UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF OXIDATIVE STARCH OF SWEET POTATO (VITATOTM) VIA HURDLE TECHNOLOGIES

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ABSTRACT

Oxidized starch is a starch derivative that is prepared by treating native starches via physical and/or chemical methods to change its characterization. However, chemical oxidations might lead to low oxidized starch yield because of the loss of small molecules produced by the starch breakdown and generate wastewater containing a high concentration of salts, causing wastewater disposal problems. For those reasons, starch oxidation via ultrasonic combined with ozone treatment is proposed. Ozone has the ability to elevate the thermodynamic oxidation potential, hence, the reactions can be performed at low temperatures. Considering that ultrasonic is a physical tool capable of mutilating the crystalline area in the starch granules resulting in the obliteration of the granular structures, which leads to an ideal method for the production of oxidised sweet potato starch. Sweet potato (VitAto™) is used in this study as there is proof that its functionality is the same as tapioca starch. Sodium metabisulfite was used as isolation medium because it is an establish method to produce oxidised starch and risk of health effects from chemical is negligible, since the concentration used is very low. Physicochemical characteristics such as solubility, swelling power, amylose content, carboxyl content, gelatinization, viscosity, morphology properties and syneresis of oxidized sweet potato starch were studied. Physicochemical characteristics such as solubility, swelling, and thermal properties of oxidized sweet potato starch was determined. The results showed low viscosity, high solubility, high swelling power and improved thermal properties. The oxidized starch obtained can be used as an emulsifier, gum arabic replacer, and binding agent in the food industry

ABSTRAK

Kanji teroksida adalah kanji yang diubah suai yang disediakan dengan kaedah fizikal atau kaedah kimia yang dilakukan kepada kanji asli untuk mengubah penciriannya. Namun begitu, pengoksidaan kimia boleh menyebabkan penghasilan kanji teroksida yang rendah kerana molekul kecil yang hilang yang terhasil oleh pemecahan kanji dan menghasilkan air sisa yang mengandungi kepekatan garam yang tinggi, menyebabkan masalah pembuangan air sisa. Oleh yang demikian, rawatan ozon dan ultrasonik dicadangkan untuk pengoksidaan kanji. Ozone mempunyai kemampuan untuk meningkatkan potensi pengoksidaan termodinamik, oleh itu, tindak balas boleh dilakukan pada suhu yang rendah. Manakala, ultrasonik pula adalah kaedah fizikal yang dapat memutilasi kristal di dalam butiran kanji yang mengakibatkan struktur butiran kanji terhapus yang menyumbang kepada kaedah yang ideal untuk menghasilkan kanji teroksida ubi keledek. Ubi keledek (Vitato™) digunakan di dalam kajian ini kerana fungsi ubi keledek dibuktikan sama dengan kanji ubi kayu. Natrium metabisulfite digunakan sebagai media pengasingan kerana ini adalah kaedah penetapan untuk menghasilkan kanji teroksida dan kesan risiko kesihatan dari bahan kimia dapat diabaikan kerana kepekatan yang digunakan sangat rendah. Sifat fizikokimia seperti daya pembengkakan, keterlarutan, kandungan amilosa, kandungan karboksil, pengelatinan kelikatan, sifat morfologi, dan sineresis bagi kanji teroksida dipelajari. Ciri-ciri fizikokimia seperti kelarutan, daya pembengkakan, dan sifat termal kanji ubi jalar teroksidasi telah dikaji. Hasilnya menunjukkan kelikatan rendah, kelarutan tinggi dan daya pembengkakan dan sifat haba yang lebih baik. Kanji terkoksida yang terhasil boleh dijadikan sebagai pengemulsi, pengganti gum arab dan ejen pengikat di dalam indsutri makanan.

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LIST OF ABBREVIATIONS

BRIS	Beach Ridges Interspersed with Swales
DOSM	Department of Statistics Malaysia
FAO	United Nation's Food Agriculture Organization
FDA	Food and Drug Administration
MARDI	Malaysia Agricultural Research and Development Institute
NIOSH	National Institute of Occupational Safety and Health
O_3	Ozone
OSHA	Occupational Safety and Health Administration
UV	Ultraviolet

CHAPTER 1: INTRODUCTION

1.1 OVERVIEW

Sweet potato or scientifically known as *Ipomoea batatas* is a root vegetable with sprawling or trailing vine which can be harvested as early as 3½-4 months after planting in the tropics, or after 5-6 months in the temperate region during the summer (Tan et al., 2015). Sweet potato is big, starchy, sweet, and rich in calories. It also contains biologically active phytochemicals such as β-carotene, polyphenols, ascorbic acid and dietary fibres (Van Hal, 2000). According to the United Nation's Food and Agriculture Organisation (FAO) report (2009), sweet potato is rank as 7th most important food crop worldwide which produce over 112 million metric tonnes of edible food products in the world annually. In Malaysia, the production of sweet potato from 2010 to 2017 are approximately 23,000 - 55,000 tons (FAO report, 2018) or 2000 ha/year (Tan et al., 2010), which is considered as high even if not in the top ten list of the world's output quantities. Malaysian Agricultural Research and Development Institute (MARDI) has introduced the VitAto™, a new hybrid of sweet potato in June 2007. The ten years breeding program were aim towards developing nutritionally-superior sweet potato variety. The cultivation of the VitAto is outstanding in its yield performance (Hanim et al., 2014).

Starch is the most important carbohydrate in human diet and is present in many of the staple foods. Cereals, tuber and root vegetables are the main sources of starch consumption worldwide. Starch is a carbohydrate composed of glucose units bound by a glycosidic connection. Starch is a glucose polysaccharide composed of two forms of α-d-glucan, amylose, and amylopectin strings where amylose is about 20-30% and amylopectin is about 70-80% (Chaplin, 2020). Starch molecules formed by each plant species have different structures and compositions, and the protein and fat content of the storage organs can differ considerably.

Starch is an important food product and biomaterial used worldwide for different purposes. While historically used in the food industry, technical innovation in many other sectors such as health and medicine, cloth, paper, fine chemicals, petroleum engineering, agriculture, and construction engineering has led to its steady importance (Egharevba, 2019). It is mostly used as consumer ingredients or additives in baked goods, confectioneries, pastas and soups for thickening, preservation and quality enhancer in the food sector. However, their specific disadvantages have often significantly restricted their use in many industrial food use, such as cold water insolubility, lack of viscosity and thickening after frying, poor shear resistance, thermal resistance, and a high tendency to retrograde (Singh et al, 2007). Thus, starch modification, which includes modifying the native starch's physical and chemical features to enhance its functional properties, may be used to adapt starch to different food applications (Cai et al, 2014). The starch modification has the function of stabilizing starch granules during processing and making them ideal for many food and industrial applications. Starch modification is usually accomplished by derivatization such as etherification, esterification, cross-linking and starch grafting; heat or moisture decomposition or physical treatment of starch.

Starch oxidation with hydrogen hypochlorite as the oxidizing agent is one of the most popular industrially used processes. Generally, the oxidation process is done in a mild to moderate alkaline solution to support the carboxyl group yield (Zhang et al, 2011). Hydrogen peroxide, permanganate also considered as oxidizing agent for starch oxidation. However, these chemical oxidizing agent have disadvantages where yield of the oxidized starch is low due to loss of small molecules produced by starch breakdown (Wing & Willett, 1997) and large amounts of wastewater produced during the process, which includes a high salt content, causing wastewater disposal problems (Kesselmans & Bleeker, 1997).

Ozone is a molecule made up of three oxygen atoms that are negatively charged. The molecule of ozone is very unstable and has a short half-life, causing it to return to its original form in a short time. Ozone is an oxygen that occurs naturally and can be created industrially. It does not leave toxic residue when it is introduced to a food product (Chan et al, 2010). It is also a powerful oxidant since it has an extra oxygen atom (Ariffin et al, 2012). Another advantage of ozone is that it is considered as an anti-microorganism agent and has the potential to destroy microorganisms and eliminate toxic contaminants and it has considerably strong prospects as an environmentally safe cleaning device. Thus, ozone can be used to treat, preserve and prepare food in the form of gases and liquids, including fresh raw materials and goods for fruit and vegetables (Khadra et al., 2001).

Ultrasound treatment is a physical method for starch modification that has many benefits, such as less usage of additives, time and environmental friendly processing (Krishnakumar & Sajeev, 2017). Ultrasound is form of energy produced by waves of frequencies that are too high for human ear to perceive. When propagated through a biological structure, ultrasound induces compressions and decompressions of the

medium particles and a significant amount of energy can be imparted. Most ultrasound applications in food technology have involved non-invasive analysis with specific reference to quality assessment. There are a wide variety of possible applications of ultrasound technology in the food industry. Emulsification was one of the early uses of ultrasound technology in processing. Ultrasound-generated emulsions are often more stable than conventionally formed emulsions and often need little or no surfactant (Mason et al. 1996).

Oxidised starch is the natural starch that has been altered chemically or enzymically, which usually includes starch derivatives. Due to its specific functional properties such as low viscosity , high durability, transparency, film forming and binding properties, oxidized starch is also becoming increasingly important in food industry. This can be used in the confectionary as a coating and sealing agent, as an emulsifier, as a bread dough conditioner, as a gum arabic replacer and as a binding agent in the batter applications (Kuakpetoon, and Wang, 2006). The oxidized starch has been widely used in many industries to provide surface sizing and coating properties, especially for the paper, textile, laundry finishing, and construction materials industries.

In this research, ozone treatment will be uses as chemical modification to develop oxidative starch because ozone is green technology that can be effectively as an alternative oxidation technique for starch modification. Ultrasound treatment also will be use as physical modification on starch where ultrasound is useful to modify the functionality of starch in terms of physicochemical and functional properties. Ultrasound modified the morphology and physical structure of the starch (Yang et al, 2019). To the best of our knowledge, only quite limited studies on the impact of

ultrasound treatment and ozone treatment on modification physicochemical properties of sweet potato have been published.

1.2 PROBLEM STATEMENT

The food industry is currently in need of innovative processing technologies in order to meet customers' demand of fresher and stable shelf life of food products. Ozone treatment and ultrasound treatment are the technologies that can extend shelf life (Golwacz et al., 2015; Cao et al., 2010).

Sweet potato was ranked among the ten most important food crops produced in the world from Asia to America. For certain countries sweet potato is a major staple food, mostly in Africa region. In 2017, Global sweet potato production amounted to 113 million tons, with China leading 64 percent of the world 's output followed by Malawi and Nigeria (FAO, 2017). In malaysia, 55000 tonnes of sweet potato output in 2017 (DOSM, 2018). Sweet potato is easy to cultivate in Malaysia because it located in the same tropical latitudes and has been influenced by similar airpath and also have high temperatures , high humidity and heavy rainfall which is suitable for sweet potato to grow that require only 3½-4 months. Sweet potato csn produce 20-30% of starch (Rahman et al., 2007). However, the physicochemical properties of vitato™ has been limited as well as its utilization in food industry.

Most starch modification in the industry are utilized chemical alteration where sodium hypochlorite, hydrogen peroxide, etc. However, this modification produce wastewater that give negative effect on the environment and society. On the other hand, ozone treatment is chemical modification that environmentally safe cleaning

device that acts as an anti-microorganism agent and has the potential to destroy microorganisms and eliminate toxic contaminants. Previous studies show that ozone treatment can give positive effect to the physicochemical properties of the starch. At this time, there is no particular method for calculating the concentration of gaseous ozone (O3) in sweet potato starch.

Ultrasound treatment is a physical method for starch modification that has many benefits, such as less usage of additives, time and environmental friendly processing (Krishnakumar & Sajeev, 2017). Ultrasound is form of energy produced by waves of frequencies that are too high for human ear to perceive. Ultrasound treatment rely on a few factors such as frequency, sonication time, temperature and sonication power (Kaur & Gill, 2019). Ultrasonic treatment, particularly in water, affected starch's physicochemical and functional properties, resulting in increased fat and water absorption, lower concentration of gelling, solubility and swelling power, and lower viscosity of starch (Sujka & Jamroz, 2013). To the best of our knowledge, only quite limited studies on the impact of ultrasound treatment and ozone treatment on modification physicochemical properties of sweet potato have been published which mean this treatment are not well known yet in the oxidation of the starch.

1.3 OBJECTIVE

The general aim of this study is to develop oxidative starch from sweet potato (VitAto™) via hurdle technologies (combination of ozone and/or ultrasonic treatment). Hence, the objectives are:

1. To study the physicochemical effects of various macerations and drying methods on starch isolation and oxidation.
2. To determine the optimum conditions (maceration and drying method) to produce the highest yield of oxidised starch.

1.4 SCOPE OF STUDY

This study is to analyse the physicochemical effects to the oxidised starch isolation from various maceration and drying methods and to determine the optimum conditions to produce the highest yield of oxidised starch from VitAto™ sweet potato. This study's limitation involves a maximum ultrasonic power of 24W and a maximum frequency of 20kHz. In addition, ozone gas generator limits the maximum processing period to 30 minutes with the concentration of ozone of 1 ppm is fixed.

CHAPTER 2: LITERATURE REVIEW

2.1 SWEET POTATO

2.1.1 VITATO

Sweet potato or scientifically known as *Ipomoea batatas* is widely grown in tropics and warm temperate regions, and is a big, starchy, sweet and root vegetable. Sweet potato is rich in calories and contains biologically active phytochemicals such as β-carotene, polyphenols, ascorbic acid and dietary fibers (Van Hal, 2000). According to the United Nation's Food and Agriculture Organisation (FAO) report (2009), sweet potato is rank as 7th most important food crop worldwide which produce over 112 million metric tonnes of edible food products in the world annually. In Malaysia, the production of sweet potato from 2010 to 2017 are around 23,000-55,000 tons (FAO report, 2018) which quite high amount even though not in top 10 production quantity in the world. The annual production of sweet potato in Malaysia was about 2000 ha/year (Tan et al., 2010). Sweet potato is a sprawling or trailing vine which can be harvested as early as 3½-4 months after planting in the tropics, or after 5-6 months in the temperate region during the summer (Tan et al., 2015). Almost half of the sweet potatoes produced in Asia are used for animal feed, with the remainder primarily used

for human consumption (Consultative Group for International Agricultural Research, 2005).

In Malaysia, sweet potato ranks second among the tuber crops next to cassava, and since the 17th century has been cultivated in small scale. In June 2007, the Malaysian Agricultural Research and Development Institute introduced the VitAto, a new sweet potato variety, the outcome of a 10-year breeding plan aimed at producing more nutritionally superior sweet potato varieties. The VitAto™'s cultivation is excellent in terms of yield performance, even on marginal soils such as tin-tailings, BRIS (Beach Ridges Interspersed with Swales) and acid sulphate soils with sufficient agronomic changes, beyond currently cultivated orange-fleshed varieties in Malaysia (Hanim et al., 2014). Table 2.1 show the proximate analysis for vitato™ where vitato™ have high crude protein and dietary fibre for adding bulk to our diet and helping control weight in human body (NIH, 2015).

Table 2.1: Proximate Analysis of Vitato™ (Adapted from Hanim et al., (2014))

Parameter	VitAto	Bukit Naga	Okinawan
Moisture (g/100g)	4.60 ± 0.00^{b}	4.40 ± 0.14^{b}	5.15 ± 0.07^{a}
Ash (g/100g)	3.15 ± 0.07^{a}	3.20 ± 0.00^{a}	2.75 ± 0.07^{b}
Crude protein (g/100g)	5.70 ± 0.00^{a}	3.85 ± 0.07^{a}	3.50 ± 0.00^{b}
Fat (g/100g)	0.20 ± 0.00^{a}	0.70 ± 0.00^{a}	0.20 ± 0.00^{b}
Dietary fiber (g/100g)	14.80 ± 0.00^{a}	11.20 ± 0.00^{c}	11.30 ± 0.00^{b}
Carbohydrate (g/100g)	86.35 ± 0.07^{c}	87.85 ± 0.21^{b}	88.40 ± 0.00^{a}
Energy (kcal/100g)	399.60 ± 0.28^{a}	395.50 ± 0.57^{b}	392.00 ± 0.00^{c}

All means present the average of two independent replications. Values followed by the different superscript in each row are significantly different ($P < 0.05$)

2.2 STARCH

2.2.1 NATIVE STARCH

Native starches are essentially pure starch types. They are available from sources such as corn, wheat, potato, rice, cassava, and tapioca. Starch is a carbohydrate composed of glucose units bound by a glycosidic connection. Plants store glucose as the starch of polysaccharides (Whistler, 1997). Such long-chain carbohydrates, depending on type and temperature, are insoluble in cold water and swell to varying degrees. In general, natural starches are used for food texture and thickening purposes. Depending on the temperature used, they are insoluble in cold water and swell to various degrees. Natural starches have very good properties for thickening, gelling, preservation of moisture and anti-staling. Natural starches have been used in the food industry for decades, but due to limitations such as breaking down when reheated or in acidic conditions, some food producers have moved to use food starches that have been changed physically, chemically or enzymatically. Figure 2.1 show the chemical structure of a native starch.

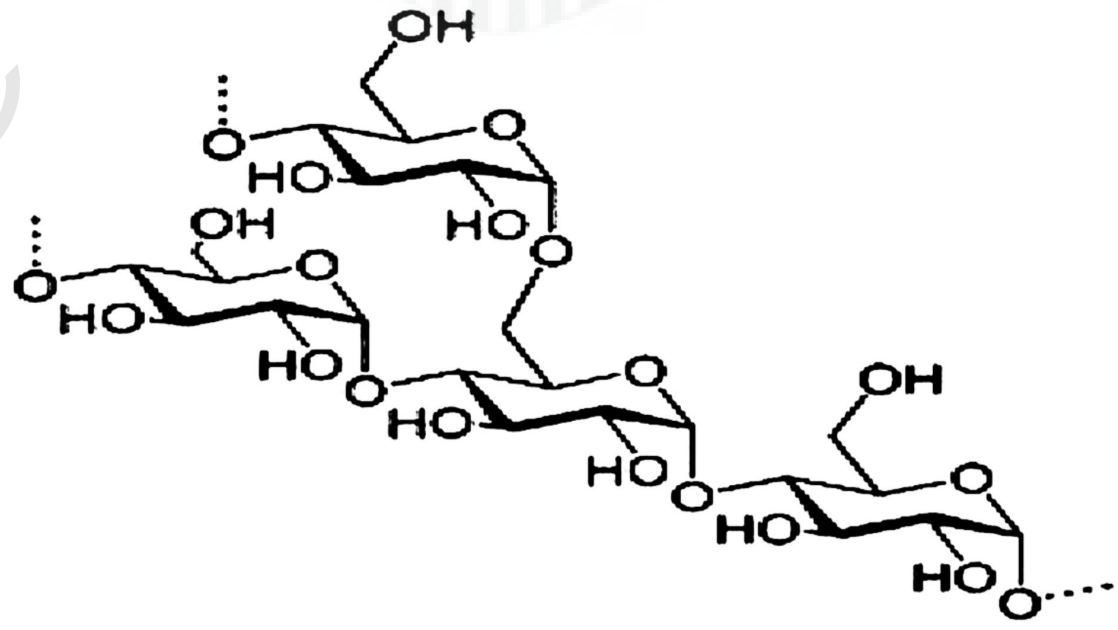


Figure 2.1: Structure of Native Starch

2.2.2 OXIDISED STARCH

Starch is an important ingredient in the food industry for various things, such as cereals for the breakfast, noodles, sauces, soups, meat products, coatings and dairy products as well as pastries (Vanier et al., 2017). Starch plays a crucial role in regulating food slurry viscosity and increases stability during processing and storage (Kaur et al., 2012). Oxidised starch or known as modified starch is the starch that has been processed from grains and vegetables to enhance its ability to maintain the food's texture and structure. Modified starch is used in many processed foods due to improved functional properties relative to natural starch (Karmakar et al., 2014). For food products that need to be microwaved, frozen, cooked at high temperatures or baked and fried, we use modified starch so that during the cooking process the texture of such food does not change. Modified starch can also be used as a thickening agent, stabilizer or emulsifier in almost all starch applications, for example in food products; as a dis-integrant in pharmaceuticals; or as a binder in coated paper. Figure 2.2 show the chemical structure of modified starch.

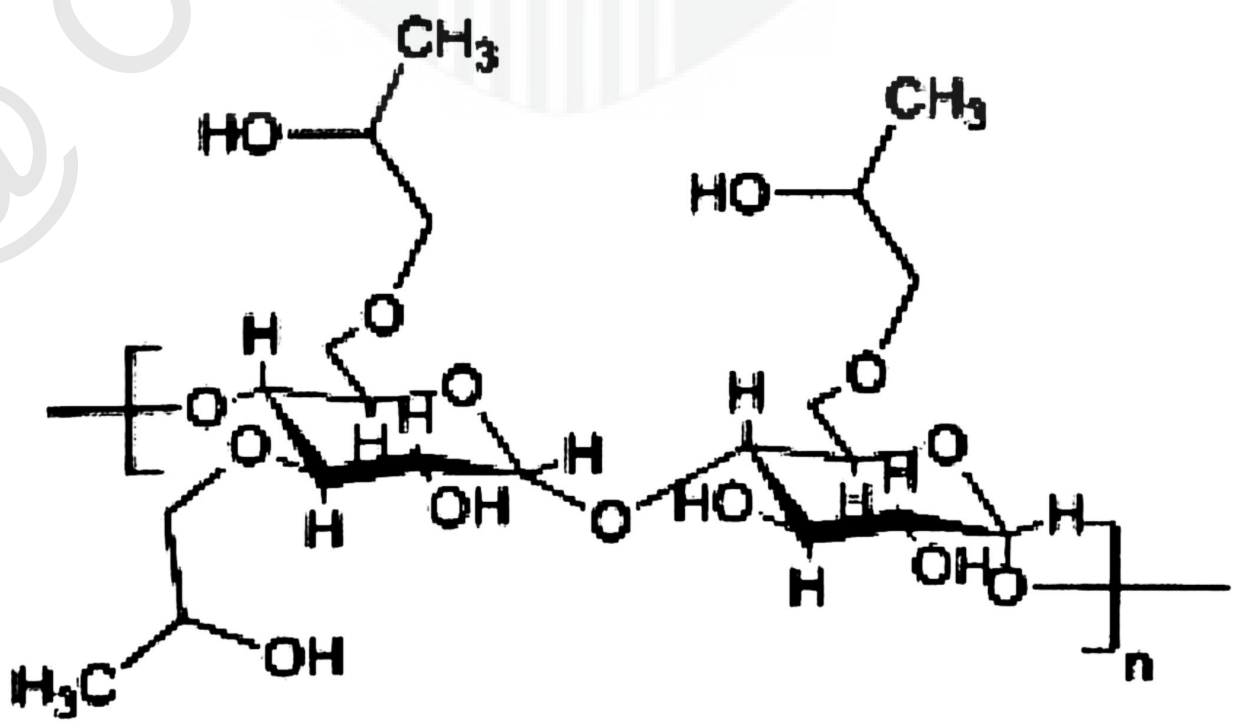


Figure 2.2: Structure of Modified Starch

2.2.3 PHYSICAL AND CHEMICAL MODIFICATION OF STARCH

Functional changes require improvements in starch anatomy and the three-dimensional structure under the control of certain physical influences. Physical improvements result in variations in particle size, surface properties, solubility index and functional properties such as water absorption of starch, swelling ability, pasting and gelation (Nawaz et al., 2020). These modifications directly affect the changed starch 's mechanical consistency and selectivity and suitability for various food, pharmaceutical, and industrial formulations. Several experiments have been documented using various methods involving physical starch alteration. The widely used methods of physical modification include superheating starch, treatment with thermal suppression, UV and gamma irradiation, treatment with microwave, high pressure, etc. Table 2.2 displays the most commonly employed and often effective methods of physical modification and their effects on the structure and properties of various starches.

The chemical alteration involves modification of starch's physiochemical properties by incorporating new chemical or functional starch groups without any physical change in the form and size of the molecule. There are three reactive hydroxyl groups in each of the amylose and amylopectin glucose classes, which are the key sites for chemical starch modification. The chemical modification improves starch's physical actions by retrogradation, salting, and gelatinization by stabilising the intermolecular and intramolecular bonding of starch granules. The commonly used methods of chemical modification of starch include oxidation by different oxidizing agents, etherification by addition of some hydroxyethyl, hydroxypropyl or

carboxymethyl moieties on hydroxyl groups of starch, esterification by condensation of some fatty acids, other carboxylic acids and phosphates with active hydroxyl groups of starch, cationization by introducing some cationic molecules, cross-linking by addition of various cross-linkers and graft-polymerization of starch with synthetic polymers (Paramo-Calderon et al., 2016; Liu, Yang & Yang, 2017; Paleos, Sideratou & Tisourvas, 2017; Zanella et al., 2019). Table 2.3 summarizes the widely used effective methods of starch chemical modification, and their impact on the structure and properties of different starc

Table 2.2: Summary of Previous Studies on Physical Modification Method of starch

Modification Method	Treatment	Sample	Change in starch granule	Change in starch properties	Reference
Thermal Modification					
Superheating	Heating starch at relatively high temperature (180–220°C)	Wheat starch	It results in the formation of spreadable gel particles with a creamy texture on cooling.	It improves the gelatinization and pasting properties of starch.	(Majzoobi et al., 2011)
Extrusion Heating	Application of mechanical force in low-temperature environment	Cassava Starch	It causes the degradation of amylose and amylopectin of the starch polymer by random chain-splitting. It also causes a high degree of granule disruption with complete loss of crystallinity.	It lowers the swelling power and viscosity and improves the water solubility and digestibility of starch.	(Acosta et al., 2016)
Hydrothermal Treatment	Heating of starch in an aqueous medium.	Corn starch	It causes physical reorganization of starch granules.	It improves the granule size, mobility, and stability which make it able to be digested easily by amylase. It also improves the gelatinization properties of starch.	(Priya et al., 2014)
Heat moisture Treatment	Heat application in the presence of limited moisture levels: 22–27% and high temperature above the glass-		It results in a change in size, shape and granular and crystalline structure of starch. It causes a partial or complete conversion of the B-type crystalline starch to A-type. It also causes disruption of helical structures within the amorphous Regions of starch granules. It	It decreases the amylose leaching, peak viscosity, and swelling capacity and enhances the solubility, thermal stability, gelatinization temperatures, pasting temperature, pasting time, interaction properties and susceptibility of starch to	(Vamadevan et al., 2013).

	transition temperature: i100 - 120°C for a specified length of time: i1 – 24 h		induces the molecular degradation of starch and increases the degree of polarization.	chemical and enzymatic attack (α-amylase and acid hydrolysis).	
Gentle Heating	Heating starch at low temperature (45–65°C)	Cassava starch	Causes slight changes in starch structure and amylopectin to amylose ratio.	It shows no significant effect on the physico-mechanical properties of starch.	(Wicaksono, Nuri & Wisudyaningsih, 2016).
Microwave Irradiation	Application of microwave radiation at different ranges of moisture and temperature to influence the dielectric constant of starch.	Corn starch Tapioca starch	It improves the granular crystallinity and surface morphology.	Microwave treatment improves the water and oil holding capacity, emulsifying activity, swelling capacity, solubility, and gelling ability. It also increases the pasting temperature and paste viscosity. It decreases the peak viscosity and gelatinization, and the degree of relative crystallinity.	(Amini et al., 2015)
Ultraviolet Irradiation	Starch granules exposed to UV light.	Cassava starch	It causes free radical-induced cross-linking and depolymerization, oxidative photodegradation, and dextrinization in starch.	UV treatment Influences the physical, chemical and functional properties of starch.	(Bertolini et al., 2001).
Gamma Irradiation	Exposure of starch granules to various doses of high energy gamma radiation.	Rice starch	Gamma irradiation causes the breakage of the amylopectin chains at the amorphous regions and decreases the amylopectin to amylose ratio. It also causes the radiolysis and radio-depolymerization of starch.	The exposure to gamma radiation decreases the pasting viscosity, and enthalpy change of starch and molecular weight and gyration radius of amylopectin. It increases the susceptibility of starch towards amylase. It also improved the rheological properties such as	(Bao, Ao & Jane 2005).

				gelatinization viscosity, swelling power, and solubility.	
Non-Thermal Modification					
pH Treatment	Addition of some acid or base to change the pH of the medium.	Cassava starch	A high pH results in partial degradation of starch granules with a decrease in molar size and radius of gyration. A low pH results in hydrolysis of starch particularly in the amorphous region of granules and decreases the molecular weight of the starch.	Increase in pH improves the solubility, swelling power, and compression properties. Low pH treatment improves the gelation properties of starch.	(Wicaksono, Nuri & Wisudyaningsih, 2016).
Grinding	It involves the grinding of starch by physical forces.	Maize starch	It decreased the crystalline/amorphous ratio, crystallinity, content of double helix of starch. It also results in a rapid increase and then a gradual decrease in surface parameters.	It reduces the viscosity and increases the susceptibility of physical and chemical factors to starch. It increases water-binding capacity, adsorptive capacity, and reactivity of starch.	(Liu et al., 2011)
Mechanical Activation by Stirring	Application of mechanical force on starch by stirring ball mill.	Cassava starch	The treatment results in the degradation of the crystal structure to amorphous particles and formation of an agglomerate of the resulting amorphous particles.	It reduces the gelatinization temperature and enthalpy, shear-thinning, and apparent viscosity of starch resulting in enhancement of cold-water solubility of the starch.	(Huang et al., 2007)
High-pressure Treatment	Treatment of starch under pressure < 400 MPa.	Barley starch	It exerts a pressure and time-dependent effect on the microstructure of starch. It causes melting of amylopectin crystals and loss of birefringence.	The pressure treatment causes changes in rheological properties of starch. It increases the hardness and chewiness and improves the freeze–thaw stability of the starch gels.	(Stolt, Oinonen & Autio, 2000)
Osmotic Pressure Treatment	Heating of starch in a hypertonic (saturated)	Corn starch	It causes distortion in the shapes of starch granules and changes the B-type crystalline starch to A-type	This modification increases the gelatinization temperature.	(Pukkahuta et al., 2008).

	solution of sodium sulphate at 100–120°C across the semipermeable membrane.				
Hydrostatic Pressure Treatment	Application of high pressure ranging from 400 to 900 MPa.		It causes the disintegration and retrogradation of starch granules.	It retards the swelling of granules or reduces viscosity with preserving the taste and nutrient of starch	(Stute et al., 1996)
Ultra-sonication	Treatment of starch with ultrasonic waves.	Potato starch	It distorts the starch granules.	It increases the solubility, viscosity and swelling capacity of granules and reduces the pasting ability and digestibility of starch. It also increases the gelatinization temperature and enthalpy and decreases the solubility.	(Nadir et al., 2015)
High Pressure Ultra-sonication	The treatment of ultrasound waves to native starch granules. at 24KHz to 360KHz frequency	Corn starch	It distorts in the crystalline region of the starch granules.	It decreases the enthalpy of gelatinization, consistency coefficient, crystallinity and molecular weight of starch granules.	(Jambrak et al., 2010)
Annealing	Modification of starch in the presence of intermediate water contents (40–50% w/w) or excess water more than 65%		It increases interaction between the amylose–amylopectin and amylose-amylose chains and the crystalline perfection. It enhances the mobility of double-helical chain segments within granules, allows subsequent recrystallization, restructuring, or	Decreases the amylose leaching and swelling of granules and increases thermal stability gelatinization temperatures, and susceptibility towards α-amylase.	(BeMiller & Huber, 2015)

	w/w at temperatures lower than the onset temperature of gelatinization.		both of starch chains, enhances molecular order and provides more homogeneity among crystallites.		
Thermal Inhibition	Dehydration of starch at a high temperature until it becomes anhydrous (<1% moisture).	Potato starch	It results in a decrease in granular size.	It increases the cohesive-texture and stabilizes the viscosity of starch.	(Lim et al., 2002)
Freeze–Thaw Treatment	Heating of starch at high temperature (59–79°C) flowed by freezing and defrosting.		An increase in the number of Free-Thaw cycles changes the complex modulus and phase angle of the starch.	Affects the rheological properties of starch. Increases the swelling power, viscosity, and thermal stability of starch. It also influences the surface properties of the starch granules.	(Lawal, 2019)
Heat-Moisture Treatment-Annealing	Heat-moisture treatment followed by annealing.	Carioca Bean	No significant damage of individual treatment ion the structure of starch granules has been observed. Heat-moisture-annealing treatment resulted in disruption of crystalline structure.	Increase in enthalpy.	(Zanella Pinto et al., 2019)
Annealing-sonication and Sonication-annealing	Annealing followed by sonication and vice versa.		Both treatments promote a synergic behaviour ion crystallite collapse and result in a decrease in relative crystallinity. The later also results in irregular surface morphologies and granule disintegration.	Both increase the pasting viscosity	

Table 2.3: Summary of Previous Studies on Chemical Modification method of starch

Modification Method	Treatment	Change in starch granule	Change in starch properties	Reference
Oxidation	Addition of carboxyl and carbonyl group to native starch by ihe use of an oxidizing agent.	It causes depolymerization of starch resulting in retardation in recrystallization due to the incorporation of carbonyl and carboxyl groups.	It increases the stability, clarity and biding properties but reduces the dispersion viscosity of starch.	(Boukhalfa et al., 2018)
Stabilization by addition of a polymer	Copolymerization with synthetic polymers	It provides the structural stability to starch and reduces the retrogradation.	It improves the freeze–thaw stability and shelf life of starch-based food products.	
Hydroxyethylation	Introduction of hydroxyethyl group to the starch.	Changes in the granular structure.	Improves the drug binding ability for some anticancer and other drugs.	(Paleos et al., 2017)
Carboxymethylation	Carboxymethyl substitution of hydroxyl groups in starch	It adds the hydrophobic groups on the starch molecule.	It increases the stability of starch in aqueous media, reduces the recrystallizing ability and prevents the damage from heat and microorganisms	(Lawal, 2019)
Acetylation	Reaction of an acetyl group with the hydroxyl group of polymeric starch.	It retards the crystallization or retrogradation in starch granules.	It inhibits the formation of intramolecular hydrogen bonds and enhances the viscosity and swelling capacity of granules. It reduces the pasting temperature and solubility.	(Thirumdas et al., 2017)
Phosphorylation	Addition of phosphate group on hydroxyl groups of starch	It results in the formation of either monophosphate or diphosphate starch. It increases the steric	It improves the viscosity, textural properties, paste clarity and Freeze-Thaw stability of starch. It also increases resistance to low pH, high	(Waliszewski et al., 2003)

		hindrance and prevents the linearity of molecular chains.	temperature, and high shear. It decreases the temperature of gelatinization.	
Cross-linking (Formation of inter and intramolecular bridges)	Etherification and esterification of granules with cross-linking polymers by reacting with a mixture (99:1) of sodium trimetaphosphate and sodium tripolyphosphate or other cross-linkers in an aqueous alkaline slurry containing sodium sulphate.	It reduces the mobility of amorphous chains in the starch granule. It introduces the inter- and intra-molecular bonds with multifunctional small molecules with hydroxyl groups ion starch to strengthen the granules against various factors. It increases the ordering of internal granule structure and stability.	It decreases the solubility of starch in water which reduces its association with lipids, moisture, and proteins. It also causes decreases in viscosity, swelling capacity, digestibility, retrogradation rate, the peak temperature of relaxation endotherm and enthalpy of starch. It increases the gelatinization temperature, glass transition temperature, melting enthalpy, free volume of starch chains, relaxation enthalpy and stability of starch to high temperature.	(Páramo-Calderón et al., 2016)

2.3 OZONE TREATMENT

2.3.1 INTRODUCTION

Ozone is a molecule made up of three oxygen atoms that are negatively charged. The molecule of ozone is very unstable and has a short half-life, causing it to return to its original form after some time. Mainly, an ozone molecule is nothing but an oxygen molecule that has provided an extra oxygen atom from high voltage electric. Ozone is naturally produced by certain forms of chemical reactions. Ozone is one of the type for oxidation that act as oxidising agent. Chemical oxidizing agents are typically unacceptable due to safety and environmental issues for starch alteration. In the hypochlorite oxidation phase, for example, large amounts of salts are produced, causing wastewater disposal problems (Kesselmans & Bleeker, 1997). On the other hand, when added into a food product, ozone does not leave a residue. Ozone treatment would therefore be a good alternative to food products being chemically processed. Ozone is an oxygen that occurs naturally and is created industrially (Dillon et al., 1992). It is also a safe oxidant with a high potential for thermodynamic oxidation, which means that the reactions can be carried out at low temperatures (Sahle-Demessie & Devulapelli, 2008).

2.3.2 PHYSICAL AND CHEMICAL PROPERTIES OF OZONE

Figure 2.3 show the resonance structure of ozone molecule. The three oxygen atoms in the ozone molecule are positioned at an obtuse angle at which two equal distance oxygen atoms are bound to a central oxygen atom. The included angle is 116.8° and the bond length is 1.278Å.

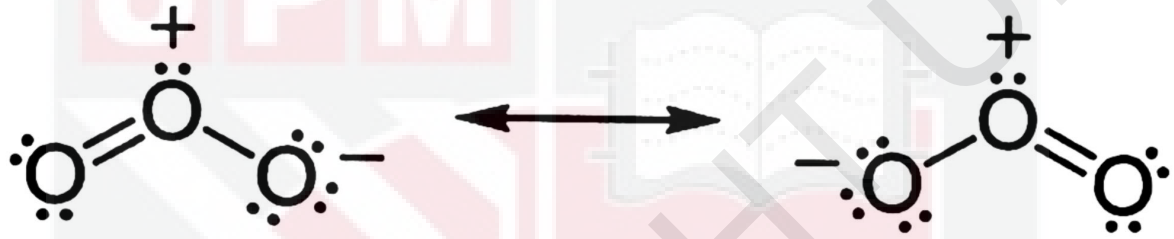


Figure 2.3: Resonance Structure of Ozone Molecule

Ozone highly absorbs radiation in the ozone spectrum ultraviolet range between 220-290 nm. It protects the Earth and its citizens from the Sun's harmful ultraviolet radiation. This show that ozone have good physical properties. Without this protective layer, more ultraviolet radiation will penetrate the Earth's surface and threaten the life of plants, animals and humans. Pure ozone is a blue gas that has a strong smell of irritation. It is approximately 1.5 times heavier than air and has a vapor density of 24, which corresponds to formula O_3.

Ozone is a substance which is unstable. Pure ozone explosively decomposes and ozonized oxygen gradually decomposes at room temperature. The decomposition at about 573 K is instantaneous. The presence of manganese dioxide, platinum black and copper oxide accelerates the decomposition. Ozone can reduce the property where

ozone reduces peroxides to oxides and in turn gets reduced to oxygen. For example, in figure 2.4, with H2O2 and it gives H_2O.

$$H_2O_2 + O_3 \longrightarrow H_2O + 2\,O_2$$

Figure 2.4: chemical reaction of hydrogen peroxide to oxides using ozone

2.3.3 HALF-LIFE OZONE

When ozone is produced it will decay rapidly, because ozone is an instable compound with a relatively short half-life. Table 2.4 shows the half-life of ozone in air and water at different temperature. From the table, half-life in water a lot shorter than in air. Ozone process analysis often includes the reactions of two species: ozone and OH-radicals. When these OH-radicals are in solution in the dominant particles it is considered an advanced process of oxidation. The decay of ozone in OH-radicals in natural waters is defined by a rapid decrease in ozone, preceded by a second phase in which ozone decreases with first order kinetics (Von Gunten, 2003). The half-life of ozone varies within the range of seconds to hours, depending on the quality of the water. Temperature , pH, environment and concentrations of dissolved matter and UV light are factors which affect the decomposition of ozone in water.

Table 2.4: Half-life of ozone in air and water at different temperature

Air		Disolved in water	
Temperature (°C)	**Half life**	**Temperature (°C)**	**Half life**
-50	3 months	15	30 min
-35	18 days	20	20 min
-25	8 days	25	15 min
20	3 days	30	12 min
120	1.5 hours	35	8 min

2.3.4 OZONE GENERATION

Ozone is created when single oxygen molecules separated by a high energy input from oxygen are combined with the oxygen available. Ultraviolet sunlight irradiation and lighting discharge are the primary source of high energy for ozone production (Suslow, 2004). Figure 2.5 show ozone generation of corona discharge cell configuration.

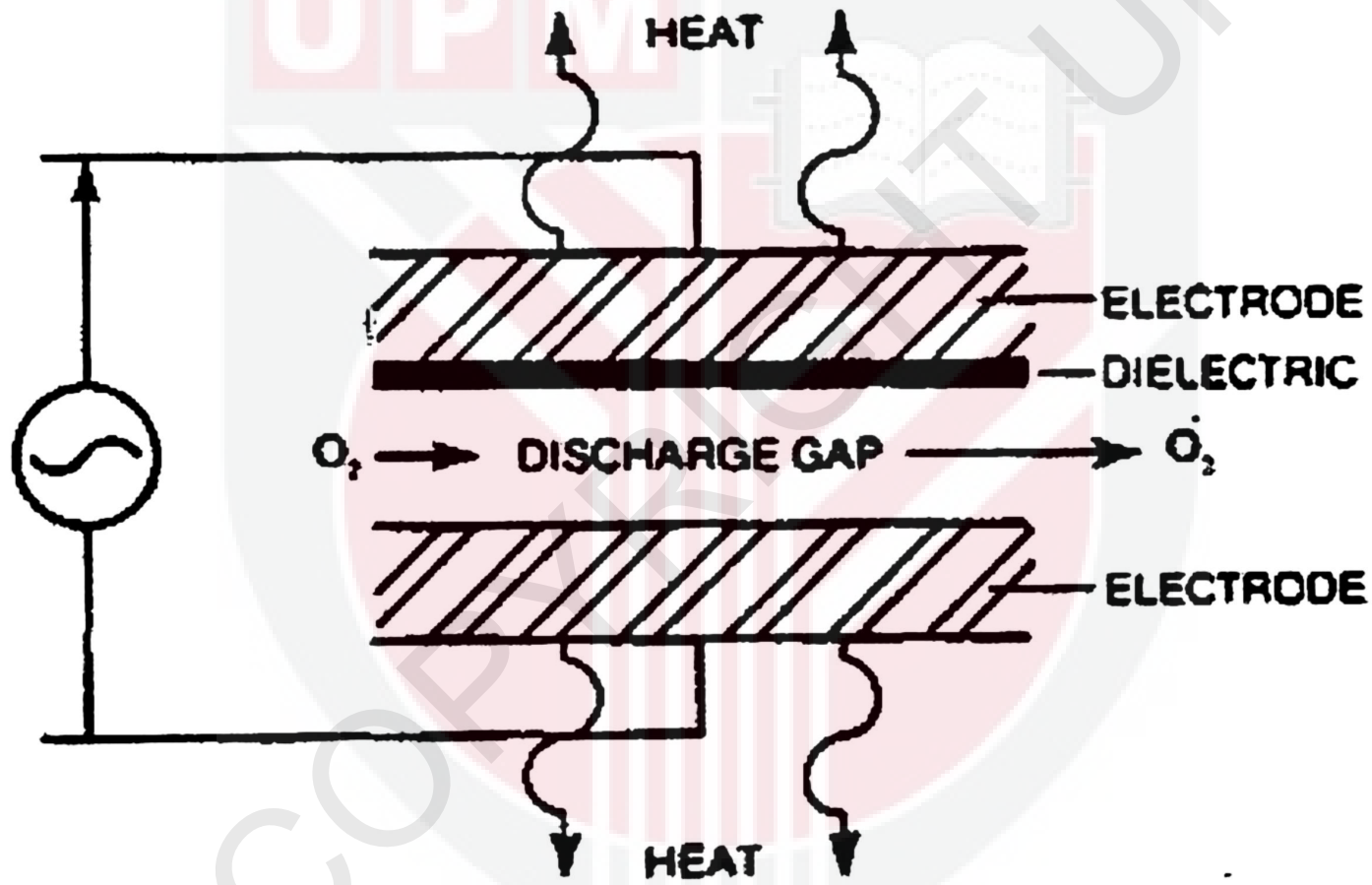


Figure 2.5: ozone generation of corona discharge cell configuration

Electrical discharge production of ozone is a common occurrence in things such as photocopiers, defective light switches, engine brushes, and electrical transmission lines. Using electrical power to produce ozone by discharge from corona has become and remains the most commercially viable. Essentially a corona is characterized at a relatively high voltage gradient by a low current electrical discharge through a gas-filled gap. The energy source

from the sun or lightning can be replaced by the 140-190 nm wavelength UV light (Halaweish et al., 2000) or use a corona discharged process involving two high-voltage electrodes that accelerate the electrons in the air to break the double bond of oxygen (Bourke et al., 2009). This results in a partial ionization of the gas and a diffused bluish light by using pure oxygen as the feed gas (Smith, 2011).

2.3.5 OZONE IN FOOD APPLICATION

Ozone gas is provided as a way to preserve food by reducing the amount of harmful bacteria and chemical pollutants that are attached to food. Ozone is an anti-microorganism compound that can be used to handle, store and process food in the form of gases and liquids, including fresh fruit and vegetable raw materials and products (Khadra et al., 2001). Ozone has the ability to kill microorganisms and remove hazardous materials, so as an environmentally friendly cleaning tool, it has considerably good prospects. In addition, ozone can be used in solid and liquid raw materials to treat, store and process food. Ozone is an effective oxidizing agent that can be used to kill bacteria (sterilization), eliminate colour and odours. Ozone was used in the cultivation of vegetables and fruits as a safer option, even before harvesting (Rice & Netzer, 1982). Table 2.5 show the summary of previous studies of ozone treatment on starch.

Table 2.5: Summary of Previous Studies of Ozone Oxidation on Starch

Sample	Treatment	Change in starch properties	Reference
Potato Starch	Ozonated 15-60 min with concentration 47mg/L and dried until 12% moisture content at 35°C.	It increase in carboxyl group, carbonyl group and reducing sugar and decrease the amylose content, pH value and molecular size.	(Castahna et al., 2019)
Rice Starch	Ozonated 0-30 min with concentration 170mL/min	It increases in pasting properties and decrease in proximate analysis than commercialize starch.	(An & King, 2009).
Cassava Starch	Ozonated 0-45 min with concentration 60-90mg O_3/g and dried at 50°C until dry	It increase in carbonyl and carboxyl group, acidity, whiteness and percentage of light transmittance.	(Sittichoke et al., 2015)
Corn Starch Sago Starch Tapioca Starch	Ozonated for 1,3,5,10 min in the vessel that connected to the generator	It decrease in molecular size and viscosity	(Chan et al., 2011)
Cassava Starch	Ozonated for 60 min with concentration of 13mg/L and dried at 40°C until 11% moisture content	It increase in carbonyl and carboxyl group and decreases in pasting properties with no significant change in morphology in starch granules.	(Klein et al., 2014)
Corn Starch	Ozonated for 1,3,5,10 min with concentration of 0.5 mol	It increases in swelling power and solubility, carboxyl and carbonyl content, pasting properties and reduce in intrinsic viscosity	(Chan et al., 2009)

2.3.6 OZONE TREATMENT LIMITATION IN FOOD INDUSTRY

Minimizing pathogenic and bacteria in fruits, vegetables and their products is a major issue for food health. Traditionally, thermal processing techniques are used to prevent pathogens. However this technique has an effect on food safety. Non-thermal processing avoids losses of product quality such as lack of original color , texture , shape, colour, quality of nutrients, etc (Barrett et al., 2010). Current sanitization technologies are generally critical to maintaining the quality and enhancing the safety of fresh agricultural commodities, but it is necessary to minimize the disadvantages and potentially hazards caused by consumer treatments. Promising results have been discovered in the treatment through ozone application to food production issues such as bacteria, rodents, mycotoxin and pesticide residues (Prabha et al., 2015).

Ozone is a strong oxidizer and a good disinfectant. Disinfecting agents have wide uses in food industry to ensure health and quality. However, some of these agents are inefficient against certain organisms , especially at high pH or against spore-forming microbes, such as chlorine. In addition, chlorine can contribute to the development of trihalomethanes which are of concern both for human dietary health and as pollutants to the environment. Therefore, the limitation of ozone treatment is important to avoid the negative effect into the environment. Table 2.6 show the ozone treatment limitation from some important institution in food industry.

Table 2.6: Ozone Treatment Limitations (Adapted from EPA, 2017))

Institution	Standard
Food and Drug Administration (FDA)	Concentration limit exposure of 0.05 ppm during 8 hours
National Institute of Occupational Safety and Health (NIOSH)	Concentration limit exposure of 0.10 ppm, not to be exceeded at any time.
Occupational Safety and Health Administration (OSHA)	Concentration limit exposure of 0.10 ppm during 8 hours.

2.4 ULTRASOUND TREATMENT

2.4.1 INTRODUCTION

Ultrasound is a form of energy produced by waves of frequencies that are too high for human ear to perceive, i.e. above 16 kHz (Jayasooriya et al., 2004). Ultrasound creates compressions and decompressions of the medium particles when propagated through a biological structure and a large amount of energy can be imparted. There are a variety of physical, chemical and biochemical effects that can be experienced depending on the frequency used and the amplitude of the sound wave (Knorr et al., 2004). Ultrasound uses are widely classified into two groups. Ultrasound applications of low energy require the use of frequencies above 100 kHz at intensities below 1 W/cm^2. Ultrasound of low intensity uses such a low power level that ultrasonic waves do not cause any physical or chemical alterations in the properties of the material through which the wave passes, i.e. it is generally non-destructive. The most common use of low-intensity ultrasound in the food industry is an analytical tool to provide information on the physicochemical properties of foods, such as composition, structure and physical condition (Knorr et al., 2004). Ultrasound applications of high energy require the use of frequencies above 100 kHz at intensities above 1 W/cm^2. For many years, high-intensity ultrasound has been used to create emulsions, kill cells, and distribute aggregated materials. More recently, different areas with greater potential for future development have been identified, for example, inactivation of enzymes, and liquid foods degassing (Knorr et. Al, 2004). The advantageous use of sound energy is understood by the various effects on the medium where it transmits the ultrasound. In this range, the physical, mechanical or chemical

effects of ultrasonic waves will alter the material properties by producing immense pressure, shear and temperature gradient in the medium through which they propagate.

2.4.2 ULTRASOUND IN FOOD APPLICATION

Within food technology, we can find nearly all the processing examples that can be applied to ultrasound. Until recently, most ultrasound applications in food technology have involved non-invasive analysis with specific reference to quality assessment. These applications use high frequency low-power ultrasound techniques that are close to those used in diagnostic medicine, or non-destructive research. There is a wide range of potential uses of power ultrasound in the food industry. Emulsification has been one of the early applications of power ultrasound in manufacturing. Ultrasound-generated emulsions are often more stable than conventionally formed emulsions and often need little or no surfactant (Mason et al. 1996). Research has shown that the use of ultrasound as a processing aid can reduce yogurt production time by up to 40%. In addition, sonication decreased the process's normal reliance on milk sources as well as enhanced the product's quality and texture. Fish egg exposure to frequency 1 MHz ultrasound for 35 min was also observed to result in a decrease in hatch time for loach from 72 to 60 hours three times a day. Several literature reports suggest that pre-sowing seed ultrasonic treatment is an effective way to improve crop yield (Mason et al. 1996). Table 2.7 show previous studies of ultrasound treatment of starch.

Table 2.7: Summary of Previous Studies of Ultrasound Treatment on Starch

Sample	Treatment	Change in starch properties	Reference
Potato Starch	Treatment of starch with ultrasonic waves.	It increases the solubility, and swelling capacity of granules and reduces the pasting ability and digestibility of starch. It also decrease the gelatinization temperature and enthalpy.	(Nadir et al., 2015)
Corn Starch	Treatment of starch with ultrasonic waves with 100% amplitude at a frequency of 25kHz and 200W power	It increase carbonyl and carboxyl content, swelling power, solubility and pasting properties.	(Chong et al., 2013)
Tapioca Starch	Treatment with ultrasonic waves at 24kHz frequency and 400W power with 50 and 100% frequency.	It increase in solubility and swelling power and reduce the the granule structure.	(Manchun et al., 2012)
Rice Starch	Treatment with frequency of 20 kHz, power 170 W at temperature range of 25–35 °C for 1 h	It increase swelling power and solubility, crystallinity and reduce the amylose content and granule structure.	Keeratiburana et al., 2020)
Corn Starch Wheat Starch Rice Starch	Treatment with ultrasounds for 30 min at 20°C at frequency of 20 kHz and power 170 W.	It decrease the granular structure.	(Sujka, 2017)
Cassava Starch	Treatment of starch with ultrasonic waves with frequency of 30kHz and 750W power	It increase the swelling power and solubility and decrease the pasting properties and granular structure.	Krishnakumar & Sajeev, 2018)

CHAPTER 3: METHODOLOGY

3.1 MATERIALS

Sweet potato (VitAto™) was obtained from Malaysian Agricultural Research and Development Institute (MARDI) Kelantan. For maceration of sweet potato (VitAto™), the sodium metabisulfite was used as a solvent obtained from Eva Chem Company.

3.2 RESEARCH DESIGN

Sweet potato (Vitato™) preparation is done to extract the starch content that content in the sweet potato. Ultrasound treatment and maceration with sodium metabisulfite can increase the sweet potato starch extraction. after that, the starch that have been extracted will undergo ozone treatment and then oven drying until the moisture content achieve 11%. Figure 3.1 show the flowchart of research design to produce oxidized starch.

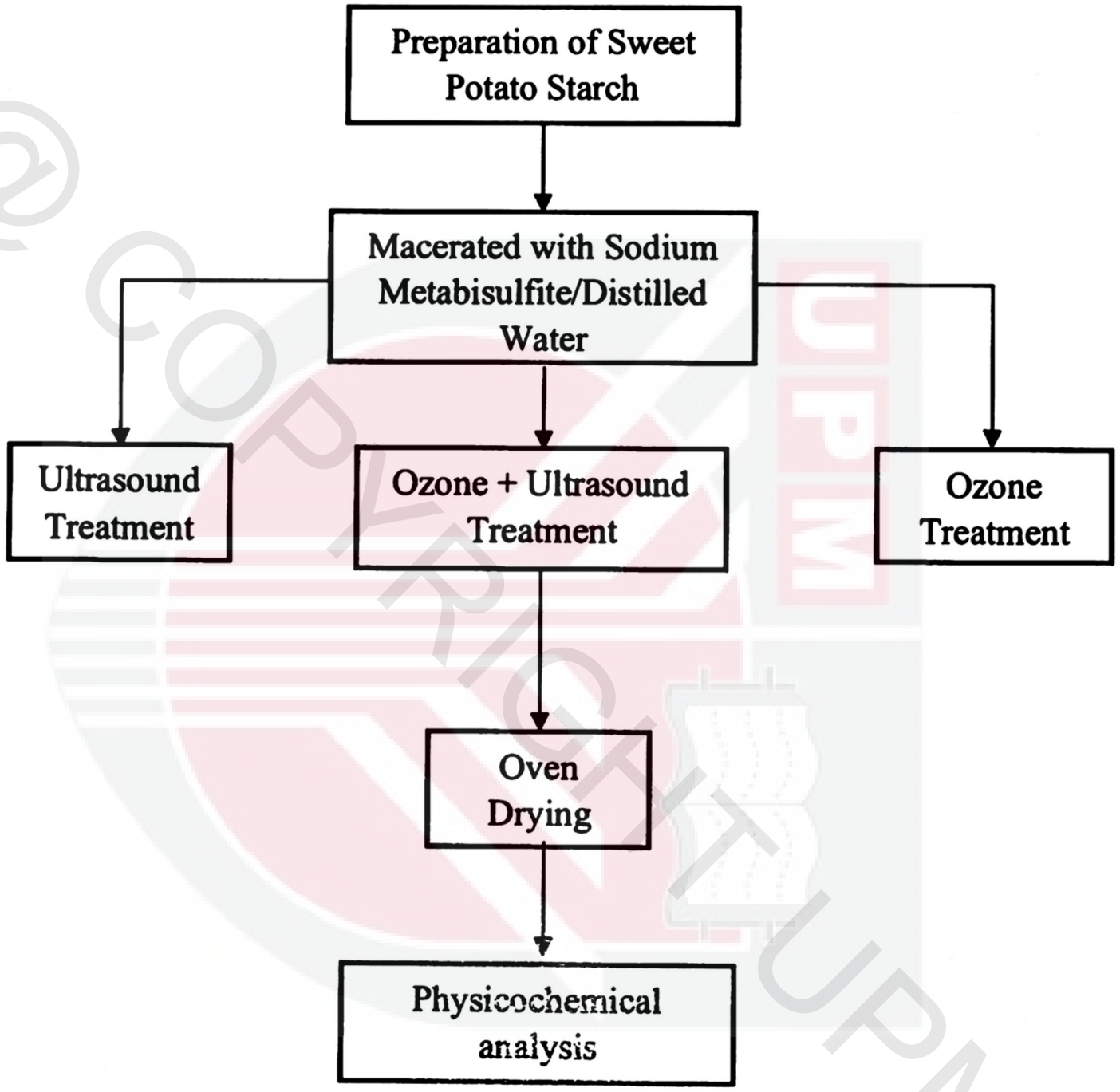


Figure 3.1: Research Design

3.3 DESIGN OF EXPERIMENT

One Factor At A Time (OFAT) method was used in this study to single out the significant factor that will produce oxidized starch with the highest yield and qualitative characteristics. Each sweet potatoes sample undergo the below mentioned method to produce oxidative starch in triplicates (Table 3.1).

Table 3.1: Description of method for each samples

Sample	Method
Sample A (control)	Maceration (Sodium Metabisulfite) + Oven dried 4 hours
Sample B	Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour
Sample C	Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour
Sample D	Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour
Sample E	Maceration (Distilled water) + Sonicate + Oven dried 4 hours

3.4 PREPARATION OF SWEET POTATO STARCH

3.4.1 SAMPLE A

The sweet potatoes were peeled and cut into small pieces. Then, they were macerated with 0.01M sodium metabisulfite ($Na_2S_2O_5$), pH=4.5 at low speed for 10 min. Next, the mixture was incubated at 40°C for 2 hours. The suspension was sieved through 140-mesh sieve for 3 times and finally washed with distilled water for several times. The slurry were stood for 15 minutes for starch sedimentation and then centrifuged at 2,500 x g for 15 minutes. The white starch was collected and dried in the oven at 40°C for 4 hours. Finally, the dried starch were ground into powder and stored at room temperature in a glass bottle.

3.4.2 SAMPLE B

Sweet potatoes were peeled and cut into small pieces. Then, the small pieces of sweet potatoes were placed in 500mL beaker filled with 0.01M sodium metabisulfite ($Na_2S_2O_5$), pH=4.5 at low speed for 10 min. A piezoelectric apparatus equipped with a VCX probe system was used for ultrasound treatment. The probe with vibrating titanium tip of 19 mm diameter was attached to the transducer, and it was immersed in a suspension that was irradiated with an ultrasonic wave directly from the horn tip. The ultrasound treatment on the starch was derived from HerCeg et al. (2010) which its application time was 30 minutes at 100W of power and 40% amplitude at frequency of 20 kHz. Next, the mixture was incubated at 40°C for 2 hour. The suspension was sieved through 140-mesh sieve for 3 times and finally washed with distilled water for several times. The slurry were stood for 15 minutes for starch sedimentation and then centrifuged at 2,500 x g for 15 minutes. The white starch was collected and undergo ozonation (Figure 1) for 4 hours before being dried in the oven at 40°C for 4 hours. Finally, the dried starch were ground into powder and stored at room temperature in a glass bottle.

3.4.3 SAMPLE C

Sweet potatoes were peeled and cut into small pieces. Then, the small pieces of sweet potatoes were placed in 500mL beaker filled with 0.01M sodium metabisulfite ($Na_2S_2O_5$), pH=4.5 at low speed for 10 min. Next, the mixture was incubated at 40°C for 2 hour. The suspension was sieved through 140-mesh sieve for 3 times and finally washed with distilled water for several times. The slurry were stood for 15 minutes for starch sedimentation and then centrifuged at 2,500 x g for 15 minutes. The white starch was collected and undergo ozonation (Figure 1) for 4 hours before being dried in the oven at 40°C for 4 hours. Finally, the dried starch were ground into powder and stored at room temperature in a glass bottle.

3.4.4 SAMPLE D

Sweet potatoes were peeled and cut into small pieces. Then, the small pieces of sweet potatoes were placed in 500mL beaker filled with distilled water. A piezoelectric apparatus equipped with a VCX probe system was used for ultrasound treatment. The probe with vibrating titanium tip of 19 mm diameter was attached to the transducer, and it was immersed in a suspension that was irradiated with an ultrasonic wave directly from the horn tip. The ultrasound application time was 30 minutes at 24W of power and 40% amplitude at frequency of 20 kHz. Next, the mixture was incubated at 40°C for 2 hours. The suspension was sieved through 140-mesh sieve for 3 times and finally washed with distilled water for several times. The slurry were stood for 15 minutes for starch sedimentation and then centrifuged at 2,500 x g for 15 minutes. The white starch was collected and undergo ozonation (Figure 1) for 4 hours before being dried in the oven at 40°C for 4 hours. Finally, the dried starch were ground into powder and stored at room temperature in a glass bottle.

3.4.5 SAMPLE E

Sweet potatoes were peeled and cut into small pieces. Then, the small pieces of sweet potatoes were placed in 500mL beaker filled with distilled water. A piezoelectric apparatus equipped with a VCX probe system was used for ultrasound treatment. The probe with vibrating titanium tip of 19 mm diameter was attached to the transducer, and it was immersed in a suspension that was irradiated with an ultrasonic wave directly from the horn tip. The ultrasound application time was 30 minutes at 24W of power and 40% amplitude at frequency of 20 kHz. Next, the mixture was incubated at 40°C for 2 hours. The suspension was sieved through 140-mesh sieve for 3 times and finally washed with distilled water for several times. The slurry were stood for 15 minutes for starch sedimentation and then centrifuged at 2,500 x g for 15 minutes. The white starch was collected and dried in the oven at 40°C for 4 hours. Finally, the dried starch were ground into powder and stored at room temperature in a plastic zip bag.

3.5 PHYSICOCHEMICAL ANALYSIS

3.5.1 SWELLING POWER AND SOLUBILITY

According to the method describe by Leach et al. (1959), swelling power and solubility can be determined by weighing 0.1 g of starch and adding 10 ml of distilled water. The suspension will be stirred and placed in water bath for 30 minutes at temperature ranging from 55°C to 95°C, increasing 10°C from time to time and centrifuging for 15 minutes at 3400 g. A 5 ml aliquot will be removed from the supernatant, placed in petri dish and placed on the stove at 105°C for 24 hrs to determine the weight of the solubilized starch. After the outer walls of the tubes were dried, the tubes will be carefully weighed. The swelling power and solubility will determined as follows:

i. Swelling Power

$$\frac{(weight\ of\ tube + residue\ after\ centrifugation) - (weight\ of\ tube + sample\ on\ dry\ basis)}{weight\ of\ sample}$$

ii. Solubility

$$(weight\ of\ plate\ with\ sample\ after\ evaporation) - (weight\ of\ plate)\ x\ 100$$

3.5.2 CARBOXYL CONTENT

The carboxyl content is measured in 30 ml 0.1 N of hydrochloric acid (HCl) by suspension of 500 mg of starch. Those samples will be washed with de-ionized water after 30 minutes, then suspended in 300 ml of de-ionized water and heated under agitation for 10 minutes at boiling temperature. Hot solution with 0.002 N of sodium hydroxide (NaOH) will be titrated using phenolphthalein as an indicator. Carboxyl content is expressed as a percentage of COOH (w/w).

3.5.3 AMYLOSE CONTENT

0.1 mg of starch sample will be dissolved in 1 ml of 95% ethanol (v / v) and 9 ml of 1 N solution of sodium hydroxide (NaOH) and put in a refrigerator at 4 ° C overnight. Distilled water will be added to make up 100 ml in a volumetric flask containing the dissolved starch sample. According to the operator's manual, the volume of amylose will be determined using an automated chemical analyser (FS3100, Ol Analytical, USA) (Zhang et al., 2016).

3.5.4 INTRINSIC VISCOSITY

The viscosity of the starches were determined using a rotational viscometer at a sample concentration of about 1.0 mg/mL (Zhang et. al, 2009).

3.5.5 GELATINIZATION

The gelatinization properties will be determined by Differential Scanning Calorimetry (DSC) using a differential exploratory calorimeter (Shimadzu, model DSC 50, coupled to computer software) in a nitrogen atmosphere at a flow rate of 50 ml.min. 6 μL of distilled water will be added to 2 mg of starch for the preparation of the samples, enclosed in tubes and weighed again. Before analysis, the samples must be kept 24 h at room temperature to ensure uniform distribution of water in starch. The temperature of the scanning ranged from 30 ° C to 150 ° C, and heating was 10 ° C / min (Lawal & Adebowale, 2005).

3.5.6 SYNERESIS

Syneresis of starch determined according to method that have been described by Singh et al (2004). Srarch suspension will be heated at 85℃ for 30 min in a water bath, followed by rapid cooling in an ice bath to room temperature. Samples of starch will be kept at 4 ° C for 24 , 48, and 120 h. Syneresis is measured as the percentage of water released for 15 min after centrifugation at 300 g (E.I. Yousif, 2012).

3.5.7 MORPHOLOGICAL PROPERTIES

The morphology of the starch granules will be examined using a scanning electron microscope (Shidmadzu,SSX-550). A small quantity of each sample is spread directly onto the stub surface and dried for 1 h in an oven at 32 ° C. Subsequently, all the samples will be gold-coated and examined under a 15 kV acceleration voltage and 5000x magnifications in the scanning electron microscope (B. Klein et al., 2014).

3.5.8 STATISTICAL ANALYSIS

A test was used to determine the optimum condition to produce highest yield of oxidised starch means from the data obtained between solubility and swelling power, viscosity and gelatinization. Design Expert Software was used to analyse the data obtained from the physicochemical properties.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 SWELLING POWER AND SOLUBILITY

Swelling power measures the water-holding capacity of starch after being heated, cooled, and centrifuged, whereas the solubility reflects the degree of dissolution during the starch swelling procedure (Zhang et al., 2018). Solubility stems from the leaching and diffusion of amylose from the starch granule during swelling (Paraginski et al., 2014). High solubility may be due to a less stable structure of the starch granules. From Figure 4.1 and 4.2, the highest swelling power and solubility of VitAto™ is Sample B (macerated starch with sodium metabisulfite, followed by sonication and then gaseous ozone treatment treated on the starch) which are 15.34 and 1.4% respectively.

Particularly in comparison to sample A and sample E, they provide different medium of starch isolation and additional physical modifying treatment on sample E, where sample E was macerated with distilled water during starch isolation and then sample E undergoes 30 minutes of sonification. Use of chemical can leading to allowing greater water penetration in the granule (Fontes et al., 2017). Thus, the different swelling power between sample A and sample E can be theorized due to

ultrasonication on the starch where sonication can increase the swelling power and solubility (Zheng et al., 2013). Such findings from previous research were consistent with our analysis where VitAto 's swelling power and solubility for sample E was higher than that of sample A. Disintegration of starch granules is caused by the cavitation forces breaking the crystalline molecular structure and starch chains by disrupting the covalent bonds. The water molecules may subsequently bind more to free amylose and amylopectin hydroxyl groups by way of hydrogen bonding, which contributes to improved swelling power and starch solubility (Jambrak et al., 2010).

Compared to Sample B and Sample D, it shows that Sample B's swelling power and solubility is higher than Sample D, where they are differentiated by the starch isolation media; Sample B was macerated with Sodium metabisulfite, and Sample D was macerated with distilled water. A previous research have stated that usage of sodium metabisulfite resulted in a higher swelling power compared to water as an isolation medium on sweet potato (Xue et al., 2018).

Compared to Sample A and Sample C, it shows that Sample C is slightly higher for sweet potato than Sample A for swelling power and solubility, where the sample is differentiated by the starch drying method; Sample A was dried using an oven while Sample C was dried using the combination of ozone treatment and oven. The starch molecules, usually amylose, leach into the continuous phase contributing to high solubility. The semi-crystalline structure of the molecules is disrupted as starch molecules are heated in excess of water. Water molecules form hydrogen bonds in amylose and the exposed amylopectin with the hydroxyl groups, increasing the swelling power and solubility of the starch (Vanier et al., 2017).

Table 4.1: Swelling power and Percentage of solubility for of VitAto™ at different conditions.

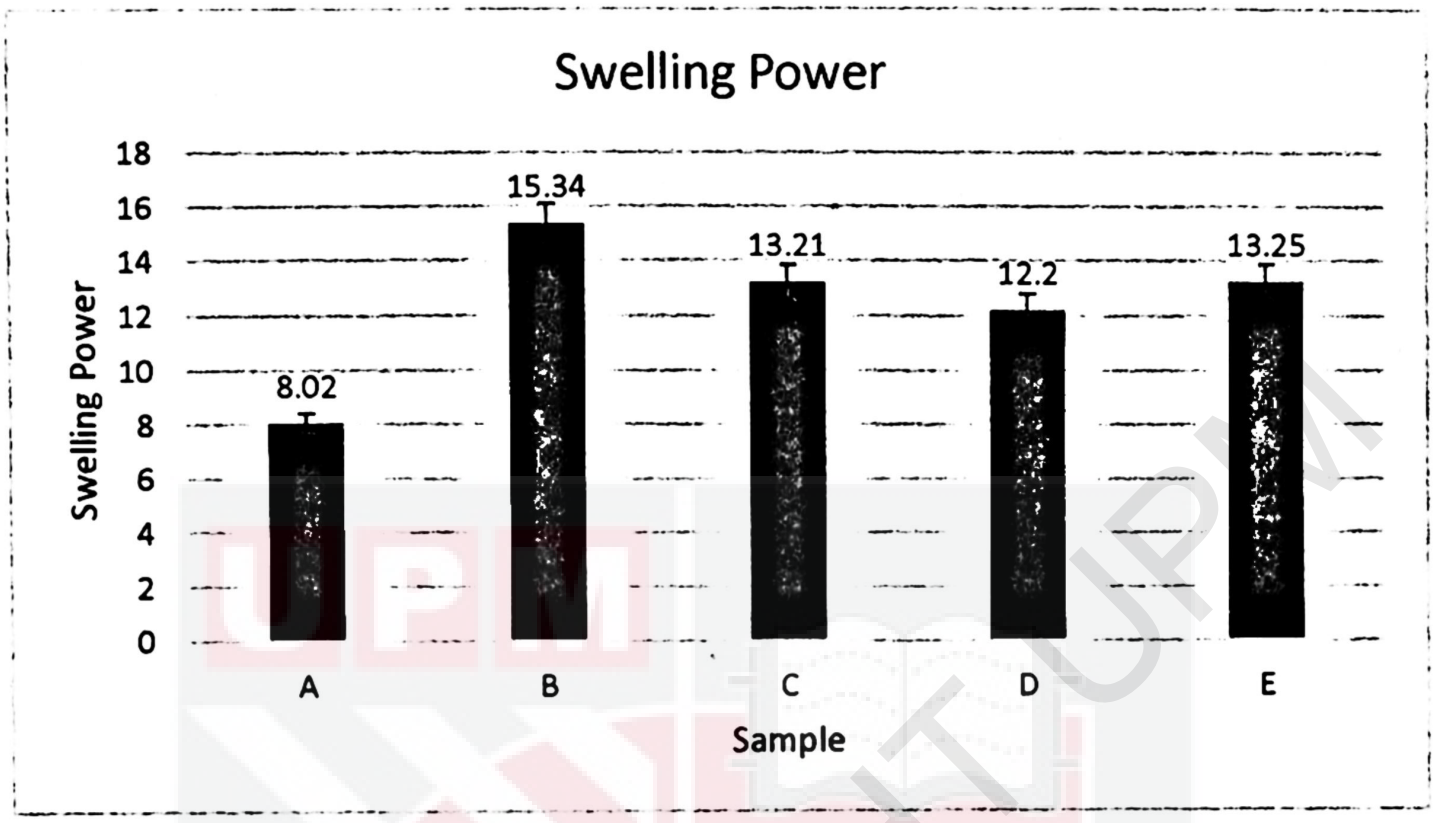


Figure 4.1: Swelling power for VitAto™ at different conditions.

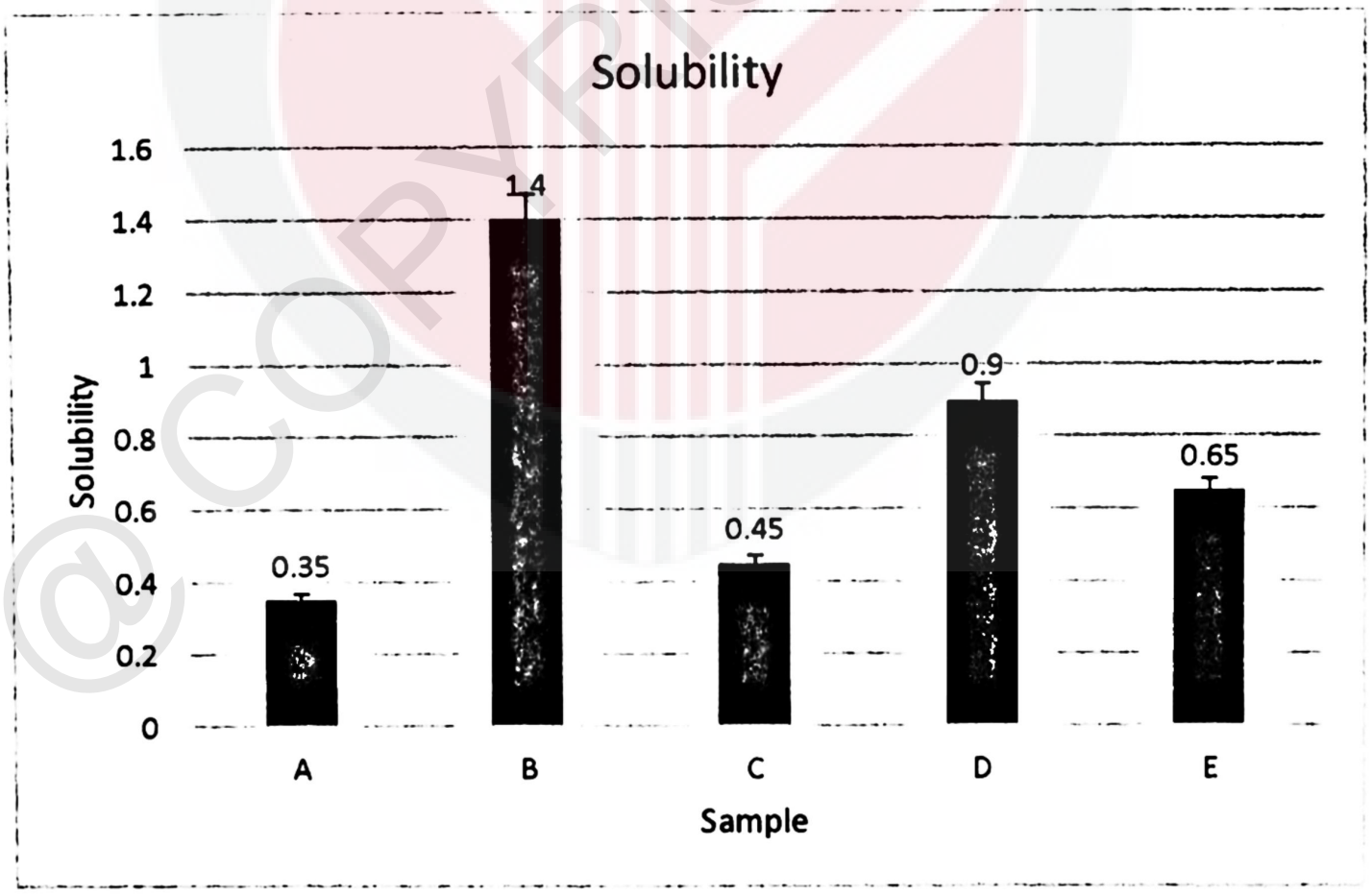


Figure 4.2: Percentage of solubility of VitAto™ at different conditions.

A- Maceration (Sodium Metabisulfite) + Oven dried 4 hours; B- Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour; C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour; D- Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour; E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours

4.2 CARBOXYL CONTENT

From Figure 4.3, where Sample A was referred as a reference to other samples, the highest carboxyl content of VitAto was obtained in Sample B, which is 0.18% where the samples were macerated with sodium metabisulfite, and then treated for 30 minutes with sonication and 4 hours with ozone oxidation. Meanwhile, the lowest carboxyl content of VitAto™ at Sample E (0.027%).

In comparison between Sample A and Sample E, they have different starch isolation media and additional physical modification treatment on Sample E, where Sample E was macerated with distilled water during starch isolation and then undergo sonication for 30 minutes. When the sonicated medium is water, H+ and -OH radicals are generated during cavitation and these highly reactive radicals may help in enhancing starch oxidation (Chong et al., 2013). According to Murphy (2000), the relatively bulky carboxyl (COOH) and carbonyl (C=O) groups are introduced at some stage in oxidation process, with partial depolymerization of the starch chains. Thus, the carboxyl content in Sample E can be correlated to the reactive radicals that enhance the starch oxidation.

In comparison between Sample B and Sample D, it shows that the carboxyl content of Sample B is higher than Sample D, where the starch isolation media that differs them; Sample B was macerated with Sodium metabisulfite and Sample D was macerated with distilled water. However, no previous studies have studied the effect of different starch isolation medium on carboxyl content of starch. A slight difference between carboxyl content on Sample B and Sample D might be due to the different rates of depolymerization of starch chains (Chan et al., 2012). In comparison between

Sample A and Sample C, they have different starch drying method where Sample A was dried using oven and Sample C was using the combination of ozone and oven drying.

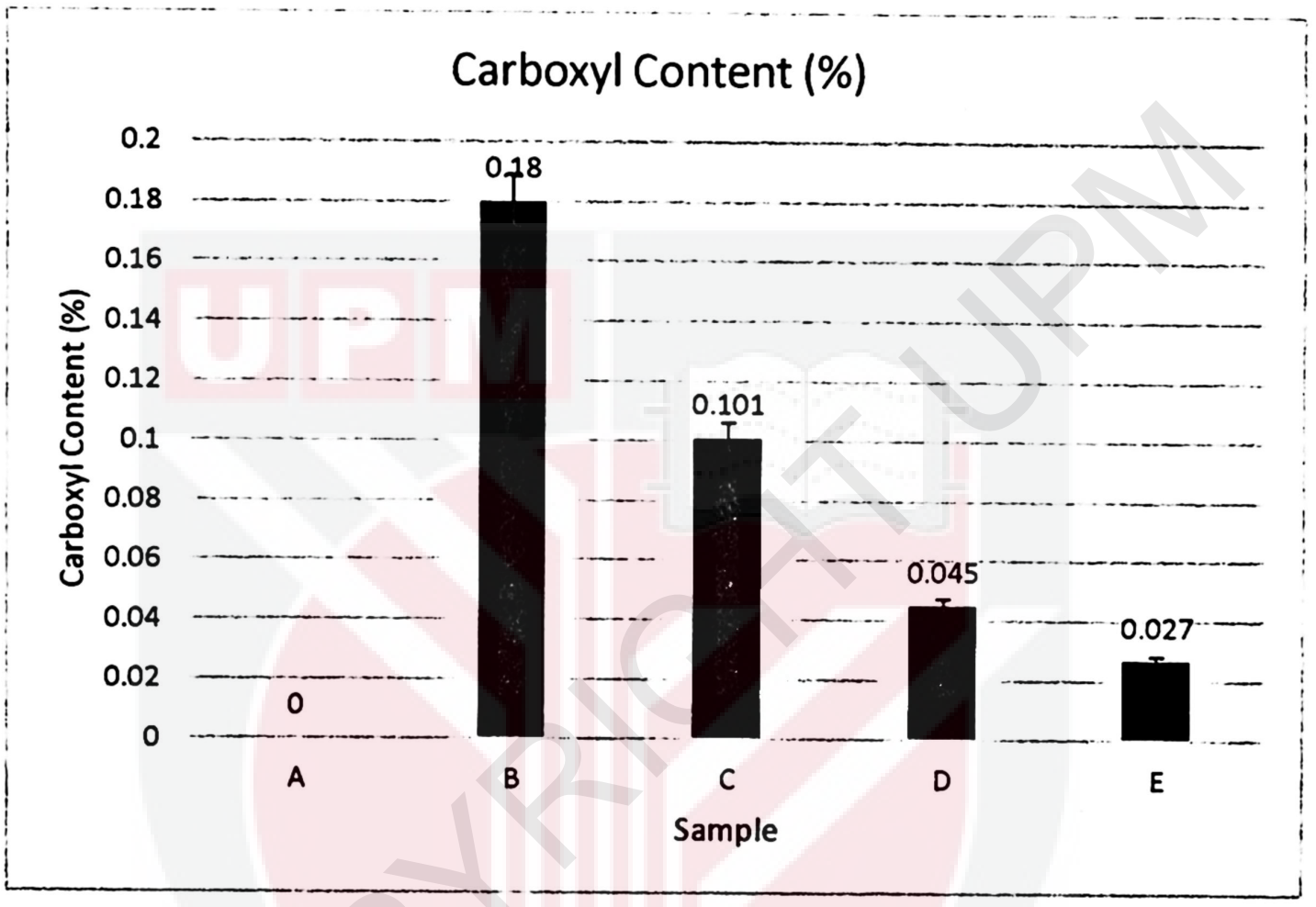


Figure 4.3: Percentage of carboxyl content for VitAto™ at different conditions

A- Maceration (Sodium Metabisulfite) + Oven dried 4 hours; B- Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour; C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour; D- Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour; E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours

4.3 AMYLOSE CONTENT

From Figure 4.4, Sample A shows the highest amylose content of VitAto™ which is 49.7% where the samples were macerated with sodium metabisulfite, and oven dried for 4 hours. Meanwhile, the lowest amylose content for VitAto™ at Sample B (42.3%). Amylose to amylopectin ratio characterizes gelatinization process, which takes place when the starch granules are heated in presence of water, and it is defined as the disintegration of molecular order within the starch granule, producing irreversible modifications (Liu, 2005). In this process, the starch granules swell and form gel particles. The swollen granules are composed via amylopectin, whereas amylose forms the continuous gel phase outside the granules.

In comparison between Sample A and Sample E, they have different starch isolation media and additional physical modification treatment on Sample E, where Sample E was macerated with distilled water during starch isolation and then undergo sonication for 30 minutes. Amylose content of VitAto™ was higher at Sample A compared to Sample E where Xue et al. (2018) stated in their study that sodium metabisulfite had no significant effect on apparent amylose content, crystalline structure, ordered degree, and lamellar structure of starches. Ultrasound primarily affects the amorphous region, while maintaining the granule's shape and size (Alcázar-Alay & Meireles, 2015).

In comparison between Sample B and Sample D, it shows that the amylose content of Sample D is slightly higher than Sample B, where the starch isolation media that differs them; Sample B was macerated with Sodium metabisulfite and Sample D was macerated with distilled water. However, the starch isolation media does not

affect the amylose content of the starch (Xue et al., 2018). The variations of amylose results between Sample B and Sample D could be due to the different structural of each sweet potato and morphological characteristics of the starch.

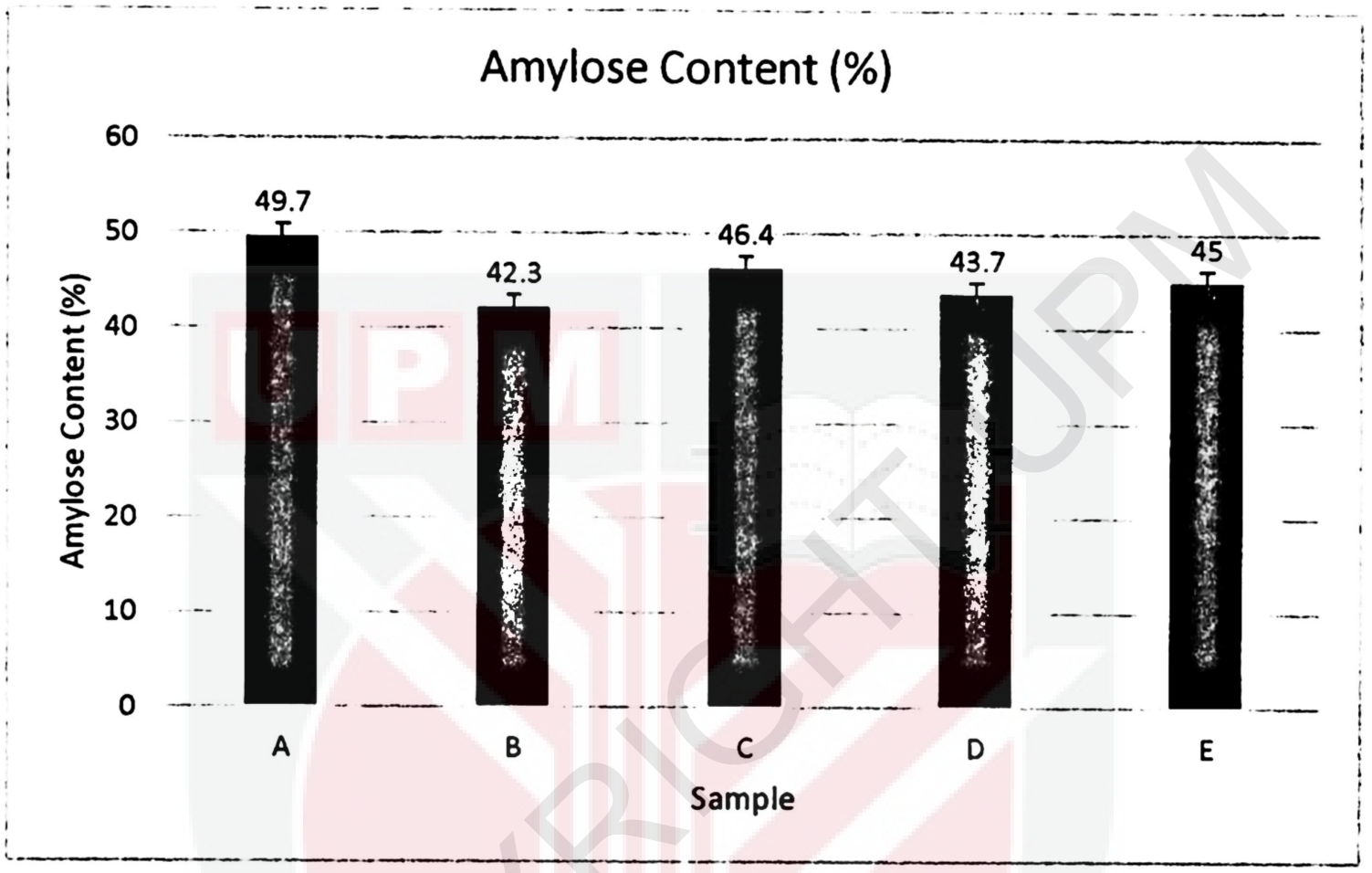


Figure 4.4: Percentage of amylose content for VitAto™ at different conditions.

A- Maceration (Sodium Metabisulfite) + Oven dried 4 hours; B- Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour; C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour; D- Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour; E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours

4.4 INTRINSIC VISCOSITY

Intrinsic viscosity is essentially a measure of the internal friction or resistance of high polymeric molecules in solution (Chan et al., 2009). Higher concentration in solution of polymeric molecules implies that the internal friction in the solution is stronger, contributing to higher intrinsic viscosity. From Figure 4.5, sample A has the highest intrinsic viscosity which is 5.067 mPa.s where the samples were macerated with sodium metabisulfite and oven drying for 4 hours. Meanwhile, sample B has the lowest intrinsic viscosity which is 4.887 mPa.s where the sample where the samples were macerated with distilled water and then treated with sonication for 30 mins and ozone-oxidation for 4 hours.

In comparison between sample A and sample E, they have different starch isolation media and additional physical modification treatment on Sample E, where Sample E was macerated with distilled water during starch isolation and then undergo sonication for 30 minutes. The intrinsic viscosity of sample A are higher than intrinsic viscosity of sample E. In comparison between sample B and sample D, it shows that the intrinsic viscosity of Sample B is lower than intrinsic viscosity of Sample D, where the starch isolation media that differs them; Sample B was macerated with Sodium metabisulfite and Sample D was macerated with distilled water. In comparison between sample A and sample C, it shows that the intrinsic viscosity of Sample A is higher than intrinsic viscosity of Sample C, where the starch drying method that differs them; Sample A was dried using oven while Sample C was dried using ozone treatment oven.

When the starch is oxidised, intrinsic viscosity decreases (Zhang et al., 2012). During extensive oxidation, the decrease in viscosity is cause by the partial cleavage of the glucosidic linkages, resulting in a decrease in starch molecular weight (Kuakpetoon & Wang, 2001). As starch is oxidized, not only the hydroxyl groups that was oxidized into carbonyl and carboxyl groups but also cleaves the carbohydrate chains (Zhang et al., 2012). Whereas, when sonication is introduced, the decrease in molecular weight decreases the amount of hydrogen bonding sites per chain contributing to a decrease in the intermolecular hydrogen bonding of polymer molecules at the same time (Cheng et al., 2002). The intrinsic viscosity decreases with ultrasonic frequency (Liu et al., 2015). Thus, sonication reduce the intrinsic viscosity of the starch.

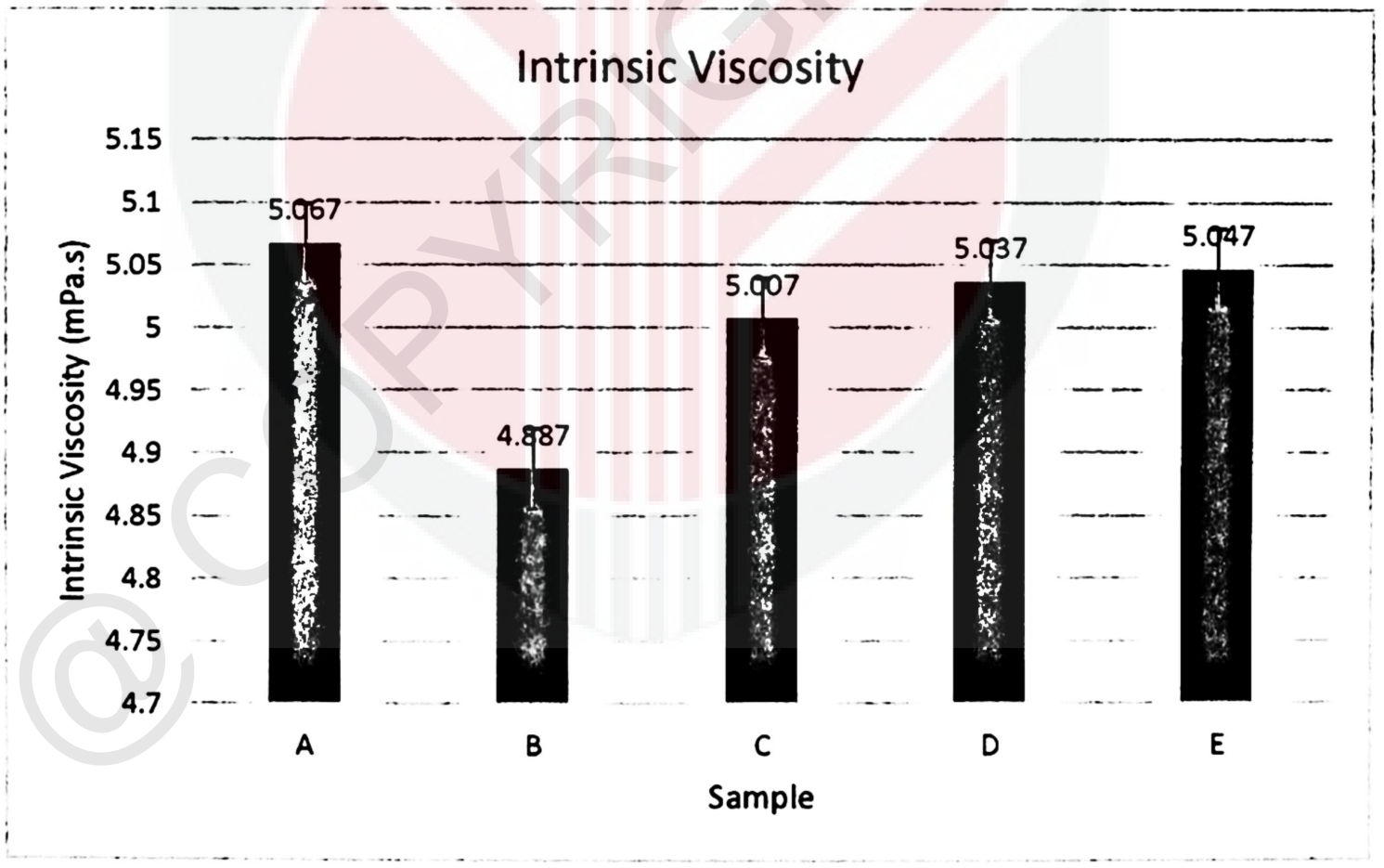


Figure 4.5: Intrinsic Viscosity for VitAto™ at different conditions.

A- Maceration (Sodium Metabisulfite) + Oven dried 4 hours; B- Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour; C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour; D- Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour; E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours

4.5 GELATINIZATION

Table 4.1 shows the temperature and enthalpy of gelatinization of VitAto™ at different conditions as measured by DSC. From the table, sample A was observed to have the highest gelatinization temperature and enthalpy where the samples were macerated with sodium metabisulfite and oven drying for 4 hours. Meanwhile, sample E has the lowest gelatinization enthalpy where the sample where the samples were macerated with distilled water and then treated with sonication for 30 mins and ozone-oxidation for 4 hours. Gelatinization temperature and enthalpy of starches depend on the microstructure and degree of crystallinity within the granules and also the granule size and the amylose-to-amylopectin ratio (Ahmad et al., 1999). During gelatinization, the crystalline structure of starch is disrupted (Vanier et al., 2017). This disturbance is triggered by relaxing hydrogen bonds and forming interactions between the water molecules and amylose and amylopectin hydroxyl groups (Vanier et al., 2017). These interactions increase the granule size, eventually leading to the rupture of granules and partial solubilization of the starch (Hoover, 2001).

In comparison between sample A and sample E, they have different starch isolation media and additional physical modification treatment on Sample E, where Sample E was macerated with distilled water during starch isolation and then undergo sonication for 30 minutes. The gelatinization temperature and enthalpy of sample A are higher than gelatinization temperature and enthalpy of sample E. Sajeev et al. (2003) reported that uses of chemical lead to deterioration of starch quality even at low concentrations that increase the gelatinization temperature and enthalpy.

In comparison between sample B and sample D, it shows that the gelatinization temperature and enthalpy of Sample B is lower than gelatinization temperature and

enthalpy of Sample D, where the starch isolation media that differs them; Sample B was macerated with Sodium metabisulfite and Sample D was macerated with distilled water. This comparison as same results as sample A and sample E where the uses of chemical lead to deterioration of starch quality even at low concentrations.

In comparison between sample A and sample C, it shows that the gelatinization temperature and enthalpy of Sample A is higher than gelatinization temperature and enthalpy of Sample C, where the starch drying method that differs them; Sample A was dried using oven while Sample C was dried using ozone treatment oven. Reduction of the gelatinization temperature was reported in oxidised VitAto™ starch. These reductions in temperature of the oxidized starches may be due to the degradation of the composition of the starch granule, contributing to the premature rupture of double helices of amylopectin (Adebowale & Lawal, 2003). The type of oxidizing agent might also affect the temperatures of transition and the enthalpy of the oxidized starch gelatinization (Vanier et al., 2017).

Table 4.1: Gelatinization of VitAto™ at different conditions

Physicochemical Properties	**Sample**	**VitAto™**			
		Onset Temperature (To) (°C)	Peak Temperature (Tp) (°C)	Conclusion Temperature (T_c) (°C)	Enthalpy (J/g)
Gelatinization	A	111.57 ± 2.053	118.85 ± 2.431	144.44 ± 2..241	1812.07 ± 2.494
	B	94.31 ± 2.341	101.89 ± 2.311	106.70 ± 2.051	1657.82 ± 2.266
	C	97.56 ± 2.124	106,81 ± 2.095	140.04 ± 2.043	1765.18 ± 2.063
	D	98.66 ± 2.013	105.85 ± 2.165	143.03 ± 2.072	1723.61 ± 2.311
	E	99.54 ± 2.034	114.52 ± 2.238	143.20 ± 2.321	1403.57 ± 2.318

A-aceration (Sodium Metabisulfite) + Oven dried 4 hours; B- Maceration (Sodium Metabisulfite) +Sonicate + Ozone 4 hours + Oven dried 1 hour; C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour; D- Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour; E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours

4.6 SYNERESIS

This process shows the removal of gels from water during refrigerated storage. Syneresis occurs by expelling or removing a substance such as water from a gel (Smitsa, 2020). Water release from cold-stored gelatinized starch is considered a starch based food problem (Simsek et al., 2012). Because of this, syneresis is an important consideration to remember when formulating refrigerated starch based foods. From Figure 4.6, sample B was observed to have the highest percentage of syneresis for all days where the samples were macerated with sodium metabisulfite and oven dried for 4 hours. Meanwhile, sample A has the lowest percentage of syneresis for all days where the sample where the samples were macerated with sodium metabisulfite and oven dried for 4 hours.

In comparison between sample A and sample E, they have different starch isolation media and additional physical modification treatment on Sample E, where Sample E was macerated with distilled water during starch isolation and then undergo sonication for 30 minutes. The percentage of syneresis of sample A are lower than gelatinization temperature and enthalpy of sample E. In comparison between sample B and sample D, it shows that the percentage of syneresis of Sample B is higher than percentage of syneresis of Sample D, where the starch isolation media that differs them; Sample B was macerated with Sodium metabisulfite and Sample D was macerated with distilled water. In comparison between sample A and sample C, it shows that the percentage of syneresis of Sample A is lower than percentage of syneresis of Sample C, where the starch drying method that differs them; Sample A was dried using oven while Sample C was dried using ozone treatment oven.

Syneresis intensity is associated with amylose content, starch chains association degree, amylopectin side chains chain length and amylose and amylopectin polymerization degree (Vanier et al., 2014). The low value of syneresis indicates slow retrogradation of starch gels due to strong interactions between amylose/amylopectin dispersed and water molecules (Liu et al., 2014). Oxidized starch shows high frozen stability where the starch structure is weak and the starch molecular weight is lower (Vanier et al., 2014). In addition, oxidation increase starch syneresis (Trinh & Dang, 2019). Hu et al. (2014) reported that increase of the syneresis after starch ultrasonication. Higher syneresis of sonicated starch gels could be associated with their weaker gel structure, with less capacity to retain water inside (Falsafi et al., 2018).

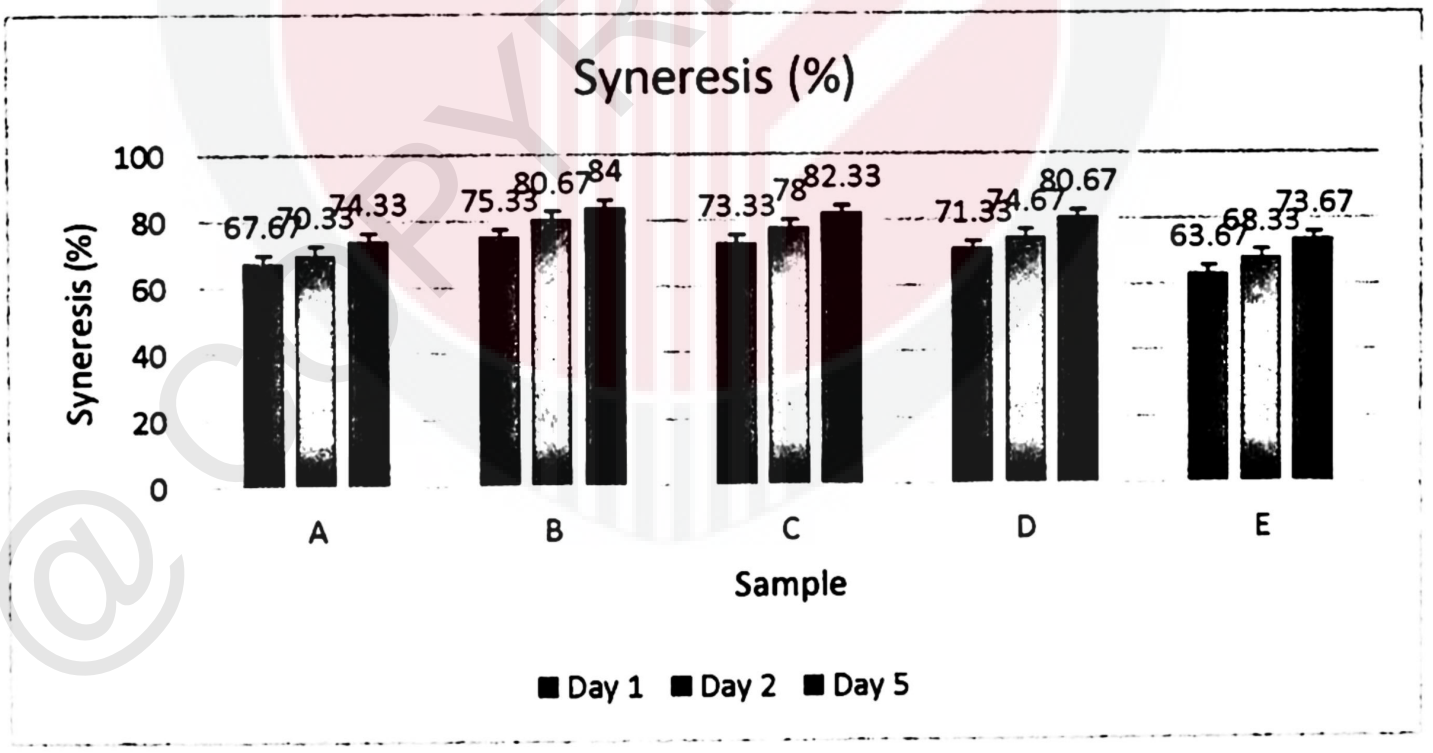


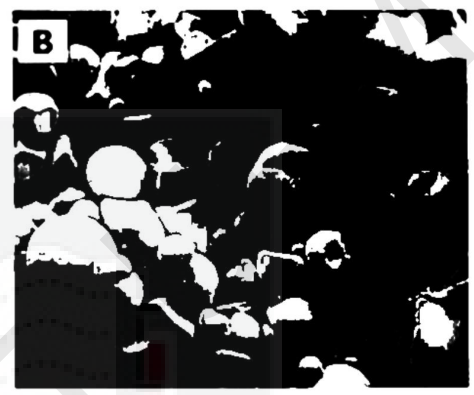
Figure 4.6: Percentage of syneresis of VitAto™ at different conditions

A- Maceration (Sodium Metabisulfite) + Oven dried 4 hours; B- Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour; C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour; D- Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour; E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours

4.7 MORPHOLOGICAL PROPERTIES

The scanning electron micrographs (SEM) of the native and ozone-oxidized sweet potato VitAto™ starch granules are shown in Table 4.7. The SEM technique was applied in order to evaluate whether the isolation media and modification method affect the surface morphology of the starch granules. The overall results showed that all methods produced the same results. The granule surface of all samples appeared to be smooth with no sign of any fissure. Zhu et al. (2011) also observed smooth granule surface of sweet potato starches without cracks. However, it highly depends on sweet potato varieties. In comparison to Sample A, where the sweet potatoes were treated with sodium metabisulfite and oven dried, the only reason behind this could be the usage of ultrasonic as maceration aids. The starch granules were expanded and collapsed openly in distilled water which acts as medium during the cavitation which lead to the breakage of starch granules. Whereas comparison between sample B and D where the difference lies in the usage of sodium metabisulfite as maceration aids (sample B) and distilled water as sonication medium (Sample D), both samples were observed to have same patterns of starch granules - definite circular shapes with even void-spaces. In comparison between Sample A and Sample C, it shows that the granule size are slightly smaller than Sample A for both sample, where the starch drying method that differs them; Sample A was dried using oven while Sample C was dried using ozone treatment oven. This might due to the better drying method where ozone have better accessibility to the granules during oxidation.

TABLE 4.2: Scanning electron micrographs of VitAto™ at 5000x magnification

Sample	Vitato™
A	
B	
C	
D	
E	

4.8 OPTIMIZATION

Table 4.3: Triplicate Data of Each Sample of Sweet Potato Starch

Std	Run	Factor 1 ATreatment	Response 1 Swelling Power	Response 2 Solubility (%)	Response 3 Viscosity (%)	Response 4 Gelatinization (J/g)
1	7	A	7.92	0.3	5.065	1814.64
2	3	B	15.14	1.4	4.907	1659.74
3	6	C	13.18	0.4	4.995	1763.42
4	2	D	12.26	0.8	5.041	1727.75
5	8	E	13.10	0.7	5.044	1401.87
6	15	A	8.12	0.4	5,062	1811.91
7	11	B	15.53	1.4	4.881	1655.32
8	13	C	13.24	0.5	5.011	1764.67
9	4	D	12.14	1.0	5.036	1723.92
10	1	E	13.40	0.6	5.048	1406.21
11	12	A	8.02	0.35	5.074	1809.66
12	9	B	15.335	1.4	4.873	1658.40
13	5	C	13.21	0.45	5.015	1767.45
14	10	D	12.20	0.9	5.034	1721.16
15	14	E	13.25	0.65	5.049	1402.63

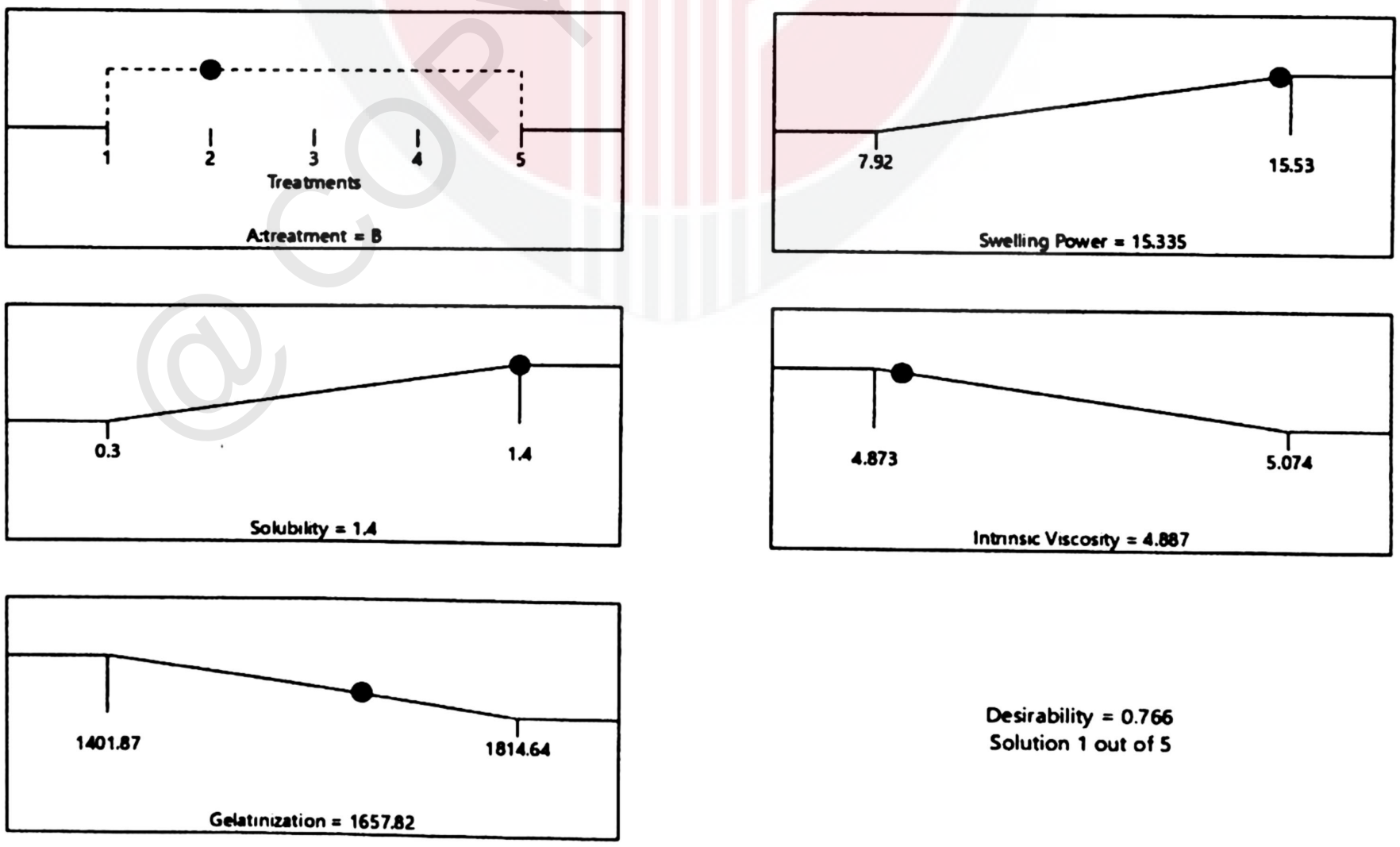


Figure 4.7: Design optimization of sample

Table 4.8 show the triplicate data of each sample of sweet potato starch. There are four responses considered to get the optimum condition to produce highest yield of oxidised starch. Figure 4.7, 4.8, 4.9 and 4.10 show the optimization of swelling power, optimization of solubility, optimization of intrinsic viscosity and optimization of gelatinization respectively. From the figures, there are two lines that showed the minimum and maximum value to get the optimum conditions and the value in the bottom is the average from the triplicate data of each sample. For swelling power and solubility, the maximum value indicate the optimum condition of the oxidised starch while for intrinsic viscosity and gelatinization, the minimum value indicate the optimum condition of the oxidised starch. From the figures, the blue circle represents the sample that have been chosen in the software as oxidised starch with the optimum conditions based on the four important responses.

From Table 4.8, the sample that represent the blue circle in the figure is Sample B with desirability is 0.766. Sample B shows the most optimum condition to produce the highest yield of oxidized starch with desirability of 0.83. Sample B was treated with maceration medium of sodium metabisulfite (0.001mole) undergo ozone oxidation (1ppm for 4 hours) and ultrasound treatment (24W power, 20kHz frequency and sonicate for 30 min). Sample B shows the optimum condition of oxidized starch in terms of swelling power, solubility, viscosity and thermal properties.

CHAPTER 5: CONCLUSION

5.1 CONCLUSION

In conclusion, oxidation via gaseous ozone and ultrasound treatment have shown positive synergistic effect to produce high yield of oxidative starch for sweet potato (VitAto™). The study also showed that the oxidised starch gave impressive results on physicochemical analysis than non-modified starch. Swelling power and solubility, amylose content, carboxyl content, intrinsic viscosity, gelatinization and morphological analysis have been conducted to determine the physicochemical effects of various macerations and drying methods on starch isolation and oxidation. Oxidation of starch in the presence of sonication degraded the starch polymer chains significantly. Ultrasound treatment was found to increase the rate of oxidation by giving high carboxyl content and low amylose content and syneresis. The modification done on the starch generally increases the starch 's properties when the starch 's swelling power and solubility increase, resulting in the starch 's better pasting properties. Furthermore, the temperature of gelatinization and enthalpy of gelatinization required for the starch also decreased as the modification has been done on the starch. However, the maceration and oxidation method do not affect the surface

characteristics of the sweet potato starch. Sodium metabisulfite was used as isolation medium because it is an establish method to produce oxidised starch and risk of health effects from chemical is negligible, since the concentration used is very low (0.001 mol). In this research, sample B shows as the highest yield of oxidised starch with desirability is 0.76 where maceration of sodium metabisulfite, ozone oxidation and ultrasound treatment are used.

5.2 RECOMMENDATION FOR FUTURE WORK

Further study should be done to investigate other extrinsic and intrinsic parameters such as temperature, and concentration of ozone treatment and ultrasound in order to study the factors that influence the highest yield of oxidised sweet potato starch.

Further investigations with relation to oxidised sweet potato starch should be done on various local species of sweet potatoes since there are inadequate studies on them. Apart from that, optimization on suitable ozone concentration and processing time without jeopardizing the nutritional value of the starch should also be considered.

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APPENDICES

Table 1: Physicochemical analysis of VitAto™ at different conditions.

Sample	Physicochemical Analysis				
	Swelling Power (g/g)	Solubility (%)	Carboxyl Content (%)	Amylose Content (%)	Intrinsic Viscosity (mPa.s)
A [Maceration (Sodium Metabisulfite) + Oven dried 4 hours]	8.02 ± 0.141	0.35 ± 0.071	0	49.7 ± 0.010	5.067 ± 0.006
B [Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour]	15.34 ± 0.276	1.4 ± 0.000	0.18 ± 0.011	42.3 ± 0.009	4.887 ± 0.002
C [Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour]	13.21 ± 0.042	0.45 ± 0.071	0.101 ± 0.006	46.4 ± 0.010	5.007 ± 0.011
D [Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour]	12.2 ± 0.085	0.9 ± 0.142	0.045 ± 0.011	43.7 ± 0.023	5.037 ± 0.018
E [Maceration (Distilled water) + Sonicate + Oven dried 4 hours]	13.25 ±0.212	0.65 ± 0.071	0.027 ± 0.002	45.0 ± 0.020	5.047 ± 0.004

Table 2: Physicochemical analysis of VitAto™ at different conditions

Sample	Physicochemical Analysis						
	Syneresis (%)			Gelatinization			
	Day 1	Day 2	Day 5	Onset Temperature (To) (°C)	Peak Temperature (Tp) (°C)	Conclusion Temperature (T_c) (°C)	Enthalpy (J/g)
A [Maceration (Sodium Metabisulfite) + Oven dried 4 hours]	63.67 ± 0.009	68.33 ± 0.011	73.67 ± 0.017	111.57 ± 2.053	118.85 ± 2.431	144.44 ± 2..241	1812.07 ± 2.494
B [Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour]	75.33 ± 0.012	80.67 ± 0.009	84.00 ± 0.006	94.31 ± 2.341	101.89 ± 2.311	106.70 ± 2.051	1657.82 ± 2.266
C [Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour]	73.33 ± 0.016	78.00 ± 0.012	82.33 ± 0.006	97.56 ± 2.124	106,81 ± 2.095	140.04 ± 2.043	1765.18 ± 2.063
D [Maceration (Distilled Water) + Sonicate + Ozone 4 hours + Oven dried 1 hour]	71.33 ± 0.010	74.67 ± 0.073	80.67 ± 0.011	98.66 ± 2.013	105.85 ± 2.165	143.03 ± 2.072	1723.61 ± 2.311
E [Maceration (Distilled water) + Sonicate + Oven dried 4 hours]	67.67 ± 0.006	70.33 ± 0.022	74.33 ± 0.009	99.54 ± 2.034	114.52 ± 2.238	143.20 ± 2.321	1403.57 ± 2.318