Effect of non-thermal ultrasound on inulin from Jerusalem artichoke and its application in dairy industry

by

Hengguang Xu

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Abstract

Jerusalem artichoke (JA) is a rich source of dietary fiber. The major dietary fiber inside of JA is inulin which is a heterogeneous collections of fructose polymers with many health benefits for humans. To investigate the effect of ultrasound on the inulin from JA, JA powder, Purified JA inulin (PJAI) was treated with 20KHz ultrasound compared with chicory inulin (CI). Ultrasound treatment time had a positive linear relationship with reducing sugar content of these samples. After ultrasound treatment, reducing sugar content in JA powder increased from 6.101g/100g up to 12.273g/100g. In PJAI, reducing sugar content increased from 10.378g/100g up to 12.274g/100g. Reducing sugar content in CI increased from 1.126g/100g up to 2.183g/100g. Also, determined by HPLC and GPC, for PJAI, there was a negative linear relationship between high degree of polymerization (DP) inulin with ultrasound treatment time (R^2=0.9169), and a positive linear relationship between low DP inulin with ultrasound treatment time (R^2=0.9738), which was not observed for inulin from chicory. Furthermore, JA powder was added into whey and milk and treated with 20KHz ultrasound to investigate the structure change. Ultrasound changed the microstructure of milk resulting in a flatter surface for milk and whey. Ultrasound combined particles of milk and whey This kind of phenomenon was much more obvious only for milk when mixed with JA powder.

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List of Abbreviations

CI	Chicory inulin
DP	Degree of polymerization
GPC	Gel Permeation chromatography
HPLC	High performance liquid chromatography
JA	Jerusalem artichoke
PJAI	Purified Jerusalem artichoke Inulin
SEM	Scanning electron microscopy
WAI	Water Absorption Index
WSI	Water Solubility Index

Chapter 1 – Introduction

Inulin in Jerusalem artichoke

1.1 Dietary fiber in Jerusalem artichoke

Jerusalem artichoke (Helianthus tuberosus L.), which belongs to Asteraceae family, is native to the central part of North American (Bock, Kane et al. 2014). The history of JA cultivation is very long. JA had been already cultivated by the Native Americans as a food source before the Age of Discovery. The tubers can be stored for years (Kays and Nottingham 2007). JA contains high amount of dietary fiber (DF), and the major DF is inulin (Li, Li et al. 2013). Commonly, most crops contain starch, a polymer of glucose, which is utilized as a carbohydrate reserve, but the storage carbohydrate of JA is inulin, a fructose polymer (Kays and Nottingham 2007). Inulin is a heterogeneous collection of fructose polymers mainly linked by β-(1-2) bonds, and, at the reducing end, there is a terminal glycopyranose unit (Causey, Feirtag et al. 2000). There exist small amount of inulin that they do not contain a terminal glycopyranose but rather a terminal fructose. β-(2-6)-linkages also exists in a small percentage of inulin chains, which is used to form a very limited degree of branching (Kays and Nottingham 2007). The degree of polymerization (DP) for inulin ranges from 2 to about 65, which is much lower than the DP for starch (Samolińska and Grela 2017).

In different kinds of plant, the DP of inulin or fructan polymers vary depending on species, production conditions and harvest time (Khuenpet, Fukuoka et al. 2017). For example, if JAs are stored at low temperature (4°C), the content of fructo-oligosaccharide (inulin with low DP) will increase (Saengthongpinit and Sajjaanantakul 2005). Jerusalem artichoke and chicory (Cichorium intybus L.) are considered as the most important plant species which contain high amounts of inulin, and companies always use these two kinds of plants to extract inulin (Kleessen, Schwarz et al. 2007). However, the inulin in these two kinds of plants

are different. In JA, most of the inulin have a low DP (i.e. GF_5), but, in chicory, most of the inulin have a very high DP (Kays and Nottingham 2007). The pathway of inulin in plant is showed in **Figure 1.1.2**. Sucrose:sucrose 1-fructosyltransferase (1-SST), fructan:fructan 1-fructosyltransferases (1-FFT), fructan: fructan 6G-fructosyltransferase (6G-FFT) and sucrose:fructan 6-fructosyltransferase (6-SFT) are involved in the formation of inulin in plant (Arkel 2013).

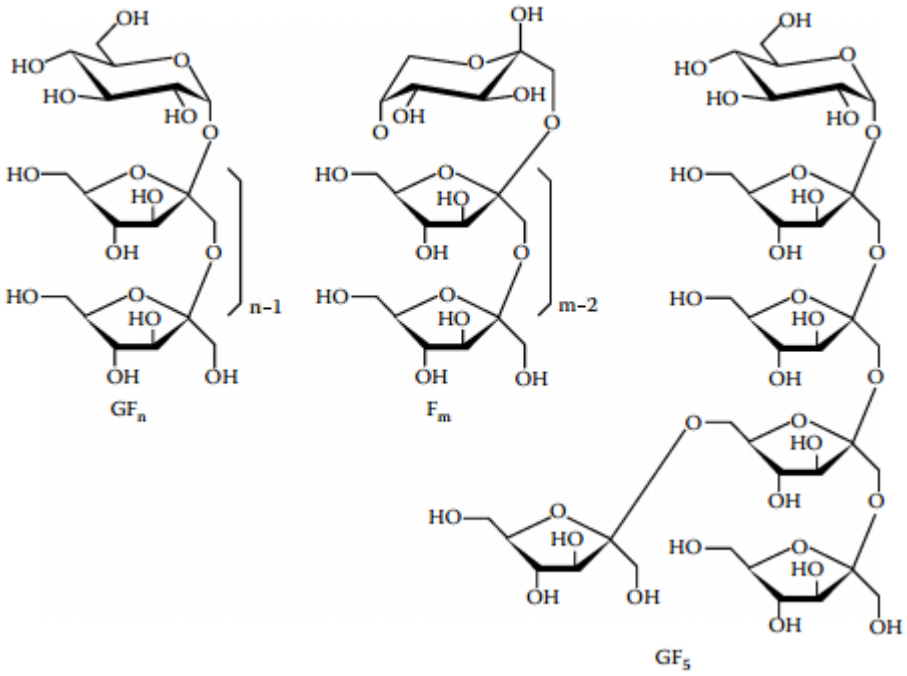


Figure1.1.1 Structure of inulin containing a terminal glucopyranose unit (GF_n), inulin with a terminal fructoside unit (GF_m) and a branched inulin (GF_5) (Kays and Nottingham 2007).*

*n-1 and m-2 is the area to calculate the degree of polymerization of inulin

Short-chain fructooligosaccharides or inulin with low DP can provide some health benefits to humans. It can be used as a sweetner. Inulin with higher DP can be used as fat replacer or used for high-fructose syrups (Khuenpet, Fukuoka et al. 2017). For functional food and nutraceuticals, short-chain fructoolioligosaccharides might be more important than high DP inulin in some area due to their health benefits, which will be introduced in **1.3**.

Table 1-1 Distribution of Fructan Polymers in the Edible Portion of Selected Crops (Kays and Nottingham 2007)

		Degree of Polymerization (%)			
Crop	**%Fructan (dm)**	**≤9**	**10-20**	**20-40**	**>40**
Jerusalem artichoke	16-20	52	22	20	6
Chicory	15-20	29	24	45	2
Globe artichoke	2-9	0	0	13	87

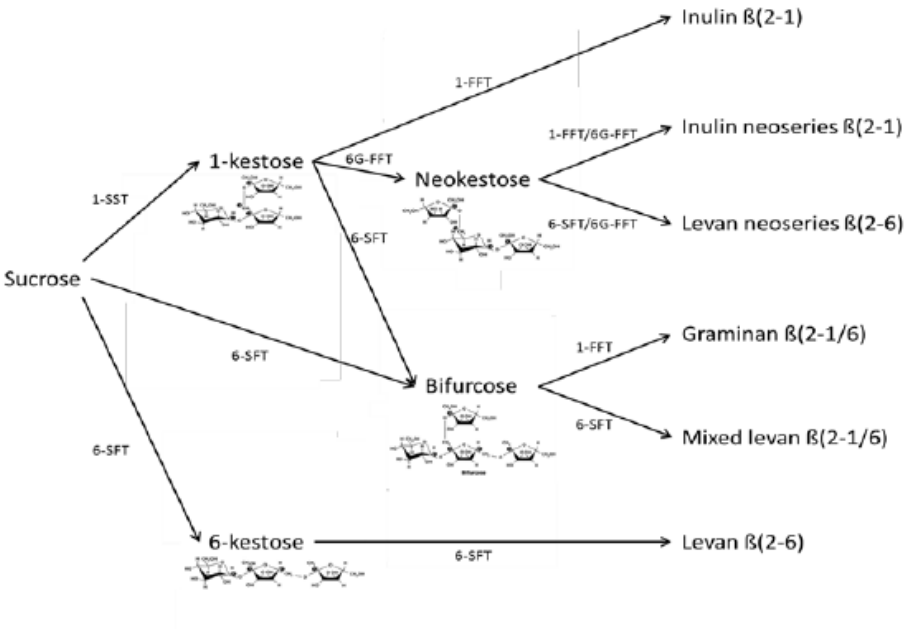


Figure 1.1.2 Schematic representation of enzymatic reaction of the mayor fructans biosynthesis in plants (Arkel 2013)

Since 2000, the interest in inulin and inulin-containing crops has been increasing especially in Europe, and one reason for that is due to its health benefits (Kays and Nottingham 2007). In Europe, inulin is considered a desirable ingredient in food processing, for example in thermal treatment of food due to the accelerating the Maillard reaction and saving time for the bread crust formation(Panchev, Delchev et al. 2011). Nowadays, JA is cultivated all over the world – North America, Northern Europe, China, Korea, Australia and New Zealand since JA is an

important source of inulin (Li, Zhang et al. 2015).

1.2 Inulin in digestive system

In the human body, inulin can hardly be decomposed by digestive enzymes, such as disaccharases from the intestine mucosa, which breaks down sucrose and α-amylase from pancreatic homogenates. These enzymes are incapable of breaking the 1,2-β-linkage, but, when inulin arrives to the large intestine, it is fermented by microflora and short chain fatty acids are produced(Causey, Feirtag et al. 2000).

Bifidobacterium is a genus of gram-positive, nonmotile bacteria, and that is a part of the colon flora, and capable of breaking down oligofructose and inulin, producing short chain fatty acids as a byproduct(Wang and Gibson 1993).

There are several pathway involved: the Embden-Meyerhof-Parnas pathway, the succinate pathway and the acrylate pathway. First, undigested carbohydrates are broken down into smaller carbonhydrates by microbial hydrolysis, which is mainly done by *Bifidobacterium* in the large intestine. The monosaccharides are then fermented to phosphoenolpyruvate (PEP) by the Embden-Meyerhof-Parnas pathway (Boets, Deroover et al. 2015). The Embden-Meyerhof-Parnas pathway is the major pathway of glycolysis, and this pathway will convert monosaccharides to PEP which are then converted into pyruvate by pyruvate kinase in glycolysis (White, Drummond et al.). Acetate, propionate and butyrate are some of the main short chain fatty acids (SCF) produced from PEP. Propionate is mainly produced from PEP by the succinate pathway and the acrylate pathway, with the succinate pathway being the most important pathway (Boets, Deroover et al. 2015). Bacteroidetes use the succinate pathway to produce propionate via methylmalonyl-CoA. The formation of acetate and butyrate is different than the formation of propionate. It requires the conversion of PEP into acetyl-coenzyme A (acetyl-CoA) (Reichardt, Duncan et al. 2014). Acetate is then produced directly from acetyl-CoA by many Firmicutes belonging to *Clostridium luster* IV and XIVa. Butyrate is produced by

butyrate kinase or butyryl-CoA-transferase (Boets, Deroover et al. 2015). These SFAs are then absorbed by the human large intestine and result in some physiological effects which are beneficial to human health (Reichardt, Duncan et al. 2014).

1.3 Health benefits of inulin

Inulin has been determined to have lots of health benefits for humans. The health benefits of inulin are mainly based in the fact that inulin can hardly be digested by humans because β(2-1) or β(2-6) linkages confer resistance towards human digestive enzymes and is instead fermented by bacteria in the large intestine which could be transferred into short chain fatty acids (Shoaib, Shehzad et al. 2016). Inulin is known as a kind of non-starch polysaccharide. It suggested that 16 to 24 grams of non-starch polysaccharides should be consumed by an adult for health benefits (Kalyani Nair, Kharb et al. 2010). These benefits include the reduction in risk of gastrointestinal diseases, the positive effect on lipid metabolism, and the enhanced absorption of minerals (Shoaib, Shehzad et al. 2016).

1.3.1 Reduction in risk of colon cancer

The main function for inulin to reduce the risk of gastrointestinal diseases is that it could help decrease the risk of colon cancer in humans (Shoaib, Shehzad et al. 2016). Fermented Synergy 1 which contains a high amount of inulin and fermented products, such as short chain fatty acids. The research results showed that inulin from fermented synergy 1 could inhibit the growth of colon cancer cells (Munjal, Glei et al. 2009). Inulin could prevent prenoplasia and inflammation by increasing the *bifidobacterua* and *lactobacilli* count and decreasing other gut microflora count. These two probiotics have shown the ability to reduce enzyme activities that produce cancer casuing substances compared with other gut microflora such as coliforms. Furthermore, inulin can be fermented into short chain fatty acids in the intestine, such as butyric acid which be protective against genotoxic carcinogens (Hijová, Szabadosova et al. 2013).

1.3.2 Effect on lipid metabolism

The main effect of inulin on lipid metabolism is that it can reduce triacylglycerol concentrations. Some research also showed that inulin can slightly decrease cholesterol levels (Kalyani Nair, Kharb et al. 2010). For the animal studies, most were done on rats (Beylot 2005). Generally, highly fermentable inulin or dietary fructans reduced the triacylglycerol levels in rats compared with poorly fermentable cellulose (Daubioul, Rousseau et al. 2002), The research also shows that 10g per day of high performance inulin for three weeks in a high-carbohydrate and low fat diet can reduce the level of lipogenesis and triacylglycerol levels in humans (Letexier, Diraison et al. 2003). A lots of the research also shows that there exists a relationship between inulin intake and triacylglycerol levels in vivo, but the whole mechanism is still unclear (Beylot 2005; Shoaib, Shehzad et al. 2016). For the effect of inulin on cholesterol, some of the research shows that inulin may slightly decrease cholesterol levels, while other studies found no effect. In one study, the daily consumption of inulin-enriched soymilk containing 2g of phytosterols and 10g of inulin resulted in a lower level of low density lipoprotein-cholesterol but had no effect on high density lipoprotein in humans, compared to standard soymilk (Kietsiriroje, Kwankaew et al. 2015). Inulin combined with Fibersol-2 with a 3:7 ratio, significantly lowered the ratio of low density lipoprotein to high density lipoprotein in hamsters (Huang, Lin et al. 2016). Some research showed that neither inulin nor oligofructose influenced the absorption of cholesterol or bile acids in dogs and human (Ellegård, Andersson et al. 1997; Flickinger, Loo et al. 2003). The mechanism by which inulin can decrease cholesterol levels in the body could be that inulin or oligofructose stimulates liver conversion of cholesterol to bile acids resulting in a reduction of cholesterol concentration in the body (Beylot 2005).

1.3.3 Enhanced absorption of minerals

Inulin is a kind of dietary fiber, and some theories predict that dietary fiber might reduce the bioavailability of minerals since polysaccharides contain free hydroxyl

groups or carboxyl groups which are weak binding and these weak binding could led fiber get some binding characteristics affecting the body's mineral balance; however, a lot of research shown that dietary fiber does not have this kind of negative effect (Harland and Oberleas 2001).

For inulin, some research shows that inulin enhances the absorption of calcium, magnesium and iron (Shoaib, Shehzad et al. 2016). A 6 week treatment with 10g/d of a chicory oligofructose and long-chain inulin mixture could significantly improved intestinal absorption of calcium and magnesium in postmenopausal women (Holloway, Moynihan et al. 2007). For iron absorption, high performance inulin and oligofructose significantly increased intestinal iron absorption in rats, which was 33% higher than the control group (Freitas, Amancio et al. 2012).
The main reason why inulin might increase the absorption of minerals may be due to the reduction of intestinal pH. As a result of colonic fermentation, inulin can be fermented into short chain fatty acids or some organic acids, leading to a pH reduction in the intestine. Taking calcium as an example, it is very important for calcium to be ionized before absorption, so a low pH can improve the bioavailability of calcium, Absorption always happens in the small intestine and the first part of large intestine (Shoaib, Shehzad et al. 2016). The short chain fatty acids produced by inulin fermentation stimulates calcium absorption across the colon in human (Scholz-Ahrens and Schrezenmeir 2002).

However, there exists some research showing that inulin has no effect on absorption of minerals. The inclusion of inulin has no influence on phosphorus, calcium or nitrogen utilization, nor on bone mineralization in pigs (Varley, McCarney et al. 2010). A low-iron diet with 40g/kg of inulin did not significantly improve the colonic of iron in iron-deficient pigs (Patterson, Rutzke et al. 2009).

Inulin also has some other health benefits to humans. For example, it may help reduce the level of serum cholesterol and improve the control of blood glucose

(Causey, Feirtag et al. 2000). Inulin also contains a very low caloric value compared to other polysaccharides, with 1.5 kcal/g or 6.3 kJ/g For other carbohydrates, the caloric value is 3.9 kcal/g or16.3 kJ/g (Roberfroid 1999).

1.3.4 Low degree of polymerization of inulin and human health

Degree of polymerization also affect the influence of inulin on human health. Some of the health benefits associated with high DP or high-performance inulin has already been mentioned above. Most studies focused on high DP inulin. For low DP inulin, in some factors, some studies showed better health benefits compared to high DP inulin.

Compared with high DP or high performance inulin, low DP inulin increased the reduction of weight gain, body fat mass, hyperglycemia, liver steatosis and also total cholesterol in high-fat diet-induced mice (Márquez-Aguirre, Camacho-Ruíz et al. 2016). Low DP inulin, DP4 and DP8, seemed to be fermented faster than high DP inulin due to increased cecal immunoglobulin A in rats. Low DP inulin also decreased cecal pH more than high DP inulin providing a better environment for acid-tolerant bacteria like bifidobacteria in rats (Márquez-Aguirre, Camacho-Ruíz et al. 2016). Similar results were observed in another study. Cecal immunoglobulin A concertation was higher with DP4 compared to DP8, and higher with DP8 compared to DP16. At the same time, low DP inulin also significantly increased the amount of immunoglobulin A-producing plasma cells, as well as cecal lactobacilli amounts in rat cecal mucosa (Ito, Takemura et al. 2011). *Bifidobacteria* and *lactobacilli* are probiotics in the human body, that help maintain intestinal health through certain activities like using bacteriocins to limit detrimental bacteria (Kleerebezem and Vaughan 2009; Kalyani Nair, Kharb et al. 2010). Increasing the amount of these probiotics might provide better intestinal health for humans as reduce the risk of colon cancer.

1.4 Processing on plants with high inulin content

Most studies focused on inulin extraction methods from plants while only a few talked about how to change the degree of polymerization of inulin or how processing effects its structure.

1.4.1 Extraction method of inulin from plants

Heating method

Several methods can be used to extract inulin from plants, mainly from chicory and JA. The most common method to extract inulin is using a hot water bath. For chicory, before extraction, it should be cut into slices. After that, the slices are diffused for 1 to 2 hours at 70-80 °C to fully extract the inulin. The ratio of solids to water should be about 1:5 to optimize the inulin extraction efficiency (Zhu, He et al. 2016). For JA, tubers should also be cut into small slices. Then, they are placed in boiling water for 5 mins to reduce enzyme activity. After that, the slices are dired at 60 °C for 7h and then grinded into power. The powder is then diffused for 40 mins at 90 °C with deionized water (Li, Zhang et al. 2015). The ratio of solids to water should be about 1:35 which is much different from the method for chicory root (Khuenpet, Fukuoka et al. 2017). Some studies modified this method, and they combined hot water extraction with pressure. They extracted inulin from JA with water at 80 °C for 20 mins with 1500 psi which resulted in more than 60% inulin from JA (Srinameb, Nuchadomrong et al. 2015).

Ethanol can also be used as a solvent for inulin extraction because it has a lower boiling temperature than water, allowing for recovery of inulin that is otherwise impacted by pH and processing. The concentration of ethanol used for extraction of inulin has a large range from 20% to 90%. (Zhu, He et al. 2016).

Besides water bath extraction, there also exists another heating method for extraction, which is less common, called the ohmic heating method. The JA powder is mixed with distilled water, and the mixture is put into an ohmic chamber. The ohmic chamber is then heated by electric energy due to the high electrical

conductivities of the solutions. However, the extraction yield for ohmic heating is similar to the yield from water bath extraction (Khuenpet, Fukuoka et al. 2017).

Ultrasound treatment

Another extraction method that is commonly investigated is the ultrasound extraction method. There are two kinds of ultrasound extraction methods: direct ultrasound extraction and indirect ultrasound extraction. For direct extraction, ultrasound works directly on sample solution. For indirect extraction, ultrasound works directly on water which becomes a propagation medium that transfers energy to the sample solution. For extraction of inulin from JA, it seems that indirect extraction might be better than direct extraction because indirect extraction may limit the reduction of the degree of polymerization of inulin, and the noise is much lower. However, the efficiency of direct extraction is much higher than indirect extraction, but for both of methods (direct ultrasound 20 kHz, 150 W& indirect ultrasound 59 kHz, no power information), after 20 mins treatment, the extraction yield of inulin is over 80% (Lingyun, Jianhua et al. 2007). For extraction of inulin from *Eremurus spectabilis* tubers, it also showed that indirect ultrasound extraction (25 kHz, 500 W) is better than direct ultrasound extraction (24 kHz, 200 W) (Pourfarzad, Habibi Najafi et al. 2015). Due to the ability of direct ultrasound treatment to destruct inulin, it could be used to reduce the degree of polymerization of inulin in JA powder. For direct ultrasound extraction of inulin from burdock root, the most suitable parameters are sonication time: 25 mins, amplitude: 83.22% and temperature: 36.76 ºC to achieve optimal extraction (Milani, Koocheki et al. 2011).

Microwave-assisted extraction

Microwave can also be used as an extraction method for inulin, but few studies have focused on this method. For JA tubers, one study treated samples using microwave power at 450 W for 6 mins with a solid to liquid ratio of 1:18, resulting in 12.2% inulin extraction from JA tubers (Xiao, Zhu et al. 2013).

Purification

After extraction, inulin is in an impure inulin-containing juice which contains a lot of impurities. These impurities include peptides, some anions and colloids. These kinds of impurities can be removed by $CaCO_3$ sludge and filtration, which is the pre-purification step. Then, after pre-purification, refinement can be done by using cationic and anionic ion-exchange resins, and a step also known as demineralization. In order to get a pure white color, active carbon can then be used for decolorization. After decolorization, the purified juice should be sent through a 0.2μm filter, and the moisture should be removed by spray drying (Zhu, He et al. 2016).

Separation of low DP inulin from high DP inulin

There are several ways for producers to obtain high performance inulin. These include chromatography, enzymatic removal and ultrafiltration. For enzymatic removal, yeast strains are used to ferment short chain length inulin. Enzymatic removal cannot be used to obtain low DP inulin, which is converted into ethanol by fermentation. In order to collect low DP inulin that is lower than DP9, a cation exchange column (Ca^{2+} form) can be used. Low DP inulin and high DP inulin can be chromatographically separated using a Ca^{2+} loaded, strongly acidic cation exchange resin with low cross linkage due to the reducing ends. In order to increase the yield of low DP inulin, endo-inulinase can be used to break down long-chain-length inulin, resulting in a yield of more than 90% for low DP (2 to 7) (Kays and Nottingham 2007).

1.4.2 Processing of standard inulin and high-performance inulin

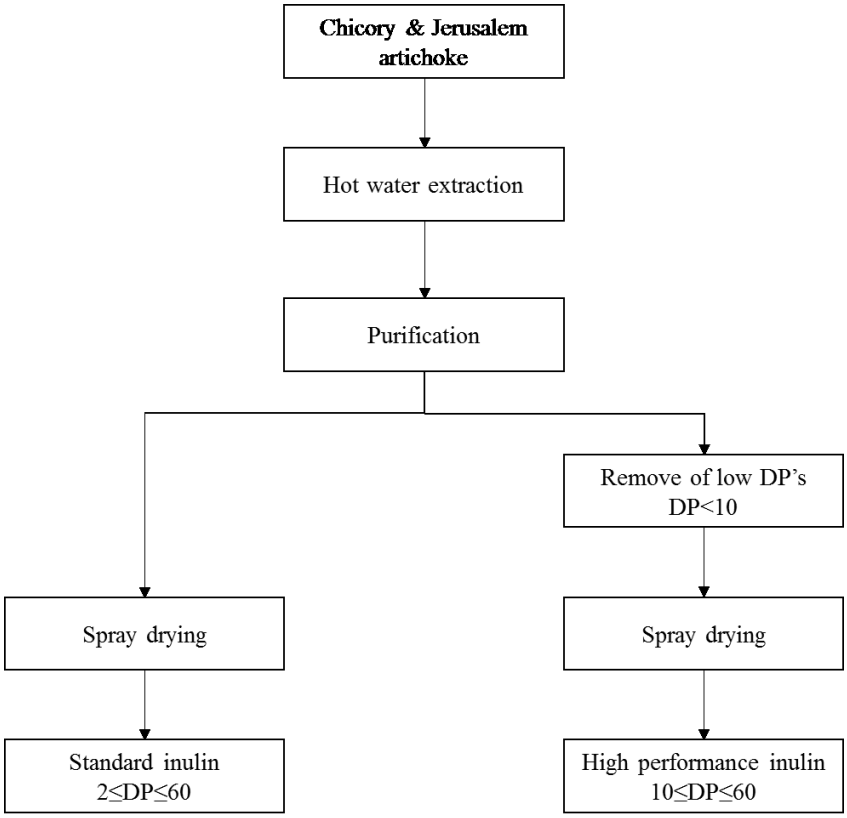


Figure 1.4.2 Schematic for production of standard inulin and high performance inulin (**Kalyani Nair, Kharb et al. 2010**).

1.5 Inulin in food application

1.5.1 Inulin as fat replacer

High degree of polymerization inulin can be used as a fat replacer. Although the inulin molecule is small, and the water holding ability is lower than hydrocolloids, it can still be used to replace fat. The reason is that this kind of inulin consists of long-chain molecules, that form a gel when inulin mixed with water providing a fat-like mouthfeel (Shoaib, Shehzad et al. 2016). Several studies mentioned that inulin can be used as a fat replacer for dairy products, like yogurt and cheese, and for bakery products like muffin and bread. When using a fat replacer, it is very important to keep, or even increase, the sensory value while also improving the nutritional quality of the food. For muffins, it is suitable to replace 50% baking fat with inulin which can reduce 45% of total fat in muffins. Inulin could also impact

the sensory value of muffins including texture and color. Inulin might result in a darker color than full fat muffins, and can lead to a higher crumb firmness. However, if all the baking fat was replaced by inulin, the quality of product would decrease a lot, such as toughness, volume and taste (Zahn, Pepke et al. 2010). For wheat bread, it showed that bread with inulin gel, and an inulin concentration of 2.5% without fat, had a similar quality to common fat containing bread. However, similar to the inulin containing muffins, bread containing inulin powder had a low volume and high firmness. The reason for this might due to a low concentration of inulin, making it hard for inulin to form gel (O'Brien, Mueller et al. 2003). In general, the main problem with using inulin as a fat replacer is that it might result in a higher firmness and a lower volume bakery product than common fat containing bread. Several studies investigated the use of inulin as a fat replacer in cheese, yogurt and ice-cream. For low-fat yogurt, made from 0.1% fat milk, a 1% inulin content provided a similar quality to common yogurt made from whole milk. Also, the inulin concertation in the yogurt did not influence its pH value or its titratable acidity, but more than 1% inulin increased whey separation and affected consistency. For sensory test, the higher inulin content resulted in a lower score, with highest score coming from whole milk yogurt. This means that the sensory evaluation of inulin containing yogurt was not as good as common yogurt (Guven, Yasar et al. 2005). Inulin could also be used as fat replacer for ice cream. Results showed that 4% inulin content, which replaced 40% fat in ice cream, provided a similar quality as common ice cream. However, using inulin to substitute fat reduced mix viscosity and increased hardness and adhesiveness of the ice cream (Tiwari, Sharma et al. 2015). Low fat cheese always suffers from problems like rubbery texture, off-flavor or poor mutability, due to more compacting protein matrix than common cheese. But using inulin as a fat replacer led to a better mouthfeel for low fat cheese. Adding 5 to 10% inulin in low fat, or fat free cheese, contributed to a smooth and creamy texture compared to low fat cheese. However, the texture was still harder than full fat cheese (Karimi, Azizi et al. 2015).

1.5.2 Inulin as sugar replacer

Inulin could also be used as sugar replacer, but only inulin with low degree of polymerization (DP 2-7), known as oligofructose (2004). The literature shows that if using 30% of the sugar replacement by inulin in cake, it will provide a similar sensory evaluation as common 100% sugar cake; However, at the same time, inulin could contribute to a wide bubble size distribution for the final product (Rodríguez-García, Salvador et al. 2014). For muffins, inulin could be used as sugar replacer, but, same as when used to replace fat, if 100% of sugar is replaced with inulin, the result is a firmer texture. This problem can be solved by replacing only 50% of sugar with inulin, which will not influence textural properties (Gao, Brennan et al. 2016). In general, inulin can be used as a sugar replacer in cereal products, resulting in an increase in the hardness of the products like crust and crumb. Also, it can increase the loaf volume of the products (Rößle, Ktenioudaki et al. 2011).

1.5.3 Application of ultrasound treatment in food industry

Recently, ultrasound equipment has become more and more popular in the food industry due to practical and reliable equipment becoming available. Ultrasound could be used in food processing in many areas like cooking, defoaming and emulsification. These kind of applications are based on the principles of ultrasound, such as uniform heat transfer and cavitation phenomenon which means that ultrasound forms vapor bubbles of flowing liquid (Sagong, Lee et al. 2011).

1.6 Food processing using Ultrasound Treatment

The major advantage of ultrasound application for food processing is that it can reduce the processing time, leading to less cost for producers while preserving the nutritional quality of food. Homogenization is one application of ultrasound that could be applied in beverage industry for milk and fruit juice, or the condiment industry for products like Ketch up. Besides food processing, ultrasound could also be used for food preservation and food fermentation (Chemat, Zill e et al. 2011). The most important advantage for ultrasound processing compared to thermal

treatment is that it is a kind of non-thermal treatment. This means that ultrasound processing could be used to increase the quality of fruit juice compared to the degradative nature of thermal treatment. The literature shows that high-intensity ultrasound (376W/cm^2 for 10 min) reduced the activity of polyphenol oxidase without significantly affecting the phenolic compounds in pineapple juice. On the other hand, thermal processing did not change the polyphenol oxidase activity. Ultrasound processing did affect the color of pineapple juice, but, at the same time, increased the color stabilization of the juice during storage (Costa, Fonteles et al. 2013). Slightly different results were observed for cantaloupe melon juice for which high-intensity ultrasound (376W/cm2 for 10min) effectively reduced the activity of peroxidase and polyphenol oxidase without changing the color of the juice. However, it did result in a 30% loss of phenolic compounds (Valdramidis, Cullen et al. 2010). In some conditions, ultrasound did not reduce polyphenoloxidase or peroxidase activity. When the temperature of processing weas higher than 60ºC, the activity of polyphenol oxidase actually increased. For peroxidase, a longer treatment time of ultrasound led to an increase of enzyme activity (Silva, Almeida et al. 2015). Ultrasound can also influence the quality of milk like texture and sensory properties. Ultrasound and high hydrostatic pressures were used to treat goat milk to test the effect on particle size of fat globules. The results showed that ultrasound and high hydrostatic pressures increased the total area of fat globules improving the stability and quality of the emulsions (Karlovic, Bosiljkov et al. 2014). For cream cheese, milk treated by thermosonication could be used to improve the textural and rheological properties of cream cheese. One study showed that the fat content and the yield of cheese increased, while the size of fat globules decreased, when using thermosonication to treat milk at 30 to 50ºC for less than 30 min. At the same time, treated milk imporved the thermostability of the cheese (Almanza-Rubio, Gutiérrez-Méndez et al. 2016). Ultrasound treatment can also be used for dairy products during fermentation. Ultrasound at 20 kHz can reduce particle size in yoghurt, resulting in more uniform size and improved gel texture to provide a smooth feeling for consumers (Tabatabaie,

Mortazavi et al. 2009). Ultrasound treatment also has an effect on tomato pulp by changing the viscoelasticity of the pulp. These kind of changes can lead to a stronger network inside the tomato pulp. However, these stronger network might decrease the bioaccessibility of lycopene (Anese, Mirolo et al. 2013).

Ultrasound can be used for food preservation and many studies have focused on this application. There are two kinds of ultrasonic waves: high-intensity ultrasonic waves and low-intensity ultrasonic waves. High-intensity ultrasonic waves can rupture cells and denature enzymes, while low-intensity ultrasonic waves can modify the metabolism of cells (Chemat, Zill e et al. 2011).

1.7 Ultrasound treatment in food safety

Most studies show that ultrasound alone does not result in a significant microorganism inactivation for food. In order to achieve satisfactory inactivation, ultrasound can be combined with other treatments, like thermal treatment or with salts which include calcium propionate, organic acids and sodium benzoate (Arvanitoyannis, Kotsanopoulos et al. 2017). This kind of combined treatment can lead to significant inactivation of microorganism in vegetables and fruit. For example, ultrasound treatment combined with organic acids can effectively inactivate the growth of microorganism and reduce pathogens in fresh lettuce, without significantly influencing color or texture during 7 days of storage. Using a higher concentration of organic acid (highest 2%) led to a better reduction of pathogens (Sagong, Lee et al. 2011). In another example, thermo-ultrasound treatment combined with calcium propionate, led to a more than 5 log reduction of microorganism on fresh-cut celery. Using 59 ºC thermoultrasound with 40 KHz frequency and 2% calcium propionate for 15 min was effective for inactivation of Escherichia coli O157:H7, while17 min was effective for Salmonella enterica serovar Typhimurim (Kwak, Kim et al. 2011). Similar to its application for vegetables and fruits, when ultrasound is used for beverages, it hardly influences microorganism growth when used alone, but combined treatment solves this

problem. For beverages like fruit juice, thermal treatment combined with ultrasound resulted in a better inactivation of pathogens than thermal treatment alone. Thermosonication can inactive Escherichia coli O157:H7 and Salmonella Enteritidis in mango juice when treated for more than 5 min at 60 ºC. Salmonella Enteritidis was more sensitive to thermosonication compared to Escherichia coli O157:H7 (Kiang, Bhat et al. 2013). However, thermosonication was not as good as high pressure when processing apple juice. A 10 min thermosonication treatment, at 24 KHz and 75 ºC did not inactive Neosartorya fischeri, While a 10 min 600 MPa high pressure treatment led to a 3.3 log reduction of N. fischeri (Evelyn, Kim et al. 2016).

Ultrasound is always combined with thermal treatment when studies focus on milk and dairy products. Pasteurization always results in a reduction of quality for dairy products which the industry wants to prevent. This is why ultrasound is of interest for the dairy industry, as it may help preserve quality while decreasing microorganism content. The literature shows that a 20 kHz ultrasound treatment can effectively kill Paeudomonas fluorescens after 6 min and Escherichia coli after 10 min, without reducing the protein, lactose and fat content of milk. However, ultrasound treatment did not significantly inactive two enzyme (Alkaline phosphatase and lactoperoxidase) which are used as indicators of effective thermal treatment by the dairy industry (Cameron, McMaster et al. 2009). When ultrasound is used in combination with thermal treatment. It can effectively reduce the activation of C. sakazakii NCTC 08155 and ATCC 11467 which are linked to infant formula. A 6.86 log reduction of C. sakazakii strain ATCC 11467 was obtained by processing for 2.5 min with an amplitude equal to 61µm and temperature at 50 ºC (Adekunte, Valdramidis et al. 2010). Ultrasound can also be combined with 0.05% hydrogen peroxide and an active lactoperoxidase system to inhibit bacteria in milk. Compared with ultrasound alone, the combined ultrasound treatment resulted in a higher microbial reduction with lower amplitude and shorter treatment time (Shamila-Syuhada, Chuah et al. 2016).

1.8 Hypothesis and objectives

Most of studies focuses are on how extraction methods or extraction factors affect the yield% of inulin from plants and some studies only focus on the health benefits of inulin. Thus, a huge gap exists between the ultrasound treatment of inulin and how this technique will affect on the physical and chemical characteristics of dairy products containing inulin.

The objectives of the research were to a) extract and purify inulin from Jerusalem artichoke, b) investigate the physical and chemical characteristics of food using ultrasound technology, c) optimize factors such as time and temperature using ultrasound technology, d) examine value-added Jerusalem artichoke inulin applications – mainly in dairy

Chapter2 – Effect of ultrasound treatment on inulin in Jerusalem artichoke powder.

2.1 Abstract

The effect of ultrasound treatment on the physical and chemical characteristics of inulin was investigated in this study. In this research, ultrasound treatment and heat treatment were used to treat purified inulin from JA (PJAI), inulin from chicory (CI) and JA powder, Results showed that physical and chemical characteristics of these samples, like water holding capacity, microstructure, reducing sugar content and depolymerization, are affected by thermal treatment and ultrasound treatment. Differences for these characteristics were observed between samples treated with heat and ultrasound. Treatment time of ultrasound also seemed to be an important factor for physical and chemical characteristics. Results show that ultrasound treatment time had a positive linear relationship with reducing sugar content of the samples. After ultrasound treatment, reducing sugar content in JA powder increased from 6.101g/100g to 12.273g/100g. In PJAI, reducing sugar content increased from 10.378g/100g to 12.274g/100g. And reducing sugar content in CI increased from 1.126g/100g to 2.183g/100g. Also, determined by HPLC and GPC, for PJAI, there was a negative linear relationship between high degree of polymerization (DP) inulin and ultrasound treatment time (R^2=0.9169), and a positive linear relationship between low DP inulin and ultrasound treatment time (R^2=0.9738), which was not observed in inulin from chicory. Moreover, compared with heat treatment, ultrasound treatment might be a good method to reduce the DP of inulin from JA.

2.2 Introduction

JA and chicory are two common plants that are often used by food companies as an inulin source, but the inulin DP in the two plants are different. Fresh JA contains 16-20% inulin, and, inside of JA, about 52% inulin has a DP lower than 9. For fresh

chicory, it contains 15-60% inulin, and about 45% inulin inside chicory has a DP between 20-40 (Kays and Nottingham 2007). It means that the DP of inulin from JA is lower than the DP of inulin from chicory. Both long degree inulin and short degree inulin have several health benefits. Compared with high DP or high performance inulin, low DP inulin has been shown to increase reduction of weight gain, body fat mass, hyperglycemia, liver steatosis and also total cholesterol in high-fat diet-induced mice (Márquez-Aguirre, Camacho-Ruíz et al. 2016). Low DP inulin, with DP4 and DP8 might be fermented faster than high DP inulin due to more cecal immunoglobulin A in rats. Faster fermentation of low DP may result in a bigger reduction of cecal pH compared to high DP inulin, providing a better environment for acid-tolerant bacteria like bifidobacteria in rats (Márquez-Aguirre, Camacho-Ruíz et al. 2016). Similar results showed in another research. Cecal immunoglobulin A concentration was higher with DP4 compared to DP8, and higher with DP8 compared to DP16. At the same time, low DP inulin also significantly increased the amount of immunoglobulin A-producing plasma cells and cecal lactobacilli amount in rat cecal mucosa (Ito, Takemura et al. 2011). Bifidobacteria and lactobacilli are probiotics in our bodies that help maintain intestinal health through certain activities like using bacteriocins to limit detrimental bacteria (Kleerebezem and Vaughan 2009; Kalyani Nair, Kharb et al. 2010). Increasing their amount of these probiotics might provide better intestinal health for humans such as reducing the risk of colon cancer.

Several methods can be used to extract inulin from plants, but the most common one used in the food industry is the heating method (Lingyun, Jianhua et al. 2007). For JA, tubers are cut into similar small slices. The slices are then placed in boiling water for 5 mins to reduce enzyme activity. After that, the slices are stored at 60 °C for 7h, and then grinded into powder. The powder is then diffused for 40 min at 90 °C with deionized water (Li, Zhang et al. 2015). For chicory, before water extraction, it should be cut into similar slices. After that, these slices are diffused for 1 to 2 hours at 70-80 °C for full extraction of inulin. The ratio of solids to water

should be about 1:5 to optimize the inulin extraction efficiency (Zhu, He et al. 2016). There are some other methods that can be used for inulin extraction like ultrasound treatment and microwave-assisted extraction. These methods are not commonly used by food processors (Xiao, Zhu et al. 2013). For ultrasound treatment, the literature shows that it can reduce the DP of inulin during extraction which might not be suitable for high performance inulin processing, but might be a good choice for depolymerization to obtain a shorter inulin (Lingyun, Jianhua et al. 2007). Most of studies focused on how different extraction methods effect the yields of inulin. There is not enough information about how ultrasound treatment effects the physical and chemical characteristics of inulin.

In this study, JA powder and inulin from chicory were treated with ultrasound and heat. The physical and chemical characteristics were investigated to find out if ultrasound treatment has an effect on inulin, like the depolymerization, which might be useful for industry to reduce the DP of inulin.

2.3 Methods and Materials

2.3.1 Materials

Inulin from chicory (Sigma-aldrich, St. Louis, MO, USA), JA powder (Action Vale, QC, Canada)

Table 2-3-1 Composition of JA powder from Action Vale

	JA powder
Moisture%	4.814%±0.143%
Ash%	6.325%±0.126%(wet basis)
	6.645%±0.123%(dry basis)
Protein%	7.493%±0.0533%(wet basis)
	7.872%±0.0507%(dry basis)
Carbohydrates%	81.368%(wet basis)
	85.483%(dry basis)
Fat%	≈0

2.3.2 Extraction of inulin from JA powder

Extraction was based on the method used by (Wu, Hu et al. 2006) with modifications. JA powder was suspended in distilled water (1g JA powder/30ml distilled water) for 1h at 80 ºC, and the solution was then filtrated with suction filtration. $CaCl_2$ was then used to remove proteins inside the solution. For discoloration, activated charcoal (0.6g activated charcoal/1.0g JA powder) was put in pre-purified juice for 1h at 70 ºC.The purified juice was then dried using freeze dryer (Bulk Tray Dryer 115V; Labconco, Kansas City, USA.) for 4 days at -50 ºC.

2.3.3 Treatment of JA powder and inulin from JA and chicory

5g, 2g, 1g of Jerusalem artichokes powder, purified inulin from JA powder and inulin from chicory was suspended in 100 ml distilled water. For heat treatment, autoclave was used at 121 ºC for 20 min. For ultrasound treatment, samples were treated with an ultrasonic processors (UIP500hdT; Hielscher, Inc. Ringwood, USA.) at 20 KHz, 90W for 1min and 2min. After treatment, all samples were transferred into plastics bag and dried using a freeze dryer (Bulk Tray Dryer 115V; Labconco, Kansas City, USA.) for 4 days at -50 ºC.

2.3.4 Determination of inulin polymerization degree for JA powder and chicory inulin

The polymerization degree of purified inulin from JA powder was determined by high performance liquid chromatography (Waters e2695, Waters Corporation, Milford, USA) and refractive detectors (Waters 2414, Waters Corporation, Milford, USA) using Waters Sugar-Pak I Column with 0.6ml/min water.

Chicory inulin was sent to University of Alberta to determine the degree of polymerization of inulin by Gel Permeation Chromatography (GPC) coupled to multiangle laser light scattering (Wyatt DAWN DSP, Wyatt Technology Corporation, Goleta, USA) and refractive index detectors (Wyatt Optilab DSP, Wyatt Technology Corporation, Goleta, USA) using Waters Ultrahydrogel Column 250 with 0.1M NaCl as eluent.

2.3.5 Scanning electron microscope (SEM) analysis

Samples of JA powder, purified inulin from JA and inulin from chicory, were coated with gold and viewed using SEM (TESCAN, Brno – Kohoutovice, Česká republika) under high vacuum conditions (10^{-3} Pa) at 10 kv voltage.

2.3.6 Water solubility index and water absorption index

The water solubility index (WSI) and water absorption index (WAI) were measured based on the method used by (Lee, Lee et al. 2014) with modification. JA powder, purified inulin from JA powder and inulin from chicory samples (1.5g) were suspended in distilled water (30ml at 30 ºC) in a 50 ml centrifugal tube. The suspension was stirred using a vortex mixer (Mini vortexer VM-3000; VWR, Radnor, USA.) intermittently over 30 min. Then, the samples were centrifuged (Sorvall Legend XTR Centrifuge 120V; ThermoFisher Scientific, Langenselbold, Germany) at 3000g for 20 min. The supernatant was decanted and dried using a freeze dryer (Bulk Tray Dryer 115V; Labconco, Kansas City, USA.) at -50 ºC for 3 days. The WAI was calculated as the remaining gel weight (g) in the centrifuge tube per dry sample weight (g). The WSI was calculated as the weight of dry solid of supernatant (g) per dry sample weight (g).

2.3.7 Determination of reducing sugar content

The method to determine the reducing sugar content in samples was based on a method by (Miller 1959) with modification. 0.5g of JA powder and 1g of chicory inulin were dissolved with distilled water and then put in a 50 ºC water bath for 20 min. After that, filtration was used to get the filtrate. The filtrate was then diluted with distilled water to get the reducing sugar extraction solution. The extraction solution was mixed with 3,5-dinitrosalucylic acid in a boiling water bath for 2min. After reaction of reducing sugar with 3,5-dinitrosalicylic acid, the absorbance was measured at 540 nm.

2.3.8 Statistical analysis

Analysis of variance (ANOVA) was determined using IBM SPSS (IBM Ltd, NY, USA) and significance was performed using Duncan's multiple range test. All analyses were carried out in separate triplicates ($p<0.05$).

2.4. Result

2.4.1 Determination of Microstructure and Particle Size by Scanning Electron Microscope Analysis

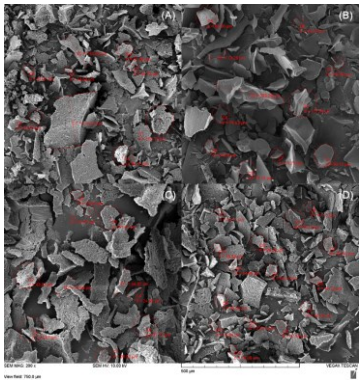


Fig.2.4.1. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 5g CI in 100ml water at magnifications of 200x

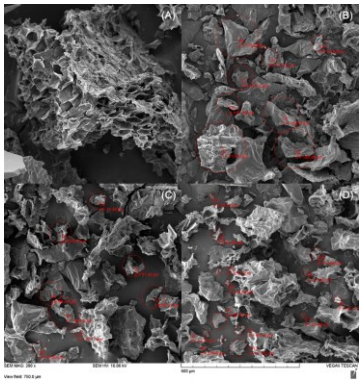


Fig.2.4.2. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 5g JA powder in 100ml water at magnifications of 200x

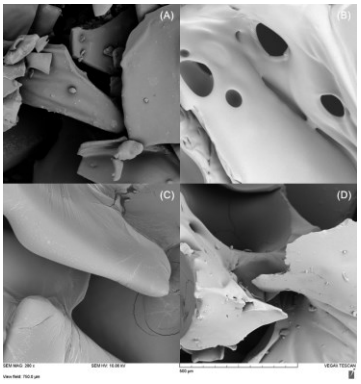


Fig.2.4.3. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 5g PJAI in 100ml water at magnifications of 200x

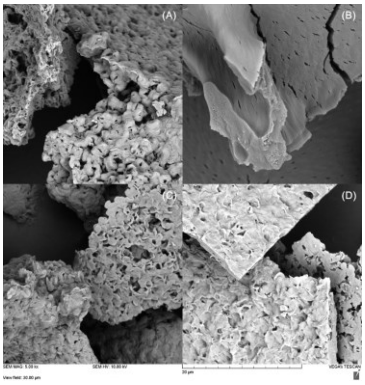


Fig.2.4.4. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 5g CI in 100ml water at magnifications of 5kx

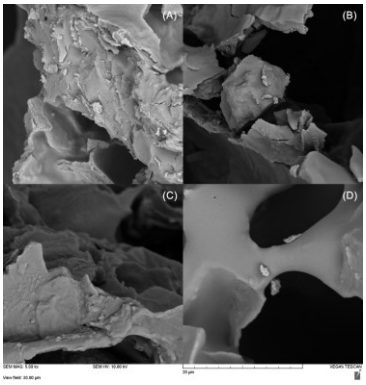


Fig.2.4.5. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 5g JA powder in 100ml water at magnifications of 5kx

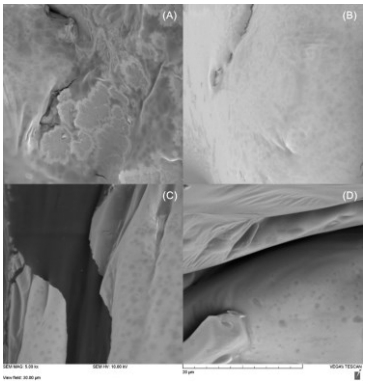


Fig.2.4.6. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 5g PJAI in 100ml water at magnifications of 5kx

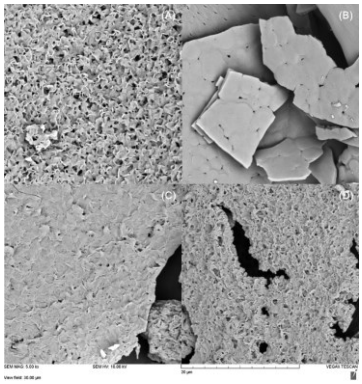


Fig.2.4.7. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 2g CI in 100ml water at magnifications of 5kx

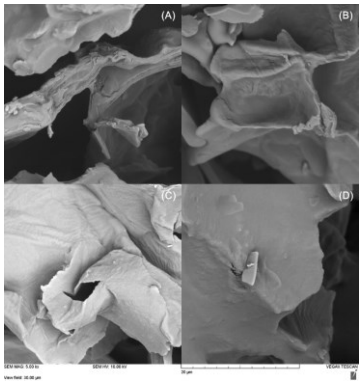


Fig.2.4.8. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 2g JA powder in 100ml water at magnifications of 5kx

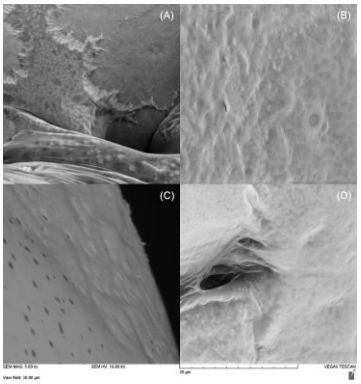


Fig.2.4.9. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 2g PJAI in 100ml water at magnifications of 5kx

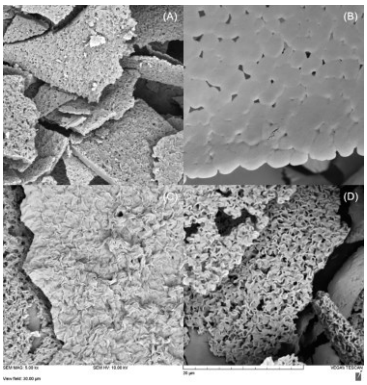


Fig.2.4.10. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 1g CI in 100ml water at magnifications of 5kx

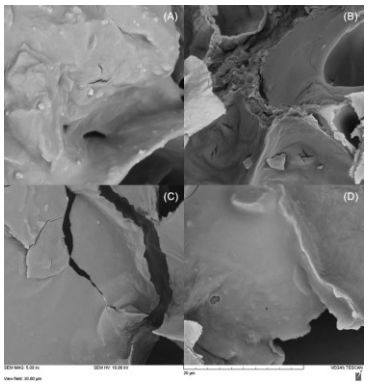


Fig.2.4.11. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 1g JA powder in 100ml water at magnifications of 5kx

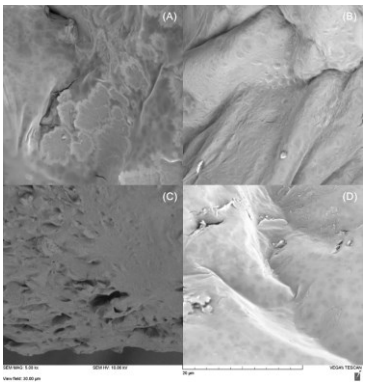


Fig.2.4.12. Scanning Electron photomicrographs of (A) untreated (B) autoclave (C) ultrasound 1min and (D) ultrasound 2min 1g PJAI in 100ml water at magnifications of 5kx

Both heat and ultrasound treatment had an effect on particle size for chicory inulin, purified JA inulin and JA powder (**Figure** 2.4.1, **Figure** 2.4.2 and **Figure** 2.4.3). In general, ultrasound treatment provided a small sized particle compared to heat treatment. This difference was much more significant in chicory inulin compared to unpurified JA powder; However, for purified inulin (**Figure** 2.4.3), due to the degree of polymerization, most particles were combined together, so processing had no effect on particle size, which will not mention in this part. As seen in figure,

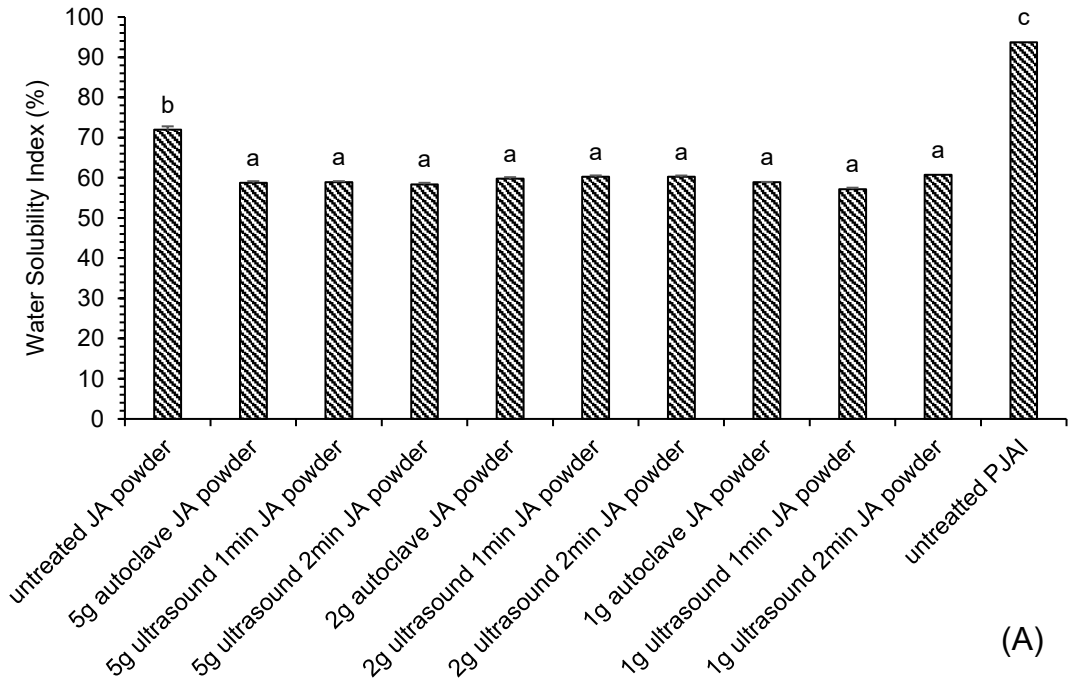
heat treatment provided a smoother surface than ultrasound treatment in both samples. For chicory inulin, the particle size was between 30 to 200 μm for untreated sample, which was quite different compared to the treated samples. After heat treatment, particle size became more uniform, and most particles were between 50 and 120 μm, with some particles over 200 μm remaining. Similar with heat treatment, using ultrasound to treat chicory samples for 1 min resulted in a smaller particle size compared to untreated samples. After ultrasound treatment for 1min, particle size of chicory inulin was around 20 to 120 μm, but still contained some particles larger than 220 μm remaining. For the samples treated with ultrasound for 2 min, particle size became more uniform with most particles around 30-80 μm. For unpurified JA powder, both heat treatment and ultrasound treatment showed a significant effect on particle size. However, no significant difference between these two kinds of treatments was observed, as well as no difference observed between ultrasound for 1min and ultrasound for 2min. The range of particle size for all unpurified JA powder samples was from 30 to 200 μm.

The microstructure of chicory inulin, and JA powder was affected by different kinds of treatment, detected with 5kx magnification by SEM. For chicory inulin, the surface of untreated chicory inulin was uneven with a lot of bumps while autoclave heat treatment provided a smoother surface for inulin. Ultrasound treatment, also provided a generally smooth surface for chicory inulin but with a lot of pores. These kinds of pores were more present in samples treated with ultrasound for 2min compared to the samples treated with ultrasound for 1min. For JA powder, untreated sample surface was uneven with some wrinkles, while samples treated with autoclave heat had a smoother surface with less wrinkle. Similar results were seen in samples treated with ultrasound for 1min, while samples with a 2min ultrasound treatment had a smoother surface with almost no bump. Solid content also effected microstructure, with differences being more significant in the 5g/100ml samples compared to 1g/100ml samples. For 1g/100ml samples, the pictures of the 4 different kinds of samples were similar, while the

5g/100ml samples were not.

Different from chicory inulin, and JA powder, the structure of purified JA inulin was affected by different kinds of treatment, detected with 200x magnification by SEM. Compared with untreated samples, the surface of treated samples was much smoother. This kind of difference was more significant in 5g/100ml samples, which means that a higher solid ratio might result a greater effect on samples. For 1g/100ml samples, no noticeable difference between samples was observed. Difference in structure were also observed between the different treatments. Autoclave treatment provided the smoothest surface for samples compared to ultrasound treatment, with some shallow wrinkles observed. For the samples treated with ultrasound for 1min, similar to the autoclave treatment, a smooth surface was observed but with a lot of tiny wrinkles. Different from the other two kinds of treatment samples treated with ultrasound for 2 min, particles were thinner, and broken into smaller parts, and each large particle had some particles attached to it

2.4.2 Determination of Water Holding Capacity



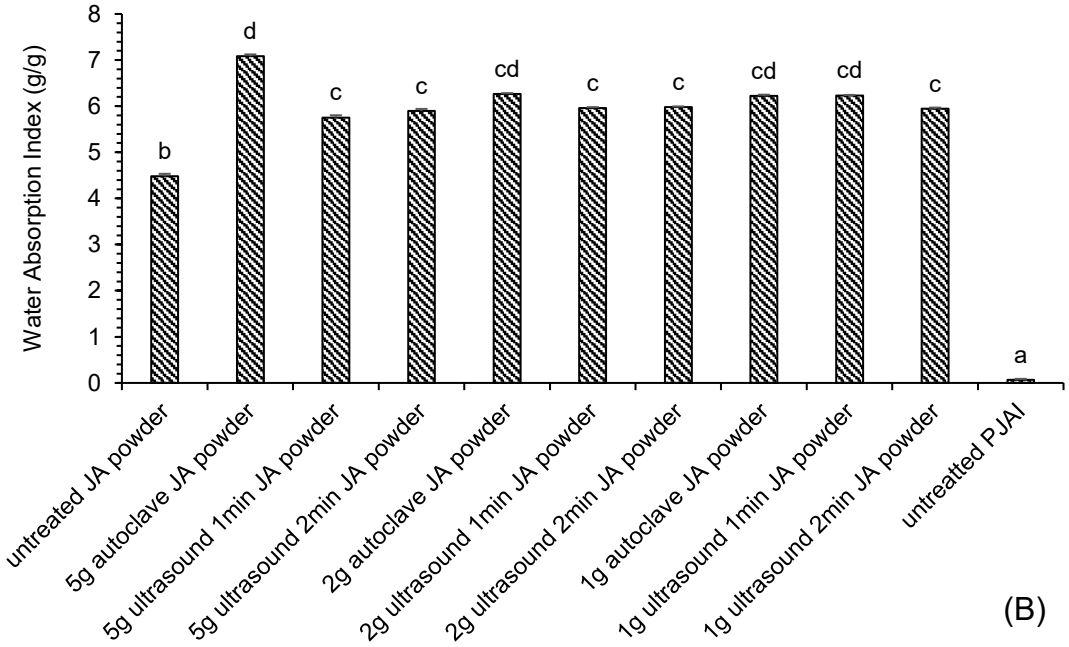
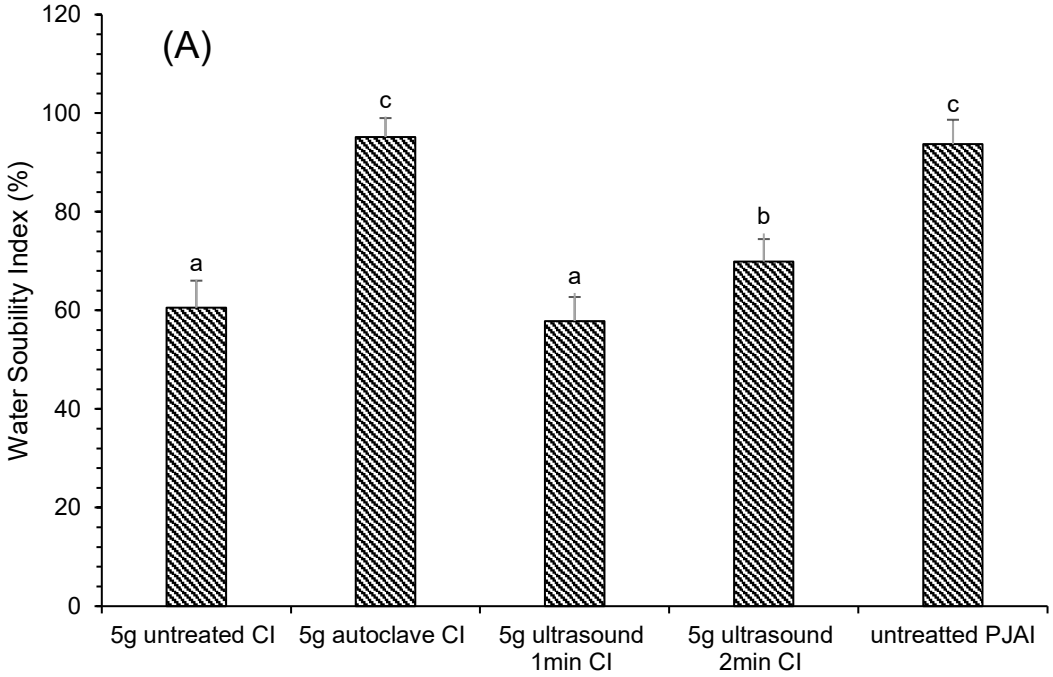


Figure 2.4.13. Water Solubility Index (A) and Water Absorption Index (B) of JA Powder and JA Inulin after Different Processing



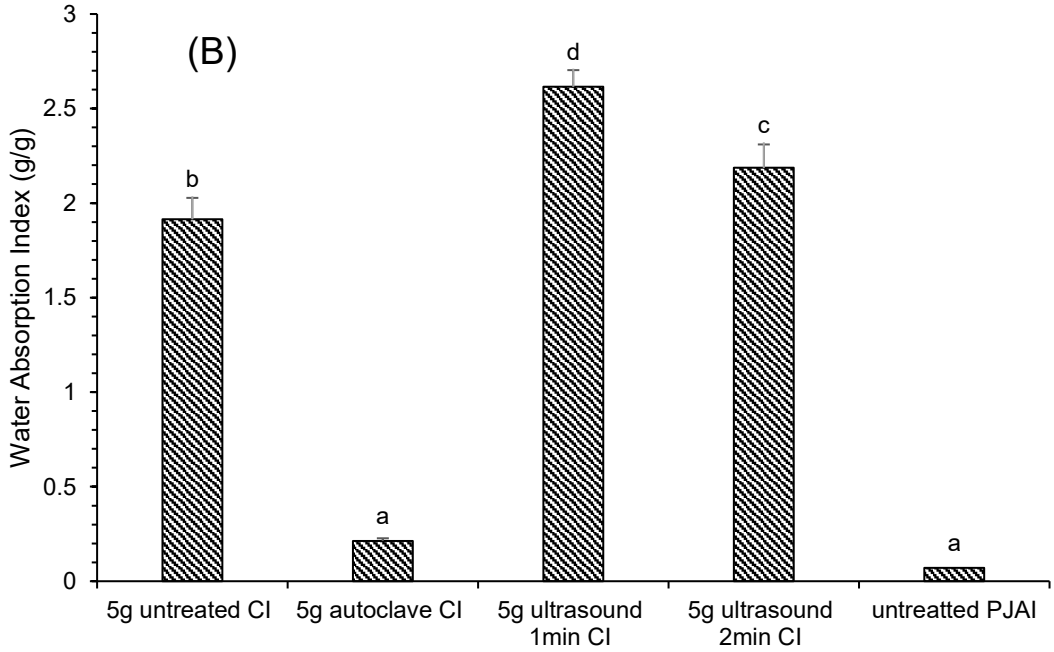


Figure 2.4.14. Water Solubility Index (A) and Water Absorption Index (B) of Chicory Inulin after Different Processing

According to **Figure 2.4.13.**, the WAI of PJAI was negligible, and the WSI of JA inulin reached 90%, so the data for JA inulin is not shown. However, results of JA powder and chicory inulin can be used as a reference. For JA powder, the WAI increased after treatment, and, at the same time, the WSI decreased after treatment. As seen in **Figure 2.4.16.**, for the autoclave treatment, sample concentration had a positive linear relationship with WAI (R^2=0.9598). And, according to **Figure 2.4.18.**, there was a positive correlation between ultrasound treatment time and WAI for the different concentrations, although no significant difference in correlation between concentration was observed (5g/100ml JA powder R^2=0.7895, 2g/100ml JA powder R^2=0.7125, 1g/100ml JA powder R^2=0.5319). For WSI, the difference between treatments, sample concentration and treatment time were negligible for JA powder (**Figure 2.4.15.** & **Figure 2.4.17.**) This means that whatever treatment is used, there is a limit value (>57.16%) for WSI and none of the treatments could result in a WSI under this value.

For CI (**Figure 2.4.14.**), autoclave treatment increased the WSI to 95.14% and

decreased the WAI to 0.21g/g. For ultrasound, the WSI was only slight increased and WAI slightly decreased for CI. The WAI and WSI of CI after autoclave treatment was similar to the WAI and WSI of untreated PJAI..

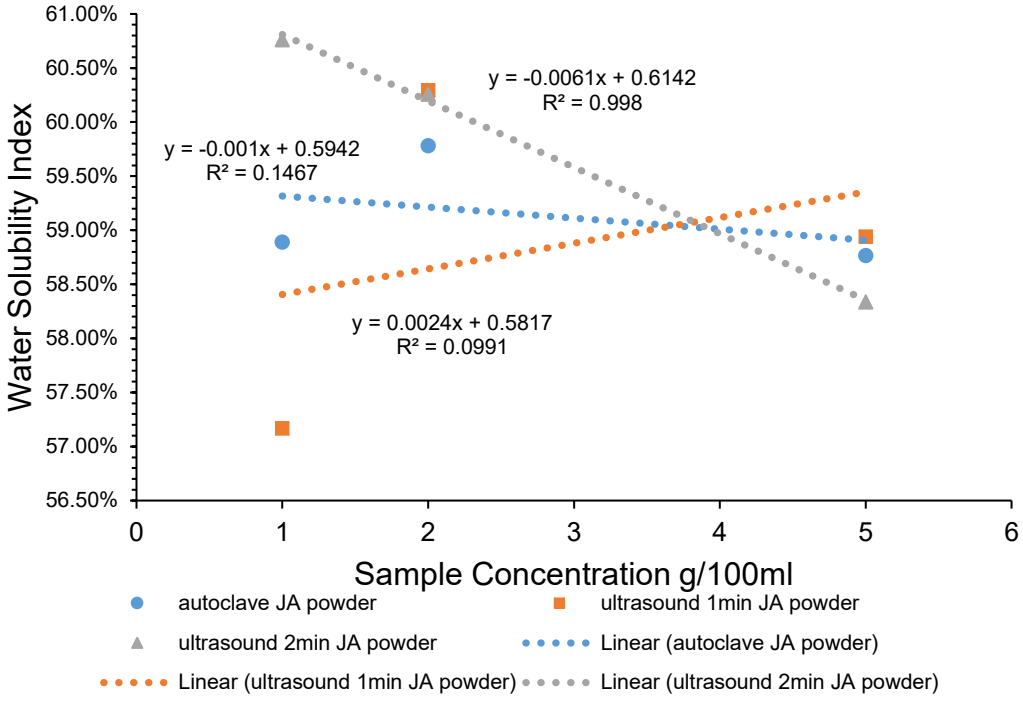


Figure 2.4.15. Correlation Between Sample Concentration of JA Powder with Water Solubility Index

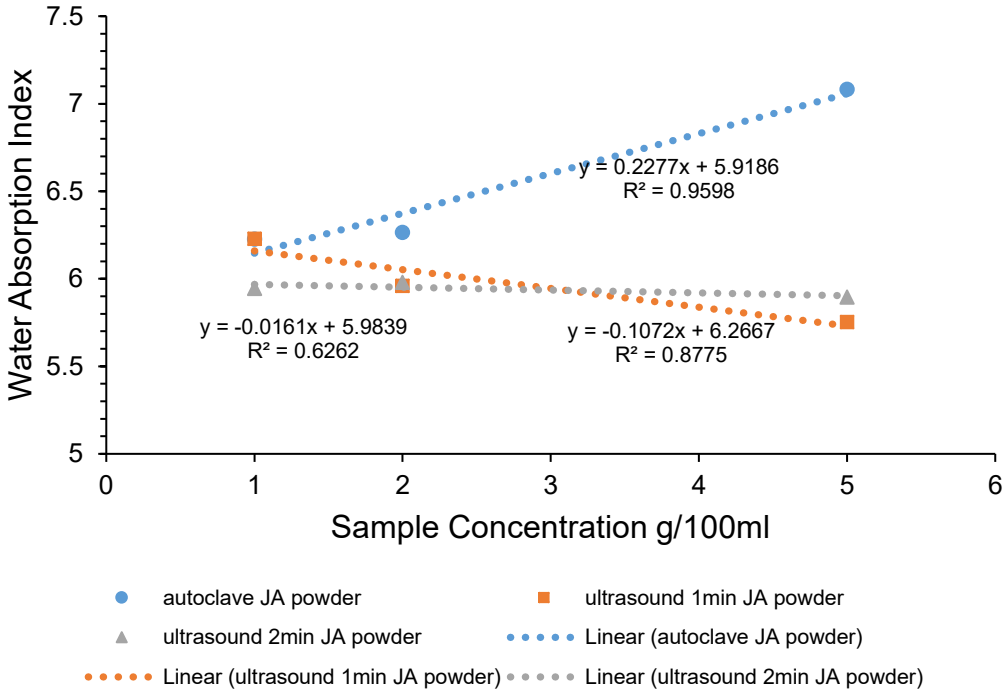


Figure 2.4.16. Correlation Between Sample Concentration of JA Powder with

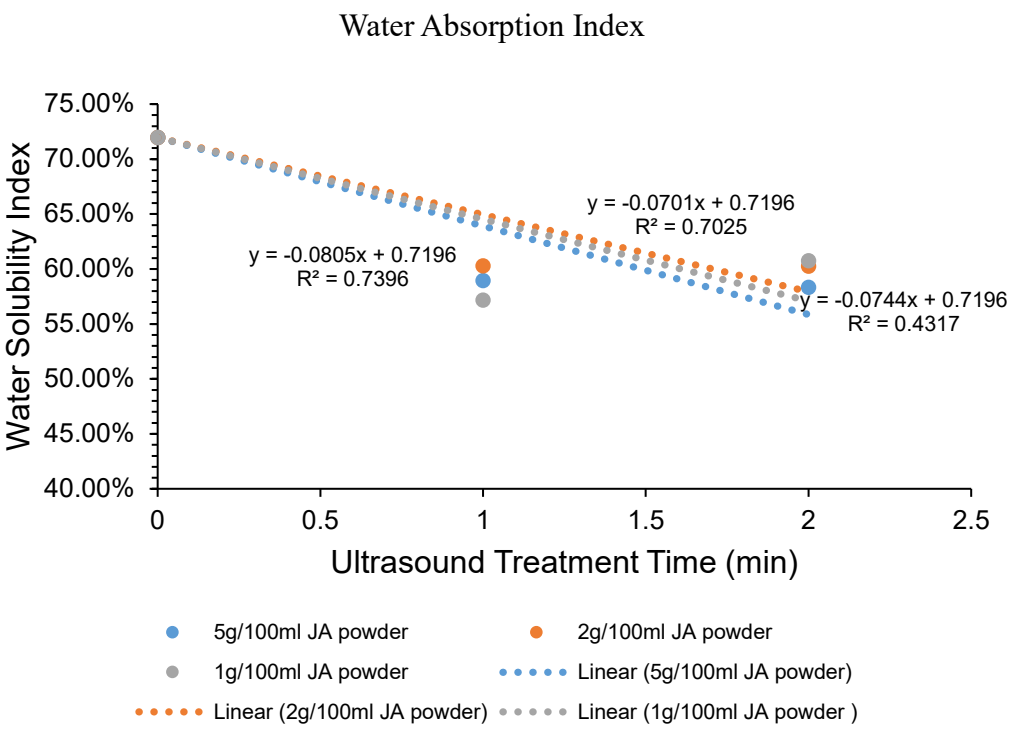


Figure 2.4.17. Correlation Between Ultrasound Treatment Time of JA Powder with Water Solubility Index

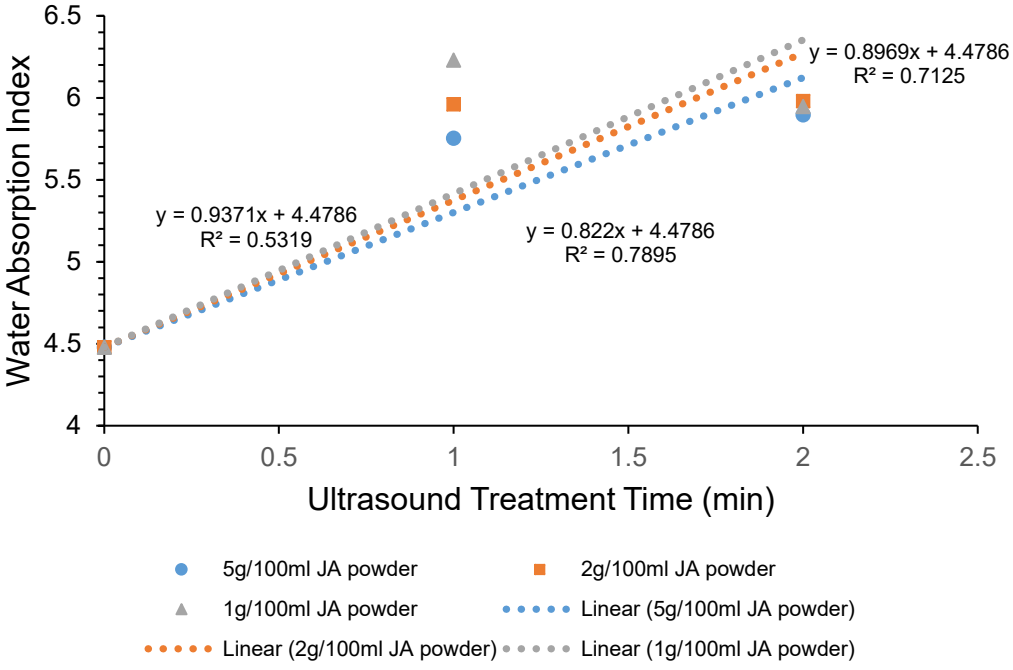
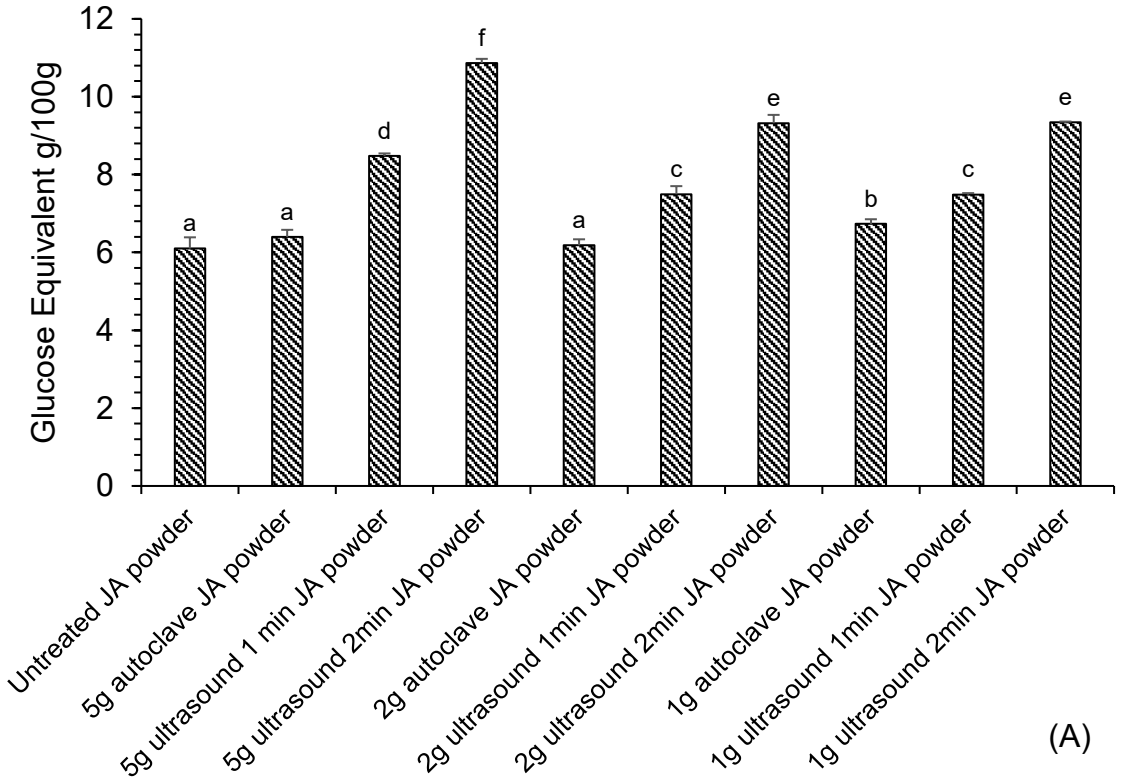
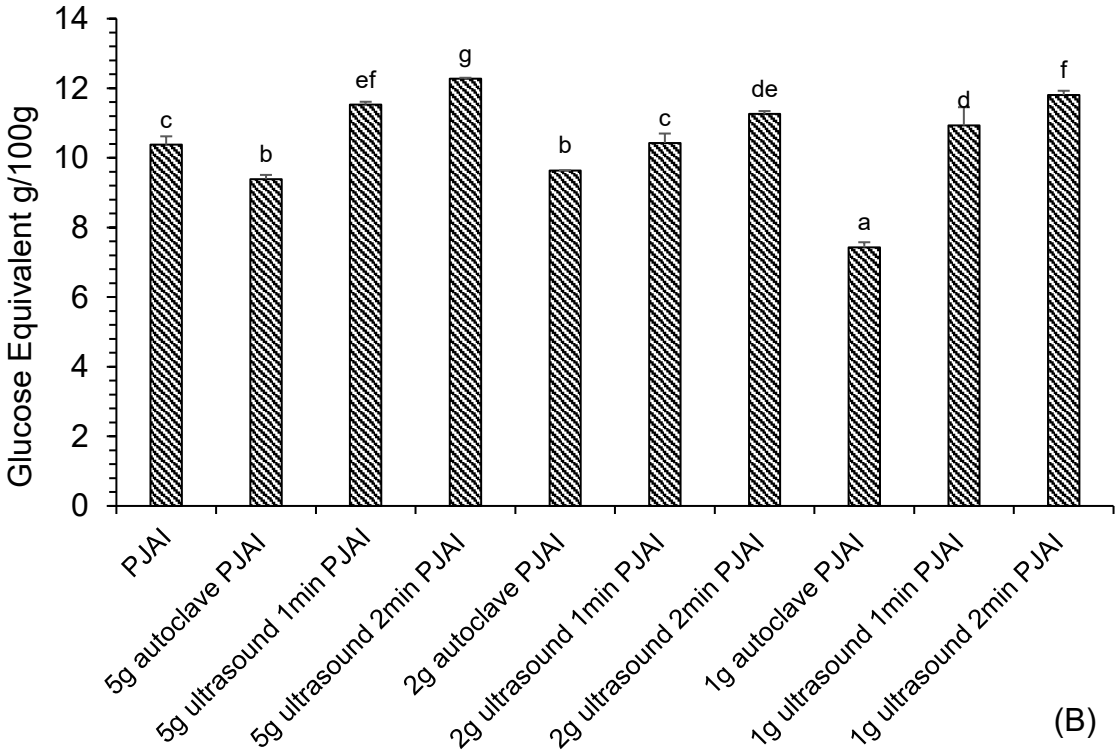


Figure 2.4.18. Correlation Between Sample Ultrasound Treatment Time of JA Powder with Water Absorption Index

2.4.3 Determination of Reducing Sugar Content





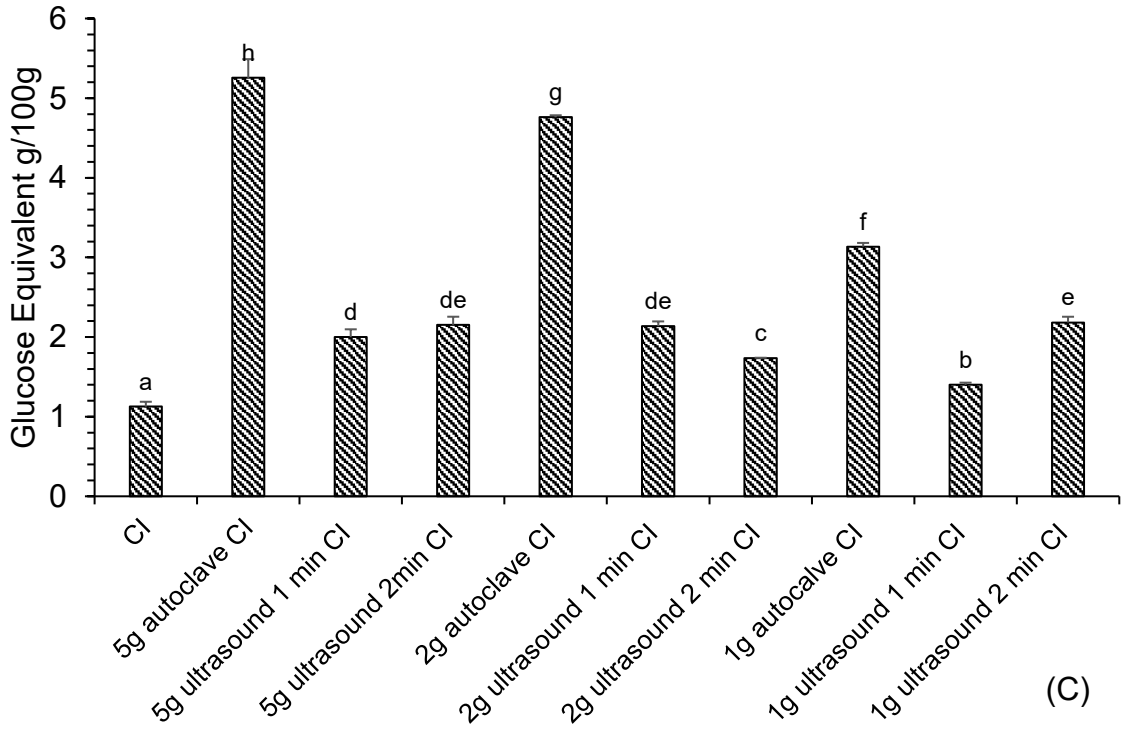


Figure.2.4.19 Reducing Sugar Content Glucose Equivalent g/100g in JA powder (A), Purified JA Inulin (B) and Chicory Inulin (C).

According to **Figure 2.4.19.**, both heat treatment and ultrasound treatment had an effect on the reducing sugar content in JA powder, purified JA inulin and chicory inulin, but the effect between the different kinds of samples was different. Glucose was used as the standard, and it showed that untreated JA powder contained 6.101g/100g reducing sugar glucose equivalent, untreated purified JA inulin contained 10.378g/100g reducing sugar glucose equivalent and untreated chicory inulin contained 1.1264g/100g reducing sugar glucose equivalent.

For JA powder, it showed that both heat treatment and ultrasound can increase reducing sugar content compared to the untreated samples. Ultrasound treatment provided a higher reducing sugar content than the autoclave treatment. Ultrasound treatment for 2min resulted in a higher reducing sugar content compared to ultrasound treatment for 1min. There also existed a linear trend with a high concentration of the samples, or high solid ratio leading to a higher reducing sugar

content for ultrasound treatment (Ultrasound 1min R^2=0.9364, Ultrasound 2min R^2=0.946), but for autoclave, no relationship was observed (R^2=0.111) (**Figure 2.4.23.**). 5g JA powder in 100ml water had a higher reducing sugar content compared to 2g JA powder in 100 ml water and 1g JA powder in 100ml water for ultrasound treatment. Also, at the same time, for all different concentrations, there exists a linear relationship between ultrasound treatment time and reducing sugar content in JA powder (1g/100ml JA powder R^2=0.9932, 2g/100ml JA powder R^2=0.992, 5g/100ml JA powder R^2=1) (**Figure 2.4.20.**). A higher ultrasound treatment time resulted in a higher reducing sugar content.

For purified JA inulin, the result was similar to JA powder, with a linear relationship between ultrasound treatment time and reducing sugar content observed (1g/100ml JA powder R^2=0.9795, 2g/100ml JA powder R^2=0.7514, 5g/100ml JA powder R^2=0.9819) (**Figure 2.4.21.**). However, the reducing sugar content for samples treated by autoclave was lower than untreated samples, and the effect of concentration of samples or solid ratio was not significant (Autoclave JA inulin R^2=0.3788, Ultrasound 1min JA inulin R^2=0.5711, Ultrasound 2min JA inulin R^2=0.4748) (**Figure 2.4.24.**). Results from HPLC, shown in **Table.**2.4.1., showed that the disaccharide and monosaccharides in purified JA inulin samples are sucrose, fructose and glucose, with no other simple sugars observed in the sample. These result also confirm the result above, that ultrasound treatment increased reducing sugar content, with longer treatment time resulting in the highest reducing sugar content (R^2=0.9927), and that autoclave treatment led to a lower reducing sugar content compared to the untreated samples. It also showed that sucrose and fructose content had no correlation with ultrasound treatment time, while glucose content showed a significant negative linear relationship with ultrasound treatment time (R^2=0.9796).

Table.2-4-1 Percentage of Saccharide and Inulin Content by Weight from PJAI Analyzed by HPLC

	% Weight			
	Sucrose	Glucose	Fructose	Total DP<2
PJAI Untreated	13.98±0.34^{ab}	0.82±0.04^{b}	3.71±0.25^{b}	18.51±0.06^{ab}
PJAI Autoclave	12.53±0.27^{a}	0.47±0.03^{a}	2.85±0.02^{a}	15.84±0.25^{a}
PJAI Ultrasound 1min	14.80±0.52^{b}	0.73±0.04^{b}	3.76±0.01^{b}	19.29±0.55^{b}
PJAI Ultrasound 2min	15.27±0.38^{b}	0.58±0.01^{a}	3.86±0.14^{b}	19.71±0.53^{b}

For chicory inulin, the results were a little different to those obtained for JA powder and purified JA inulin. Autoclave treatment provided a higher reducing sugar content compared to ultrasound treatment and untreated samples. Similar to JA inulin, there was some difference between ultrasound treatment for 1min and ultrasound treatment for 2min. The results also show that for 5g/100ml chicory inulin samples and 2g/100ml chicory inulin samples, there is a positive linear relationship between ultrasound treatment and reducing sugar content(5g/100ml chicory inulin R^2=0.8345, 2g/100ml chicory inulin R^2=0.9133), but not for 1g/100ml chicory inulin sample which might due to the low sample concentration (**Figure 2.4.22.**). Both autoclave treatment and ultrasound treatment led to a higher reducing sugar content compared to untreated samples. However, only autoclave treatment showed that a higher concentration of samples, or solid ratio, led to a higher reducing sugar content (R^2=0.6995) (**Figure 2.4.25.**).

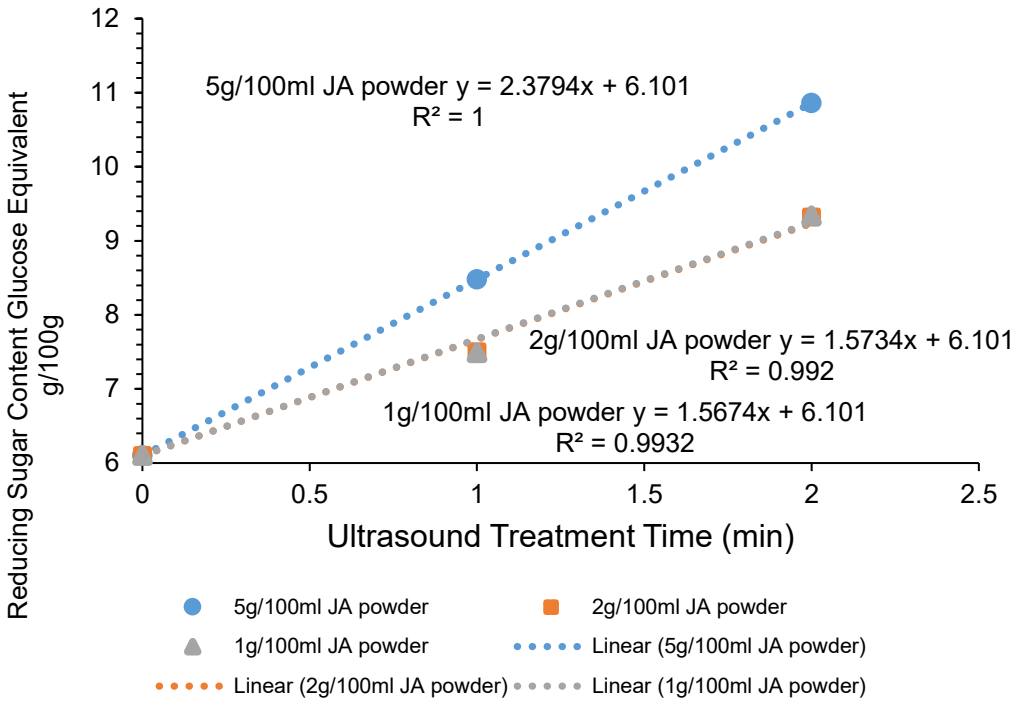


Figure 2.4.20. Correlation Between Ultrasound Treatment Time of JA Powder with Reducing Sugar Content

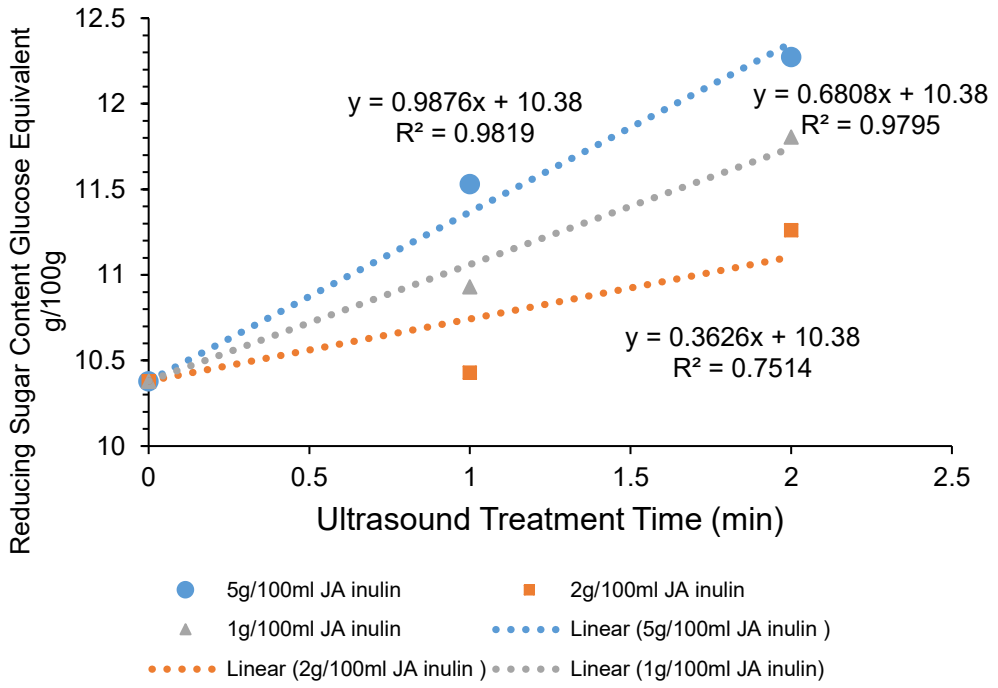


Figure 2.4.21. Correlation Between Ultrasound Treatment Time of JA Inulin with Reducing Sugar Content

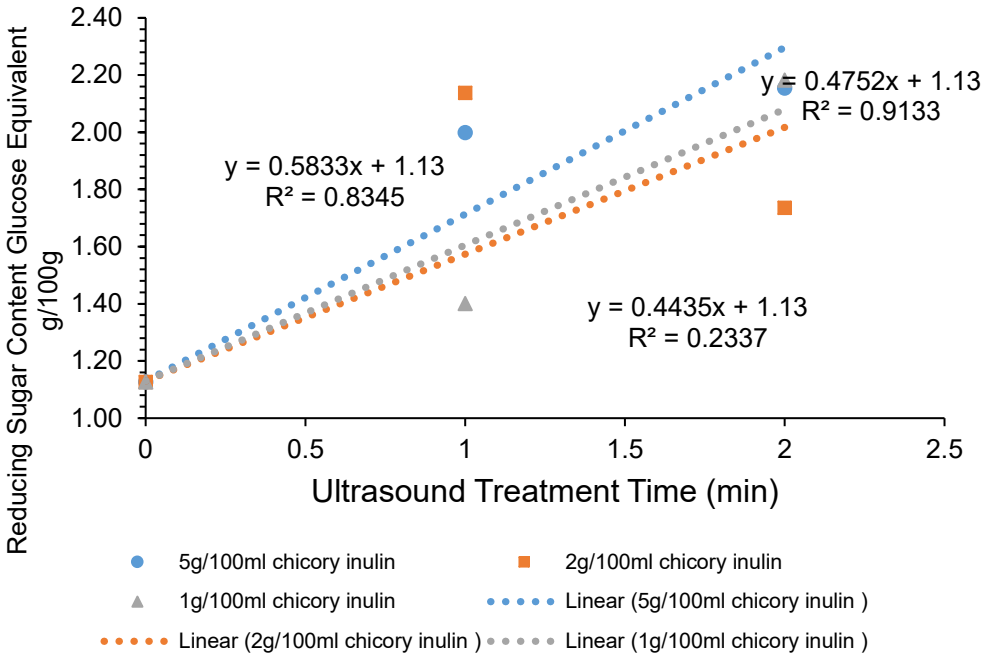


Figure 2.4.22. Correlation Between Ultrasound Treatment Time of Chicory Inulin with Reducing Sugar Content

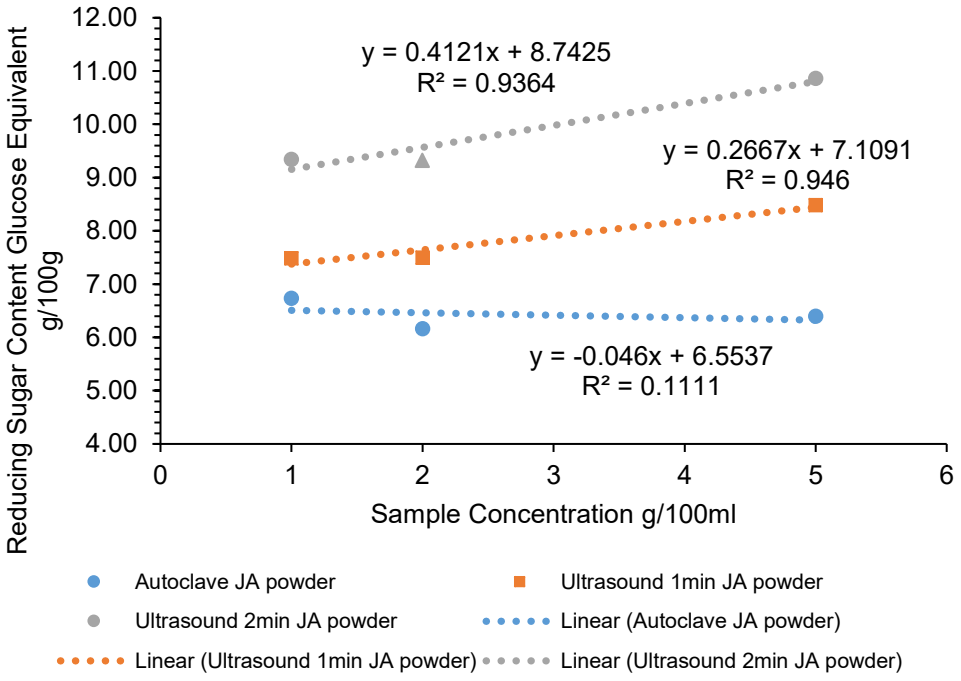


Figure 2.4.23. Correlation Between Sample Concentration of JA powder with Reducing Sugar Content

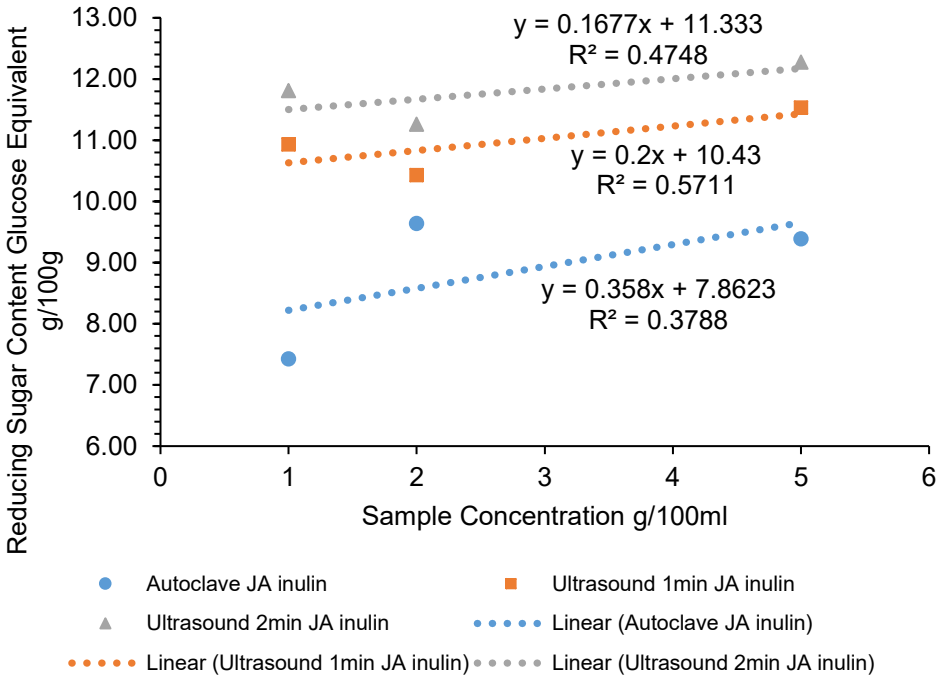


Figure 2.4.24. Correlation Between Sample Concentration of JA Inulin with Reducing Sugar Content

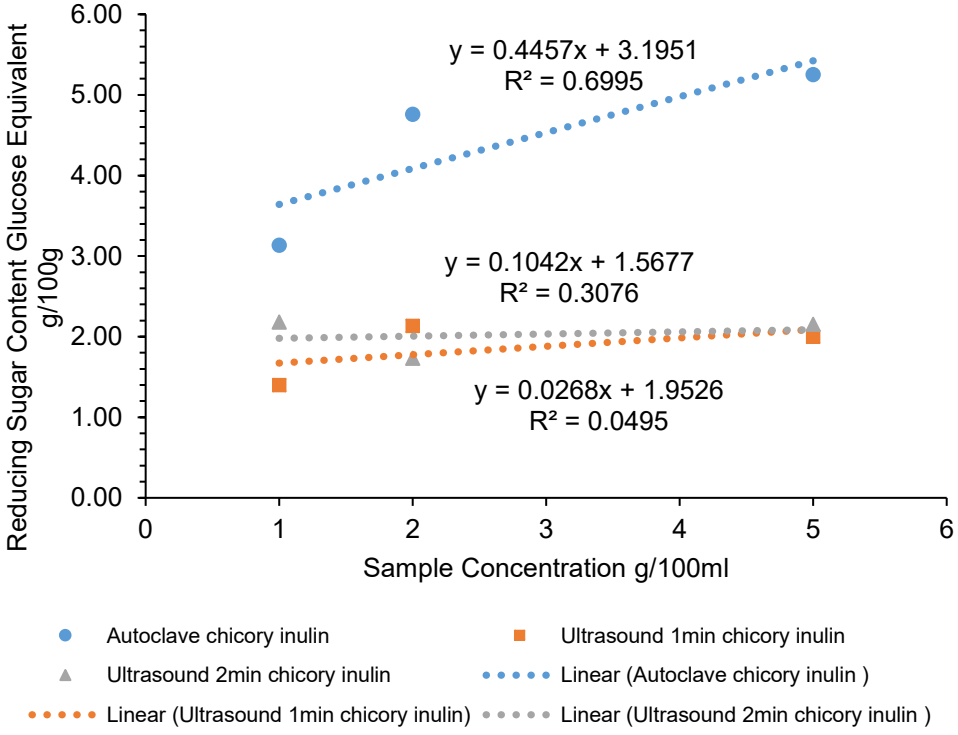


Figure 2.4.25. Correlation Between Sample Concentration of Chicory Inulin with Reducing Sugar Content

2.4.4 Determination of Inulin content and Degree of Polymerization by High Performance Liquid Chromatography and Gel Permeation Chromatography

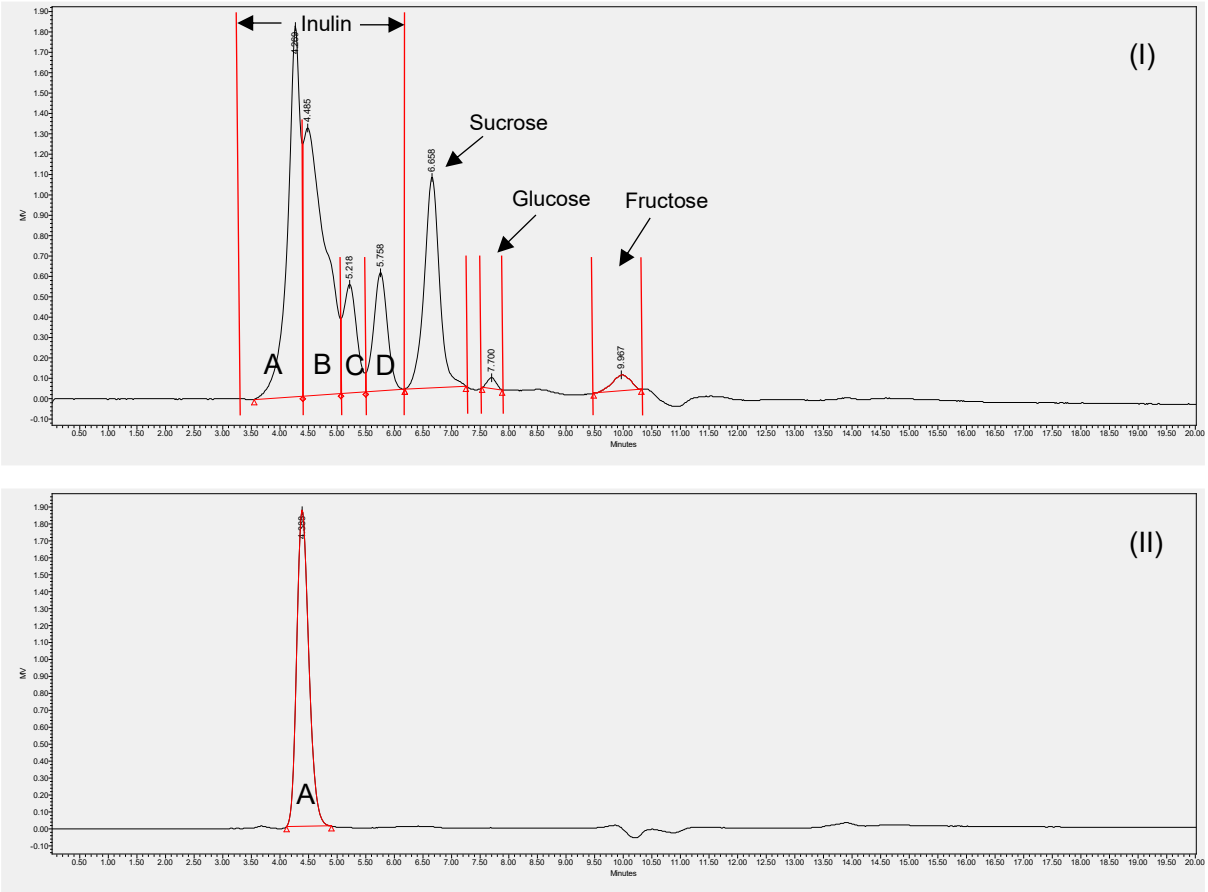


Figure. 2.4.26. High Performance Liquid Chromatogram – Refractive Index of Inulin from JA Powder (I) and Inulin from Chicory (II).

Table 2-4-2 Percentage of Area for Different Parts of Inulin from PJAI Showed on HPLC Graph

	% Area				
	Inulin Part A	Inulin Part B	Inulin Part C	Inulin Part D	Total Inulin
PJAI Untreated	27.65±0.22^{c}	33.24±0.24^{a}	8.89±0.32^{a}	9.04±0.39^{a}	78.82±0.53^{c}
PJAI Autoclave	28.65±0.20^{d}	32.97±0.15^{a}	8.23±0.71^{a}	8.54±0.14^{a}	78.38±0.27^{bc}
PJAI Ultrasound 1min	21.83±0.18^{b}	32.95±0.15^{a}	9.49±0.51^{a}	10.93±0.34^{a}	75.19±0.20^{a}
PJAI Ultrasound 2min	20.00±0.12^{a}	33.85±0.14^{a}	8.87±0.68^{a}	14.32±1.43^{b}	77.03±0.50^{b}

Inulin can only be crudely separated by sugar Pak I, but it can still provide a general grape about the distribution of different degree of polymerization for inulin (Beirão-da-Costa, et al., 2009). Based on this research, inulin were divided into four parts on HPLC graph (**Figure**. 2.4.26 (I)), according to the degree of polymerization. It showed that there is no significant change for each part of inulin after autoclave treatment, while total inulin slight decreased between PJAI untreated and PJAI autoclave which might due to the loss of reducing sugar. However, it also showed that Inulin Part A and Inulin Part D could be influenced by ultrasound treatment. There exists a negative linear relationship between Inulin Part A area which is a high degree of polymerization inulin concentration and ultrasound treatment time (R^2=0.9277). At the same time, there is a positive linear relationship between Inulin Part D area which is lowest degree of polymerization inulin concentration and ultrasound treatment time (R^2=0.9976). In general, for total inulin concentration, there is no significant correlation with ultrasound treatment, but it seems that ultrasound treatment might reduce the total inulin percentage in samples which was not observed for the autoclave treated samples. The chicory inulin samples are high purified inulin with high degree of polymerization. For these samples, only inulin part A was seen on the HPLC-RI graph. As a result, GPC was used to analyze the degree of polymerization for chicory inulin. It showed that the DP of untreated chicory inulin samples was 22.18, which contained 55.2% DP 5-20 inulin. Only autoclave treatment significantly changed the distribution of DP for chicory inulin, as the DP became more uniform after autoclave treatment with the average DP being 18.96 and the sample contained 65.9% DP 5-20 inulin. For ultrasound treatment (**Figure. 2.4.17.**), the DP of chicory inulin was not significantly different than the untreated samples (Ultrasound 1min average DP = 22.05, 55.7% DP 5-20 inulin; Ultrasound 2min average DP = 21.04, 59.6% DP 5-20 inulin). This means that ultrasound treatment hardly effected DP of chicory inulin, while autoclave treatment slightly affected it. The retention time for chicory inulin samples analyzed by HPLC-RI were 4.38 min, but the retention time for JA inulin part A was 4.22 min and the retention time for

inulin part B was 4.60 min, This means that JA inulin contains some higher DP inulin compared to chicory inulin, and ultrasound can break it down. The same result was not observed for chicory inulin.

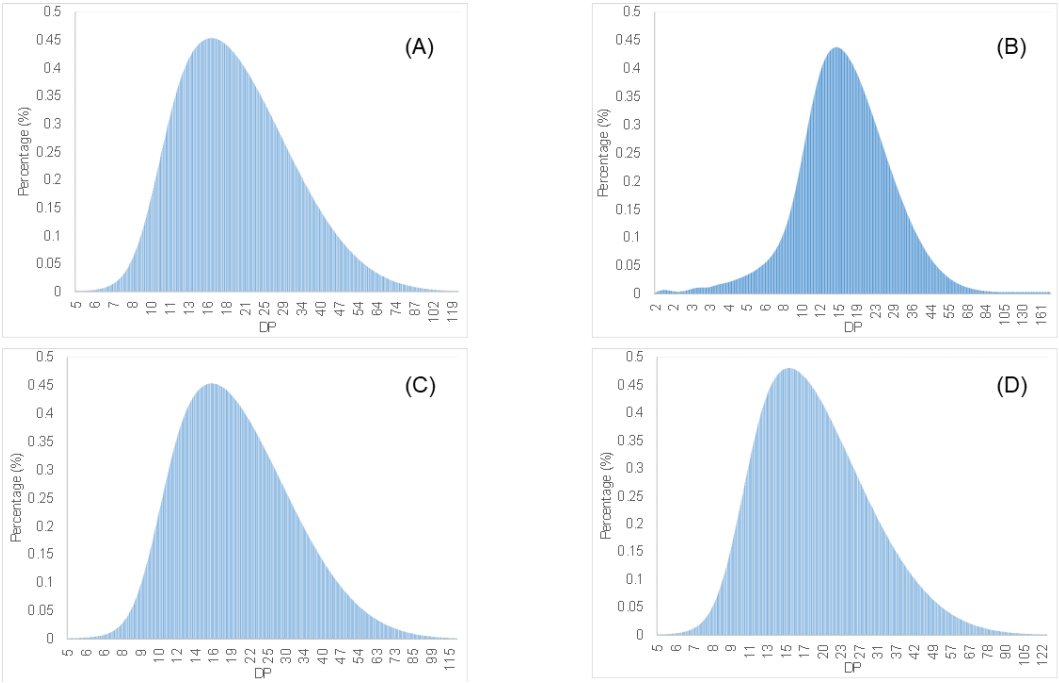


Figure. 2.4.27. Polymerization Profile of Chicory Inulin from Untreated (A), Autoclaved (B), Ultrasound 1min (C), Ultrasound 2min (D).

2.5 Discussion

Ultrasound treatment time was limited to 2min because the research focus on no-thermal ultrasound. If ultrasound treatment time is longer than 2min, the temperature of the solution will be higher than 40 ºC. As a result, ultrasound treatment time was limited to 2min due to the temperature control.

For the WSI and WAI from JA powder, (Takeuchi and Nagashima 2011) reported that blanched JA can absorb more water than unblanched JA, and the reason might be that blanching helps release free sugars, like sucrose and fructose, from inside the samples. At the same time, higher DP of saccharides leads to more absorption of water. However, according to **Figure. 2.4.13.** and the reducing sugar content, which will be discussed later (**Figure. 2.4.19.A**), after the autoclave treatment, the free sugar content of JA powder was similar to the free sugar content of the

untreated samples, while the WAI the JA powder autoclaved of samples was increased. It is therefore hypothesized that the microstructure and particle size could influence the water holding capacity of JA powder. As (Robertson and Eastwood 1981) mentioned, for fiber, water holding capacity is more related to fiber structure than chemical composition. After treatment, the particle size became smaller and the microstructure changed (**Figure. 2.4.2.**), which provided more surface area for water to bind. At the same time, unlike purified inulin, JA powder contained other components like protein, which may bind to low DP inulin, reducing the WSI. The same was observed for ultrasound treatment, although ultrasound treatment did increase the free sugar content. This might be the reason why the WAI of samples with ultrasound treatment was different than the samples with autoclave treatment and untreated sample. For CI, it suggested that the WAI and WSI of purified inulin could be easily affected by heat treatment compared to ultrasound treatment. If inulin is combined or mixed with other compound, it might be hard for treatment to affect the WAI and WSI of samples. Without the protection of other components, after heat treatment, a lot of free sugar is produced, resulting in a low WAI and a high WSI, which is further confirmed by the reducing sugar content of CI.

For reducing sugar content, the reason why the result of autoclave treatment of JA powder and purified JA inulin were different might be due to the linkage of inulin with other components, like protein, which is removed in PJAI. These kinds of linkages might protect reducing sugars, like glucose, fructose and sucrose from reacting. This could explain why the color of JA changed after the autoclave treatment as the reducing sugars were less protected than in JA powder. At the same time, (**Figure 2.4.19**) the highest reducing sugar content of purified inulin with ultrasound treatment for 2 min was 12.274% which was about 1.9% higher than untreated purified JA inulin. However, for JA powder, it showed that the highest reducing sugar content of treated samples was 10.865% which was about 4.466% higher than the reducing sugar content of untreated samples. This means that

ultrasound treatment is more capable of breaking saccharides into reducing sugar in JA powder compared to purified JA inulin. This suggests that other components inside the JA powder may help the sample absorb more energy from the ultrasound treatment. Also, the microstructure of JA powder and JA inulin samples were different which might also be a reason for these differences. The principle of ultrasound energy absorption could also explain the difference in results between JA powder and JA inulin. The principle is mainly related to viscosity, which will be discussed later. The reason why the reducing sugar content after autoclave treatment of purified JA inulin was lower than the ultrasound treatment and the sugar content after autoclave treatment of chicory inulin was higher than the ultrasound treatment might due to the degree of polymerization. Chicory inulin samples consist of high purified inulin, and little low DP inulin and reducing sugars compared to JA inulin. It might be harder for ultrasound and heat to break down saccharides of high DP inulin into reducing sugars. That might be the reason why reducing sugar content of chicory inulin samples were lower than JA inulin samples. Autoclave treatment contains more energy than ultrasound treatment, which might lead to a higher reducing sugar content than ultrasound treatment. However, with a high temperature and high pressure treatment, a lot of reactions can happen that might use reducing sugar as reactants. Untreated JA inulin contained 10.38 g/100g reducing sugar glucose equivalent and low DP inulin. The reducing sugar and low DP inulin might be involved in reactions during high temperature and high pressure treatment, resulting in a lower reducing sugar content in the autoclave samples compared to the untreated samples and the ultrasound treated samples of JA inulin. However, the autoclave treatment might help break down the high DP inulin, in chicory inulin, into low DP inulin and reducing sugars. Due to the high energy of autoclave treatment, chicory inulin samples treated by autoclave contained more reducing sugar than untreated samples and ultrasound treated samples. That might help to explain why the results for autoclave treated PJAI was lower than the ultrasound treated samples, while the result of autoclave treated chicory inulin were higher than the ultrasound treated samples. The reason why ultrasound treatment

for 2min resulted in a higher reducing sugar content in all samples compared to ultrasound treatment for 1min is because that the longer treatment lead to more energy absorbed in the sample, causing inulin to be broken down further into reducing sugar.

For inulin content, the reason why inulin part A decreased and inulin part D increased might due to the breakdown of inulin by ultrasound treatment shifting the degree of polymerization from high to low. That could explain why inulin part A and part D changed, and why both of them had a linear correlation with ultrasound treatment time, while part B and part C did not change significantly.

The possible reason why ultrasound had more of an effect on the DP of JA compared to the DP of CI might be due to the principle of ultrasound energy absorption. There is a positive relationship between sound absorption coefficient and viscosity coefficient. When ultrasonic waves go through a solution, the medium particles will produce internal friction, also known as viscous resistance, due to relative motion. Higher viscosity of the medium could lead to higher sonic energy absorbed (Shutliov, Alferieff et al. 1990). The viscosity of JA inulin solution was higher, leading to an increase in energy absorbed compared to chicory inulin. As a result, higher energy absorbed, more high DP inulin broke down. This can also explain why higher sample concentration could resulted in a higher reducing sugar content for JA powder. Also, inulin is a kind of polymer, higher molecular weight polymers in solution can increase the viscosity of the solution due to the internal friction from randomly coiling (Flory 1953), so the JA inulin samples may absorb more energy.

2.6 Conclusion

In general, ultrasound treatment had more influence on PJAI and JA powder compared with CI. Both ultrasound treatment and heat treatment changed the particle size and microstructure of PJAI, JA powder and CI. For CI, heat treatment

broke down more inulin than ultrasound treatment; However, for PJAI, it showed that longer ultrasound treatment time can result in more inulin breaking down into reducing sugar decreasing the DP of inulin, while heat treatment did not have a significant influence on PJAI. This was also proved by the reducing sugar content. The research suggests that viscosity and the DP of inulin in the samples might be very important for ultrasound treatment by affecting the absorption of energy.

Future studies need to investigate the relationship between the viscosity of inulin solution and the depolymerization of inulin caused by ultrasound treatment. Adding some other components might help to decrease the DP of inulin inside the solution.

Chapter 3 – Effect of Ultrasound on Structure of whey and Milk mixed with JA powder

3.1 Abstract

The effect of ultrasound on the structure of whey and milk mixed with or without JA powder was investigated this study. Whey and milk was mixed with 2% JA powder and treated with 20KHz ultrasound. The structure of the samples was determined by SEM and compared with original whey and milk without JA powder. In visual inspection, the structure of whey was not influenced by ultrasound, while milk particles of milk were combined after ultrasound treatment. The microstructure of milk became flatter after ultrasound treatment. This kind of change was much more significant for the samples mixed with JA powder when observed under SEM at 200x and 1kx magnification, while the same results were not seen for whey.

3.2 Introduction

Milk is a kind of health beverage for the human body. It is rich in protein. Dairy is an important part of the food industry. Milk safety and nutrition have been a research topic of great interest for decades. Researchers are interested in keeping or adding more nutrition in milk, while at the same time, making sure milk is safe to consume by preventing the presence of harmful microorganism.

Ultrasound has been used in the dairy industry for a few years, and a lot of studies have been conducted to investigate its application on milk base products. The main use of ultrasound in the dairy industry is for safety purposes as it can break down microbial cells with its high energy and high frequency. At the same time, ultrasound is a low temperature treatment. Compared with thermal treatment like pasteurization, ultrasound might help to preserve more nutrition or functional components inside of milk-based products more efficiently (Gao, Hemar et al. 2014). Also, ultrasound can decrease the fat globules and casein micelle size in

milk. Lower diameter of fat globules can increase the velocity of fat skimming (Chandrapala, Martin et al. 2012; Karlovic, Bosiljkov et al. 2014). However, there is no information about how ultrasound affects milk the components when mixing dairy product with plant-based material.

The objectives of this study were to find out whether ultrasound treatment can change the structure of whey and milk and whether adding JA powder can influence the structure of whey and milk combined with ultrasound treatment which might be useful for dairy industry.

3.3 Methods and Materials

3.3.1 Material

Whey, Milk (Winchester, ON, Canada); JA powder (Action Vale, QC, Canada)

Table 3-3-1 Composition of Whey and Milk from Winchester

	Whey	Milk
Moisture%	84.463±0.032%	84.445±0.002%
Ash%	1.568±0.035%(wet basis) 10.088±0.206%(dry basis)	1.144±0.031%(wet basis) 7.356±0.199%(dry basis)
Fat%	≈0(wet basis) <0.2%(dry basis)	≈0(wet basis) <0.2%(dry basis)
Protein%	0.511±0.005%(wet basis) 3.291±0.030%(dry basis)	0.475±0.016%(wet basis) 3.060±0.106% (dry basis)

3.3.2 Sample Preparation

3.3.2.1 Raw material

300ml of whey and milk were transferred into plastic bag and dried with a freeze dryer (Bulk Tray Dryer 115V; Labconco, Kansas City, USA.) for 4 days at -50 ºC.

3.3.2.2 Raw material mixed with JA powder

6g (2%) of JA powder were mixed into 300ml whey and milk and dried with a freeze dryer (Bulk Tray Dryer 115V; Labconco, Kansas City, USA.) for 4 days at -50 ºC.

3.3.2.3 Ultrasound Treatment for Whey and Milk

300ml of whey, milk, whey mixed with JA powder, and milk mixed with JA powder were treated with an ultrasonic processors (UIP500hdT; Hielscher, Inc. Ringwood, USA.) at 20 KHz, and 90W for 10min. After treatment, all samples were transferred into plastics bag and dried with a freeze dryer (Bulk Tray Dryer 115V; Labconco, Kansas City, USA.) for 4 days at -50 ºC.

3.3.4 SEM Analysis

Dried whey, dried milk, dried whey after ultrasound treatment, dried milk after ultrasound treatment, dried whey mixed with JA powder, dried milk mixed with JA powder, dried whey mixed with JA powder after ultrasound treatment and dried milk mixed with JA powder after ultrasound treatment were coated with gold and viewed using SEM (TESCAN, Brno – Kohoutovice, Česká republika) under high vacuum conditions (10^{-3} Pa) at 10 kv voltage.

3.4 Result

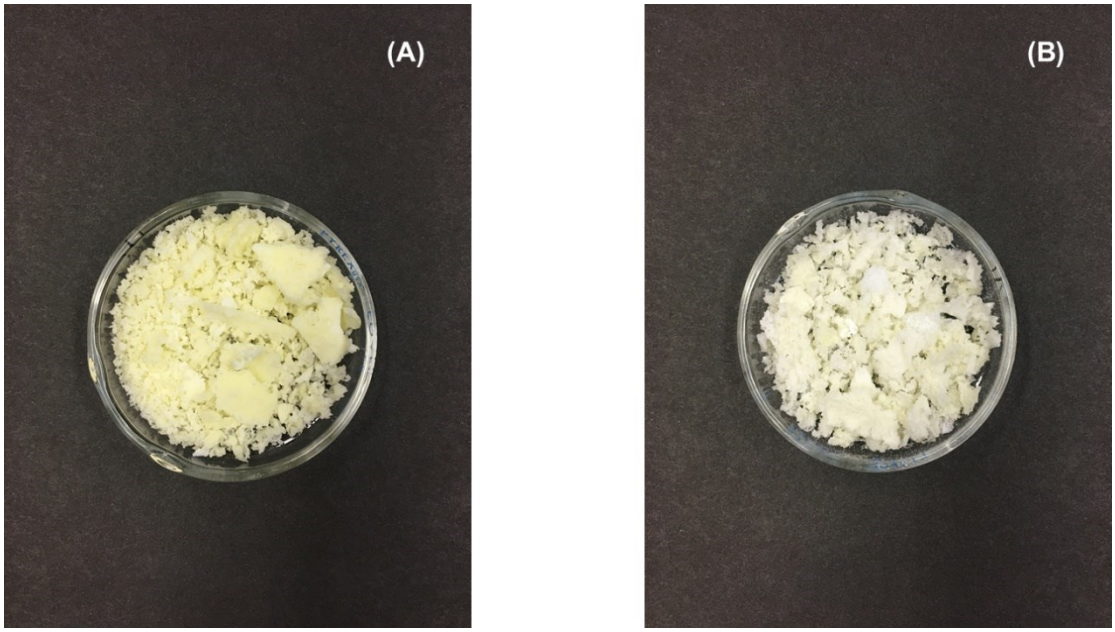


Figure 3.4.1. General Structure and Color of (A) untreated and (B) Ultrasound 10 min 300ml Whey Solution after Freeze Dry

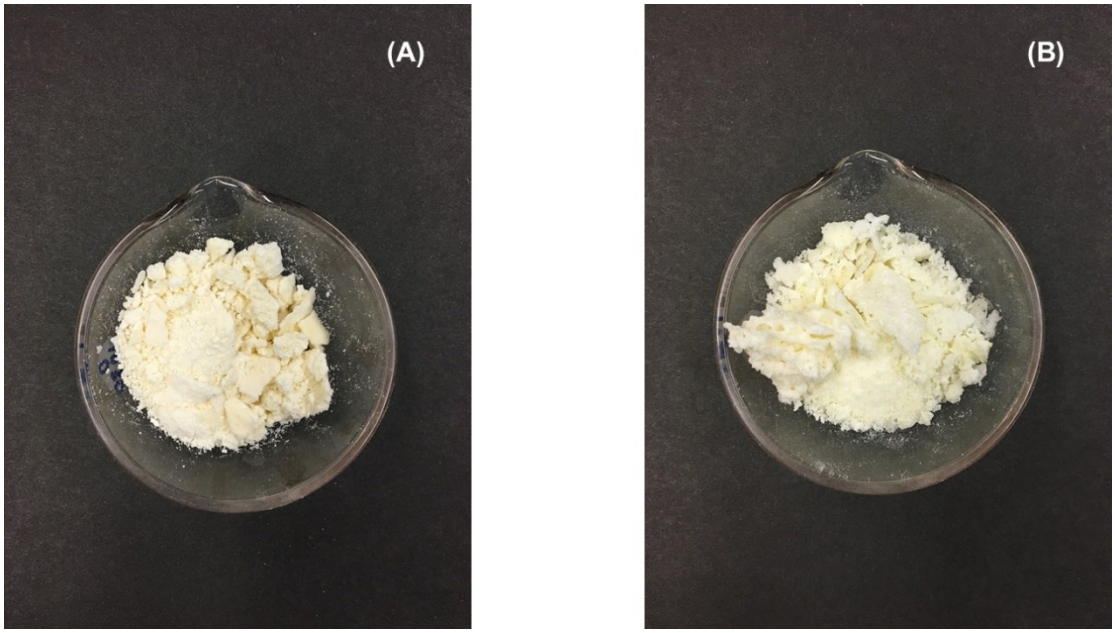


Figure 3.4.2. General Structure and Color of (A) untreated and (B) Ultrasound 10 min 300ml Milk Solution after Freeze Dry

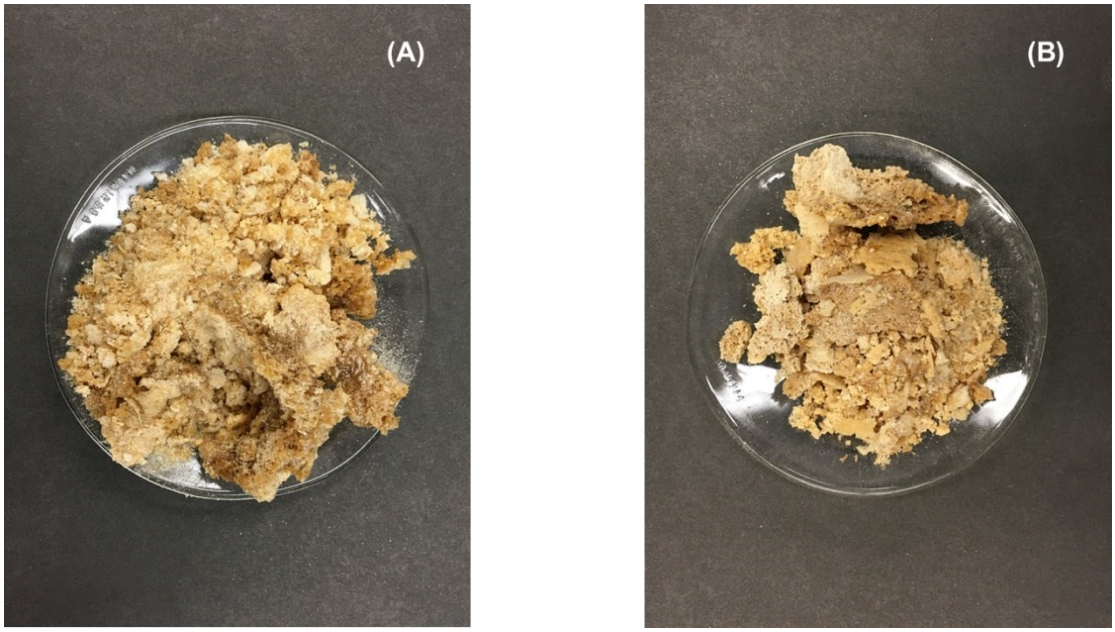


Figure 3.4.3. General Structure and Color of (A) untreated and (B) Ultrasound 10 min 300ml Whey Solution Mixed with 2% JA Powder after Freeze Dry

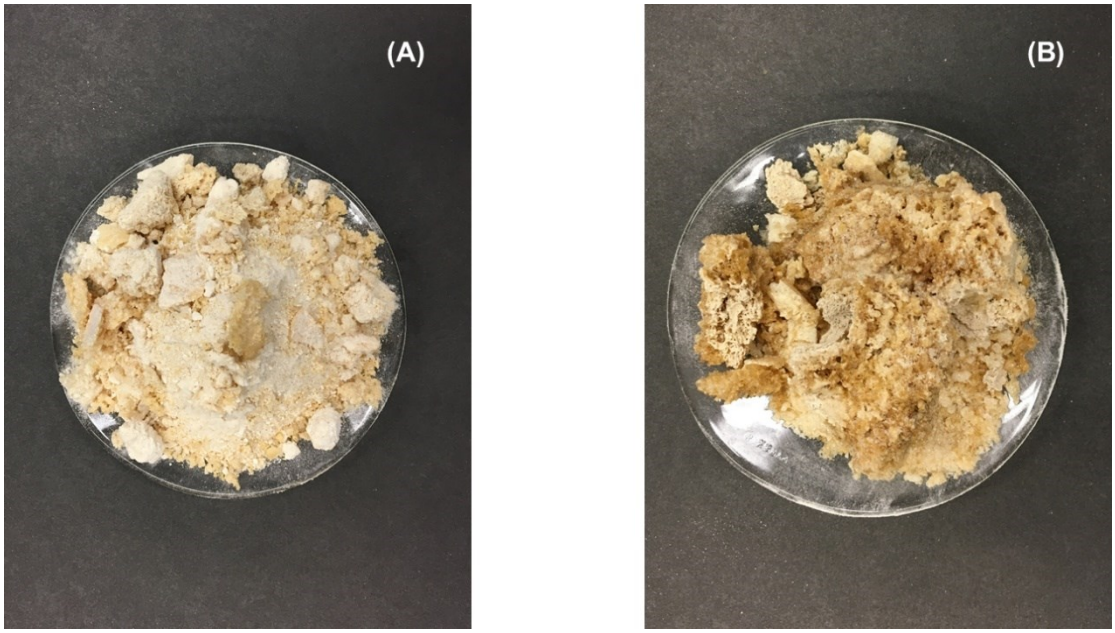


Figure 3.4.4. General Structure and Color of (A) untreated and (B) Ultrasound 10 min 300ml Milk Solution Mixed with 2% JA Powder after Freeze Dry

After ultrasound treatment, the structure of whey after freeze drying did not change a lot. The particles became larger with more large holes on the material. For milk, it seems that ultrasound could have more of an effect for changing the structure of milk. It showed that the milk particles after ultrasound treatment were flatter than the samples without ultrasound treatment.

For the sample with 2% JA powder, the color and structure were different from the samples without JA. The most important difference is the color. The color for the samples with JA powder were much darker than the original samples due to the color from JA. Comparing the samples with JA powder with and without ultrasound treatment, whey with JA powder did not change a lot after ultrasound treatment, but the particles did become more schistose. For milk, for both samples before and after ultrasound treatment, the particles were heterogenous. Some particles were very large, while some were like powder. There still exited some differences between these two kinds of samples. After ultrasound treatment the surface of large particles contained more holes compared to milk without

ultrasound treatment.

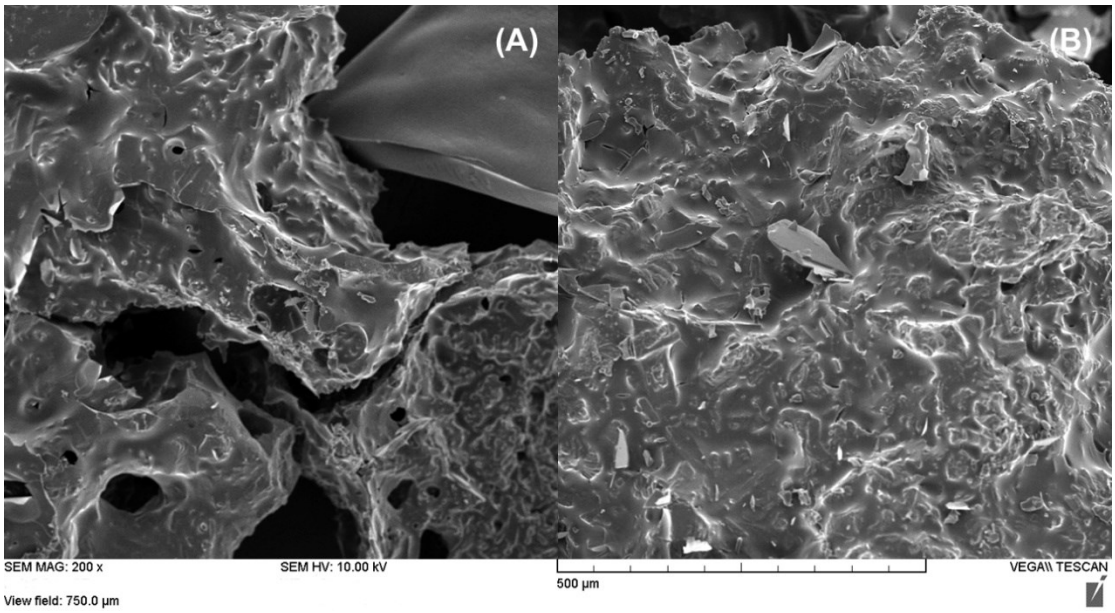


Figure 3.4.5. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Whey Solution after Freeze Dry at Magnification of 200x

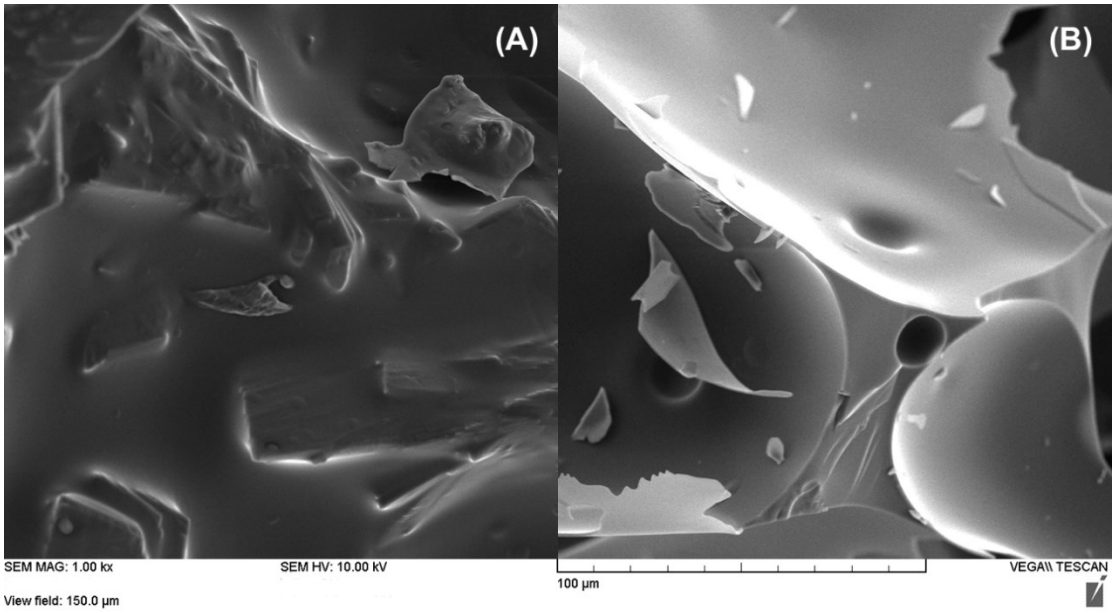


Figure 3.4.6. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Whey Solution after Freeze Dry at Magnification of 1kx

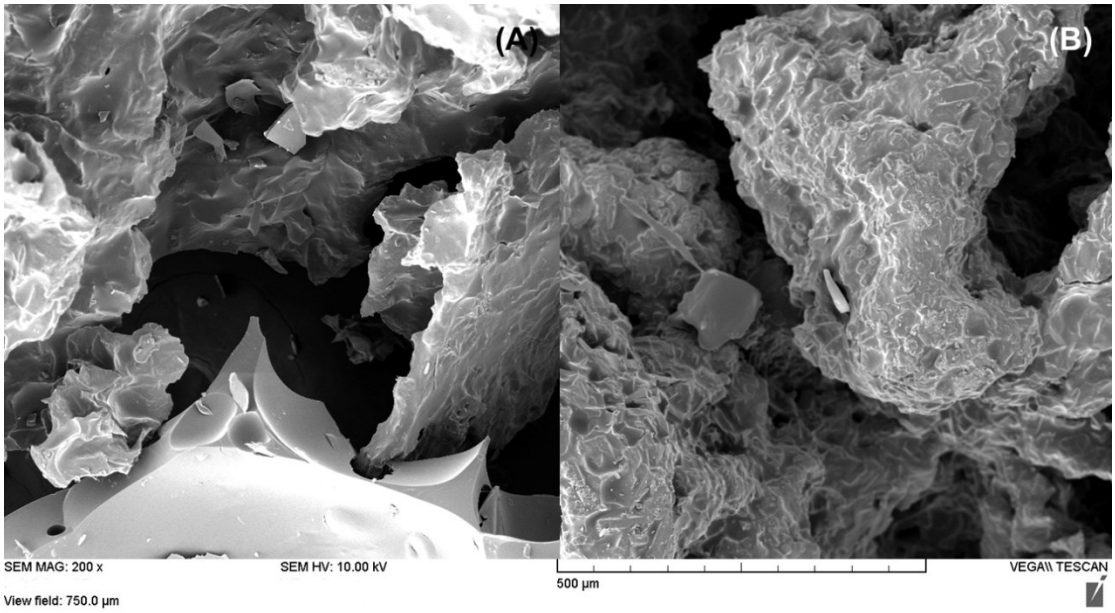


Figure 3.4.7. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Whey Solution Mixed with 2% JA Powder after Freeze Dry at Magnification of 200x

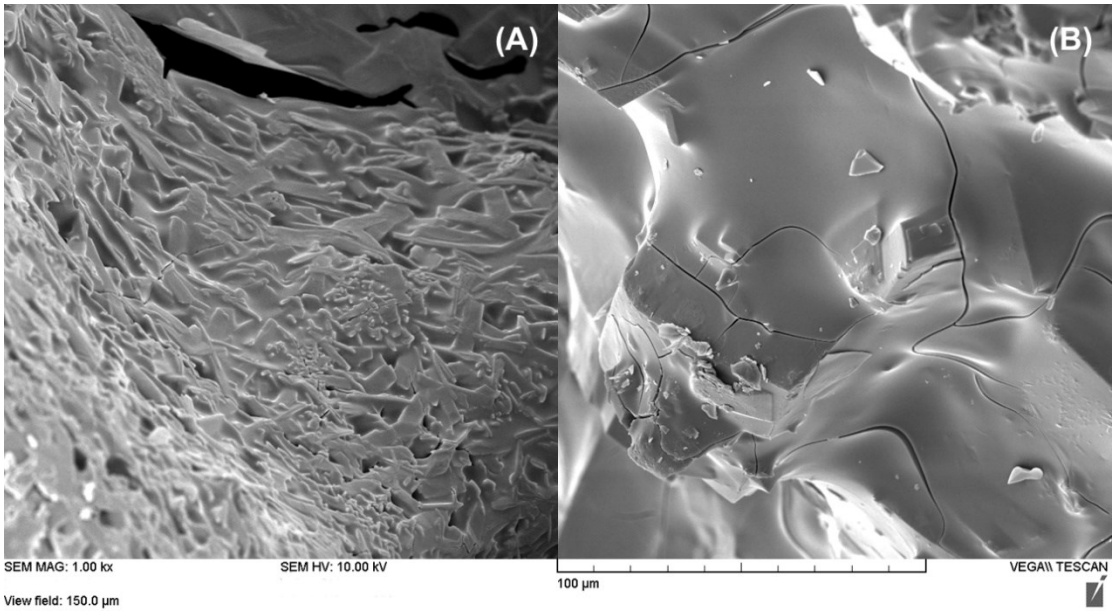


Figure 3.4.8. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Whey Solution Mixed with 2% JA Powder after Freeze Dry at Magnification of 1kx

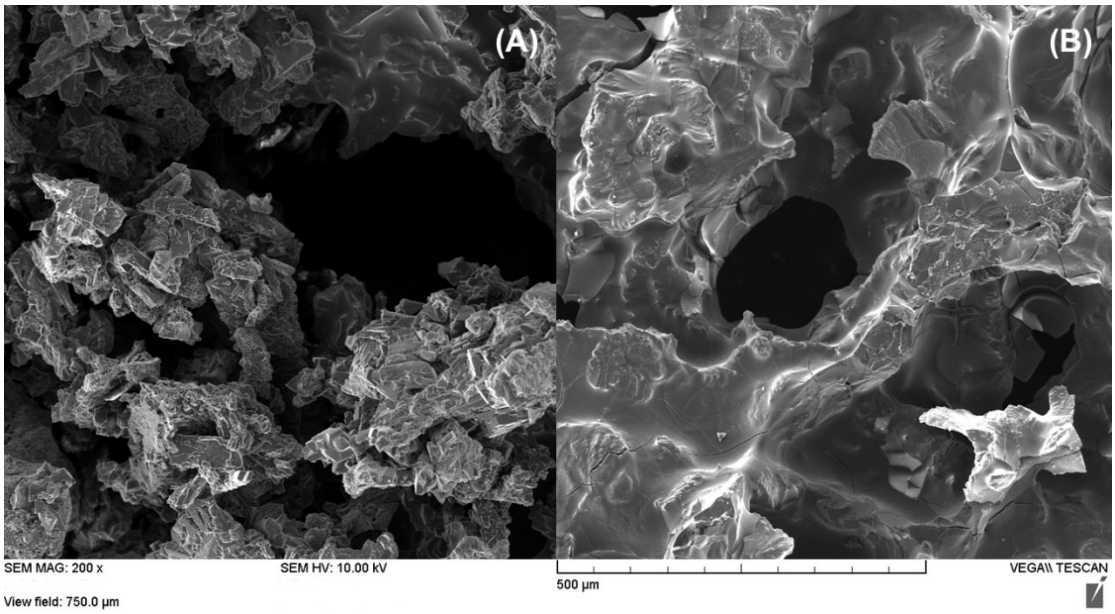


Figure 3.4.9. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Milk Solution after Freeze Dry at Magnification of 200x

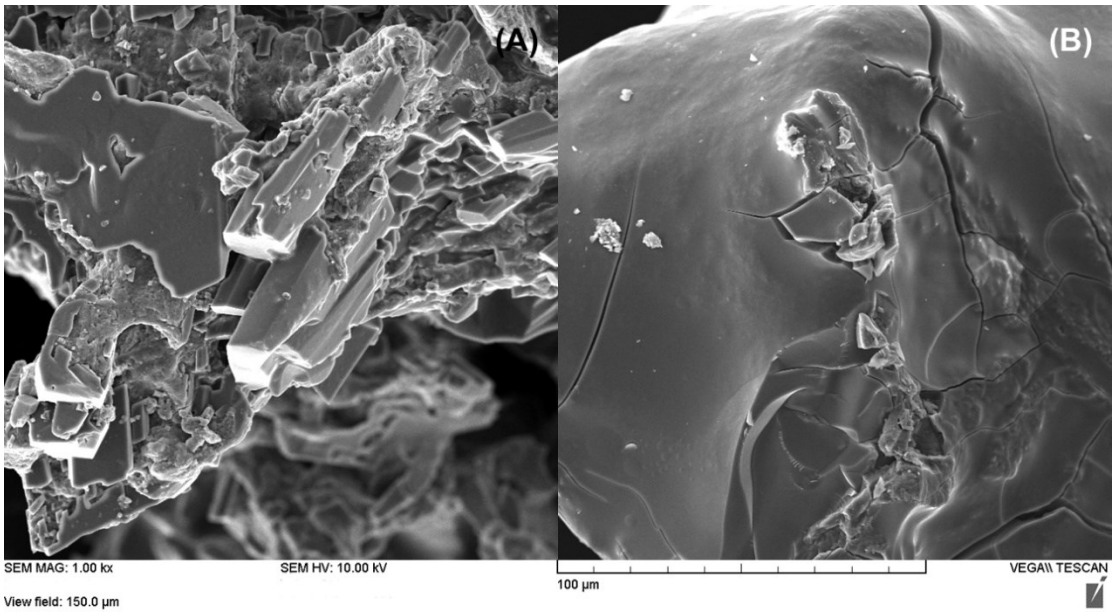


Figure 3.4.10. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Milk Solution after Freeze Dry at Magnification of 1kx

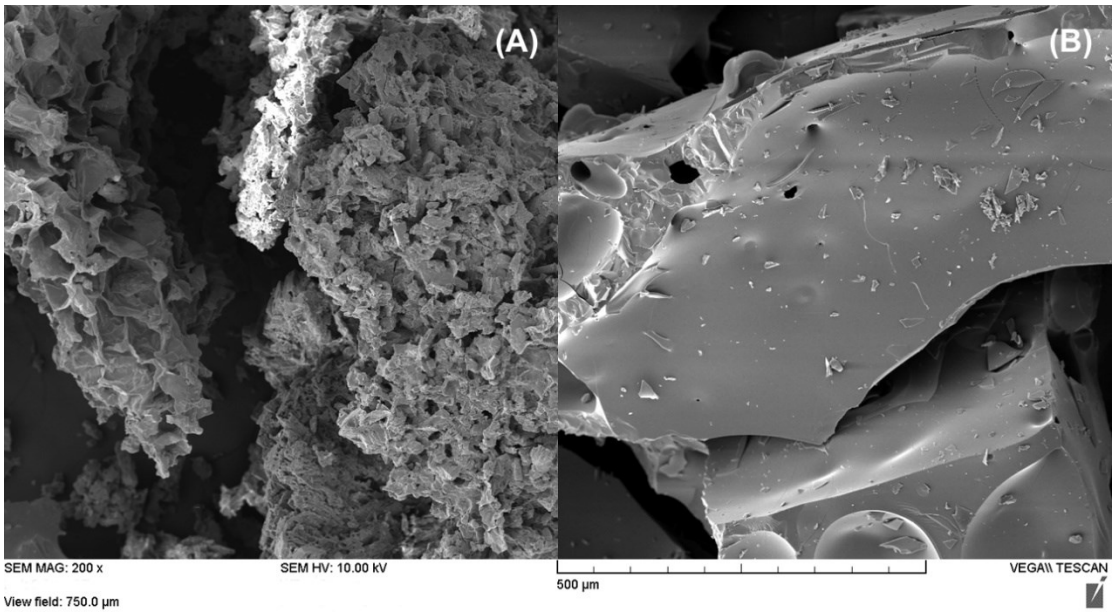


Figure 3.4.11. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Milk Solution Mixed with 2% JA Powder after Freeze Dry at Magnification of 200x

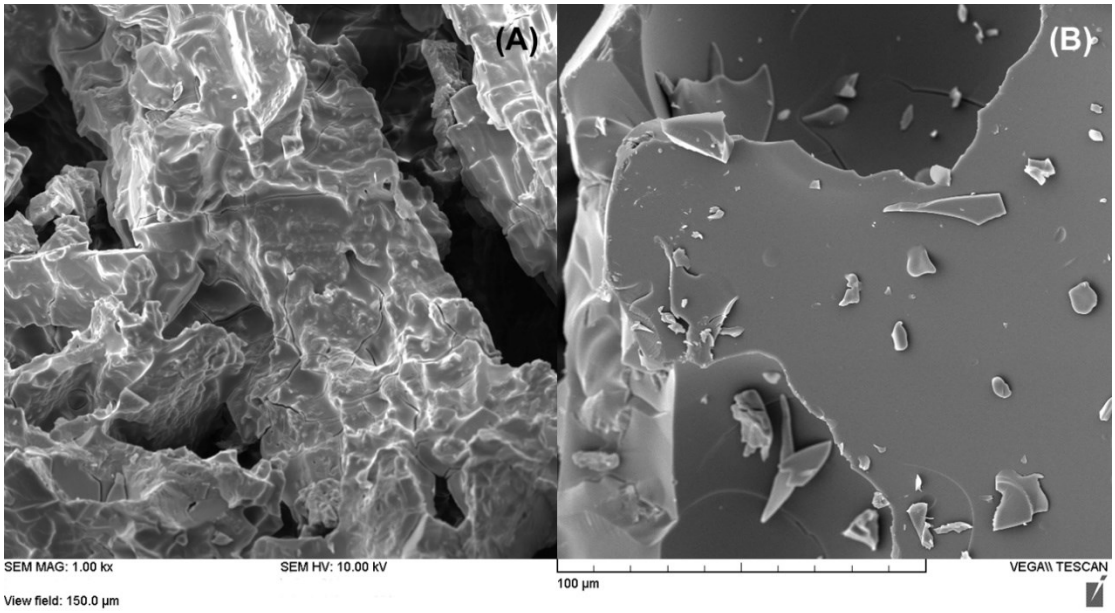


Figure 3.4.12. Scanning Electron Photomicrographs of (A) Untreated and (B) Ultrasound 10min 300ml Milk Solution Mixed with 2% JA Powder after Freeze Dry at Magnification of 1kx

The microstructure of both whey and milk were affected by the ultrasound treatment. For whey, at a magnification of 200x, it can be seen that the surface structure of whey became flatter, holes became smaller, and less gaps are seen

between particles. When the magnification went to 1kx, the surface of whey without ultrasound treatment was uneven with lot of lumps on it. However, after ultrasound treatment, the surface of whey became smooth with no lump on it. The same thing happened for milk. At magnifications of 200x, the surface of milk seems very broken, and, after ultrasound treatment, it became more combined and flat. For 1kx magnification, the surface of milk was irregular with some crystals. However, after ultrasound treatment, the surface became flat without lumps on it.

The same results were seen for samples with JA powder. It showed that ultrasound treatment can change the microstructure of the samples. However, the surface structure after ultrasound treatment were different from the original whey and milk samples. For whey, when magnification was 200x, it showed that the surface of whey samples after ultrasound treatment contained high density irregularity wrinkles compared to the samples without JA powder. When the magnification went to 1kx, it showed the same results as the samples without JA powder. The surface of whey became smoother after ultrasound treatment. For milk, it seems that ultrasound treatment had more of an effect on the samples with JA powder compared to the samples without JA powder. At magnification of 200x, the surface of the samples was much flatter than the samples without JA powder after ultrasound treatment. For 1kx magnification, the difference between samples with and without JA powder was not very significant.

3.5 Discussion

In general, it seems that ultrasound had more of an influence on milk samples than whey samples, while the microstructure of milk became flatter if mixed with JA powder.

The possible reason why the structure of milk seems more affected by ultrasound than the structure of whey might be due to the casein inside of milk. The main difference between milk and whey is the casein content. After processing, casein is

removed from milk, and the remaining solution is the whey solution. As stated, ultrasound treatment can change casein micelles in yoghurt. It can decrease the particle size of casein micelles and provide a smoother surface to the microstructure of milk. In general, ultrasound treatment increases the gel texture of yoghurt (Tabatabaie, Mortazavi et al. 2009).

The possible reason why JA powder increased the effect of the ultrasound treatment on milk might be due to the increased viscosity of the solution. As mentioned in Chapter 2., a higher viscosity of the solution can result in a higher amount of ultrasound energy absorbed by the solution. JA powder increases the solid content of the solution and it has lots of polymer, so the viscosity of the solution is higher than original milk.

These results may be of interest for dairy industry. They are applicable for yoghurt producers and ice-cream producers. As mentioned in Chapter 2, the low DP of inulin can provide a good environment for probiotics in the human body. According to the results seen in Chapter 2, ultrasound treatment can reduce the DP of inulin in JA powder, resulting in an increased amount of low DP in JA powder after ultrasound treatment. If milk mixed with JA powder and treated with ultrasound is used for making yogurt, more low DP inulin will be providing a good environment for the probiotics inside the yoghurt. Ultrasound treatment can also improve the texture of yoghurt.

3.6 Conclusion

Ultrasound treatment can change the general structure of milk after freeze drying which was not observed for whey. Ultrasound combines particles and results in holes being formed on the surface of freeze dried milk. For the microstructure, ultrasound resulted in a flatter surface for both whey and milk. This kind of change was much more significant if the samples were mixed with JA powder.

Future studies should aim to investigate how ultrasound affects the DP of inulin inside of JA powder when mixed with milk and whey solution compared with the original JA powder tested in Chapter 2.

Chapter 4: General Conclusion and Future Directions

In the present study, ultrasound treatment had more influence on PJAI and JA powder compared with CI indicated by reducing sugar content, SEM and inulin content assays. Both ultrasound treatment and heat treatment changed the particle size and microstructure of PJAI, JA powder and CI. Some differences existed between the samples. For CI, heat treatment broke down more inulin compared to ultrasound treatment. For PJAI, it showed that longer ultrasound treatment time resulted in more inulin breaking down into reducing sugar, while decreasing the DP of inulin, Heat treatment did not have a significant influence on PJAI, which was also proved by the reducing sugar content. As a result, ultrasound can be used to reduce the DP for inulin from JA. Meanwhile, the relationship between the viscosity of inulin solution and the polymerization of inulin would be an interesting topic for future studies.

In this study, the structure of whey and milk was changed after ultrasound treatment. Ultrasound led to a flatter surface of the microstructure of whey and milk. For milk, this kind of phenomenon was more significant when milk was mixed with JA powder. For general structure, ultrasound combined particles and led to the creation of some holes on the surface of freeze-dried milk. The results from whey did not show any significant difference after ultrasound treatment. This suggests that ultrasound might be a usefully method for the dairy industry if a strong texture is required, and JA powder could be a functional additive for milk-based products. Future directions should focus on how the DP of inulin inside of JA powder is changed when mixed with milk and whey.

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