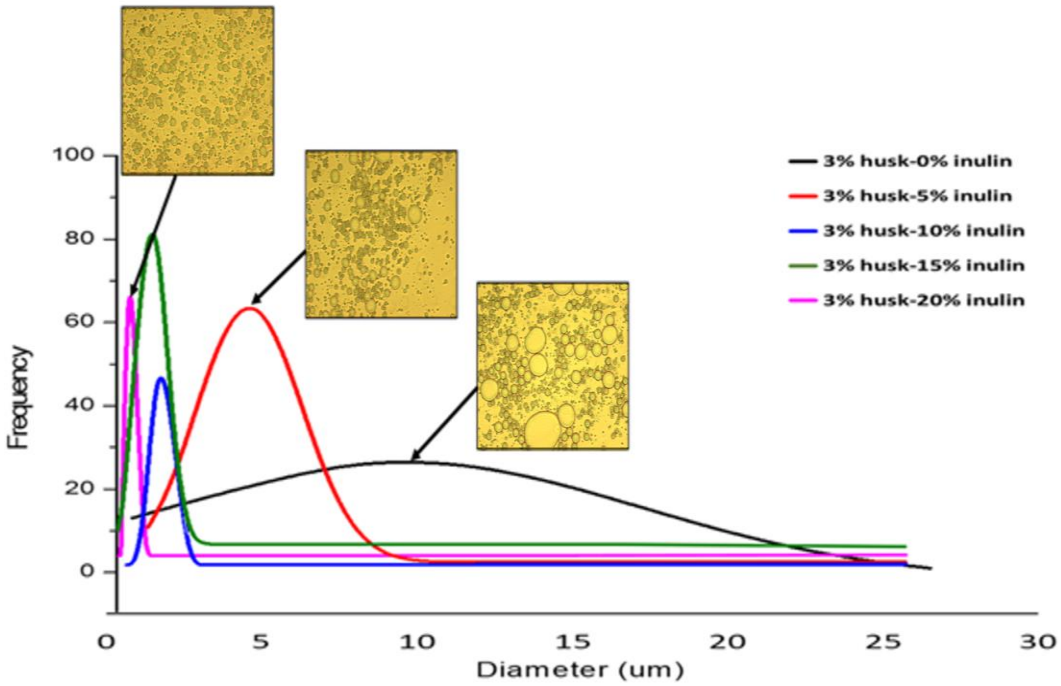


**Figure 3.4 Particle size analysis of ultrasonic-treated 3% psyllium husk emulsion gel with different concentration of inulin.**

The particle size analysis of the emulsion gel system indicated one peak which tended to become sharper and shifted progressively to the left towards smaller droplet size with increase in inulin concentration (Figure 3.4). The result revealed that emulsion droplets were more uniformly distributed, and the number of smaller droplets kept increasing in the emulsion gels. This result was also confirmed by the average particle size (table 3.3), which showed that husk-0% inulin emulsion gel had the largest average particle

size, 14.98 ±9.84 µm, while husk-20% inulin emulsion gel had the smallest average particle size, 1.58 ±1.46 µm.

Reduction of droplet size could be because the increase in polysaccharide concentration made the gel-network stronger and more compact, which entrapped emulsion oil droplets and reduced droplet aggregation (Xu et. al., 2020). In addition, inulin was available to wrap the surface of the oil droplet which limited the movement of emulsion droplets and prevented the aggregation of the droplets, so resulted in smaller oil droplets and more stable emulsion (Sarkar et al., 2018 & Hu et al., 2020). These results were further confirmed by the result of PLM and SEM image.

### 3.33 Cryo-Scanning Electron microscopic observation of emulsion gel

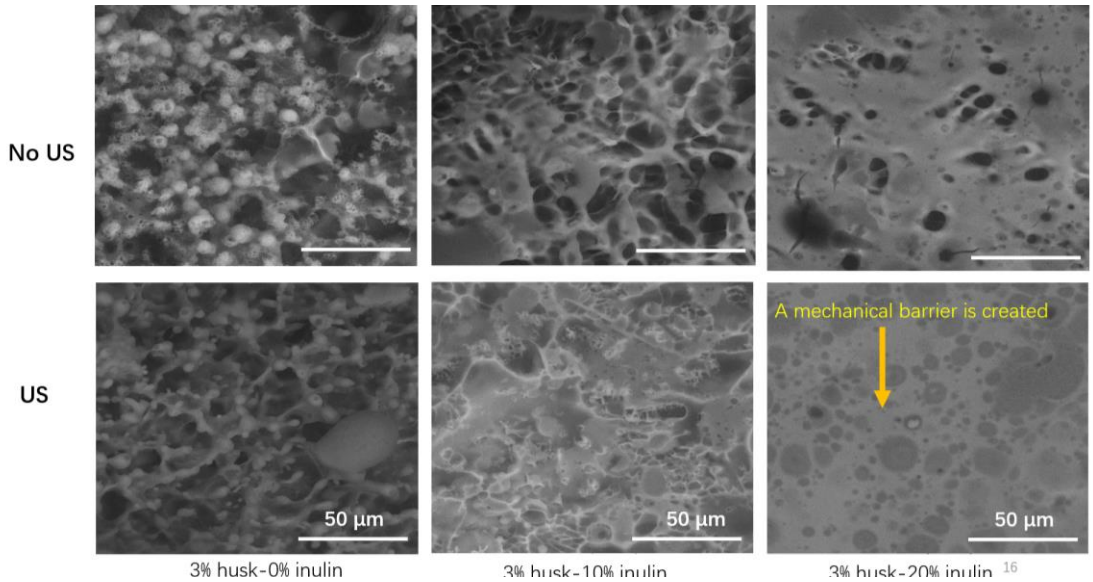


**Figure 3.5 SEM observation of ultrasonic-treated and untreated 3% psyllium husk emulsion gel with different concentration of inulin. Dark arrow indicates length of 50 µm.**

The SEM images (Figure 3.5) revealed that inulin addition and ultrasonic treatment affected the morphology of the husk emulsion gel system. The microstructure of the emulsion gel without inulin was heterogeneous and porous. When the inulin concentration increased, the surface of droplets changed from irregular to smooth, and less cavities were observed, suggesting the inulin was in a packed arrangement at the interface of droplets, and could also form an interfacial layer that coat the surface of oil droplets. Similar result was observed by Zhu et. al. (2019), who developed an emulsion with zein/corn fiber gum complex., Their study reported that the polysaccharide complex was adsorbed at the oil-water interface, and the polysaccharide complex also created a mechanical barrier to prevent the rupture of interfacial film. Adsorption of polymers at the interface could enhance mechanical properties of the interfacial films, which can inhibit coalescence, therefore, droplets with bigger size tend to have weaker interfacial films (Mao & Miao, 2015),

Compared to the untreated emulsion, the surface of the droplets in ultrasonic-treated emulsions showed a higher integrity of the coating layer. At 20% inulin concentration, the dark area in the untreated emulsion gel (Figure 3.5) showed droplets were not completely covered by the inulin layer. On the other hand, the surface of the ultrasonic treated emulsion was very smooth, and no gap was observed, suggesting that the droplet is completely covered by the inulin layer in at 20% inulin concentration. Similar result

was observed by Xiong et al. (2019), who reported that more emulsifier molecules were adsorbed on the surface of the oil droplets in the ultrasonic-treated xanthine gum emulsion compared to that of emulsion without ultrasonic treatment. The increased particle adsorption on the surface of emulsion droplet by ultrasound is suggested to occur from momentum transfer from the fluid to the particles and droplets caused by random cavitation events. The momentum transfer could overcome the stabilizing energy barriers that would normally prohibit nanoparticles from spontaneously adhering to the oil-water interface (Lee et al.,2019).

Overall, the results of SEM images showed that an innovative edible husk (up to 3%)-inulin (up to 20%) (O/W/O) emulsion gel was developed, in which the inulin molecule acted as fat and formed a protective layer to cover the husk (O/W) emulsion gel. Ultrasonic treatment further improved the formation of inulin layer which is beneficial for encapsulation of emulsion droplet. An illustration of the phenomenon is depicted in Figure 3.6.

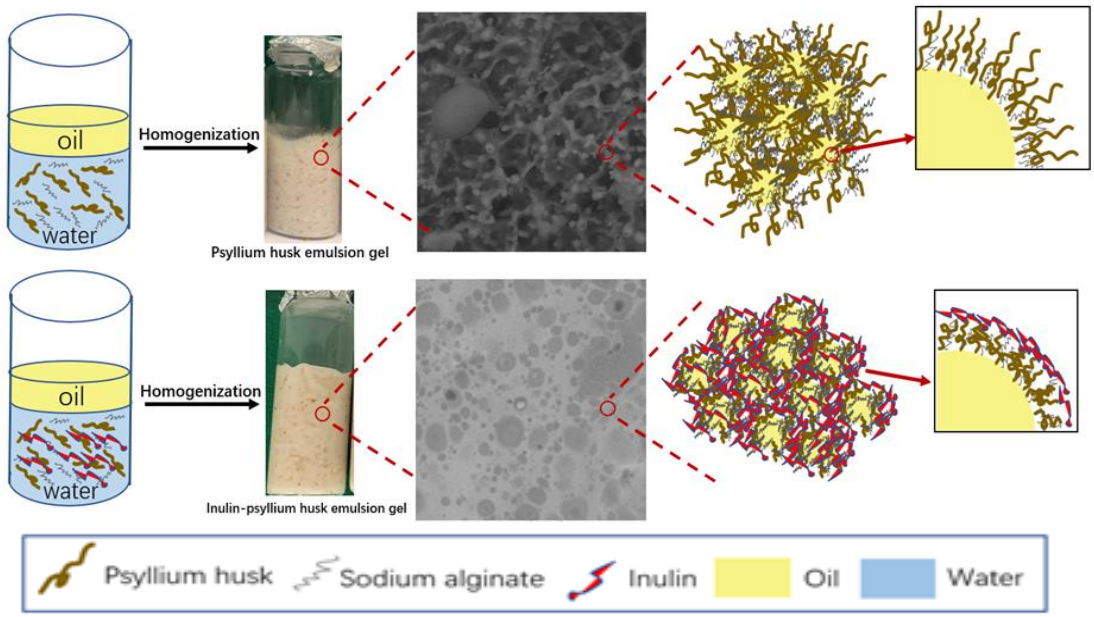


**Figure 3.6 Schematic model of inulin molecule acted as fat and formed a protective layer to cover the husk (O/W) emulsion gel to form inulin-husk (O/W/O) emulsion gel**

## 3.34 Total expressible fluid (TEF) determination

Figure 3.7 and Figure 3.9 represents the results of TEF of different purees by water bath heating, and by microwave oven heating, respectively.

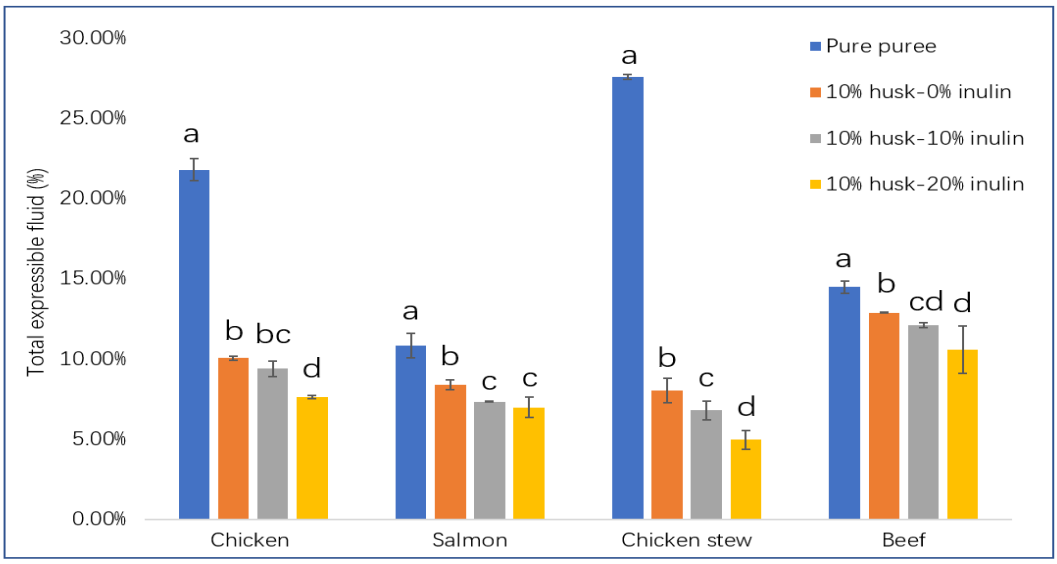

**Figure 3.7 Effect of incorporation of 10% psyllium husk (PH) emulsion gel with 0%, 10% and 20% inulin in puree samples at 70°C water bath heating for 30 min and then centrifugation at 3750 g for 3 min. Different letters represent significant different at $p<0.05$.**


**Figure 3. 8 Effect of incorporation of 10% psyllium husk emulsion gel with 0%, 10% and 20% inulin in puree samples at microwave oven heating with power 700W for 2 min and then centrifugation at 3750 g for 3 min. Different letters represent significant difference at $p<0.05$**

The TEF of water bath-heated or microwave-heated pure puree (chicken, salmon, chicken stew and beef without emulsion gel incorporation) was significantly higher than that of puree with 10% w/w husk-0% inulin emulsion gel incorporation. Water bath heating and microwave oven heating methods were selected in this study because they are common household heating methods. With increased concentration of inulin in emulsion gel (from 0% to 20%), incorporation of the emulsion gel in the purees further reduced the TEF of puree. So, the puree with husk-20% inulin emulsion gel had

the lowest TEF among the same puree group. For example, within the chicken puree group in Figure 3.7, the pure puree (no emulsion gel incorporation) had the highest TEF, with the mean value of 31.77%, while the puree with 10% w/w replacement by husk-20% inulin emulsion gel had the lowest TEF, with the mean value of 17.22%. A similar result was observed in the microwave-heating method (Figure 3.8), the puree with husk-20% inulin emulsion gel incorporation still had the lowest TEF within the same puree group. The reduction of TEF with the inclusion of husk emulsion gel may be because ingredients in the emulsion gel could crosslink with the polar groups of matrix protein. The cross-linking could make the puree have stronger three-dimensional network structure, and lead to stronger binding properties and a smaller release of fluid during heating (Colmenero et al., 2005 & Kumar, 2021). Previous study (Kumar, 2021) has shown inulin has water and fat retention capacity), which also help to decrease TEF.

In comparison to water bath heated-meat puree, the microwave-heated meat puree had higher TEF, which agrees with Colmenero et al., (2005). This may be because the microwave heating was internal which created higher center temperature of the puree, and the high steam pressure drove more water from within the sample to the surface (Wang et. al., 2020).

### 3.35 Microstructural properties of purees

Figure 3.9 and Figure 3.10 shows micrographs of the purees by PLM and SEM, respectively.

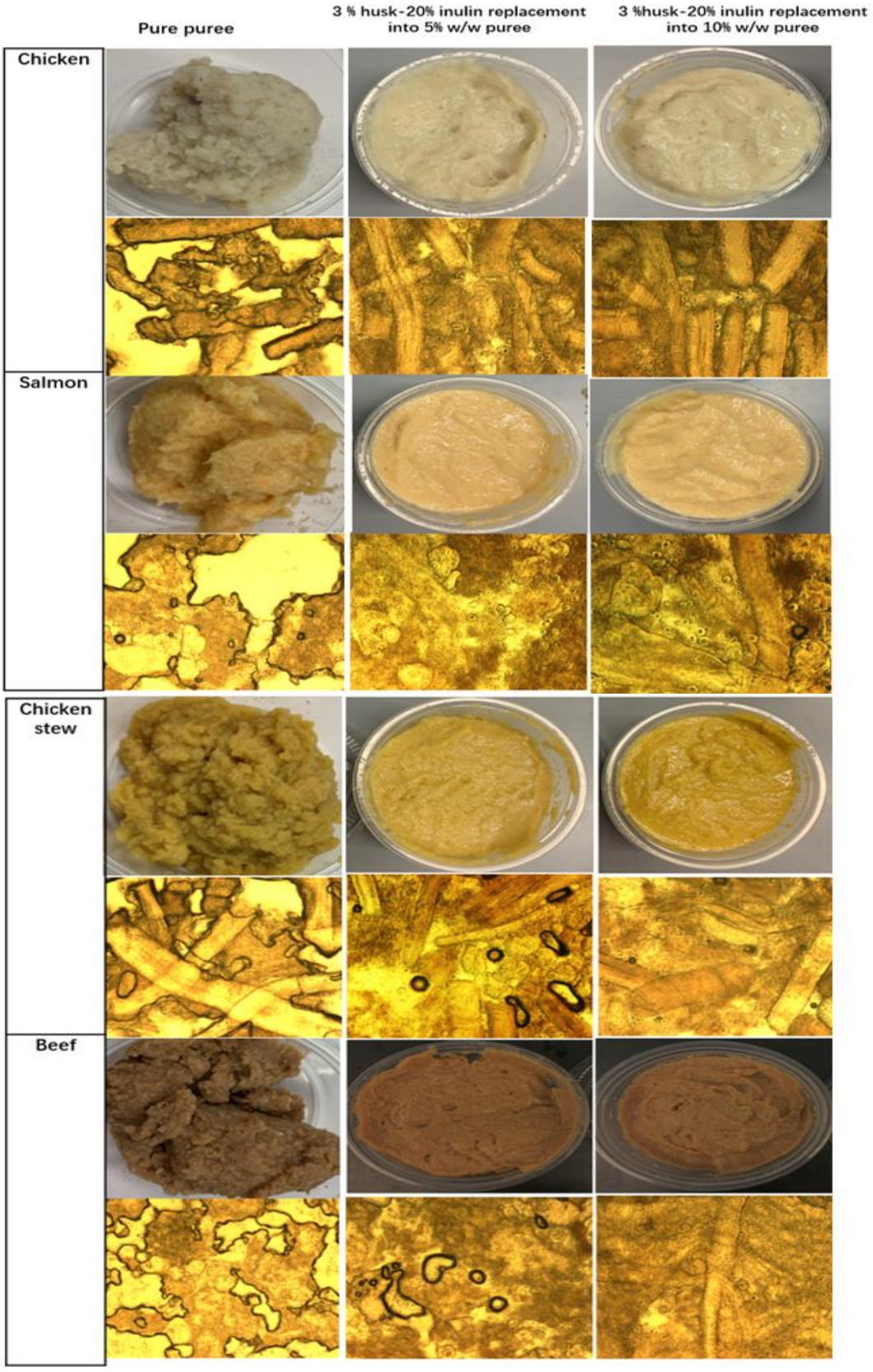


**Figure. 3.9 Visual and microscopic observation (20X) of puree samples before and after**

**incorporation with 5% or 10% w/w husk-20% inulin emulsion gel**

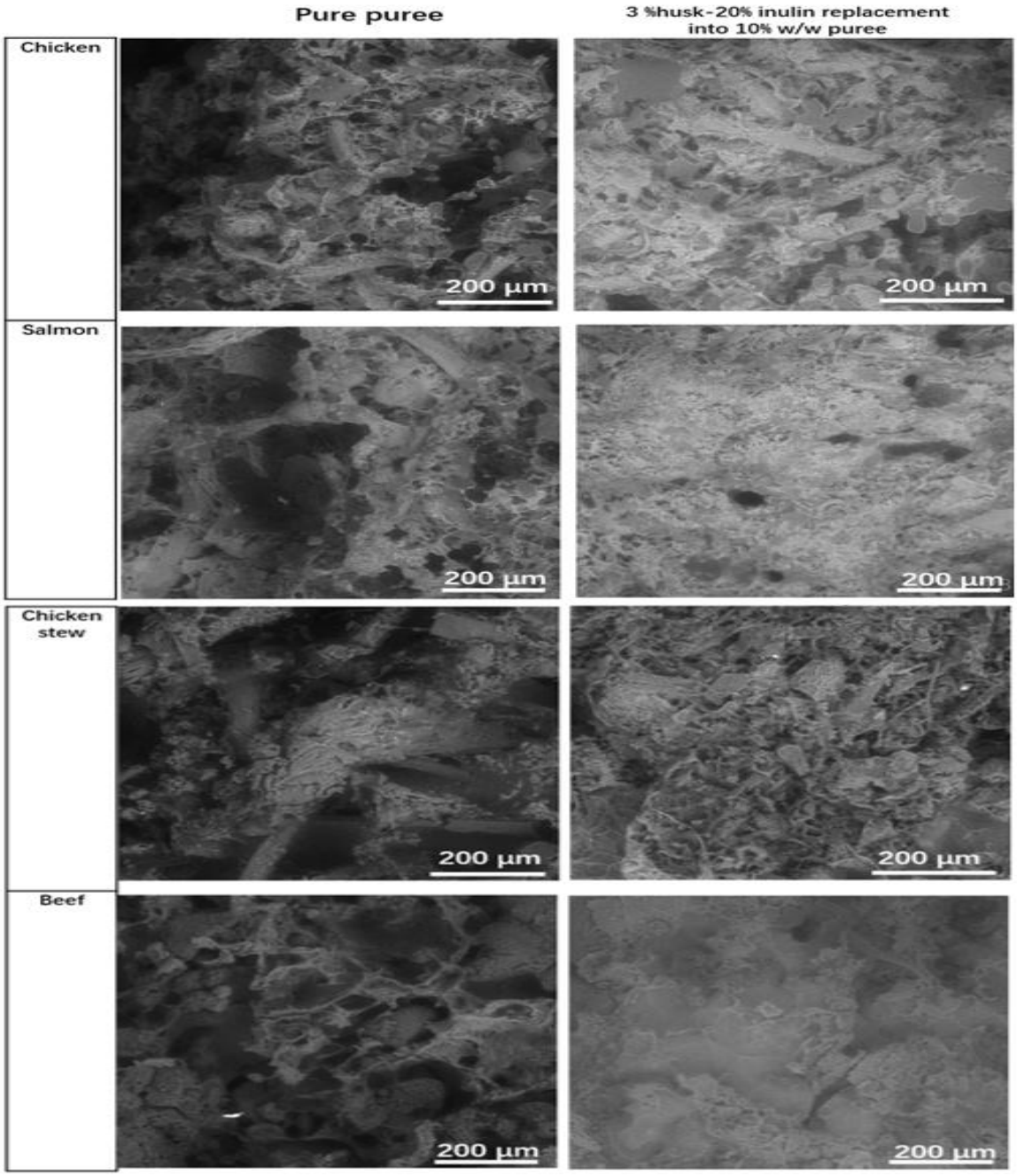


**Figure. 3.10 SEM observation of meat puree samples before and after incorporation with 10% w/w husk-20% inulin emulsion gel**

In PLM images (Figure 3.9), the morphology of the control purees showed larger voids.

However, with increased concentration of emulsion gel (from 0% to 10%) in meat puree, less voids were observed, and when 10% emulsion gel was added in meat puree, no void was observed in all puree samples. Similar results were also observed in the SEM images (Figure 3.10). In SEM images, all pure puree samples exhibited the formation of cavities of different sizes creating structures with a rough and porous appearance. The high proportion of cavities can be interpreted as the expansion of water, fat, and air in the protein network (Paglarini et.al. 2022). In the puree with 10% emulsion gel incorporation, less cavities and more compact structure compared to pure puree was also observed because the emulsion gel contained high amount of inulin, (Felisberto et al., 2015). The change in the structure was likely owing to the long chain length of inulin and degree of polymerization, which could cause the puree to become firmer and more viscous. Hence, inulin was suggested to be employed to enhance food texture (Felisberto et al., 2015).

According to Colmenero et al. (1995), meat matrices with homogeneous and more compact (less and smaller cavities) structures, similar to the structure of puree sample with 10% emulsion gel (Figure 3.10), could be classified as strong meat gels with good water and fat binding properties. On the other hand, meat matrix with irregular and less compact (more and larger cavities), and aggregate structures, similar to the structure of pure puree samples (Figure 3.10), could be classified as poor meat gels with weak binding properties.

### 3.36 Texture analysis

The results of the textural analysis in puree samples are shown in Figure 3.11.

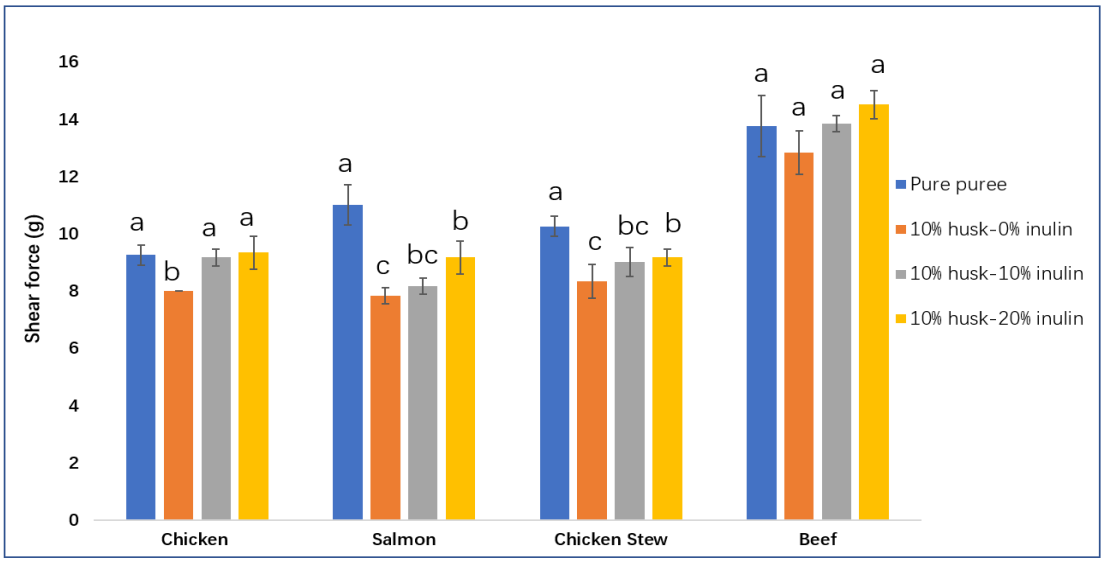


**Figure 3.11 Effect of incorporation of 10% psyllium husk emulsion gel with 0%, 10% and 20% inulin in puree samples on texture analysis. Different letters represent significant different at $p<0.05$.**

When husk-0% inulin emulsion gel was replaced at 10% w/w in purees, all except beef puree, had significantly ($P < 0.05$) lower values for hardness when compared to the control puree. Lower hardness values indicate that adding 3% husk-0% inulin emulsion gel makes the puree softer. This result was similar to studies by Grigelmo-Miguel et. al. (1999) and López-López et al. (2010), who reported that the inclusion of insoluble fibre could decrease the hardness of cooked meat. The insoluble three-dimensional network

of the insoluble dietary fibre within the meat system could have disrupted the protein–water or protein–protein gel network, resulting in a reduced meat gel strength and a softer texture. (Comer & Dempster, 1981, Grigelmo-Miguel et. al., 1999 & López-López et al., 2010).

When husk-10% inulin emulsion gel was replaced at 10% w/w in purees (chicken, salmon and chicken stew), it had significantly ($P < 0.05$) higher values of hardness compared to that of with 10% w/w replacement by 3% husk-0% inulin emulsion gel. Purees (chicken, salmon and chicken stew) with husk-20% inulin emulsion gel had higher values of hardness compared to those with 3% husk-10% inulin emulsion gel and reached similar ($P>0.05$) value of hardness with the control puree. This indicates that the addition of inulin is useful in compensating for the changes in texture caused by adding husk emulsion gel alone in meat puree. Similar results by Paglarini et.al (2022), demonstrated that addition of inulin emulsion gel in cooked sausages could increase their firmness, resulting in a higher hardness.

Although there was a study proposing that inulin could transfer its creamy characteristic to meat puree and make it softer (Luisa, Cáceres & Dolores, 2006), the different outcome could be differences in chain length of inulin, degree of polymerization, and the amount of inulin (Nourbehesht et al., 2018 & Felisberto et al., 2015). At increasing levels of inulin concentration, inulin absorbs more water and creates a crystalline

network with greater flexibility, which inhibits creaming phenomenon (Nourbehesht et al., 2018).

Interestingly, pure beef puree had the highest hardness among all pure purees, but there were not significant ($P > 0.05$) differences observed for its hardness after incorporation of emulsion., That could be because the gel strength of pure beef puree was too strong to be affected by the addition of husk-emulsion gel.

### 3.37 Alpha-amylase inhibition test

Alpha-amylase is a key enzyme in the digestion of dietary starch, producing oligosaccharides that can be broken down further into glucose. Results of the α-amylase inhibition activity by 3% husk emulsion gel are shown in Figure 3.12.

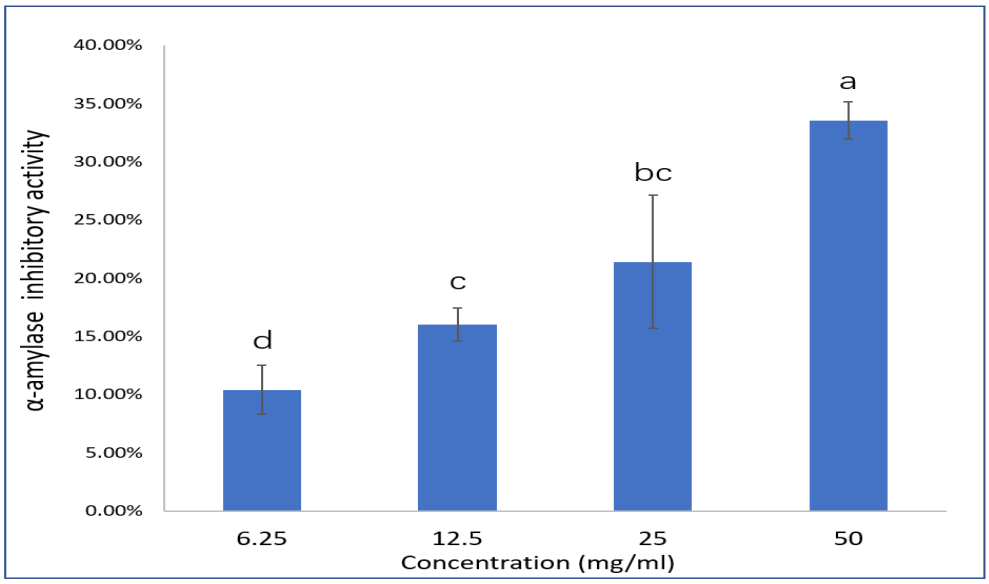


**Figure 3.12 Inhibitory potency of 3% husk emulsion in the absence of inulin at 20X, 40X, 80X**

**and 160X against α-amylase (1 U/mL) activity at 1% starch concentration. Different letters represent significant different at p<0.05.**

The inhibitory activity of the 3% husk emulsion gel was conducted at concentrations of 6.25, 12.5, 25 and 50 mg/ml with the highest inhibitory activity at 50 mg/ml. Results indicated that inhibitory activity increased with increasing husk emulsion gel concentration, demonstrating a dose-dependent effect. The inhibitory activity of 3% husk emulsion gel at 50 mg/ml on α-amylase was 33.54 ± 1.57%.

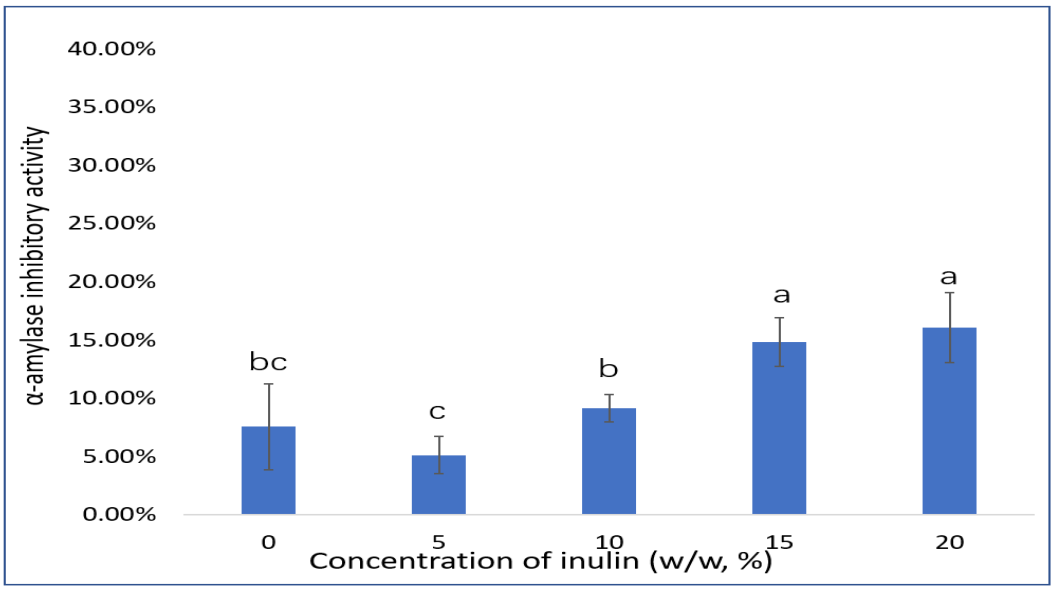


**Figure 3.13 Inhibitory potency of 3% psyllium husk emulsion in the presence of different concentration of inulin at 200X dilution against α-amylase (1 U/mL) activity at 1% starch concentration. Different letters represent significant different at p<0.05.**

The inhibitory effect of 3% husk emulsion with addition of 0%, 5%, 10%, 15% and 20% w/w inulin at 5 mg/ml on the activity of α-amylase are shown in Figure 3.13. Inhibitory

activity increased with increasing inulin concentration from 0% to 15%. When the inulin concentration reached 20%, the emulsion gel had the same inhibitory activity ($p>0.05$) with that of 15% inulin concentration. The inhibitory activity of 3% husk emulsion gel with 20% inulin at 5 mg/ml on α-amylase was 16.06 ± 3.03%.

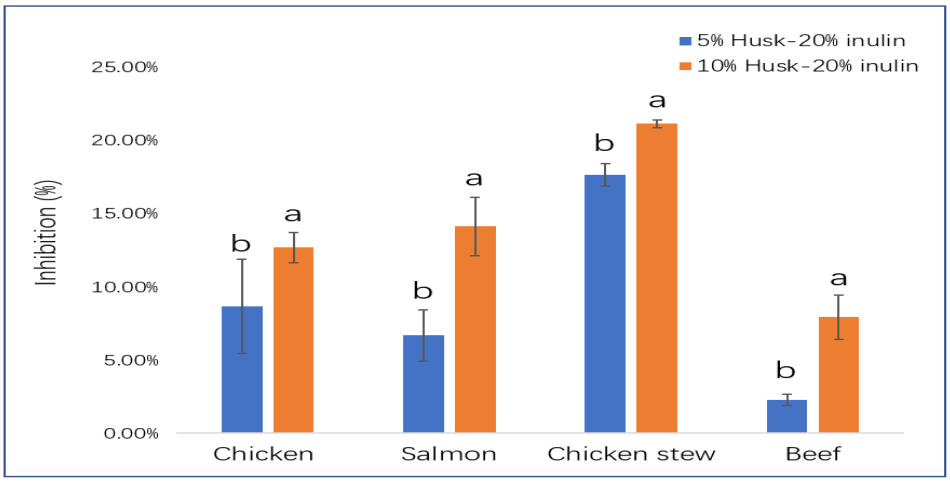


**Figure 3.14 Inhibitory potency of puree samples with incorporated 5% or 10% husk-20% inulin emulsion gel against α-amylase (1 U/mL) activity at 1% starch concentration. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. Different letters represent significant different at $p<0.05$.**

The inhibitory effects of puree samples (salmon, chicken, chicken stew or beef) with 5% w/w and 10% w/w husk-20% inulin emulsion gel on the activity of α-amylase in (Figure 3.14), show all puree samples can delay α-amylase activity. The inhibitory activity of puree samples with 10% w/w replacement by the emulsion gel was higher than that of puree with 5% w/w replacement. Amongst the purees with 10% w/w replacement by husk-20% inulin emulsion gel, the chicken stew had the highest

amylase inhibition of 21.14%, followed by salmon and chicken with the inhibition of 14.12 and 12.68%, respectively. The beef had the lowest amylase inhibition of 7.93%. Guo et. al. (2020) reported that the hydroxyl (-OH) and carboxyl groups (-COOH) on the branched chain of polysaccharides could interact with the amino acid residues of digestive enzymes (α-amylase and α-glucosidase), thus inhibiting digestive enzyme activity. In addition, Ahmed & Urooj (2010) concluded that dietary fiber, such as wheat bran and psyllium husk, could bind glucose, and thereby inhibit amylase activity. The inhibition of amylase activity was also observed by Ou et. al. (2001), who suggested that soluble fiber might capsulate α-amylase and starch, and so reduce accessibility of starch to the enzyme.

From the results, it can be concluded that incorporation of husk-20% inulin emulsion gel will be beneficial to delay breakdown of dietary starch in food product and thereby contribute for maintaining the consistency of the food product with addition of starch-based thickener during oral digestion.

### 3.38 Alpha-glucosidase inhibition test

Alpha-amylase digests dietary starch and other related carbohydrates to a large number of oligosaccharides, which is further digested by alpha-glucosidase to glucose.

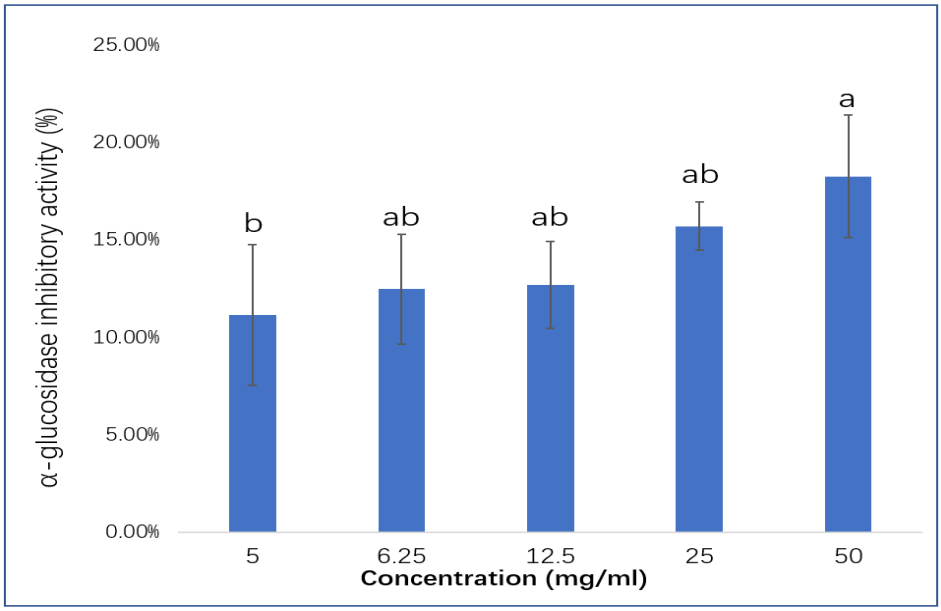


**Figure 3.15 Inhibitory potency of 3% psyllium husk emulsion in the absence of inulin at 20X, 40X, 80X, 160X and 200X dilution against α-glucosidase (1 U/mL) activity at 1mM PNPG concentration. Different letters represent significant different at $p<0.05$.**

The inhibitory activity of the 3% husk emulsion gel conducted at concentrations of 5, 6.25, 12.5, 25 and 50 mg/ml (Figure 3.15.) resulted in the 50 mg/ml concentration having the highest mean inhibitory activity. Inhibitory activity increased with increasing husk emulsion gel concentration, indicating a dose-dependent effect. The inhibitory activity of 3% husk emulsion gel at 50 mg/ml on α-amylase was 18.26 ± 3.15%.

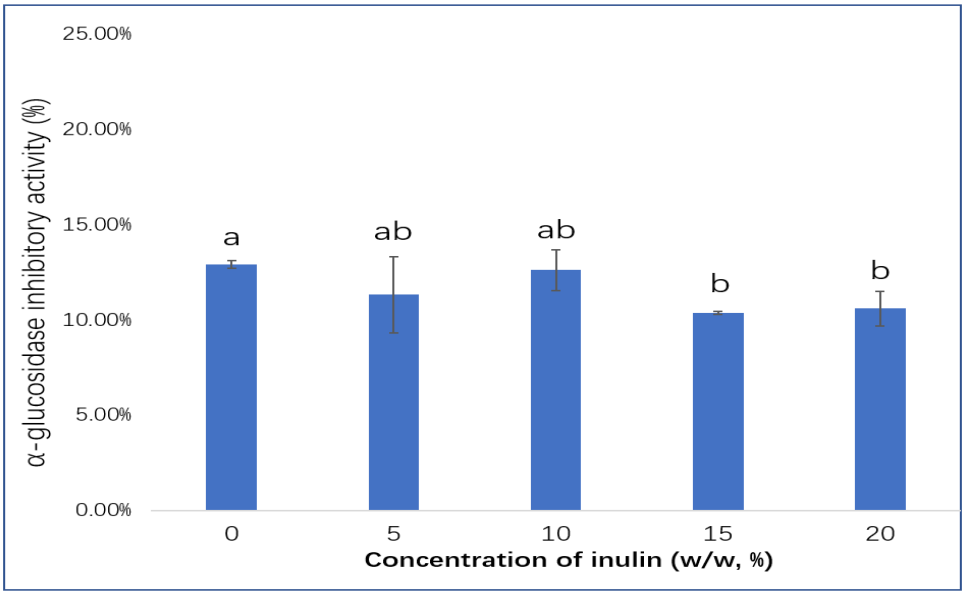


**Figure 3.16 Inhibitory potency of 3% psyllium husk emulsion in the presence of different concentration of inulin at 200X dilution against α-glucosidase (1 U/mL) activity at 1mM PNPG concentration. Different letters represent significant different at $p<0.05$.**

The inhibitory effects of 3% husk-emulsion with addition of 0%, 5%, 10%, 15% and 20% w/w inulin at 5 mg/ml on the activity of α- glucosidase are shown in Figure 3.16. Inhibitory activity slightly decreased with increasing inulin concentration from 0% to 20%. The husk-emulsion gel without inulin addition had the highest glucosidase inhibition of 12.93 ± 0.19%, while the husk-emulsion gel with 20% w/w inulin addition had the lowest glucosidase inhibition of 10.6 ± 0.91%,

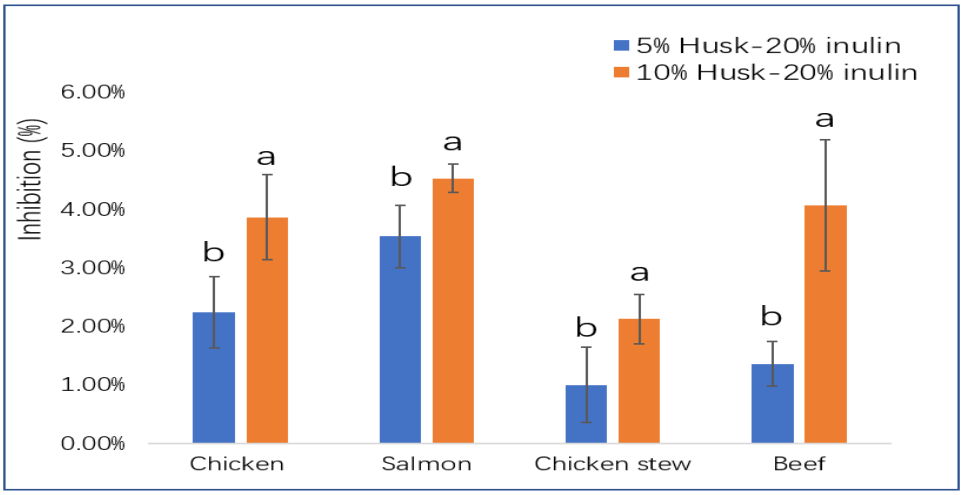


**Figure 3.17 Inhibitory potency of puree samples with incorporated 5% or 10% husk-20% inulin emulsion gel against alpha-glucosidase (1 U/mL) activity at 1mM PNPG concentration. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. Different letters represent significant different at $p<0.05$.**

The inhibitory effects of puree samples (salmon, chicken, chicken stew or beef) with 5% w/w and 10% w/w. husk-20% inulin emulsion gel on α- glucosidase activity (Figure 3.17), show that all puree samples have ability to delay α-glucosidase activity. The inhibitory activity of puree samples with 10% w/w replacement of the emulsion gel was higher than that of puree with 5% w/w replacement. The results suggest that the addition of emulsion improved the α- glucosidase inhibitory activity of the puree samples. Among the purees with 10% w/w replacement with husk-20% inulin emulsion gel, salmon had the highest glucosidase inhibition of 4.54%, followed by beef and chicken with 4.08 and 3.87%, respectively. The chicken stew had the lowest glucosidase

inhibition of 2.13%.

Previous study also suggested psyllium mucilage could delay α-glucosidase activity and entrap glucose in vitro and in vivo (Palanuvej, 2009 & Gibb et. al., 2015), but this mechanism has not been explored. Overall, it can be concluded that incorporation of husk-20% inulin emulsion gel in food product will be beneficial to delay the activity of α- glucosidase.

### 3.39 Xanthine oxidase inhibition test

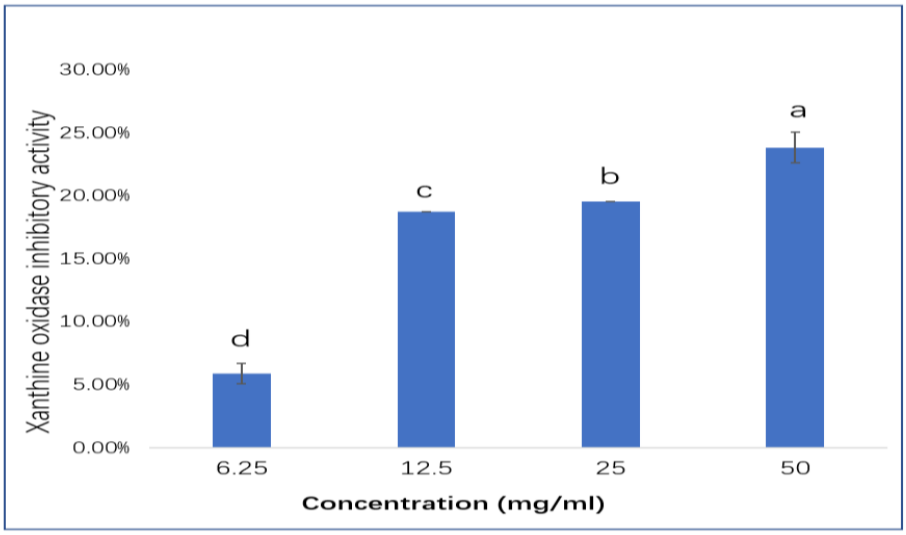


**Figure 3.18 Inhibitory potency of 3% psyllium husk emulsion in the absence of inulin at 20X, 40X, 80X and 160X dilution against xanthine oxidase (0.2 U/mL) activity at 0.6mM xanthine concentration. Different letters represent significant different at p<0.05.**

The inhibitory activity of the 3% husk emulsion gel was conducted at concentrations of 6.25, 12.5, 25 and 50 mg/ml with the highest inhibitory activity at 50 mg/ml (Figure

3.18). Inhibitory activity increased with increasing husk emulsion gel concentration, indicating a dose-dependent effect. The inhibitory activity of 3% husk emulsion gel at 50 mg/ml on xanthine oxidase was 23.86 ± 1.23%.

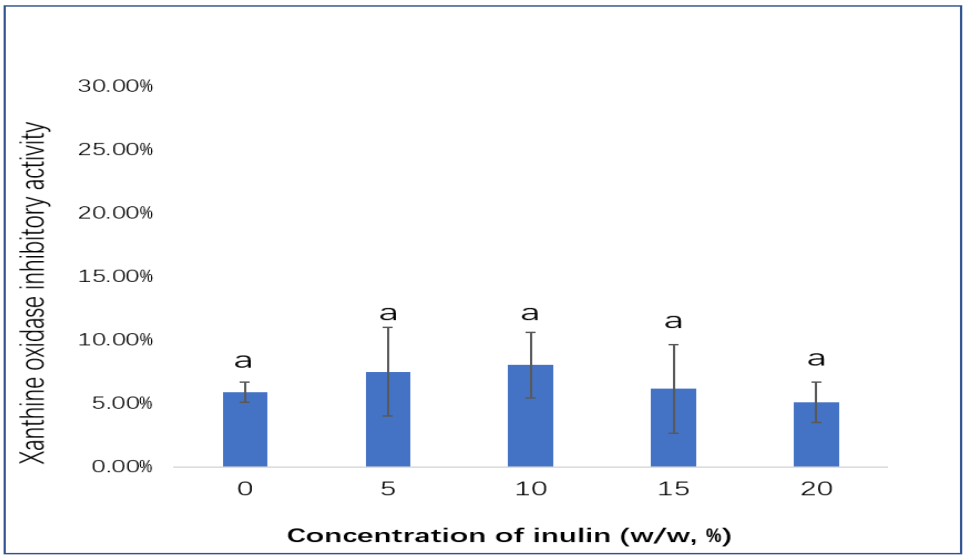


**Figure 3.19 Inhibitory potency of 3% psyllium husk emulsion in the presence of different concentration of inulin at 200X dilution against xanthine oxidase (0.2 U/mL) activity at 0.6mM xanthine concentration. Different letters represent significant different at $p<0.05$.**

The inhibitory effects of 3% husk emulsion with addition of 0%, 5%, 10%, 15% and 20% w/w inulin at 5 mg/ml on the activity of xanthine oxidase are shown in Figure 3.19. It showed increasing inulin concentration from 0% to 20% w/w do not affect the activity of xanthine oxidase, and they all showed the same inhibitory activity ($p>0.05$). The inhibitory activity of 3% husk emulsion gel with 20% inulin at 5 mg/ml on xanthine oxidase was 5.09 ± 1.61%.

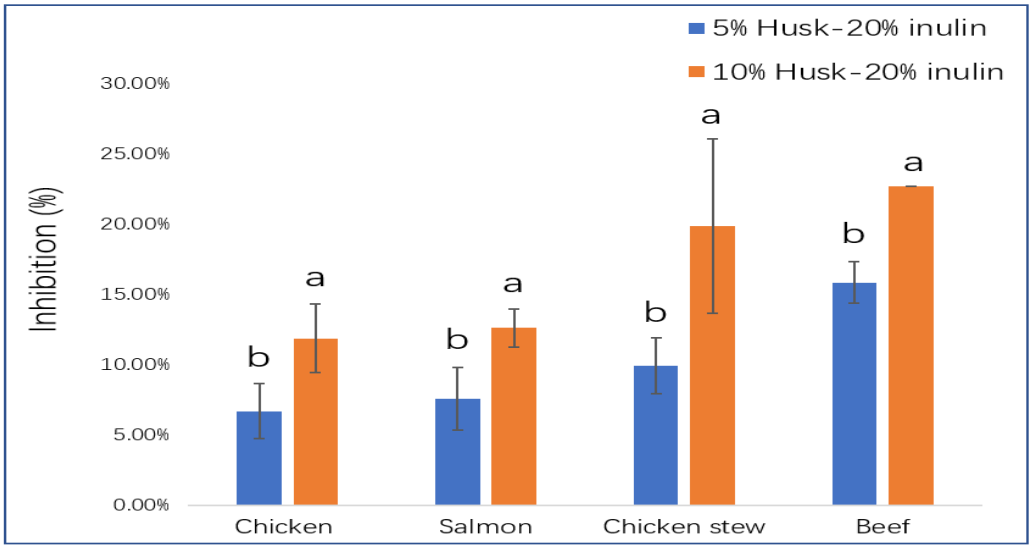


**Figure 3.20 Inhibitory potency of puree samples incorporated with 5% or 10% husk-20% inulin emulsion gel against xanthine oxidase (0.2 U/mL) activity at 0.6mM xanthine concentration. Puree sample (salmon, chicken, chicken stew or beef) without emulsion incorporation was representative of the 100% enzyme activity. Different letters represent significant different at $p<0.05$.**

From Figure 3.20, all puree samples could delay xanthine oxidase activity after incorporation with husk-20% inulin emulsion gel. The inhibitory activity of puree samples with 10% w/w replacement with the emulsion gel was higher than that of puree with 5% w/w replacement. The results indicate that incorporation of emulsion improved the xanthine oxidase inhibitory activity of the puree samples. Among the purees with 10% w/w replacement with husk-20% inulin emulsion gel, the beef stew had the highest mean xanthine oxidase inhibition of 22.70%, followed by chicken stew and salmon with 19.85 and 12.62%, respectively. The chicken had the lowest xanthine oxidase inhibition

of 11.87%. Overall, it can be concluded that incorporation of husk-20% inulin emulsion gel in food product will be beneficial to delay the activity of xanthine oxidase. Previous study has confirmed psyllium mucilage have ability to delay xanthine oxidase in vivo (Ebadollahi-Natanzi & Arabrahmatipour, 2020 & Ebadollahi-Natanzi & Arabrahmatipour, 2017), The inhibition of xanthine oxidase by psyllium seeds could be attributed to flavonoid compounds in the seeds, which have structural similarity to the substrates of xanthine oxidase, and so can attach to the active site of the enzyme and inhibit its activity (Ebadollahi-Natanzi & Arabrahmatipour, 2020).

### 3.4 Conclusion

An optimized food-grade psyllium husk (3 % w/w) emulsion gel was developed that had better stability after two weeks of storage. The emulsion gel was redeveloped with inulin to ascertain the effects of inulin addition. Increasing inulin percentage resulted in reduced particle size and improved emulsion stability with the optimum at 20% w/w inclusion. Ultrasonic homogenization further enhanced the emulsion stability by reducing the size of emulsion droplet and improving encapsulation of emulsion droplet. Increasing inulin concentrations in the emulsion gels added into purees also corresponded with decreasing total expressible fluid (TEF). The optimum was realized at 20% w/w inulin addition to the emulsion.

Inulin inclusion also had the added benefit of improved puree texture which was evident

in PLM and SEM images with less to no voids. At 10% w/w addition of husk-20% inulin, the SEM images showed a compact and smoother appearance.

The addition of the psyllium husk gel alone in purees resulted in a decrease in texture. However, with the addition of inulin in the gel, puree texture increased and became similar to the pure puree.

The stability of puree against the action of hydrolyzing enzymes α-amylase and α-glucosidase as well as xanthine oxidase was improved with the addition of psyllium husk emulsion gel. While the addition of inulin in the gel did not have significant effect on enzyme inhibition, the husk-inulin gel incorporated purees exhibited enzyme inhibition. These results imply that the husk was the primary inhibiting compound, and the effect was similar in all three enzyme tests. Better enzyme inhibition by puree was obtained at 10% w/w inclusion of the husk-inulin emulsion gel.

In this study an innovative allergy-free psyllium husk (up to 3%)-inulin (up to 20%) (O/W/O) emulsion gel was developed, in which the inulin molecule formed a protective layer on (O/W) droplets. Ultrasonic treatment further improved the formation of inulin layer for encapsulation of emulsion droplet. The inclusion of the husk-inulin emulsion gel in foods improved the texture and could delay the action of carbohydrate-hydrolyzing enzymes (α-glucosidase and α-amylase) and xanthine oxidase. Thus,

making it useful in food preparation for people with dysphagia.

Known for its use as a bulking agent, this study showed that the gelling properties of psyllium husk can be used in forming emulsion gels with positive impact on food texture. As such, the results present another ingredient that could be explored in emulsion-based foods.

# Chapter 4 Future work

In this research, emulsions gels formulated with wheat alkyresorcinols and psyllium husks were made. This a first-time report of such emulsion gels and their use in pureed foods to modify texture, improve cohesiveness and decrease syneresis. This research also reports the ability of the formulated gels to inhibit alpha-amylase, alpha-glucosidase, and xanthine oxidase. The gels and their activity are an innovative approach for addressing the food intake issues experienced by people with dysphagia. The baby food industry could also utilize the emulsion gels in formulating shelf-stable baby foods with natural preservatives such as polyphenols thus limiting the use of synthetic additives. To further develop the applications of the formulated gels, other food matrices such as sausages, cookies, bread, cakes can be explored.

In the drug industry, the ability of the gels to withstand hydrolyzing enzymes can be utilized to develop drug delivery systems. The ability of the emulsion gels to form protective coating of drug molecules can be investigated by using in-vitro cell membrane model to see if the emulsion gels can be used in drug delivery system to control drug release and to improve drug efficacy.

The bioactive compounds in psyllium husk were not explored in this research. This area would be an interesting avenue to explore. Other than the fiber that is obtained from the husk, the presence of bioactive compounds could broaden the application of the husk.

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