# Effect of structuring psyllium (Plantago ovata) husk emulsion gels by ultrasound for the application in dysphasia food

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## Abstract

The aim of this paper was to investigate effects of inulin and ultrasonic homogenization on particle size distribution and microstructure of oil-in-water emulsion gels stabilized by psyllium husk were investigated. The emulsion gel was assessed their utility in meat pure prepared for people with dysphagia. The results showed that 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 stability of puree against the action of carbohydrate-hydrolyzing enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) was improved with the addition of emulsion gels. Therefore, these developed emulsion gels could be beneficial in formulating modified-texture food prepare for people with dysphagia.

# 1. INTRODUCTION

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 (Sasegbon & Hamdy, 2017). In Canada, dysphagia affects about 35% of the elder population. 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, hindering food movement in the mouth for chewing and transferring meals to the stomach (Sasegbon & Hamdy, 2017). 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 that are naturally or mechanically altered so that the original food becomes moist, smooth and homogeneous requiring minimal oral preparation (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). Thickened fluids delay flow than liquids (e.g., water), providing an appropriate reflex reaction time when swallowing (Hadde et al., 2021).

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 form viscous dispersions and/or gels in water (Saha & Bhattacharya, 2010). Starch is the most commonly used thickener in texture modified foods because it is relatively cheap and abundant, and does not impart any foreign taste (Saha & Bhattacharya, 2010). Therefore, starch-based thickeners are traditionally used in the management of dysphagia (Newman et al., 2016). However, starch-thickened liquid decreases viscosity by 90% in 10 seconds of oral processing (Hanson et al., 2011). This sudden viscosity reduction in starch-thickened food during oral processing is due to the action of  $\alpha$ -amylase, an enzyme present in saliva. Amylase catalyzes the hydrolysis of  $\alpha$ -1,4 glycosidic linkages between glucose units in starch, resulting in amylose and amylopectin breakdown. (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).

The main carbohydrate-hydrolyzing enzymes are  $\alpha$ -amylase and  $\alpha$ -glucosidases. Alpha-amylases are hydrolytic enzymes that act upon the  $\alpha$ -(1,4)- and/or  $\alpha$ -(1,6)-linkages of starch polymers (Goesaert et al., 2009). When food is ingested,  $\alpha$ -amylase in saliva randomly hydrolyses  $\alpha$ -(1–4) glycosidic bonds of starch components to produce oligosaccharides of various lengths and a different  $\alpha$ -limit dextrins with  $\alpha$ -(1–6) bonds. As a result, the digestion process also breaks down the mechanical food structure, which is created by starch granules. It can 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  $\alpha$ -glucosidases which catalyze the hydrolysis of  $\alpha$ -1,4 and  $\alpha$ -(1,6) bonds (Tomasik & Horton, 2012).

*Plantago ovata* (Psyllium) husk polysaccharides are obtained after milling psyllium seeds and is well-known for its strong hydrophilic and gelling properties, enabling its use as stabilizers and thickeners in food industry (Zhou et al., 2022; Franco et al., 2020). Psyllium husk is an excellent source of both soluble and insoluble fiber, and 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). In addition, psyllium husk can bind with glucose, and thereby inhibit amylase activity (Ahmed & Urooj, 2010). Moreover, psyllium husk can absorb water more than 50 times its initial weight, expanding and creating a smooth bulky gel (Masood & Miraftab, 2010). However, due to its extremely strong gel-forming ability and water-absorption property, psyllium husk rapidly forms a solid gel when it binds with water, impeding its incorporation into food homogeneously and limiting its food product application (Yu et. al., 2003).

These limitations can be overcome by developing psyllium husk emulsion gel for use in modified-texture foods to enhance consistency and cohesiveness, and to reduce syneresis. Emulsion gels are formed by gelling the continuous phase of emulsions or by aggregating the emulsion droplets through the addition of hydrophilic polymers (Fig. 1). With the gelling of the continuous phase, the resulting medium is a soft solid that can entrap emulsified lipid droplets inside the gel matrix. As a result, functional compounds incorporated into emulsion gels often have better storage stability compared to those incorporated into emulsion (Cofrades et al., 2017).

(Place for figure 1)

Fig. 1. The formation of an emulsion gel (O/W), modified from Lu et.al. (2019).

The formation of emulsions requires energy to agitate the two immiscible phases together. Ultrasonic homogenization is a high-energy, cost-effective, energy-efficient, easy, and environmentally friendly emulsification process to split 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 untreated emulsion. However, the effect of ultrasound on emulsion gels needs to be further explored and assessed.

In this study, we first developed and optimized food-grade psyllium husk emulsion gel. Then, the stability of ultrasonic-treated and untreated emulsion gels was investigated by polarized light microscopic, cryo-scanning electron microscopic observations and particle size analysis. We also studied the total expressible fluid and the inhibitory effects of psyllium husk emulsion gel on alpha-amylase and alpha-glucosidase activities in puree samples.

# 2. MATERIALS AND METHODS

## 2.1. Materials

Sunflower oil was 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). Alpha-glucosidase,  $\alpha$ -amylase, P-NPG (p-Nitrophenyl- $\alpha$ -D-glucopyranoside), 3,5-Dinitrosalicylic acid and potassium sodium tartrate tetrahydrate were from Sigma-Aldrich (St Louis, MO, USA). Salmon puree, chicken puree, chicken stew puree and beef puree were kindly provided by Apetito HFS Ltd. (Orléans, ON, CA).

## 2.2. Formulation and preparation of psyllium husk emulsion gels

Psyllium husk (3%) was dissolved completely at 78.4% of water phase (80) then cooled to room temperature (23). Emulsions were prepared with 0%, 5%, 10%, 15% and 20% (w/w) inulin to investigate suitable inulin concentration. In preparation, inulin was dissolved in water phase to replace the same amount of water in the formulation. Then 1% sodium alginate and 17.6% sunflower oil were added to the water phase, followed with mechanical stirring using a Tissuemiser (Fisher Scientific, Ontario, CA).

#### 2.3. Ultrasound-treated emulsion gel

Ultrasound was applied to prepare emulsion gel with above formulation (section 2.2.) based on the method of Leong et al. (2017) with modification. Husk (1.5 g) was first dissolved completely in 13.7g of water (80) then cooled to room temperature (23). Inulin (0, 2.5, 5, 7.5 or 10 g) was mixed in the husk solution by ultrasound at 2 W calorimetric power for ~10-20 seconds. Sunflower oil phase (8.8 g) was then mixed with the aqueous phase by ultrasound at 10 W calorimetric power for ~ 60-120 seconds. The mixture was then remixed with 25 g of alginate solution (0.5 g sodium alginate in 24.5 g water) at 10 W until the emulsion appeared homogenous. The formulated emulsions were stored at 4 for further analysis.

## 2.4. Droplet size measurement

Droplet size of emulsion gels 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.

#### 2.5. Meat sample preparation

Puree samples (salmon, chicken, chicken stew and beef) were stored at least -20 oC until needed and thawed to room temperature for the experiment. Different puree sample (5% and 10% w/w) were replaced with ultrasonic-treated 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.

## 2.6. Total expressible fluid (TEF) determination

The total expressible fluid (TEF) was determined according to 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 degC) 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, ON, CA) for 1 min at 700W and immediately recentrifuged. The supernatant was removed and the residue weighed. The total expressible fluid (TEF) was calculated according to the following equation (Ismail et al., 2021):

 $\text{TEF}(\%) = \frac{Weight \ of \ the \ sample \ before \ heating- \ Weight \ of \ the \ sample \ after \ heating}{\text{Weight of the sample before heating}} \ge 100 \ (1)$ 

#### 2.7. Cryo-Scanning Electron microscopic observation of emulsion gels and puree samples

Emulsion gels and puree samples 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 and puree samples were cut into blocks of 0.5 cm, the blocks were then frozen on a metal plate surrounded 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.

## 2.8. Alpha-amylase inhibitory activity

The  $\alpha$ -amylase inhibitory activity of emulsion gels and puree samples was 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  $\alpha$ -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; DNS color reagent (80 µl) was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a Multiplate Reader (BioTek Cytation 5, Ottawa, Canada).

Psyllium husk (3%) and inulin emulsion gels (0, 5, 10, 15, and 20%) were diluted in buffer to give a final concentration of 5 mg/ml. Diluted emulsion samples were centrifuged (10,000 rpm, 2 min, room temperature), and the clear supernatant was applied to the test. For 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 centrifuged (10,000 rpm, 2 min, room temperature). 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 (%) =  $[(Abs_{bank} - Abs_{sample})/Abs_{bank}] \times 100$  (2)

Where Abs<sub>bank</sub> is the absorbance of the bank and Abs<sub>sample</sub> is the absorbance of the sample.

## 2.9. Alpha -glucosidase inhibitory activity

The  $\alpha$ -glucosidase inhibitory activity of emulsion gels and pure samples was 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  $\mu$ l Na<sub>2</sub>CO<sub>3</sub> (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). Psyllium husk (3%) and inulin emulsion gels (0, 5, 10, 15, and 20%) were diluted in buffer to give a final concentration of 5 mg/ml. For meat sample, a blank was prepared without emulsion sample and another without the glucosidase 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, calculated using the formula:

Inhibitory activity (%) =  $[(Abs_{bank} - Abs_{sample})/Abs_{bank}] \times 100$  (3)

Where Abs<sub>bank</sub> is the absorbance of the bank and Abs<sub>sample</sub> is the absorbance of the sample.

### 2.10. 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 ( $\alpha$ =0.05). Differences were significant at p < 0.05.

## 3. RESULTS AND DISCUSSION

### 3.1. Emulsion gel characterization

#### 3.1.1. Microscopy

Microscopic images showed that inulin concentration significantly influenced morphologies of emulsion gels prepared with 3% psyllium husk (Fig. 2).

(Place for figure 2)

Fig.2. microscopic observation (500X) of ultrasonic-treated and untreated 3% psyllium husk emulsion gel with different concentration of inulin. Dark arrow indicates length of 20  $\mu$ m.

At 0% inulin concentration, emulsion droplets in close proximity to each other were large and polydispersed, which increased the flocculation rate. High flocculation of emulsion droplets indicates low emulsion stability (Yvonne and Victoria, 2018). The oil droplets in the emulsion gels became smaller and uniform with increased inulin concentration suggesting that the husk-emulsions were stable at higher inulin concentration. A similar result was reported previously (Xu et al., 2020), where the particle size of oil-in-water emulsion decreased with increasing inulin concentration. The droplet decrease may be due to high inulin concentration that forms a secondary interfacial layer enhancing surface coating of oil droplets to prevent against droplet aggregation and coalescence; and this result in smaller oil droplets and highly stable emulsion (Sarkar et al., 2018). These results were further confirmed by the particle analysis and SEM image.

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

3.1.2. Particle size distribution

Fig.3. shows the droplet size distribution of ultrasonic-treaded husk emulsion gel with different inulin concentration.

(Place for figure 3)

Fig. 3. 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 sharp peak that shifted progressively to the left towards smaller droplet size with increase in inulin concentration (Fig. 3). The result revealed that emulsion droplets were more uniformly distributed, and the number of smaller droplets kept increasing in the emulsion gels. Reduction of droplet size may be due to increased polysaccharide concentration resulting in stronger and more compact gel-network, which entrapped emulsion oil droplets and reduced droplet aggregation (Xu et al., 2020). In addition, inulin was available to wrap the oil droplet surface which limited the movement of emulsion droplets and prevented their aggregation, resulting in smaller oil droplets and more stable emulsion (Sarkar et al., 2018; Hu et al., 2020). These results were further confirmed by PLM and SEM image.

3.1.3. Cryo-Scanning Electron microscopic observation

(Place for figure 4)

Fig. 4. SEM observation of ultrasonic-treated and untreated 3% psyllium husk emulsion gel with different concentration of inulin. Dark arrow indicates length of 50  $\mu$ m.

The SEM images (Fig. 4) 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. Droplets surface changed with increased inulin concentration from irregular to smooth and less cavities, suggesting that inulin was in a packed arrangement at the droplets interface, and could also form an interfacial layer coating the surface of oil droplets. Similar result was observed for an emulsion developed with zein/corn fiber gum complex (Zhu et al., 2019). Their study reported that the polysaccharide complex was adsorbed at the oil-water interface, and that complex also created a mechanical barrier to prevent interfacial film rupture. Polymers adsorption at the interface can enhance mechanical properties of the interfacial films, which can inhibit coalescence, therefore, bigger size droplets have weaker interfacial films (Mao & Miao, 2015),

Compared to the untreated emulsion, the droplets surface in ultrasonic-treated emulsions showed higher integrity of the coating layer. At 20% inulin, the dark area in the untreated emulsion gel (Fig. 4) showed that droplets were not completely covered by the inulin layer. On the other hand, the surface of the ultrasonic treated emulsion was very smooth, without gap, suggesting that the droplet is completely covered by the inulin layer at that concentration. Similarly, Xiong et al. (2019) reported that more emulsifier molecules were adsorbed on the surface of the oil droplets in the ultrasonic-treated xanthan gum emulsion compared to untreated emulsion. The increased particle adsorption on the surface of emulsion droplet by ultrasound apparently occurs from momentum transfer from the fluid to the particles and droplets caused by random cavitation events. The momentum transfer can overcome the stabilizing energy barriers that normally prohibit nanoparticles from spontaneously adhering to the oil-water interface (Lee et al., 2019).

### 3.2. Effect of emulsion gel addition in meat puree

## 3.2.1. Total expressible fluid (TEF)

Figures 5A and B represent the results of TEF of different purees by water bath, and microwave oven heating, respectively.

#### (Place for figure 5)

Fig.5. Effect of incorporation of 10% psyllium husk (PH) emulsion gel with 0%, 10% and 20% inulin in puree samples (A) at 70 water bath heating for 30 min, and (B) at microwave oven heating with power 700W for 2 min and then centrifugation at 3750 g for 3 min. Different letters represent significant different at p<0.05.

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 and microwave oven heating methods were selected in this study because they are common household heating methods. Increased inulin concentration in emulsion gel (from 0% to 20%) incorporation in the purees further reduced their TEF. 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 (Figure 3.7), the pure puree (no emulsion gel incorporation) had the highest TEF (31.77% mean value), while the puree with 10% w/w replaced by husk-20% inulin emulsion gel had the lowest TEF (17.22% mean value). Microwave-heating method (Figure 3.8) produced similar result; the puree with husk-20% inulin emulsion gel incorporation still had the lowest TEF within the same puree group. TEF reduction with the inclusion of husk emulsion gel may be due to ingredients in the emulsion gel crosslinking with the polar groups of protein matrix. The cross-linking can strengthen the puree three-dimensional network structure, leading to stronger binding properties and smaller fluid release during heating (Colmenero et al., 2005; Kumar, 2021). Previous study (Kumar, 2021) has shown that inulin has water and fat retention capacity, which also help TEF reduction.

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 due to the internal microwave heating creating higher center temperature of the puree, and the high steam pressure driving more water from within the sample to the surface (Wang et al., 2020).

3.2.2. Cryo-Scanning Electron microscope

(Place for figure 6)

Fig. 6. SEM observation of meat pure samples before and after incorporation with 10% w/w husk-20% inulin emulsion gel

In SEM images, all pure puree samples exhibited the formation of different size cavities creating structures with a rough and porous appearance. The high proportion of cavities can be interpreted as water, fat, and air expansion in the protein network (Paglarini et al. 2022). The 10% emulsion gel incorporated puree, had less cavities and more compact structure compared to pure puree because the emulsion gel contained high amount of inulin, (Felisberto et al., 2015). The structural change was likely due to inulin's long chain length and degree of polymerization, producing firmer and more viscous puree. Hence, inulin was suggested for use 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 (Fig. 6), can 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 those of pure puree samples (Fig. 6), can be classified as poor meat gels with weak binding properties.

#### 3.2.3. Alpha-amylase inhibition

Figure 7A shows the  $\alpha$ -amylase inhibitory effect of 3% husk emulsion with 0, 5, 10, 15 and 20% w/w inulin addition at 5 mg/ml. Inhibitory activity increased with increasing inulin concentration from 0% to 15%. The  $\alpha$ -amylase inhibitory activity of the emulsion gel at 20% inulin was not significantly different (p>0.05) from that of 15% inulin concentration. The inhibitory activity of 3% husk emulsion gel with 20% inulin at 5 mg/ml on  $\alpha$ -amylase was 16.06  $\pm$  3.03%.

## (Place for figure 7)

Fig. 7 Inhibitory potency of (A) 3% psyllium husk emulsion in the presence of different concentration of inulin at 200X dilution, and (B) puree samples with incorporated 5% or 10% husk-20% inulin emulsion gel against  $\alpha$ -amylase (1 U/mL) activity at 1% starch concentration. Different letters represent significant different at p<0.05.

The  $\alpha$ -amylase inhibitory activity of puree samples (salmon, chicken, chicken stew or beef) with 5% w/w and 10% w/w husk-20% inulin emulsion gel (Fig. 7B), showed that all puree samples can delay  $\alpha$ -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 (21.14%), followed by salmon and chicken (14.12 and 12.68% inhibition, 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 polysaccharides branched chain can interact with the amino acid residues of digestive enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase), thus inhibiting their activity. In addition, Ahmed & Urooj (2010) concluded that dietary fiber, such as wheat bran and psyllium husk, can bind glucose, and thereby inhibit amylase activity. Previous  $\alpha$ -amylase inhibitory activity indicated that soluble fibers can capsulate  $\alpha$ -amylase and starch, thereby reducing starch accessibility to the enzyme (Ou et al., 2001).

Our result indicates that incorporation of husk-20% inulin emulsion gel will be beneficial to delay dietary starch breakdown in food product and thereby maintain their consistency with addition of starch-based thickener during oral digestion.

#### 3.2.4. Alpha-glucosidase inhibition

Figure 8A illustrates  $\alpha$ - glucosidase inhibitory effects of 3% husk-emulsion with inulin addition (0, 5, 10, 15 and 20% w/w) at 5 mg/ml. All emulsion gel samples delayed  $\alpha$ -glucosidase activity, however inhibitory activity decreased slightly with increasing inulin concentration from 0% to 20%. The husk-emulsion gel without inulin had the highest  $\alpha$ -glucosidase inhibition (12.93  $\pm$  0.19%), while the husk-emulsion gel with 20% w/w inulin addition had the lowest glucosidase inhibition of (10.6  $\pm$  0.91%).

## (Place for figure 7)

Fig. 8. Inhibitory potency of (A) 3% psyllium husk emulsion in the presence of different concentration of inulin at 200X dilution, and (B) puree samples with incorporated 5% or 10% husk-20% inulin emulsion gel against  $\alpha$ -glucosidase (1 U/mL) activity at 1mM PNPG concentration. Different letters represent significant different at p<0.05.

All puree samples delayed  $\alpha$ -glucosidase activity (Fig. 8B). The inhibitory activity of puree samples with 10% w/w replacement of the emulsion gel was higher than that with 5% w/w replacement. The results suggest that emulsion addition improved  $\alpha$ - 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 (4.54%), followed by beef and chicken (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  $\alpha$ -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 husk-20% inulin emulsion gel incorporation in food product will beneficially delay  $\alpha$ - glucosidase activity.

## 4. Conclusion

In this study an innovative allergy-free psyllium husk-inulin (up to 20%) emulsion gel was developed, in which the inulin molecule formed a protective layer on emulsion droplets. Ultrasonic homogenization further enhanced the emulsion stability by reducing emulsion droplet size and improving the formation of inulin layer to encapsulate emulsion droplet. The inclusion of the husk-inulin emulsion gel in meat puree reduced the fluid loss during water bath and microwave oven heating and can delay the action of carbohydrate-hydrolyzing enzymes ( $\alpha$ -glucosidase and  $\alpha$ -amylase). It enables the use in food preparation for people with dysphagia. This study demonstrates the gelling properties of psyllium husk used in forming emulsion gels with positive impact on meat product. As such, the results present another ingredient that can be explored in emulsion-based foods.

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3% husk-0% inulin

3% husk-10% inulin

3% husk-20% inulin





