
**UNIVERSITI PUTRA MALAYSIA**

***DEVELOPMENT OF OXIDATIVE STARCH OF SWEET POTATO (VAR ANGGUN) VIA HURDLE TECHNOLOGIES***

**NURULAMIRA SABRI**

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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 in this study. Ozone has the ability to elevate the thermodynamic oxidation potential, hence, the reactions can be performed at low temperatures. Whereas, ultrasonic is a physical method that able to mutilate the crystalline region in the starch granules resulting in the obliteration of the granular structures, which contributes to an ideal method to develop oxidized sweet potato starch. Sweet potato (var Anggun) was used in this study because it has the ability to replace other sources of starch utilized in industry as the production supply of Anggun in Malaysia is over the demand. 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 further used as an emulsifier, gum arabic replacer, and binding agent in the food industry.

# ABSTRAK

Kanji beroksidasi adalah gantian kanji yang disediakan dengan merawat kanji asli melalui kaedah fizikal dan/atau kimia untuk mengubah penciriannya. Walau bagaimanapun, pengoksidaan kimia mungkin menyebabkan hasil kanji teroksidasi rendah kerana kehilangan molekul kecil yang dihasilkan oleh pemecahan kanji dan menghasilkan air sisa yang mengandungi kepekatan garam yang tinggi, menyebabkan masalah pembuangan air sisa. Atas sebab-sebab itu, pengoksidaan kanji melalui ultrasonik digabungkan dengan rawatan ozon dicadangkan dalam kajian ini. Ozon memiliki kemampuan untuk meningkatkan potensi pengoksidaan termodinamik, oleh itu, tindak balas dapat dilakukan pada suhu rendah. Manakala, ultrasonik adalah kaedah fizikal yang dapat memutilasi kawasan kristal dalam butiran kanji yang mengakibatkan penghapusan struktur butiran, yang menyumbang kepada kaedah yang ideal untuk mengembangkan kanji ubi jalar teroksidasi. Ubi jalar (var Anggun) digunakan dalam kajian ini kerana ia mempunyai kemampuan untuk menggantikan sumber kanji lain yang digunakan dalam industri kerana bekalan pengeluaran Anggun di Malaysia melebihi permintaan. Ciri-ciri fizikokimia seperti kelarutan, 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 teroksidasi yang diperoleh dapat digunakan sebagai pengemulsi, pengganti gusi arab, dan agen pengikat dalam industri makanan.

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## LIST OF ABBREVIATION & ACRONYMS

| Abbreviations | Acronyms |
|---|---|
| FAO | Food and Agriculture Organization |
| GRAS | Generally recognized as Safe |
| FDA | Food and Drug Administration |
| USFDA | United States Food and Drug Administration |
| OGTs | Ozone generation times |
| OFAT | One Factor At A Time |
| DSC | Differential Scanning Calorimeter |
| SEM | Scanning Electron Microscope |
| $T_o$ | Onset temperature |
| $T_p$ | Peak temperature |
| $T_c$ | End temperature |
| $T_g$ | Gelatinization temperature |
| ppm | Parts per million |
| $O_2$ | Oxygen |
| $O_3$ | Ozone |
| $Na_2S_2O_5$ | Sodium metabisulfite |
| HCl | Hydrochloric Acid |
| NaOH | Sodium Hydroxide |
| C=O | Carbonyl groups |
| COOH | Carboxyl groups |
| $H^+$ | Hydrogen ion |

| | |
|---|---|
| $OH^-$ | Hydroxide |
| $\Delta H$ | Enthalpy of gelatinization |

# CHAPTER 1

# INTRODUCTION

## 1.1 Overview

Sweet potato *(Ipomoea batatas L.)* is a dicot perennial plant belonging to the *Convolvulaceae* family which has high resistance to drought and high moisture, as well as pests and diseases (Xie et al., 2012). Sweet potato is considered as a major staple crop especially in developing countries due to its high yielding potential and wide adaptability. It is one of the root tubers that has high starch content and has an excellent source of energy with various valuable by-products such as carbohydrates, $\beta$-carotene, dietary fibre and minerals. In addition, the purple-flesh sweet potato also rich in anthocyanin and have a strong antioxidant activity for health purposes (Oki et al., 2002). There are varieties of sweet potato that may differ in its flesh colour, ranges from beige to white, red, pink, violet, purple, yellow and orange (Yusoff et al., 2018). The starch of sweet potato is composed of two main glucose polymers which are linear amylose and highly branched amylopectin (Vanier et al., 2017). Normally, 20 – 30% of amylose and 70 – 80% of amylopectin were found in starch harvested products, and this amylose to amylopectin ratio are significant in determining starch physicochemical properties (Schwall et al., 2000). According to Tan et al. (2010), the

annual production of sweet potato in Malaysia was about 2000 ha/yr. For many years, starch has been widely used in food industries such as snacks, sauces and soups, and non-food industry like pharmaceuticals, textiles and coatings.

Modification of starch has added approximately an evolution of latest innovation and marketplace trends. These highly functional derivatives have been adapted to generate a competitive gain in a new product, enhance product aesthetics, lower the production costs and prolong shelf-life whilst clearly making starch relevant in all stages of a food product's lifecycle. Modification of starch is carried out to overcome the shortcomings of the native starch and to perform the distinct properties required for various industrial applications. Physical modifications apply the friction, collision, shear and other mechanical actions to alter the molecular structure and properties of starch. Physical modification includes ultrasound treatment, annealing, ultra-high pressure treatment, microwave etc. It can be safely used in modification process of food products as it does not involve any chemical reactions by the food. Meanwhile, chemical modification involves acid or base at high concentration to destroy hydrogen bond intermolecular interactions and crystallization areas of the starch. Chemical modification includes oxidation, cross-linking, esterification etc. Starch was modified physically or chemically to produce a heat stable or resistant heat product, stable during freeze-thaw process and simply dissolve in hot or cold suspension (Syahariza & Yong, 2017).

One of the common chemical modification that has been applied on starch was oxidation process. Oxidation of starch is done by reacting the starch with a specified amount of oxidizing reagent under controlled temperature and pH. The hydroxyl groups of starch molecules are oxidised to carbonyl and carboxyl groups during starch oxidation process (Wang & Wang, 2003). The oxidizing agent penetrates deeply into

the amorphous regions and slightly affect the crystalline regions of the starch (Zia-ud-Din, Xiong & Fei, 2017). The main oxidizing reagents used in oxidation of starch are sodium hypochlorite and hydrogen peroxide, but ammonium persulfate, sodium bromate, sodium and potassium permanganate and ozone gas have also been utilized (Dias, Elias, Oliveira, & Helbig, 2007; Wang & Wang, 2003; Zhang, Zhang, Wang, Chen, & Wang, 2009). The oxidation reaction causing the loosening of intermolecular bonds and partial depolymerisation of the starch polymer chains.

Ultrasound treatment is a physical method for modification of starch that has many advantages such as less usage of chemicals and processing time and environment - friendly processing (Krishnakumar and Sajeev, 2017). Ultrasound creates compressions and depressions of the medium particles when propagated through a biological structure and a large amount of energy can be imparted. Ultrasound mainly affects the amorphous region while maintaining its granular shape and size. According to Luo et al. (2008), the starch surface becomes porous and some properties of starch are modified such as the swelling capacity, solubility and viscosity of the paste. Zuo et al. (2009) reported that ultrasound modification depends on the frequency, temperature, process time and the starch suspension properties. 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. Ultrasound treatment is useful to modify the functionality of starch in terms of physicochemical and functional properties (Luo et al., 2008).

## 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. Native starches are prone to undergo retrogradation process and high gelling tendency of pastes besides easily undergoing syneresis (Kaur & Karim, 2012). Therefore, starch modification not only decreases retrogradation, gelling tendencies of pastes and gel syneresis but also improves paste clarity and sheen, paste and gel texture, film formation and adhesion (BeMiller, 1997).

The molecular structures of amylose and amylopectin in the starches are modified via physical, chemical or enzymatic method. Different modification methods result in different properties of starch. The common chemical methods used to modify the native starch properties are acetylation, cross-linking, oxidation and etherification. However, there has been inadequacy of chemical modification as this type of modification may arise some issues concerning consumers and environment.

At the moment, there is no specific method to measure gaseous ozone ($O_3$) concentration in sweet potato starch. Most industries were utilizing the chemical modification of starch using the sodium hypochlorite, hydrogen peroxide and sodium periodate. On the other hand, the gaseous ozone treatment as a mean of improving the starch characteristics in food application is still new and now well-known among the customers. Past studies have shown good effects of gaseous ozone treatment in food application in terms of gelatinization, better coating properties, low retrogradation etc. However, consumers' acceptance on gaseous ozone treatment is still in deliberate. Thus, this study focuses on the reaction of gaseous ozone to be treated on sweet potato starch and how it affects the physicochemical and quality characteristics of the modified starch. With that, hopefully, it can convince the consumers that ozone

treatment would therefore be a good alternative to food products as ozone does not leave residue compared to other chemical oxidizing agent.

Ultrasound treatment is classified as one of the methods for physical modification on starch. It defines the sound waves at high frequency above the threshold of human ear which is above 15kHz. Zuo et al. (2009) stated that the results of ultrasound treatment on starch granules depends on many factors such as the starch dispersions, power and frequency of sonicator, temperature and time of the treatment. It is normally cited that the cells of the vegetal material will break and cells' substance will leach out into the extraction medium because of the impact of ultrasonic waves, however Toma et al. (2001) have discovered no announced proof yet. It is remarkable that an ultrasonic cell disrupter is a sufficient device for the interruption of vegetal cells, yet again no detailed data exists for the impact of ultrasound on vegetal tissues.

Sweet potato ranks as the world's seventh most vital food crop after rice, wheat, cassava, potato, maize and barley (Food and Agriculture Organization of the United Nations [FAO], 2009). Furthermore, sweet potato (*Ipomoea batatas*) ranks second among other tuber crops in Malaysia after cassava. It covers about 2000 ha/year of its plantation in Malaysia (Tan et al.,2010) which are mostly found in the states of Perak, Kelantan and Terengganu. Sweet potato (*Ipomoea batatas*) has a high nutritional value due to the presence of anthocyanin pigment derived from the purple-coloured flesh of the sweet potato. Moreover, peonidins and cyanidins in the anthocyanin pigment are good for digestion purposes as they have an anti-oxidant and anti-inflammation properties. Thus, it will be suitable to produce an oxidized starch with some functional properties.

## 1.3 Objectives

The objectives of this study are:

a) To compare the physicochemical effects of macerations and physical/chemical modification on modified starch.

b) To determine the optimum condition to produce the highest yield of oxidized starch.

## 1.4 Scope of Study

The scope of this study is limited to sweet potato variety Anggun. This study also limited to the gaseous ozone and ultrasound oxidation. Gaseous ozone is considered as chemical modification on starch, while ultrasound is physical modification done towards the starch. The concentration of gaseous ozone used in this study is 1 ppm only. The ultrasound treatment done on the starch using the VCX probe was applied at 40% amplitude at a frequency of 20kHz. Other types of modifications does not involved in this study. Other than that, the maceration medium used in this study is limited only to sodium metabisulfite and distilled water. Other chemicals or reagent do not used as the maceration medium.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Sweet potato

Sweet potato (*Ipomoea Batatas L*) is a vital food crop in several parts of the globe and it is being cultivated in more than 100 countries. It belongs to the *convolvulacae* family which are highly nutritious and effectively edible. Sweet potato is also a simple-to-develop crop with great flexibility in different natural conditions which has high yielding capacity and high energy content (Ravindran et al., 1995). Survey reports found that Nigeria is the top producer of sweet potato in Africa with annual output production of 3.56 million metric tonnes, making it second world producer with China taking the lead (FAO, 2003). Figure 2.1 shows sweet potato variety Anggun which is purple in colour.

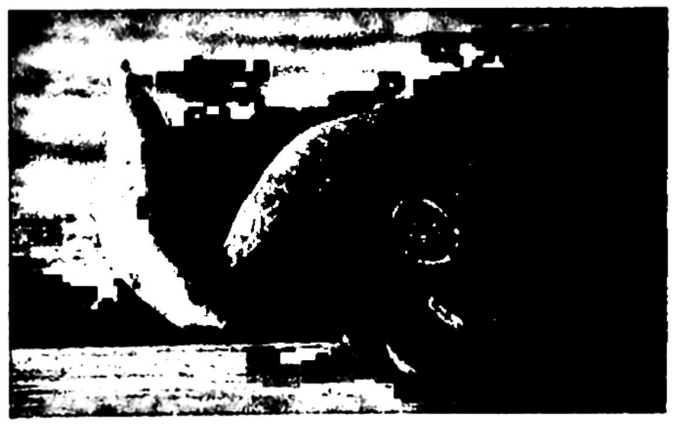


**Figure 2.1.** Sweet Potato (Anggun)

During post-harvest, the tubers are best to keep around 29°C and 85% humidity for 4 to 20 days. These storage conditions can optimize healing of the sweet potato after harvesting. During storage, the sugar content of the sweet potato is increased and thus making the starch content is decreased (Ellong et al., 2014). Sweet potato has an advantage of being high starch production up to 30-50% greater starch yield than rice, corn and wheat starch (Rahman et al., 2003). Starch flour is more stable intermediate product, which can minimize the post-harvest losses and increase the utilization of the sweet potato (Eleazu at al., 2013). The flour can be utilized as a pre-material for manufacturing of juice, bread, sweets, noodles, snacks, dough and alcohol (Eleazu and Ironua, 2013). Sweet potato variety Anggun has an abundant content of dietary fiber, minerals, vitamins and antioxidants, as well as anthocyanins, phenolic acids, beta-carotene and tocopherol (Bengtssona et al., 2008; Kim et al., 2007; Van Jaarsveld et al., 2006; Yildirim et al., 2011). Table 2.1 shows the previous research studies on properties on starch, which can be used as reference to analyze the physicochemical properties of sweet potato starch in this study. Whereas, Table 2.2 shows the chemical composition of purple flesh sweet potato.

**Table 2.1.** Previous research studies on properties of starch

| References | Studies |
|---|---|
| Eleazu, C. O., & Ironua, C. (2013) | Physicochemical composition and antioxidant properties of a sweet potato variety (*Ipomoea batatas L*) commercially sold in South Eastern Nigeria. |
| Ellong, E. N., Billard, C., & Adenet, S. (2014). | Comparison of physicochemical, organoleptic and nutritional abilities of eight sweet potato (*Ipomoea batatas*) varieties. |

| | |
|---|---|
| Jambrak, A. R., Herceg, Z., Šubarić, D., Babić, J., Brnčić, M., Brnčić, S. R., & Gelo, J. (2010). | Ultrasound effect on physical properties of corn starch. |
| Kaur, M., Oberoi, D. P. S., Sogi, D. S., & Gill, B. S. (2011). | Physicochemical, morphological and pasting properties of acid treated starches from different botanical sources. |
| Krishnakumar, T., & Sajeev, M. S. (2018). | Effect of ultrasound treatment on physicochemical and functional properties of cassava starch. |
| Oladebeye, A. O., Oshodi, A. A., Amoo, I. A., & Abd Karim, A. (2013). | Functional, thermal and molecular behaviours of ozone-oxidised cocoyam and yam starches. |
| Sandhu, K. S., & Singh, N. (2007). | Some properties of corn starches II: Physicochemical, gelatinization, retrogradation, pasting and gel textural properties. |
| Simsek, S., Ovando-Martínez, M., Whitney, K., & Bello-Pérez, L. A. (2012). | Effect of acetylation, oxidation and annealing on physicochemical properties of bean starch. |

**Table 2.2.** Proximate Composition (%) of Purple Flesh Sweet Potato

| Content (%) | Purple Flesh Sweet Potato |
|---|---|
| **Moisture** | 12.65 ± 0.07 |
| **Protein** | 0.10 ± 0.01 |
| **Lipid** | 0.5 ± 0.15 |
| **Ash** | 0.10 ± 0.01 |
| **Amylose** | 33.76 ± 0.89 |

Source: (Lee & Lee, 2017).

## 2.2 Starch

Starch is the main source of energy in human diet. Starch is gaining the greatest attention as compared to other carbohydrate polymers due to its practicality in

application of food products (Santoso, 2018). Breakfast cereals, bread, cookies, pasta, pastries and noodles are among the most common starch containing products available in Malaysia. Typically starches are added to enhance the quality of products in food industries. The ability of starch in nature to react with itself or other compounds leads to several usage in food industries such as thickener, gelling agent, stabilizing agent or as filler (Gilbert, Witt & Hasjim, 2013). Different starches source fulfilling numerous industrial demands. Therefore, Table 2.3 shows commercially available starch and its functions reported by previous studies between 2012 and 2017.

**Table 2.3.** Commercially available starch and its functions.

| Starch source | Functions |
|---|---|
| Corn | Increased fibre content in cake production.<br><br>Corn syrup production.<br><br>Thickener in infant formula. |
| Wheat | Gelling properties |
| Potato | Pasting and gelling properties.<br><br>Production of potato starch film |
| Tapioca/ Cassava | Thickener in fruit filling |
| Rice | Emulsion stabiliser.<br><br>Improved gel properties. |

Source: Yazid et. al (2018)

Starch is the most abundant carbohydrate found in plants and stored naturally in the plant cell in the form of granule. Potato, tapioca and wheat are the main available source of commercial starches in the market. Starch products are recently have been utilized for various applications in food manufacturing industries to accomplish specific technological properties such as solubility, viscosity, swelling and pasting

properties. Starch compound is made up of two main components which are amylose and amylopectin. Normally, a starch molecule is composed of 20% to 25% of amylose and 75% to 80% of amylopectin. The ratio of amylose to amylopectin molecules and the size of starch granules are depends on the source of starch, either plant or tuber (Jambrak et al., 2010). Low molecular weight with several long branches belongs to amylose, whereas a vast number of short branches and highly branched polymers belong to amylopectin (Syahariza et. al, 2013). Moreover, amylose and amylopectin are found in the form of water soluble lamellae and semi-crystalline structure within the starch granule (Ahmed et. al, 2016).

Starch has wide applications in food industries. However, starch in native state is unable to attain specific industrial requirement. Thus, modifications can be made either physically or chemically. Modification of starch using chemical method is the most frequently used method in order to improve starch pasting properties and shelf life extension (Zhang et. al, 2017). Starch processing quality can be improved by altering pasting, gelatinizing and retrogradation properties of starch (Park et. al, 2018). Commercialized starch such as potato and other type of cereals are normally undergo physical modification or simple chemical modification to enable them to be used by food or other industry (Waliszewski et. al, 2003). Starch was modified physically or chemically in order to obtain heat stable or resistance towards heat product, stable during freeze-thaw process and easily dissolve either in hot or cold suspension (Syahariza & Yong, 2017). The most common function of modified starch in food industry are as agent of gelling, stabilising and thickening.

Upon heating within the sight of abundance water, starch granules lose their crystallinity, retain a lot of water, and leach out amylose, which confer consistency to the starch or water framework (Che et al., 2007b, Che et al., 2007a, Evans and

Haisman, 1979). When heat is applied to the starch, it undergoes gelatinization where the molecular structure within the starch granule is broken down and results in irreversible changes in properties such as granule swelling, native crystallite melting, and starch solubilization. Previous research results reported that starches can be gelatinized by high temperature at room temperature (Maaruf et al., 2001). Figure 2.2 shows the difference in scanning electron microscopy photographs of sweet potato starch by acid modification method.

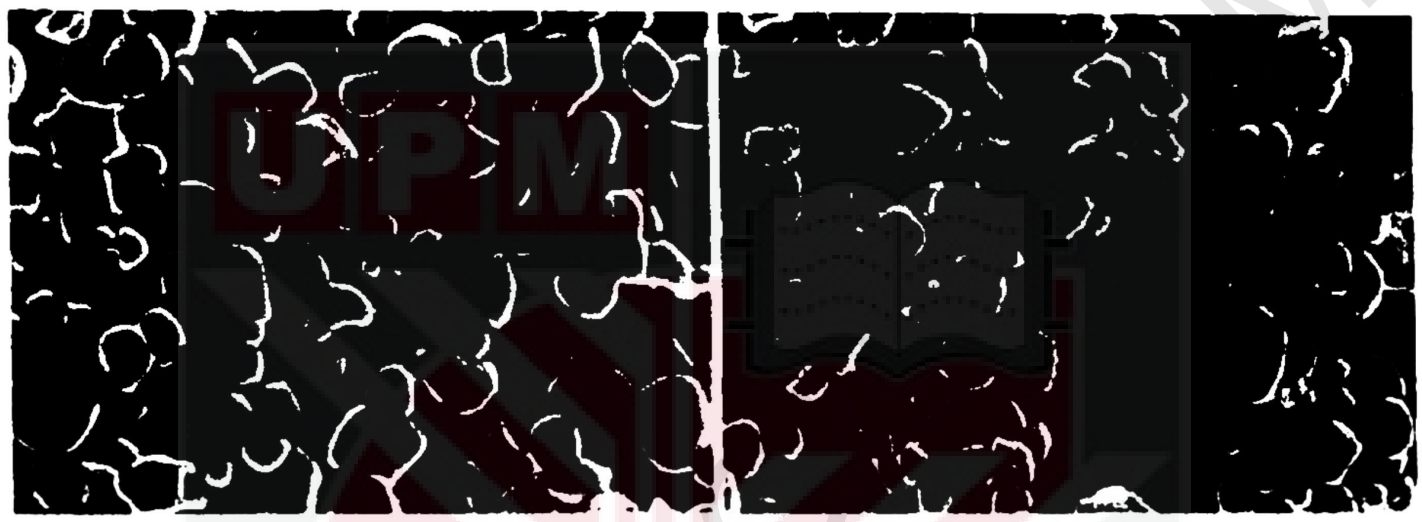


**Figure 2.2.** Scanning electron microscopy photographs of sweet potato starch (Left- Native starch, Right- Acid modified starch)

## 2.3 Ultrasound Treatment

Ultrasound is a non-thermal processing method that produce high frequency of sound waves above the threshold of the human ear (above 18 kHz). It has been utilized in 10 years ago and the application of power ultrasound offers the opportunity to modify and improve some technologically important compounds which are often used in food products. Ultrasound is an alternative method to prevent the utilization of chemical solvents during the extraction of sweet potato starch. Ultrasound treatment also is a structured procedure for the reduction of molecular weight of both polysaccharides; amylose and amylopectin (Jambrak et al., 2010). Recent study

reported that ultrasound is gaining recognition due to its various benefits such as low cost, efficient and eco- friendly as no chemical usage involved as compared to other chemical modification method (Li, Li & Zu, 2018).

Ultrasound is commonly divided into three regions of frequency which are the region from 16 to 100 kHz (1 Hz is 1 cycle/s) is known as power ultrasound, 100 kHz to 1 MHz is a high-frequency ultrasound and 1 to 10 MHz is known as diagnostic ultrasound. Ultrasound is produced by high-energy vibrations from piezoelectric or magneto-strictive transducers. The vibrations are then intensified and move to a sonotrode or probe, which is in direct contact with the fluid. The ultrasonic procedure has been established to be relevant to numerous sorts of starches (corn, potato, custard, and sweet potato) and polysaccharides (Iida et al., 2008).

The potential advantages of ultrasound in extraction are mass transfer intensification, cell disturbance, improved penetration and capillary effects. Toma et al. (2001) reported that the volume of extractable compounds from fennel, hops, marigold, mint and lime were increased around 20–40% by ultrasound extraction in contrasts with the traditional extraction technique.

The breakdown of cavitation bubbles close to cell wall is required to deliver cell disturbance together with a decent penetration of the dissolvable into the cells, through the ultrasonic jet. Ultrasound disruption towards the starch granule causing the higher facility for water entrance in a corn starch granule and leads to a higher water uptake and retention (Kim et al., 2006, Sandhu and Singh, 2007).

According to Luo et al. (2008), who sonicated maize starches of different amylose content, the ultrasonic treatment attacks mostly on the amorphous region of starch granule. The structure and size of the starch granule remain identical as before

the treatment. However, the surface of the starch turns out to be more porous and it does change the physicochemical structure of the starch such as solubility, swelling power and pasting properties. Table 2.4 shows the degree of granule disintegration and the degree of solubilisation of different types of starch samples at different sonication time.

**Table 2.4.** The degree of granule disintegration and the degree of solubilisation at different sonication time

| Samples | Sonication time (min) | Degree of granule disintegration | | | Degree of solubilization | | |
|---|---|---|---|---|---|---|---|
| | | 65 °C | 75 °C | 85 °C | 65 °C | 75 °C | 85 °C |
| Waxy maize | 0 | 3.5 | 25.7 | 22.6 | 0.004 | 0.282 | 0.483 |
| | 1 | 2.9 | 0.6 | 0.5 | 0.115 | 0.965 | 0.941 |
| | 5 | 2.5 | 0.5 | 0.4 | 0.161 | 0.993 | 0.956 |
| Potato | 0 | 28.8 | 104.5 | 110.2 | 0.084 | 0.000 | 0.000 |
| | 1 | 2.9 | 1.8 | 2.3 | 0.630 | 0.894 | 0.908 |
| | 5 | 1.1 | 0.5 | 1.2 | 0.710 | 0.939 | 0.942 |
| Tapioca | 0 | 22.0 | 26.9 | 31.0 | 0.130 | 0.171 | 0.241 |
| | 1 | 1.7 | 0.8 | 0.8 | 0.806 | 0.962 | 0.965 |
| | 5 | 0.6 | 0.5 | 0.8 | 0.843 | 0.993 | 1.027 |
| Sweet potato | 0 | 2.5 | 6.5 | 19.7 | 0.010 | 0.056 | 0.153 |
| | 1 | 2.1 | 2.4 | 0.5 | 0.021 | 0.388 | 0.911 |
| | 5 | 2.0 | 1.7 | 0.3 | 0.037 | 0.437 | 0.909 |
| Corn | 0 | 4.4 | 9.1 | 9.5 | 0.025 | 0.053 | 0.089 |
| | 1 | 4.7 | 6.4 | 7.7 | 0.027 | 0.093 | 0.220 |
| | 5 | 4.2 | 4.8 | 3.1 | 0.039 | 0.451 | 0.718 |

Source: Iida et al. (2008)

## 2.3 Gaseous Ozone

Ozone is generated by breaking down the oxygen molecules, releasing oxygen fragments and combines with other oxygen molecules to produce ozone ($O_3$). In other words, the bond between oxygen molecules are splits into two oxygen radicals which then rapidly reacts with another available $O_2$ to form a very reactive ozone using high energy input. However, ozone is an unstable gas that transforms to oxygen which will leave no residual contamination to environment. Ozone acts as an oxidizing agent that has been utilized in natural water treatment, washing and disinfecting of fruits and vegetables. In recent years, starch modified by oxidation has had great use in the food industry to form adherent surfaces and coatings (Lawal et al., 2005). Meanwhile, dual starch modification which involves the combination of chemical and physical agents (e.g., oxidation assisted by ultrasound treatment) had great use in the food industry as emulsifiers, agglutinants and thickeners.

Research on the use of ozone has increased as ozone was declared as a GRAS product (Generally recognized as Safe) by a team of experts from FDA (Habibi & Haddad, 2009). Ozone has been broadly utilized and known as powerful disinfectant for application in food industries. Ozone also refers to a powerful broad-spectrum antimicrobial specialist against parasites, microbes, infections, protozoa, bacterial and spores growth (Muthukumaparappan at al., 2000). In 2001, United States Food and Drug Administration (USFDA) has established that gaseous and aqueous ozone treatment as an antimicrobial agent for treatment, storage and processing of food (Khadre et al., 2001).

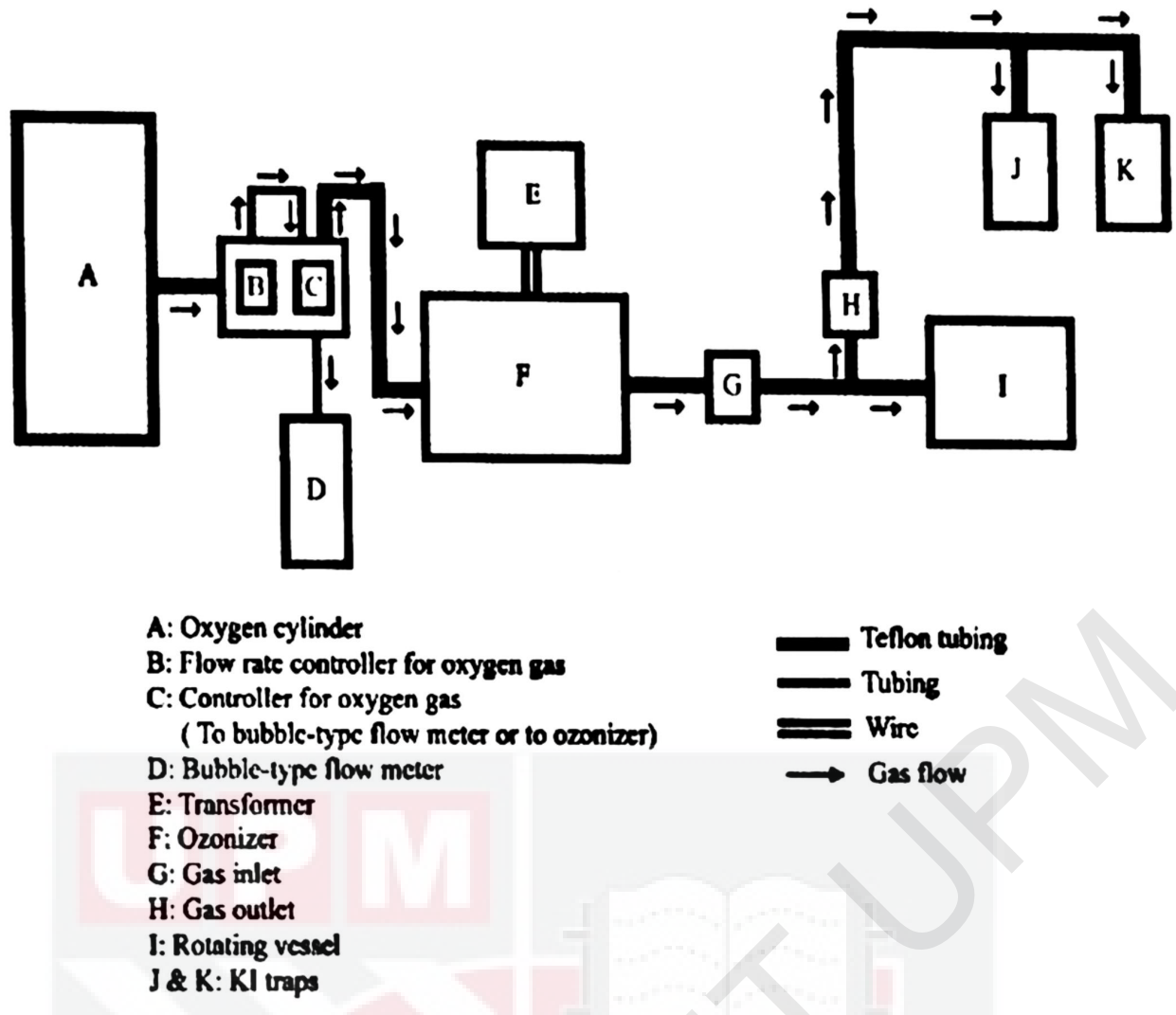



**Figure 2.3.** Schematic diagram for the setup of ozone oxidation system

According to the experimental work by Chan et al. (2009) as shown in Figure 2.3, they set up an experiment to study the effects of oxidation by ozone gas on some physicochemical and functional properties of starch (corn, sago, and tapioca) at different ozone generation times (OGTs). It is proven in Table 2.5 as the OGTs increase, the amount of ozone reacted (mmol) with the starch also increase.

**Table 2.5.** Amount of Ozone Reacted with Corn, Sago, and Tapioca Starches

| | starch | | |
|---|---|---|---|
| OGT (min) | corn | sago | tapioca |
| 1 | 0.47 ± 0.03 d | 0.39 ± 0.04 c | 0.41 ± 0.03 c |
| 3 | 0.62 ± 0.02 c | 0.67 ± 0.11 b | 0.58 ± 0.02 b |
| 5 | 0.71 ± 0.04 b | 0.76 ± 0.05 b | 0.66 ± 0.08 b |
| 10 | 1.30 ± 0.04 a | 1.37 ± 0.24 a | 0.99 ± 0.09 a |

[a] Results are expressed as means ± standard deviations ($n$ = 3). Values in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

## 2.4 Carboxyl Content

The relatively bulky carboxyl and carbonyl groups are introduced together upon oxidation, with partial depolymerization of the polymer chain of starch. There are two important reactions occurred during starch oxidation; the first step is the oxidation of hydroxyl groups to carbonyl and carboxyl groups, and the second step is the de-polymerization of starch molecules by cleavage of (1 - 4)-α-D and (1 - 6)-α-D glucosidic linkages. Table 2.6 shows the carboxyl content of chemical oxidized starch is higher than native starch. The massive amount of carboxyl content in starch granules leads to a lower gelatinization on starch paste upon oxidation (Pandiselvam et. al, 2019).

**Table 2.6.** Carboxyl content of native and oxidized starch

| Sample | Carboxyl Content (%) |
|---|---|
| Control Potato Starch | 0 |
| Oxidized Potato Starch by H2O2 | 0.20 |
| Oxidized Potato Starch by ClO2 | 0.20 |
| Oxidized Potato Starch by NaClO | 0.20 |

Source: Han (2016)

## 2.5 Amylose Content

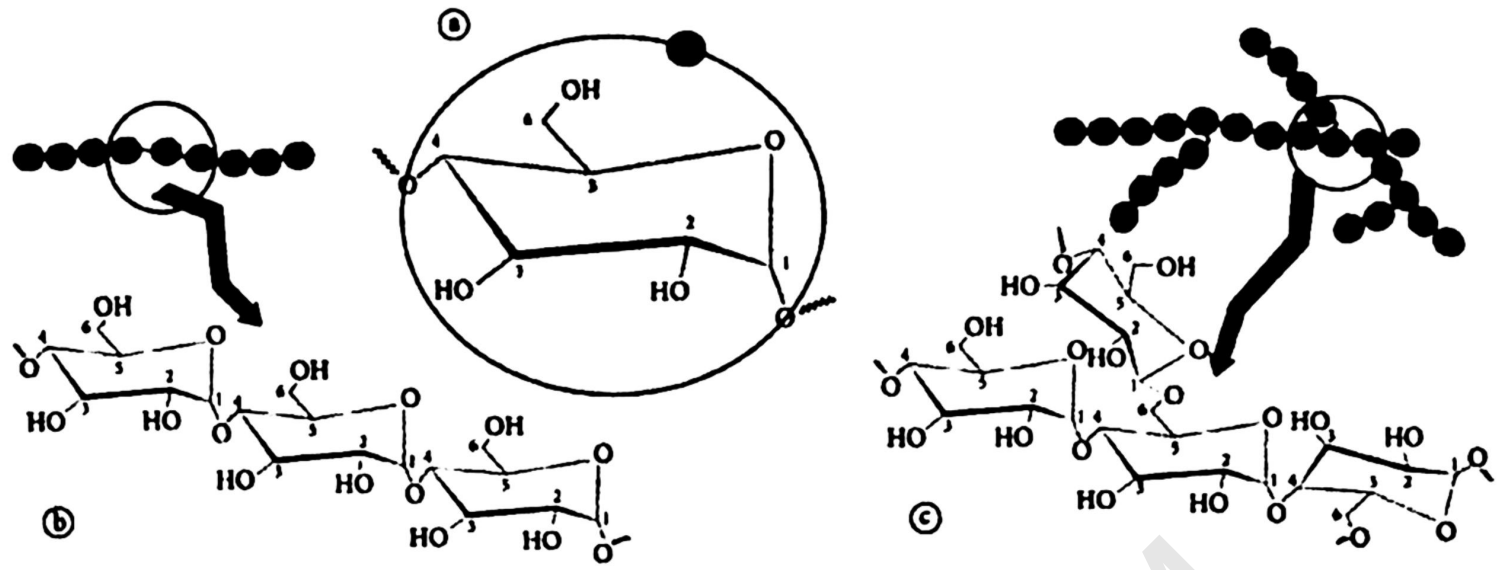


**Figure 2.4.** Basic structure of starch

(a) glucose units, (b) amylose and (c) amylopectin

The amylose content of the starch granule significantly affects the physicochemical and functional properties of starch. The amylose content can vary within the same botanical variety due to differences in geographic origin and culture conditions (Gao et al., 2014). Researchers have highlighted the role of amylose in accordance to resist swelling and solubility of starch granule, as swelling proceeds rapidly after leaching of amylose molecules. Amylose has important characteristics for industrial applications, where it is anhydrous and can form excellent films. Films formed by amylose are very strong, colorless, odorless and tasteless (Campos et al., 2011).

Amylose associated with large branches of amylopectin molecules comprise the amorphous region of granules, while amylopectin molecules with short branches comprise the crystalline region. Therefore, a higher proportion of amylopectin in starch granules results in greater crystallinity (Alcázar-Alay & Meireles, 2015). A research study by Karmvlr et. al (2018) reported that the amylose content of modified white sorghum starch were decreased compared to native starch, as shown in Table

2.7 below. There is also a study by Babu & Ramanathan (2014) whose reported that the amylose content of sweet potato starch using different isolation medium as shown in Table 2.8. The isolation of sweet potato in sodium metabisulfite shows lower amylose content compared to sodium chloride and distilled water. The amylose content of starch may be of importance since amylose content is one of the important factors affecting starch pasting and retrogradation behaviors (Chen at al., 2003).

**Table 2.7.** Amylose Content (%) of native and modified white sorghum starch

| Sample | Amylose Content (%) |
|---|---|
| Control | 22.4 |
| Oxidized | 15.1 |
| Cross-linked | 11.3 |
| Oxidized cross-linked | 4.10 |

Source: Karmvlr et. al (2018)

**Table 2.8.** Amylose Content (%) of sweet potato starch in different maceration medium

| Starch | Amylose Content (%) | | |
|---|---|---|---|
| | Sodium metabisulfite | Sodium chloride | Distilled water |
| Sweet Potato | 18.21 ± 3.06 | 18.17 ± 1.54 | 18.56 ± 1.06 |

Source: Babu & Ramanathan (2014)

## 2.6 Swelling Power and Solubility

One of the most significant structural characteristics of starch is that it passes through several different stages from water absorption to granule disintegration. Water absorption and consequent swelling of the starch granule contribute to amylopectin-

amylose phase separation and crystallinity loss, which results to the leaching of amylose to the inter-granular space (Conde-Petit et al., 2001). When starch is heated in excess amount of water, starch takes up water and the semi-crystalline structure of starch is broken. This condition causes swelling inside the starch granule and increases granule size and solubility (Singh et al., 2003). Amylose and amylopectin are disappear during this condition. This is called as gelatinization process. The swelling capacity of starch is directly associated with the amylopectin content because the amylose acts as a diluent and inhibitor of swelling (Singh et al., 2003).

Solubility is the percent measure of starch drained out into the supernatant in the swelling volume determination (Singh et al. 2005). One of the important measures in commercialized starch is its water binding capacity because it stabilize the quality and texture of the food products against syneresis (Baker et al., 1994). Kaur et al. (2011) studied the effect of acid on properties of various types of starches and found that the swelling power decreased for all types of starches as shown in Table 2.9.

**Table 2.9.** Swelling power and solubility of native and acid modified starches

| Starch | Swelling Power (g/g) | | Solubility (%) | |
|---|---|---|---|---|
| | Native | Acid Modified | Native | Acid Modified |
| Sweet Potato | 21.5 ± 0.15 | 0.72 ± 0.14 | 7.8 ± 0.76 | 67.7 ± 0.83 |

Source: (Kaur et al., 2011)

**Table 2.10.** Swelling Power (g/g) of Ozone-Oxidized Starches

| | starch | | |
|---|---|---|---|
| OGT (min) | corn | sago | tapioca |
| unmodified | 9.45 ± 0.05 c | 9.98 ± 0.19 a | 15.27 ± 0.35 a |
| 1 | 9.68 ± 0.06 ab | 9.27 ± 0.52 b | 12.49 ± 0.33 b |
| 3 | 9.52 ± 0.16 bc | 9.21 ± 0.14 b | 12.03 ± 0.29 b |
| 5 | 9.59 ± 0.09 bc | 9.03 ± 0.45 bc | 9.23 ± 0.31 d |
| 10 | 9.78 ± 0.11 a | 8.46 ± 0.12 c | 10.51 ± 0.19 c |

[a] Results are expressed as means ± standard deviations ($n$ = 4). Values in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

**Table 2.11.** Solubility (%) of Ozone-Oxidized Starches

| | starch | | |
|---|---|---|---|
| OGT (min) | corn | sago | tapioca |
| unmodified | 2.81 ± 0.63 a | 0.78 ± 0.00 b | 4.382 ± 0.00 a |
| 1 | 2.74 ± 0.30 a | 1.33 ± 0.08 a | 3.089 ± 0.47 b |
| 3 | 2.90 ± 0.50 a | 1.26 ± 0.09 a | 3.330 ± 0.36 b |
| 5 | 3.19 ± 0.45 a | 1.30 ± 0.40 a | 3.068 ± 0.51 b |
| 10 | 3.24 ± 0.34 a | 1.30 ± 0.31 a | 3.878 ± 1.27 ab |

[a] Results are expressed as means ± standard deviations ($n$ = 4). Values in the same column with the same lowercase letters are not significantly different ($P > 0.05$).

Table 2.10 and Table 2.11 shows the swelling power and solubility of different starch samples treated by ozone oxidation by Chan et al. (2009). According to Chan et al. (2009), the swelling power of the starches are significantly decrease due to the disintegration of the starch granule during the modification process. According to Hodge and Osman (1996), the solubility is increase after oxidation due to the depolymerization and structural weakening of the starch granule. Parovuori et al. (1995) also found that solubility of oxidized potato starch increased as the oxidation time is increased. Fannon et al. (1992) reported that the existence of channels in the starch granule is accountable to support and increase the potential surface region available for reaction and penetration of reagents in granules. The presence of cross-

links prevent the amylopectin molecules from leaching out and thus results a decrease in solubility (Wang and Wang, 2003).

## 2.7 Syneresis

Syneresis is a process in which the starch molecules are rearranged into a more ordered structure, and thus excreting undesirable liquid. The water excreted from cold-stored gelatinized starches is considered a problem of starch-based foods (Simsek et al., 2012). Although syneresis is a common physical characteristic of most gels, it can be used to assess the freeze–thaw stability of starch by measuring the water exuded from a gel on standing or after freezing and thawing (Hoover & Ratnayake, 2002). Freeze-thaw stability is commonly expressed as syneresis percentage which acts as an indicator of starch retrogradation. The measurement of freeze-thaw stability involves the freezing of a starch gel for a particular period, during which phase separation occurs. The frozen gel is then thawed and the water expelled from the gel is gravimetrically determined and expressed as percentage of the starch gel (Karim, Norziah, & Seow, 2000). Low syneresis value is an indicative of slow retrogradation of starch gels due to strong interactions between dispersed amylose/amylopectin and water molecules (Liu et al., 2014). The highest liquid liberation from the pastes of oxidized starches could be associated with the weak starch structure and lower molecular weight of the starch polymers (Matsuguma et.al, 2009).

The intensity of syneresis is associated with amylose content, the degree of association of the starch chains, the chain length of the amylopectin side chains and degree of polymerization of the amylose and amylopectin. In most cases, the liquid liberation is undesirable and has negative effects on the texture and appearance of food products.

## 2.8 Gelatinization

Gelatinization occurs when starch is heated in the presence of excess water and undergoes a transition phase. During the gelatinization process, water diffuses into the starch granule, which then swells substantially due to hydration of the amorphous phase causing loss of crystallinity and molecular order of starch structure (Jiménez et al., 2012). Gelatinization occurs initially in the amorphous region because of the weak hydrogen bonds in this area. Then, the process then extends to the crystalline region of the starch.

There is a characteristic of temperature interval for gelatinization which are onset temperature ($T_o$), peak temperature ($T_p$) and end temperature ($T_c$). High transition temperatures correspond to a high degree of crystallinity, high stability and resistance of the granule structure to gelatinization (Tester et al., 2004). Gelatinization of starch granules is associated with the loss of crystalline order due to the breaking of the double helix in the crystalline region and the leaching of amylose structure (Alcázar-Alay & Meireles, 2015).

Gelatinization is necessary for particular processes such as hydrolyzed starch industries. Starch gelatinization happens due to the manufacturing of products from starch based raw materials, especially cereals. The progress of starch gelatinization along the process is determined by the physicochemical properties of the starch, the presence of other ingredients, the availability of water and process parameters applied such as temperature and time (Schirmer et al., 2015). Table 2.10 shows gelatinization properties of ozonated and control (unozonated) starch samples.

**Table 2.12.** Gelatinization properties of ozonated and control (unozonated) starch samples.

| Sample | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | ΔH (J/g) |
|---|---|---|---|---|
| Control Corn Starch | 70.1 ± 0.3 | 74.8 ± 0.2 | 80.5 ± 0.7 | 13.6 ± 1.8 |
| Ozonated Corn Starch | 72.6 ± 0.1 | 76.5 ± 0.1 | 82.6 ± 0.3 | 9.1 ± 0.8 |
| Control Potato Starch | 64.1 ± 0.3 | 68.6 ± 0.5 | 75.9 ± 1.1 | 15.5 ± 1.2 |
| Ozonated Potato Starch | 65.3 ± 0.2 | 70.3 ± 0.1 | 77.4 ± 0.3 | 9.7 ± 1.3 |

Source: Çatal, H., & İbanoğlu, Ş. (2012)

## 2.9 Texture Properties

Rheological behaviour of a starch involves studying its viscosity, elasticity and plasticity. Food processors measure starch viscosity as a quick assessment of product performance. Viscosity is an important property of starches and it indicates its utility in specific applications. Starch viscosity refers to its thickness, or resistance to shear, agitation or flow. When starches are heated in an aqueous suspension, they gelatinize, i.e., they absorb water and swell irreversibly, creating a thick or viscous paste. Measuring the viscosity of starches in foods gives a direct assessment of their processability in terms of pumping and mixing (Brandt, 2003).

Table 2.13 and Table 2.14 show the viscosity of native and modified starch. The decrease in starches' viscosity is caused by the partial cleavage of the glucosidic

linkages during the extensive oxidation, resulting in a decrease in molecular weight of starch molecules (Zhang et. al, 2012).

**Table 2.13.** Viscosity of native and ozonated starch

| Sample | Viscosity (cP) |
|---|---|
| Control Corn Starch | 5998 ± 12 |
| Ozonated Corn Starch | 7 ± 1 |
| Control Potato Starch | 4972 ± 5 |
| Ozonated Potato Starch | 10 ± 1 |

Source: Çatal, H., & İbanoğlu, Ş. (2012)

**Table 2.14.** Viscosity of native and oxidized potato starch

| Sample | Viscosity (Pa.s) |
|---|---|
| Control Potato Starch | 5.255 |
| Oxidized Potato Starch by H2O2 | 0.11 |
| Oxidized Potato Starch by ClO2 | 0.074 |
| Oxidized Potato Starch by NaClO | 15.69 |

Source: Han (2016)

## 2.10 Morphological Properties

Microscopy has played an imperative role in increasing perceptive of granular structure of modified starches. It has been widely used to detect structural changes

caused by ozone modifications. The granular surface of different starch sources was reported to be rough, irregular shapes, fissures, and pores, and it became more heterogeneous with increasing ozonation time (Catal and Ibanoglu, 2012). The structure of starch granule is very complex. The complexity of the starch granule is due to variations in their component structure, starch composition (moisture, lipids and proteins) and variations between amorphous and crystalline regions. Starch granules have microscopic sizes with diameters ranging from 0.1 to 200 μm. Its morphology varies between different shapes such as oval, ellipsoidal, spherical, smooth, angular and lenticular, depending on the botanical source (Hoover, 2001; Singh et al., 2003). Normally, starch granules are isolated before microscopic observation, and the isolation method is crucial because it can affect the original size of the starch (Gao et al., 2014; Lawal et al., 2011).

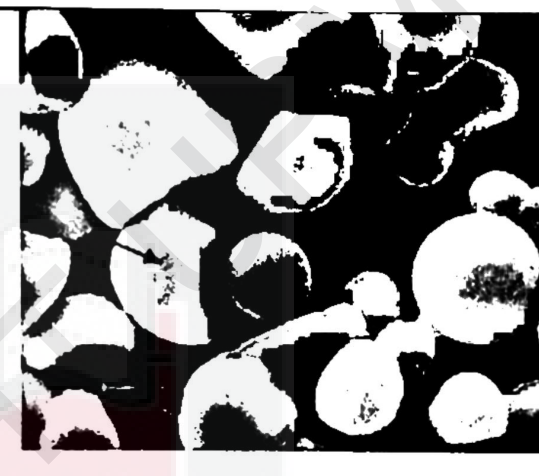
Table 2.15 shows that morphological properties of native sweet potato starch. Meanwhile, Table 2.16 shows the effect of ozone-oxidized on the morphological properties on cassava starch. Klein at. al (2014) reported no morphological differences were observed in the granules surface. Çatal and Ibanog˙lu (2012) reported changes in the shape and the surface of potato starch granules after ozonation in aqueous solution, but changes in corn starch granules were difficult to see. Differences in ozone concentration, starch concentrations in water during ozonation, starch source, pH of reaction and time of reaction are responsible for divergence in literature data on ozone-oxidation of starch in aqueous solution.

**Table 2.15.** Morphological properties of sweet potato starch

| Starch source | Granule shape | Diameter (μm) |
|---|---|---|
| Sweet potato | Polygonal | 5-25 |

Source: Alcázar-Alay & Meireles (2015)

**Table 2.16.** Scanning electron morphology of native and ozone-oxidized cassava starch

| Cassava starch | SEM (2000x) |
|---|---|
| Native |  |
| Ozone-oxidized at pH 3.5 |  |
| Ozone-oxidized at pH 6.5 |  |
| Ozone-oxidized at pH 9.5 |  |

Source: Klein at. al (2014)

# CHAPTER 3

# METHODOLOGY

## 3.1 Research Design

The design of this research is an experimental work on the sweet potato starch (var Anggun). The preparation of the sweet potato is done to extract the starch content that present in the sweet potato. There are two methods to maximize the extraction of the sweet potato starch either maceration with sodium metabisulfite or ultrasound treatment. Those two methods are utilized to aid the extraction process of the starch from sweet potato.

Once the starch has been collected, they will undergo ozone oxidation process and then oven drying to achieve 11% moisture content. Then, the starch properties will be analysed in terms of its swelling power and solubility, carboxyl content and amylose content.

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 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 |

## 3.2 Preparation of Sweet Potato Starch

### 3.2.1 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 plastic zip bag.

### 3.2.2 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 3.1) 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 plastic zip bag.

### 3.2.3 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 3.1) 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 plastic zip bag.

### 3.2.4 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 3.1) 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 plastic zip bag.

### 3.2.5 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.

### 3.3 Ozonation of Starch

Sample B, Sample C and Sample D were exposed to the ozone gas that was obtained from an ozone generator. The generator had an integrated oxygen production unit using the atmospheric air. The ozone gas was directed from the generator to a container that was evenly spread with the sample on a strainer. Details of ozone parameters are described in Figure 3.1.

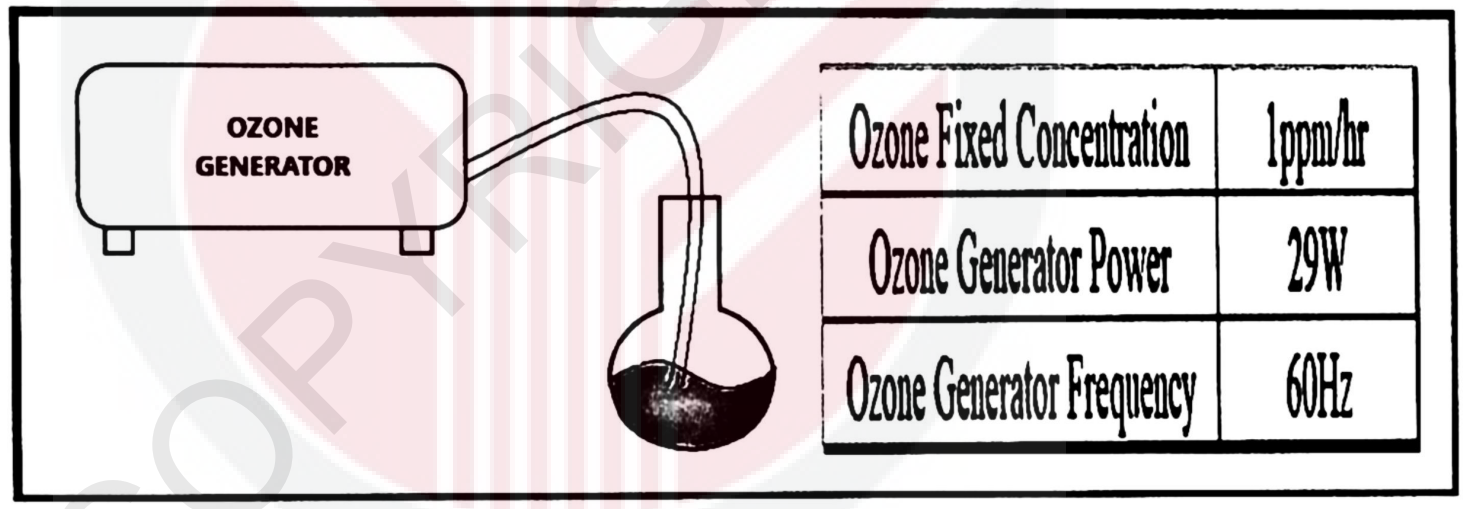



**Figure 3.1.** Set up for ozonation of starch

### 3.4 Physicochemical Analysis

#### 3.4.1 Carboxyl Content

The carboxyl content was determined following titration method adapted from (Gozé et al., 2016). 500mg of starch granules were mixed with 30 mL of 0.1N HCl. After 30 minutes, these samples will be washed with de-ionized water, then suspended in 300 mL de-ionized water and heated at boiling temperature under agitation for 10 minutes. Hot solution will be titrated with 0.002N NaOH, using phenolphthalein as

indicator. The carboxyl content will be calculated using Eq. 1 and expressed as % COOH (w/w).

$$\text{COOH (\%)} = \frac{(Vs-Vb) \times M \times 0.045 \times 100}{W} \qquad \text{(Eq.1)}$$

where Vs is the volume of NaOH required for the sample (mL), Vb is the volume of NaOH used to test the blank (mL), M is the molarity of NaOH and W is the weight of sample (d.b.).

### 3.4.2 Amylose Content

Amylose content is sweet potato starch samples were determined based on iodine-binding procedure adapted from Thomas et al. (2013). 100mg of starch sample will be dissolved in 1 mL of 95% ethanol (v/v) and 9 mL of NaOH solution (1 N) followed by thorough mixing. Further, the samples were heated on a boiling water bath (100°C) for 10 mins to gelatinize the starch and then cooled to room temperature. 5mL of gelatinized starch was then transferred into a 100 mL volumetric flask followed by addition of 1 mL of 1N acetic acid and 2 mL of iodine solution. Distilled water will be added to make up 100 mL in a volumetric flask containing the dissolved starch sample. The absorbance was measured at 700 nm using a UV-Visible Spectrophotometer (Model AA-6650, Shimadzu Co. Japan). The amylose content in samples was determined based on the standard curve prepared using potato amylose.

### 3.4.3 Swelling Power and Solubility

Swelling power and solubility was determined according to the method described by Sandhu et al. (2012). 0.1 g of starch was weighed in a beaker and 10 ml of distilled water was added. The suspension was stirred and placed in a water bath for 30 minutes at temperature ranging from 55°C to 95°C, increasing 10°C from time to time and centrifuged for 15 min at 3400 g. A 5 ml aliquot was removed from the

supernatant, placed in petri dish and placed on the stove at 105℃ for 24 hour to determine the weight of the solubilized starch. After the outer walls of the tubes were dried, the tubes were weighed carefully, and the swelling power and solubility will be determined using Eq. 2 and 3, respectively;

$$\text{Swelling Power} = \frac{(A+B)-(A+C)}{\text{Weight of sample}} \qquad \textbf{(Eq. 2)}$$

Where A is the weight of tube, B is the weight of the residue after centrifugation and C is the weight of the sample on dry basis.

Solubility (%) = (Weight of plate with sample after evaporation) – (weight plate) x 100 **(Eq. 3)**

### 3.4.4 Syneresis

The syneresis of the starches were determined according to the method described by Simsek et al (2012) with some modifications. Starch suspension (2% w/w) were heated at 85°C for 30 minutes in water bath, and then the starch suspension were cooled down to room temperature. The starch samples were stored for 24, 48 and 120 hours at 4°C. The syneresis were measured as the percentage of water released after centrifugation at 300 g for 15 minutes.

### 3.4.5 Gelatinization

The gelatinization characteristic of the starches were investigated using the method described by Oladebeye et al (2013) with some modifications. The gelatinization properties were determined using a Differential Scanning Calorimeter (DSC) in a nitrogen atmosphere at a flow rate of 50 ml.min. For the preparation of the samples, starch slurries were prepared at 1:3 dry starch to water ratio. The starch slurries were hermetically sealed and reweighed on the aluminium pan. To provide the uniform distribution of water in the starch, the samples were maintained 24 hours at

room temperature before analysis. The samples were heated at a rate of 10°C/min from 30°C to 150°C. Onset temperature ($T_o$), peak temperature ($T_p$), end temperature ($T_c$) and enthalpy of gelatinization ($\Delta H$) were obtained from the curve shown in the computer software.

### 3.4.6 Texture Properties

The viscosity of the starches were determined using a rotational viscometer (ViscoQC 100, Anton Paar, Malaysia) at a sample concentration of about 1.0 mg/mL (Zhang et. al, 2009).

### 3.4.7 Morphological Properties

The morphological properties of the starch granules was examined using a scanning electron microscope (Shimadzu, SSX-550). A small quantity of each sample was spread directly onto the surface of the stub. Subsequently, all of the samples were coated with gold and examined in the scanning electron microscope with magnifications of 2000x.

## 3.5 Experimental Design and Statistical Analysis

All analysis were carried out in triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyzed using Microsoft Excel XP. The correlation and design optimization were done using RSM-Design Expert Software.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 General

In the following discussion, five different samples on sweet potato var Anggun will be discussed in terms of their carboxyl content, amylose content, swelling power, solubility, syneresis, gelatinization, texture properties and morphology of the starch granules. All the samples shown in Table 4.1 below were dried until 11% moisture content was achieved (Klein et. al, 2014). When all the starch samples were ready in powder form, the analysis was done to determine the properties of the starch.

**Table 4.1.** 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 |

## 4.2 Carboxyl Content

The percentage of carboxyl content in sweet potato var Anggun at different conditions were shown in Figure 4.1. The carboxyl content of Anggun varies from 0.099% to 0.261%. According to Murphy (2000), the hydroxyl group of starch were oxidized to carbonyl (C=O) group first, and then to carboxyl (COOH) groups during starch oxidation, with partial depolymerization of the starch chains.

Sample A is considered as a control sample, hence a reference to other samples. Thus, the result for sample A is 0. From Figure 4.1, it shows that the highest carboxyl content of Anggun was obtained at Sample B which is 0.26%, where the sample was macerated with sodium metabisulfite, and then treated with sonication for 30 min and ozone-oxidized for 4 hours. Meanwhile, the lowest carboxyl content of Anggun was at Sample E (0.099%). 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. A report by Chong et al (2013) stated that H+ and -OH radicals are generated when water was used as the sonicated medium and these highly reactive radicals may help in enhancing starch oxidation. Thus, this finding is corresponding to sample E, where the usage of distilled water along with sonication of starch have a slight effect on the carboxyl content of starch.

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. At the moment of writing, no previous studies have been done and proved on the effect of different starch isolation medium on the carboxyl content of oxidized starch. However, it can be observed that sonication-

oxidized starches (Sample B and Sample D) showed higher carboxyl contents compared to other samples. This shows that sonication was effective in enhancing starch oxidation, where, more hydroxyl groups are being converted to carbonyl groups and promptly oxidized to carboxyl groups, especially for Sample B and Sample D.

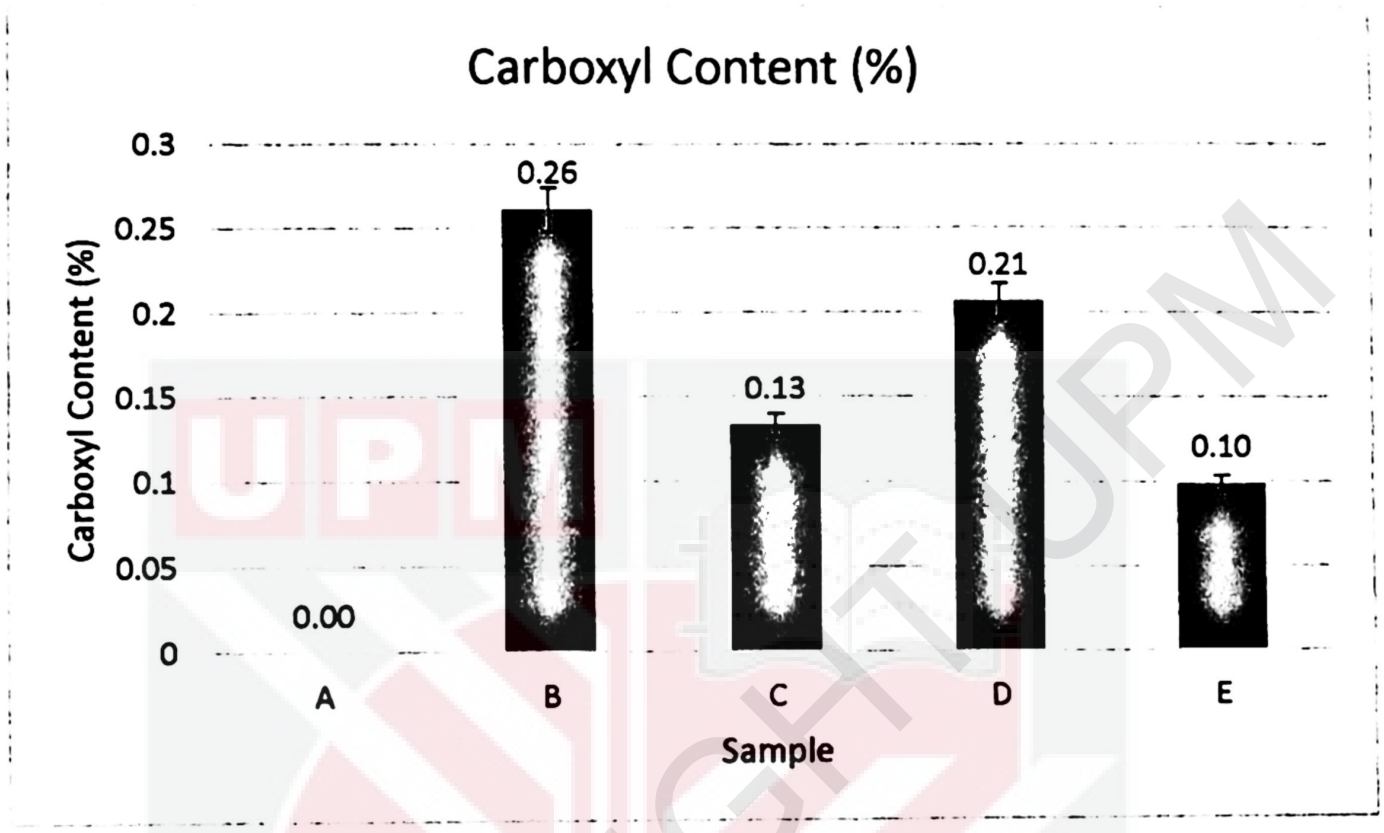



**Figure 4.1.** Carboxyl content of each samples.

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

Amylose content determined by iodine affinity method ranged from 26.1 to 39.98% for Anggun as shown in Figure 4.2. Sample A was observed to have the highest amylose content of Anggun which is 39.98%. Meanwhile, Sample E shows the lowest amylose content which is 26.07%.

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 Anggun was lower at Sample E compared to Sample A. Xu et al., (2018) stated that the sodium metabisulfite had no significant effect on apparent amylose content, crystalline structure, ordered degree, and lamellar structure of starches. Whereas, ultrasound primarily affects the amorphous region, while maintaining the granule's shape and size (Alcázar-Alay & Meireles, 2015). A lower amylose content in Sample E could be due to sonication process that affects the amorphous region and disrupts the molecular order of the starch, which makes the amylose to leach out.

In comparison between Sample B and Sample D, it shows that the amylose content of Sample B is slightly lower 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. Again, for this situation, there is no previous study stated that the isolation media does affect the amylose content of the starch. However, these results were corresponding to a study by Babu & Ramanathan (2014), where the isolation medium of sodium metabisulfite resulted in a lower amylose content of the starch.

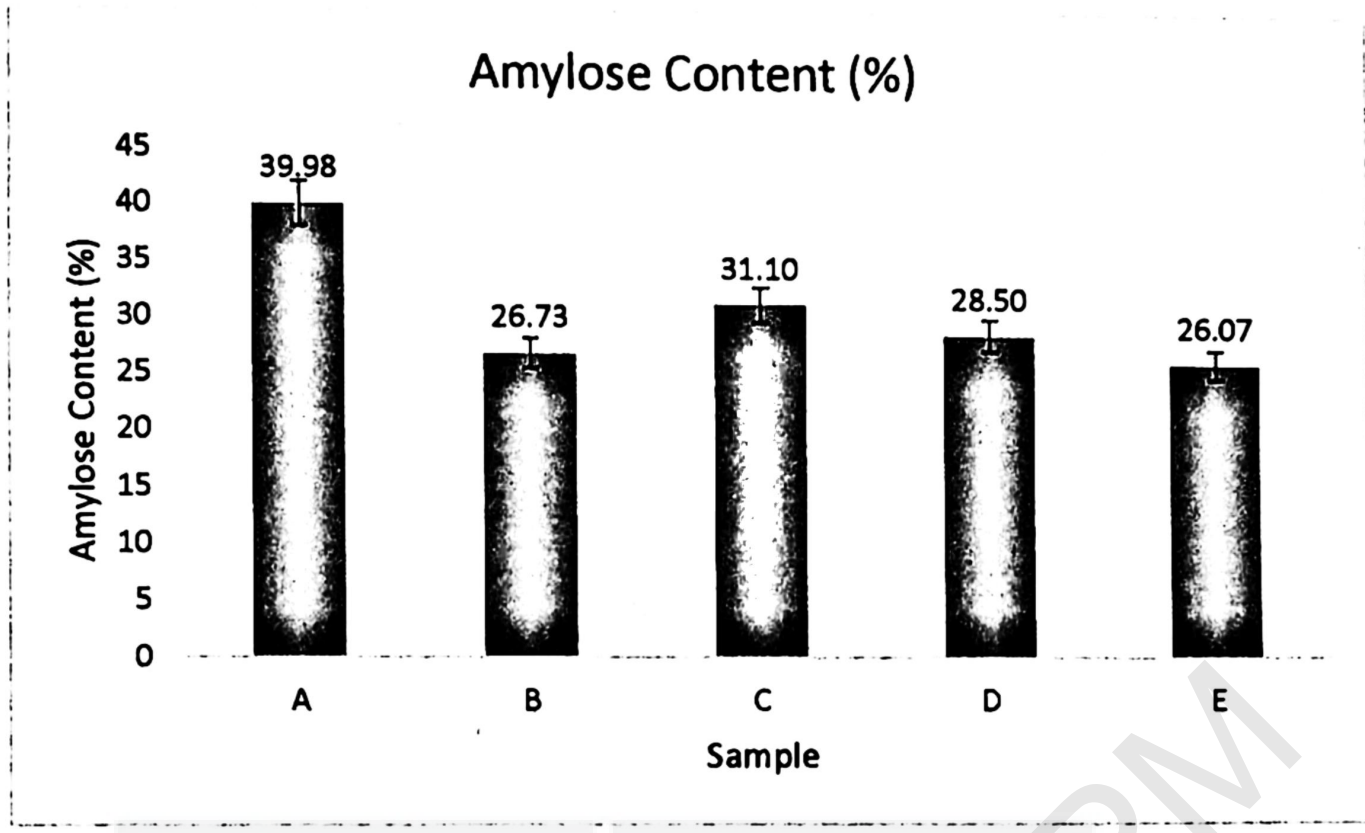



**Figure 4.2.** Amylose content (%) for each sample.

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 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). From Figure 4.3, the highest swelling power of Anggun was oserved at Sample C, where the sample was macerated with sodium metabisulfite and ozone-oxidation for 4 hours. From Figure 4.4, sweet potato var Anggun shows the highest solubility at Sample B which is 2.70%. Generally, swelling power and solubility increases as the samples undergo oxidation process. 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. Sample E shows higher swelling power compared to Sample A. However, the solubility of Sample A is greater than Sample

E. The higher swelling power of Sample E compared to Sample A could be due to ultrasound treatment that disrupts of the starch granule and results in a higher facility for water entrance in starch granule and lead to a higher water uptake (Manchun et al., 2012). The significant effect on the starch granule disintegration is caused by the cavitation forces from the sonication that break the crystalline molecular structure and chains of starch by disrupting the covalent bonds.

Moreover, the higher solubility of Sample A compared to Sample E could be due to the usage of sodium metabisulfite as the maceration medium during starch preparation. These results were correlated to the research by Xu et al. (2018), which reported in their study that the usage of sodium metabisulfite as isolation media on the sweet potato results to a higher swelling power and solubility compared to water.

In comparison between Sample B and Sample D, it shows that the swelling power and solubility 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. These results were also corresponded to Xu et al. (2018) which reported that the swelling power and solubility of the sweet potato increase when the starch were macerated in the sodium metabisulfite. However, the high swelling power of sample B and sample D could also be due to the gaseous ozone and ultrasound treatment, which causes the breakdown of starch molecules and thus allowing more water molecules to form hydrogen bonding with exposed hydroxyl groups of amylose.

In addition, the increase in swelling power and solubility were probably influenced by the leaching of amorphous region of the starch granule. Increased solubility upon oxidation arises as a result of structural weakening and

depolymerization of the starch granule. Subsequently, the water molecules could bind more to free hydroxyl groups of amylose and amylopectin by hydrogen bonds, which leads to an increase in swelling power and solubility of the starch (Jambrak et al., 2010).

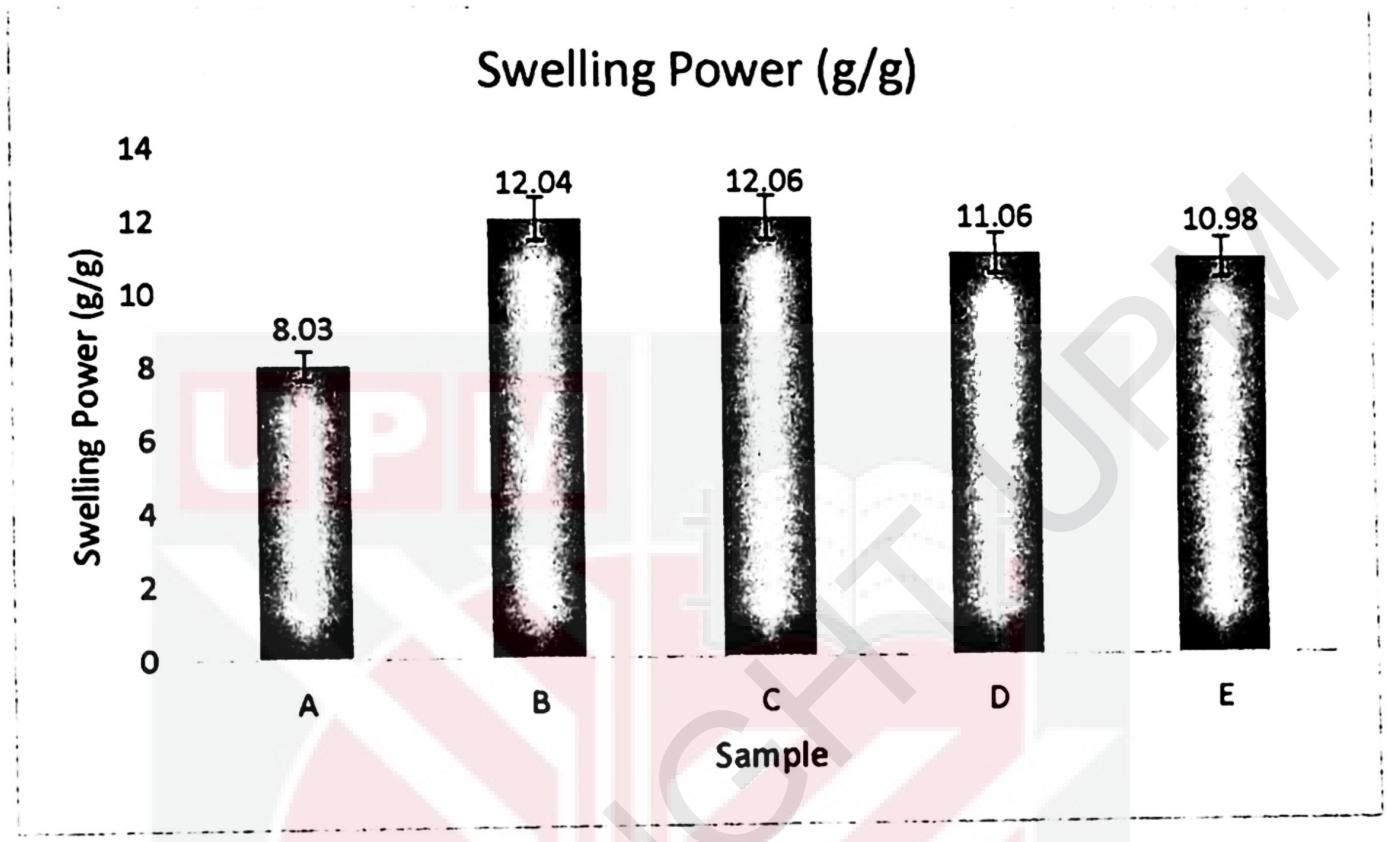



**Figure 4.3.** Swelling power (g/g) for each sample.

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.

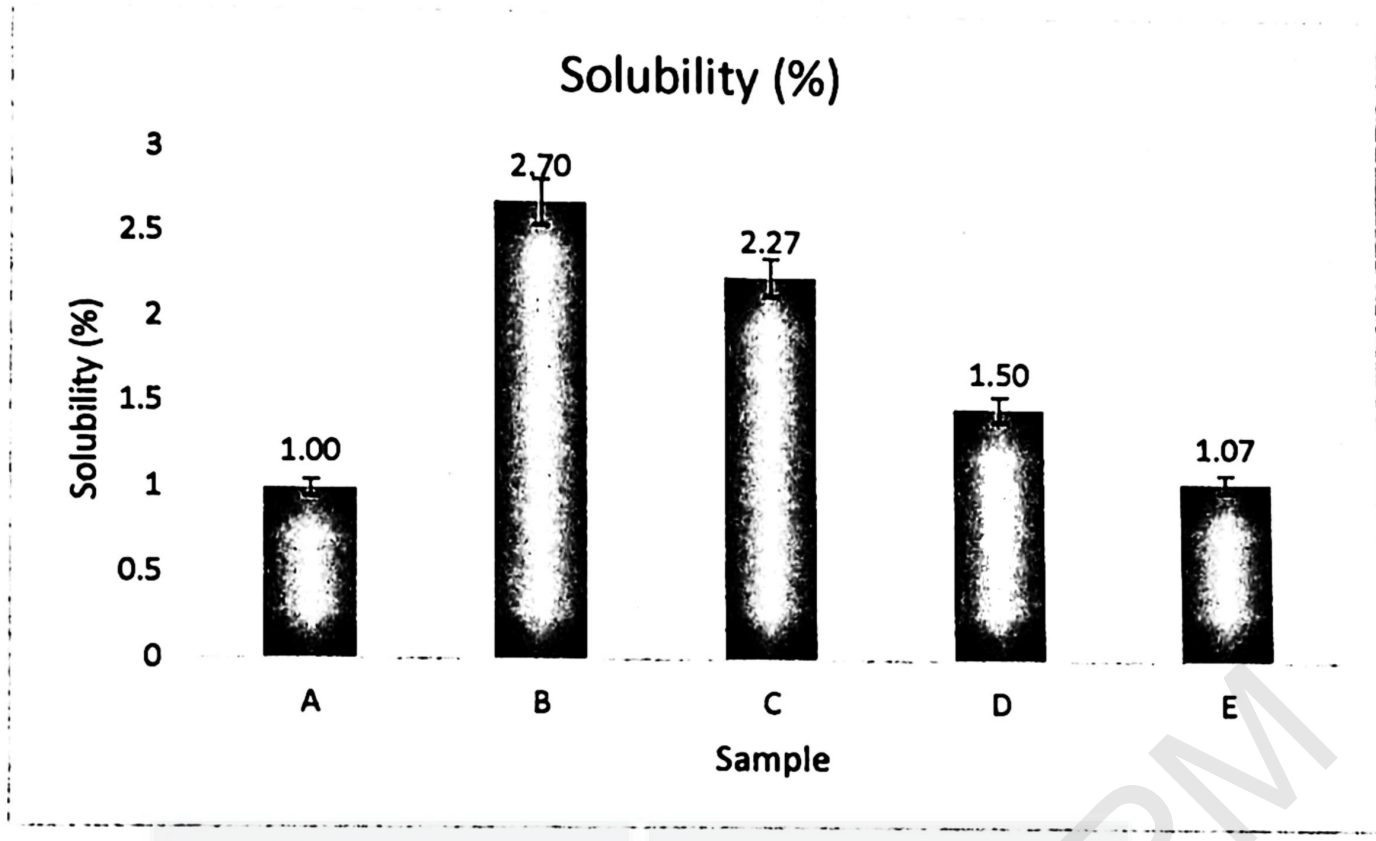



**Figure 4.4.** Solubility (%) for each sample.

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 Syneresis

Syneresis is a phenomenon that is related to freeze-thaw stability and it is commonly used as an indicator of the starch retrogradation. In other words, the starch molecules rearranged to a more ordered structure and release undesired water from the starch during refrigerated storage. Thus, syneresis is a significant factor to take into account in order to formulate or create starch-based foods.

The results of syneresis in the samples are shown in Figure 4.5, where an increase in syneresis was observed with respect to the storage time. As the storage time increase, the percentage of syneresis also increase for all samples. However, Sample B shows the highest percentage of syneresis as the storage time increases, compared to the other samples. This indicates that Sample B; where the samples were macerated with sodium metabisulfite, and then treated with sonication for 30 mins and ozone-oxidation for 4 hours, is not stable during refrigerated storage than the native and other

modified starches. Therefore, the starch modification via gaseous ozone and ultrasound treatment did not improve the functionality of these starches for their uses in refrigerated foods. The high amount of liquid excreted from the oxidized starches could be associated with the weak structure and lower molecular weight of the starch polymers. In most cases, the liquid liberation is undesirable and has negative effects on the texture and appearance of food products (Matsuguma et. al, 2009).

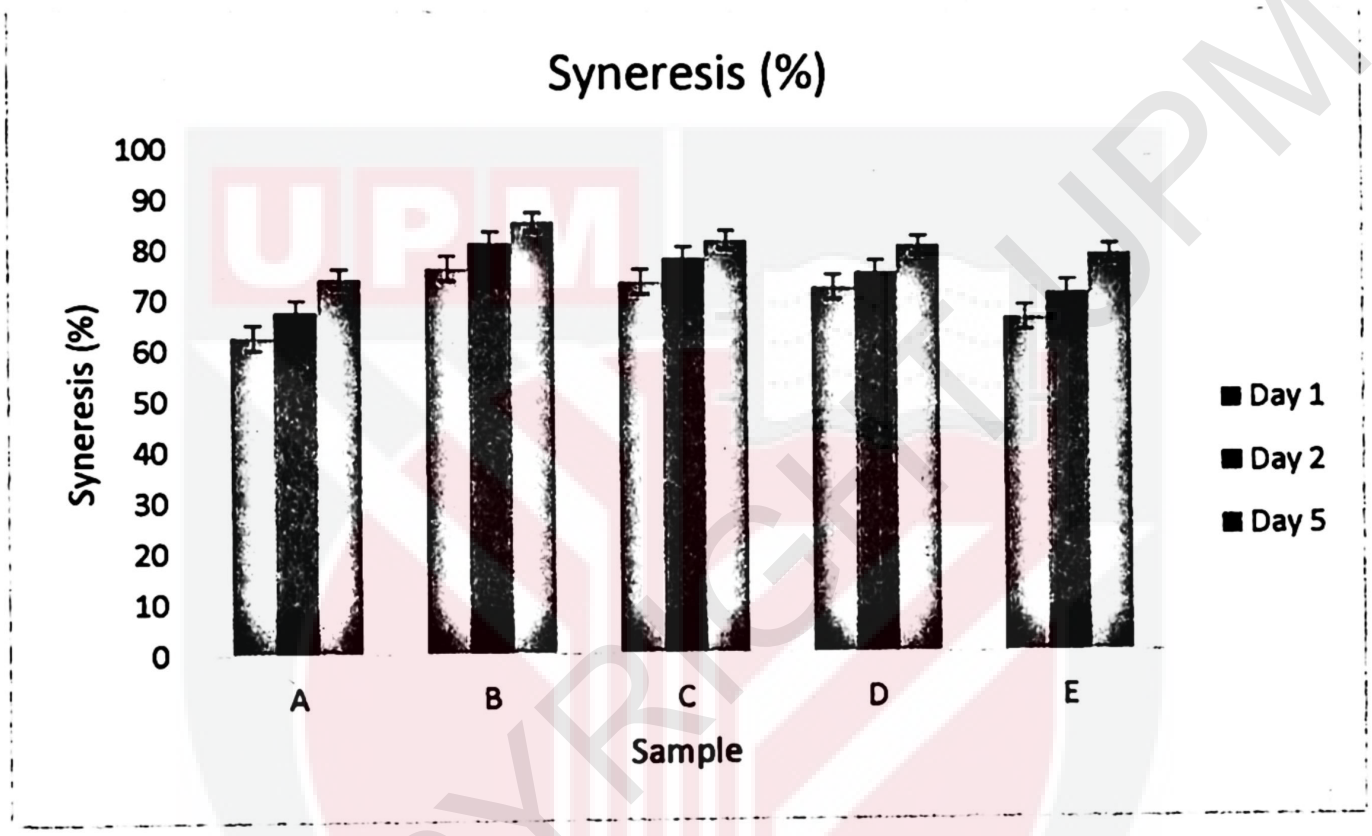



**Figure.4.5.** Syneresis (%) for each sample.

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.

## 4.6 Gelatinization

Starch gelatinization refers to the disruption of molecular orderliness within the starch granule, which give results on the solubilization, swelling of granules, and development of viscosity of starch. The profiles of starch gelatinization are shown in Table 4.1, which are onset temperature ($T_o$), peak temperature ($T_p$), end temperature ($T_e$) and enthalpy of gelatinization ($\Delta H$). During heating, $T_o$ is the temperature at which

the paste viscosity starts to increase, $T_p$ is the maximum viscosity temperature and $T_c$ is the final temperature of the viscosity increment. ΔH indicates of the amount of thermal energy needed throughout the gelatinization process.

Based on Table 4.1, Sample C shows the lowest gelatinization temperature which is 108.98 °C. Whereas, Sample E shows the highest gelatinization temperature at 119.13 °C. According to Mizuno et. al. (1998), the gelatinization temperature ($T_g$) was referred to $T_p$, where the temperature of the starch starts to cook. Apart from that, Sample E shows the lowest enthalpy energy which is 692.29 J/g while Sample A shows the highest enthalpy energy at 1641.95 J/g.

The gelatinization temperature and enthalpy of starches depends on the microstructure, granule size, degree of crystallinity within the granules and amylose-to-amylopectin ratio of the starch (Sandhu et al., 2008). In comparison among all the samples, it can be seen that Sample B, Sample D and Sample E are having lower enthalpy energy compared to Sample A and Sample C. From my observation, Sample B, Sample D and Sample E were treated with ultrasound treatment and some were undergo oxidation via gaseous ozone. Ultrasonication can induced cracks and pores in the granular structure of starch in which further provide assistance in the modification of starch (Zia-ud-Din et. al, 2017). Oxidation generally leads to the weakening of starch granules via partial degradation of starch molecules in crystalline lamella. The massive content of carboxyl group in starch granules results in lower gelatinization of starch paste upon oxidation (Pandiselvam, 2019). Thus, sonication-oxidized starches may require lower energies for gelatinization. The lower energy of gelatinization is due to the less ordered of the molecular starch structure.

The disruption of starch molecules occurs due to the relaxation of hydrogen bonds and the establishment of interactions between water molecules and the hydroxyl groups of amylose and amylopectin. These interactions increase the granule size, eventually leading to the rupture of granules and partial solubilization of the starch (Vanier et. al, 2017). When the starch granules are heated in presence of water, the molecular order within the starch granule were disintegrates and 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.

**Table 4.2.** Profiles of starch gelatinization for each sample.

| Sample | Gelatinization temperature (°C) | | | ΔH (J/g) |
|---|---|---|---|---|
| | To | Tp | Tc | |
| **A** | 97.58 | 116.52 | 137.73 | -1641.95 |
| **B** | 108.19 | 118.27 | 137.89 | -1017.92 |
| **C** | 105.83 | 108.98 | 135.13 | -1370.59 |
| **D** | 95.4 | 116.68 | 140.1 | -1340.23 |
| **E** | 106.93 | 119.13 | 138.18 | -692.29 |

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 Texture Properties

Viscosity refers to the resistance of fluid to flow due to internal friction of a moving fluid. In other words, viscosity determines the ease of movement for fluids or their fluidity. A fluid with high viscosity resists motion, while a low viscosity of fluid flows freely. Rheology is widely recognized for its effect on the quality of food and its

sensory characteristics. The rheological properties of starch determine its potential application as a thickener or gelling agent (Berski et al., 2011).

The texture properties of starch are shown in Figure 4.6 in terms of viscosity. It can be seen that starch samples subjected to oxidation and sonication showed a greater decrease in its viscosity as compared to those samples prepared with conventional method. Reduction of the viscosity could be attributed to the degradation of starch molecules and greater amount of carboxyl formed during oxidation (Chong et. al, 2013). Thus, even under mild conditions the starch was degraded because of the lower molecular weight of oxidized starch and disruption of glycosidic bonds during oxidation. Hence, lower viscosity of fluid results in lower resistance to flow and took a shorter time to travel through the viscometer. High viscosity of starch as resulted in sample A and sample E having high resistance to flow as the fluid takes time to move.

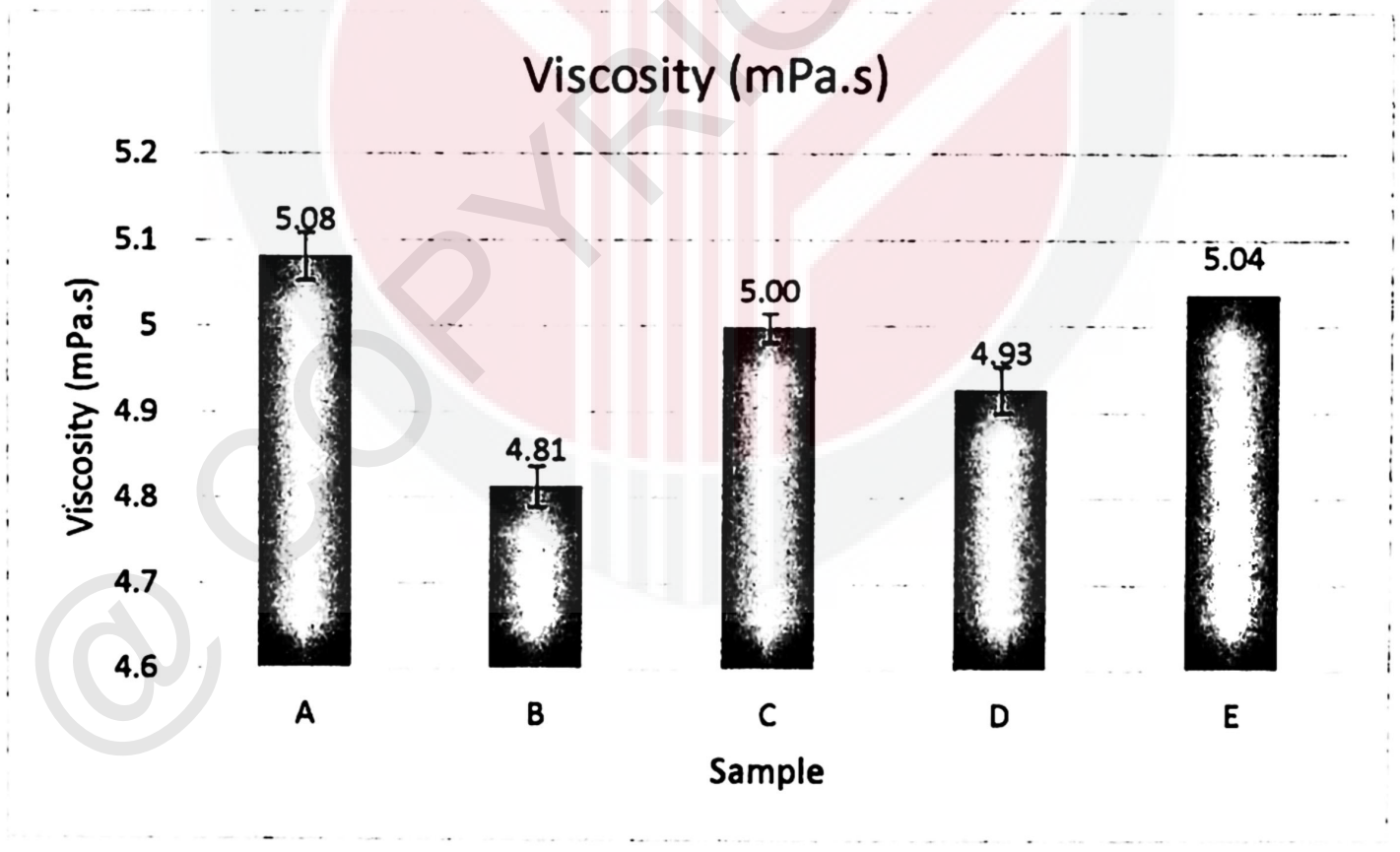



**Figure 4.6.** Viscosity for each sample.

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.

## 4.8 Morphology of Starch Granules

The scanning electron micrographs (SEM) of the native and ozone-oxidized sweet potato var Anggun starch granules are shown in Table 4.2. 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, where 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 is highly depends on sweet potato varieties. Anggun (Sample A) produced slight firmer and larger starch granules that were more pronounced in shape than other samples. With the exception of sample E, where it was treated with ultrasonication for 30mins with distilled water as the medium and oven dried, Anggun (Sample E) seemed to have smaller starch granules and less pronounced shapes. Fragments of starch granules were seen filling the void spaces between the granules. In comparison to Sample A, where the sweet potato 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. However, it can be seen that the size of starch granules for Anggun in Sample D is smaller in comparison to Anggun in Sample B. This could be reflected by the usage of distilled

water as maceration aids which was also the same of Sample E in both varieties of sweet potatoes. The reason for this phenomenon is uncertain and should be further studied.

The overall results showed that all methods produced a slightly same result among all the samples. However, it can be seen that sample C and sample D resulted in higher number of small size granules than other samples. According to Babu et. al (2014), the small size granules of starch might be suitable to be used as fat substitute in food industry. Hence, the modification done in this study might be not effective enough to change the surface characteristics of the samples.

**Table 4.3.** Morphology of starch granules for each sample

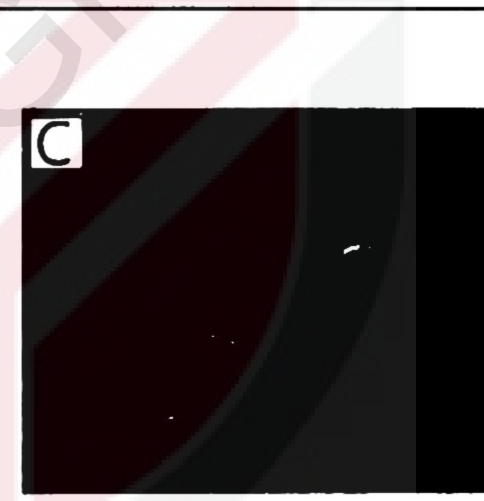
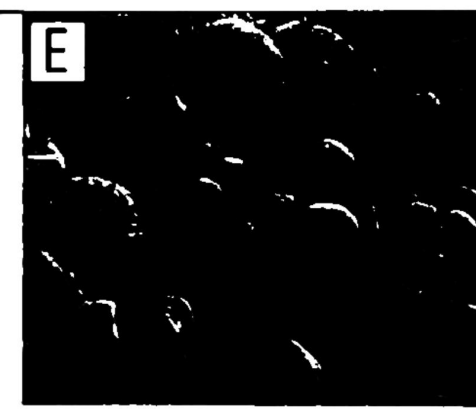
| Sample | Sweet Potato (Anggun) |
|---|---|
| **A - Maceration (Sodium Metabisulfite) + Oven dried 4 hours** |   |
| **B - Maceration (Sodium Metabisulfite) + Sonicate 30 mins + Ozone 4 hours + Oven dried 1 hour** |   |
| **C - Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour** |   |
| **D - Maceration (Distilled Water) + Sonicate 30 mins + Ozone 4 hours + Oven dried 1 hour** |   |
| **E - Maceration (Distilled water) + Sonicate 30 mins + Oven dried 4 hour** |   |

**Table 4.4.** Table of Summary of all analysis

| Sample | Carboxyl Content (%) | Amylose Content (%) | Swelling Power (g/g) | Solubility (%) | Viscosity (mPa.s) | Syneresis (%) | | | Gelatinization | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Day 1 | Day 2 | Day 5 | $T_p$ (°C) | ΔP (J/g) |
| **A** | 0 | 39.98 ± 0.035 | 8.03 ± 0.066 | 1.00 ± 0.265 | 5.037 ± 0.03 | 62.67 ± 0.252 | 67.67 ± 0.153 | 74.33 ± 0.153 | 116.52 | -1641.95 |
| **B** | 0.261 ± 0.0175 | 26.73 ± 0.061 | 12.04 ± 0.078 | 2.70 ± 0.200 | 4.813 ± 0.02 | 76.33 ± 0.551 | 81.33 ± 0.208 | 85.67 ± 0.208 | 118.27 | -1017.92 |
| **C** | 0.133 ± 0.0200 | 31.10 ± 0.032 | 12.06 ± 0.076 | 2.27 ± 0.306 | 4.926 ± 0.03 | 73.67 ± 0.208 | 78.33 ± 0.252 | 82.00 ± 0.100 | 108.98 | -1370.59 |
| **D** | 0.207 ± 0.0115 | 28.50 ± 0.010 | 11.06 ± 0.085 | 1.50 ± 0.200 | 4.998 ± 0.02 | 72.67 ± 0.252 | 75.67 ± 0.208 | 81.00 ± 0.100 | 116.68 | -1340.23 |
| **E** | 0.099 ± 0.0115 | 26.07 ± 0.023 | 10.98 ± 0.081 | 1.07 ± 0.153 | 5.081 ± 0.03 | 66.67 ± 0.252 | 71.67 ± 0.153 | 79.33 ± 0.208 | 119.13 | -692.29 |

A- Maceration (Sodium Metabisulfite) + Oven dried 4 hours;

B- B- Maceration (Sodium Metabisulfite) + Sonicate + Ozone 4 hours + Oven dried 1 hour;

C- C- Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 1 hour;

D- D- Maceration (Distilled Water ) + Sonicate + Ozone 4 hours + Oven dried 1 hour;

E- E- Maceration (Distilled water) + Sonicate + Oven dried 4 hours.

## 4.9 Optimization Analysis

The modification done on the starch via hurdle technologies have been proven to affect the physicochemical properties of starch. However, previous results in this study corresponds with results reported by Xu et. al (2018), where the different isolation medium had a slight effect on the physicochemical properties of the starch. Hence, most of the starch physicochemical changes were due to the physical or chemical modification done on the starch, e.g. ozone oxidation and ultrasound treatment.

Therefore, an experimental design using Design Expert Software has been carried out to study the effect of various maceration medium, ozone oxidation and ultrasound treatment on the physicochemical properties of sweet potato starch (var Anggun). Table 4.1 shows the utilization of Design Expert Software experimental design on this study with all its dependent variables; swelling power, solubility, viscosity and gelatinization.

The dependent variables of the experimental design were chosen based on the desired properties of starch, especially in industrial application. Rheological behaviour of starch involves studying its viscosity is an important factor in starch properties and it indicated its utility in industry applications. Measuring the viscosity of starches in food industry application gives a quick assessment of the product performance and their processability in terms of pumping and mixing (Brandt, 2003). Other than that, starch gelatinization properties is also an important factor for starch development. It indicates the cooking temperature and energy needed for the starch to cook. When starches are heated in an aqueous suspension, they gelatinize, i.e., they absorb water and swell irreversibly, creating a thick or viscous paste. Next, one of the important

measures in commercialized starch is its water binding capacity because it stabilize the quality and texture of the food products against syneresis (Baker et al., 1994).

Based on Figure 4.7, 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 0.01M sodium metabisulfite, undergo ozone oxidation at 1 ppm/ hr for 4 hours and ultrasound treatment for 30 minutes at 100W of power and 40% amplitude at frequency of 20 kHz. Sample B shows the optimum condition of oxidized starch in terms of swelling power, solubility, viscosity and thermal properties.

**Table 4.5.** Experimental Design of dependent variables

| Std | Run | Factor 1 A:treatment | Response 1 Swelling Power | Response 2 Solubility (%) | Response 3 Viscosity (mPa.s) | Response 4 Gelatinization (J/g) |
|---|---|---|---|---|---|---|
| **1** | 12 | A | 7.97 | 1.3 | 5.007 | 1641.95 |
| **2** | 4 | B | 12.13 | 2.9 | 4.837 | 1017.92 |
| **3** | 14 | C | 12.13 | 2.6 | 4.899 | 1379.59 |
| **4** | 13 | D | 11.15 | 1.7 | 4.98 | 1340.23 |
| **5** | 15 | E | 10.99 | 1.2 | 5.089 | 699.29 |
| **6** | 8 | A | 8.02 | 0.9 | 5.067 | 1639.35 |
| **7** | 3 | B | 12.08 | 2.5 | 4.79 | 1019.64 |
| **8** | 9 | C | 11.98 | 2.2 | 4.927 | 1380.93 |
| **9** | 10 | D | 11.06 | 1.3 | 5.001 | 1337.03 |
| **10** | 6 | E | 11.05 | 1.1 | 5.051 | 702.21 |
| **11** | 2 | A | 8.1 | 0.8 | 5.037 | 1644.23 |
| **12** | 5 | B | 12.02 | 2.7 | 4.811 | 1023.4 |
| **13** | 7 | C | 11.98 | 2 | 4.953 | 1383.49 |
| **14** | 1 | D | 10.98 | 1.5 | 5.013 | 1339.28 |
| **15** | 11 | E | 10.89 | 0.9 | 5.104 | 699.58 |

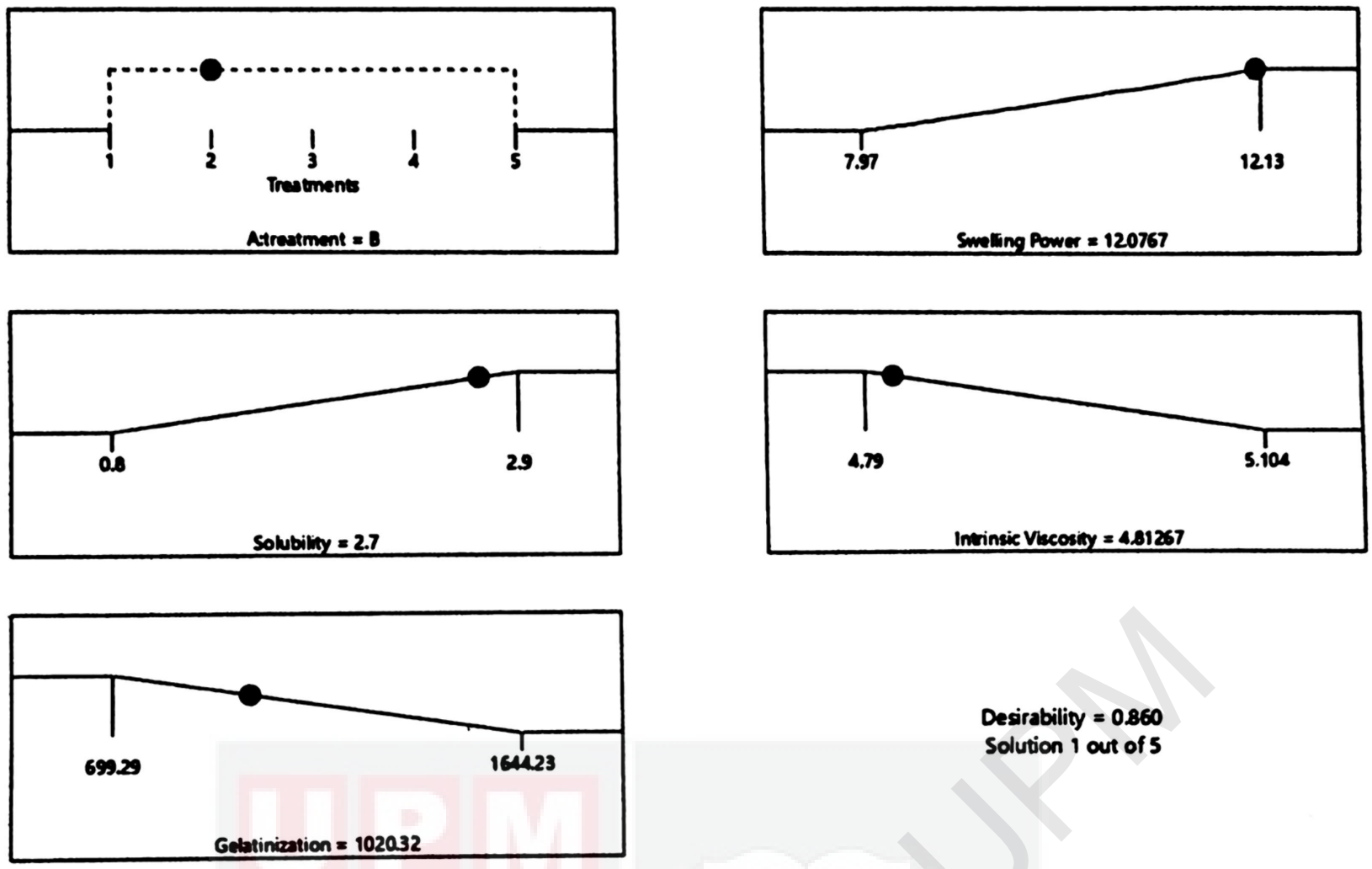



**Figure 4.7.** Design optimization of samples

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

Starch has a major role in the food industry for its nutritional value and broad technological functionality. Starch in native form is rarely used in industry because native starches have restricted solubility in water, which limits industrial applications. Thus, starch modification via physical or chemical modification can greatly improve the characteristics of native starch by altering its physicochemical properties and structural attributes and thus increasing its technological value.

Oxidation of starch in the presence of sonication degraded the starch polymer chains significantly. Sonication was found to accelerate the rate of oxidation by giving higher carbonyl and carboxyl contents. The amount of carboxyl content was found to be higher in oxidized starch compared to native starch. However, the higher amount of carboxyl content results to lower amylose content in oxidized sweet potato starch.

The modification done on the starch generally improves the properties of the starch as the swelling power and solubility of the starch increase, which resulted in better pasting properties of the starch. In addition, the gelatinization temperature and

gelatinization enthalpy needed for the starch also decreasing as the modification were done on the starch. The syneresis percentage was found to be higher in oxidized sweet potato starch, which makes them not suitable for refrigerated based food products. Moreover, the maceration and oxidation method do not affect the surface characteristics of the sweet potato starch.

In conclusion, the modification on the sweet potato starch via hurdle technologies do affect the physicochemical properties of the starch. The experimental design using the Design Expert Software shows that Sample B; maceration of sodium metabisulfite, ozone oxidation and ultrasound treatment is the optimum condition to produce the highest yield of sweet potato starch with desirability of 0.83.

## 5.2 Recommendations

This project has clearly identified the need for further experimental work to confirm the effect of different macerations and physical or chemical modifications on the starch on the surface characteristics of sweet potato starch. In present study, the particle shape measurement was not included in this study. The analysis should be included to ensure the particle size of the starch powder, and hence the properties of starch such as swelling power, solubility and viscosity can be studied and correlated. Further in-depth study can be carried out by varying the parameters on the samples and further analysis on the samples such as chemical and pasting properties of starch upon oxidation.

Apart from that, the statistical analysis using ANOVA software can be done in this research to determine whether the factors are significant. Other than that, a correlation and regression study also can be done using Minitab Software.

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# APPENDICES

Table 1: The carboxyl content (%) of each sample

| Sample | Carboxyl Content (%) |
|---|---|
| A | 0 |
| B | 0.261 ± 0.0175 |
| C | 0.133 ± 0.0200 |
| D | 0.207 ± 0.0115 |
| E | 0.099 ± 0.0115 |

Table 2: The amylose content (%) of each sample

| Sample | Amylose Content (%) |
|---|---|
| A | 39.98 ± 0.035 |
| B | 26.73 ± 0.061 |
| C | 31.10 ± 0.032 |
| D | 28.50 ± 0.010 |
| E | 26.07 ± 0.023 |

Table 3: The swelling power (g/g) of each sample

| Sample | Swelling Power (g/g) |
|---|---|
| A | 8.03 ± 0.066 |
| B | 12.04 ± 0.078 |
| C | 12.06 ± 0.076 |
| D | 11.06 ± 0.085 |
| E | 10.98 ± 0.081 |

Table 4: The solubility (%) of each sample

| Sample | Solubility (%) |
|---|---|
| A | 1.00 ± 0.265 |
| B | 2.70 ± 0.200 |
| C | 2.27 ± 0.306 |
| D | 1.50 ± 0.200 |
| E | 1.07 ± 0.153 |

Table 5: The syneresis (%) of each sample

| Sample | Syneresis (%) | | |
|---|---|---|---|
| | Day 1 | Day 2 | Day 5 |
| A | 62.67 ± 0.252 | 67.67 ± 0.153 | 74.33 ± 0.153 |
| B | 76.33 ± 0.551 | 81.33 ± 0.208 | 85.67 ± 0.208 |
| C | 73.67 ± 0.208 | 78.33 ± 0.252 | 82.00 ± 0.100 |
| D | 72.67 ± 0.252 | 75.67 ± 0.208 | 81.00 ± 0.100 |
| E | 66.67 ± 0.252 | 71.67 ± 0.153 | 79.33 ± 0.208 |

Table 6: The viscosity (mPa.s) of each sample

| Sample | Viscosity (mPa.s) |
|---|---|
| A | 5.037 ± 0.03 |
| B | 4.813 ± 0.02 |
| C | 4.926 ± 0.03 |
| D | 4.998 ± 0.02 |
| E | 5.081 ± 0.03 |

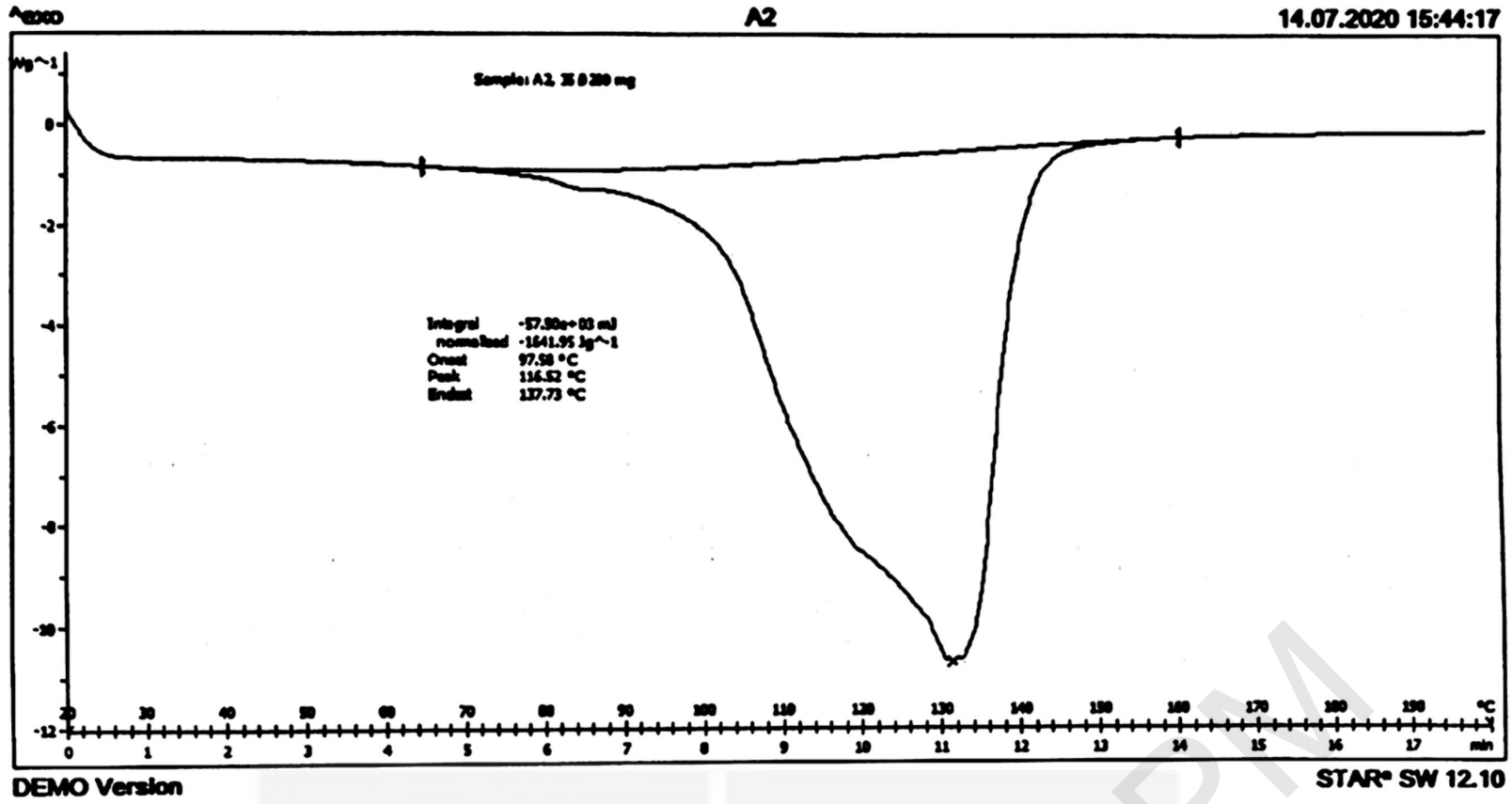


Figure 1: Starch gelatinization profiles of Sample A

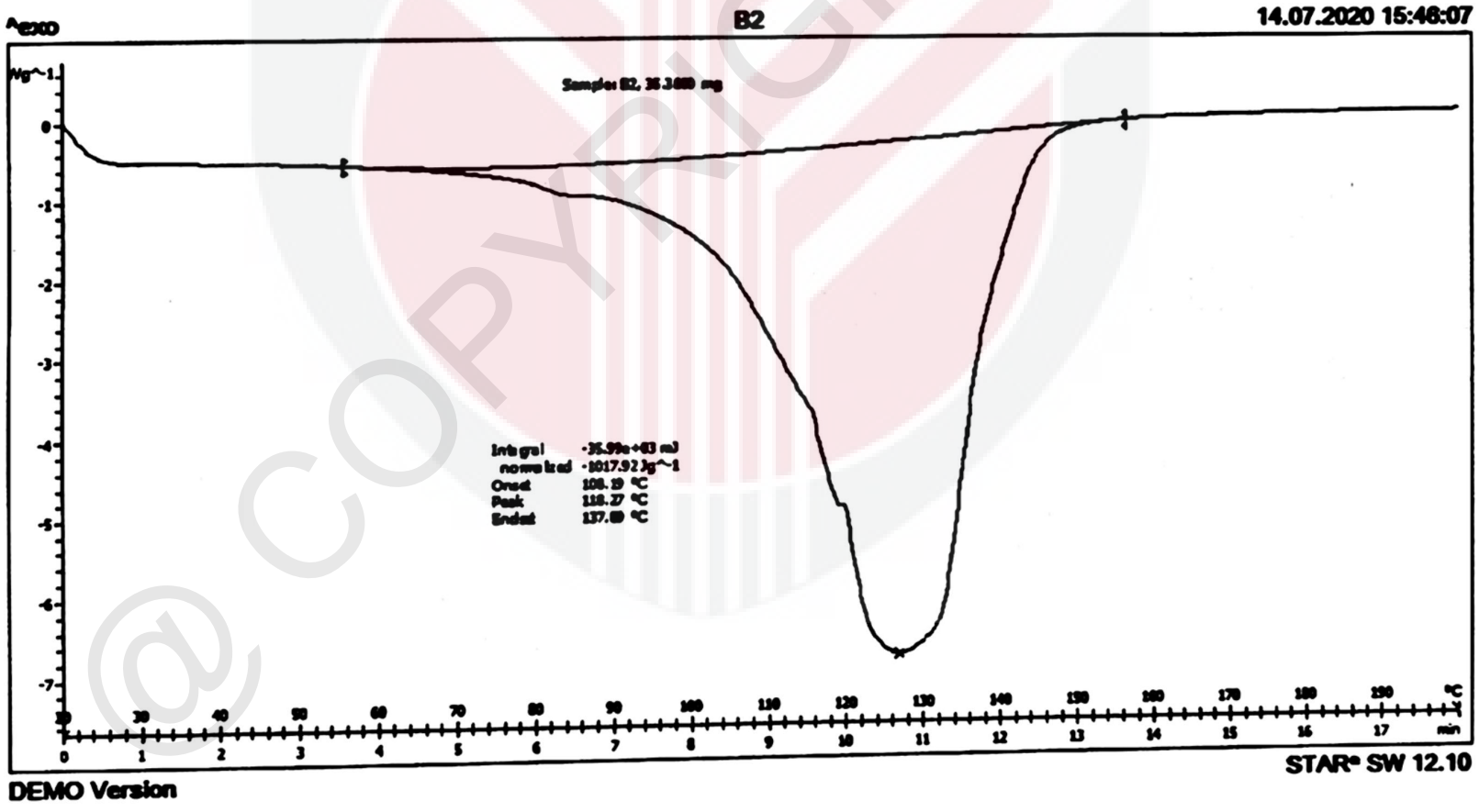


Figure 2: Starch gelatinization profiles of Sample B

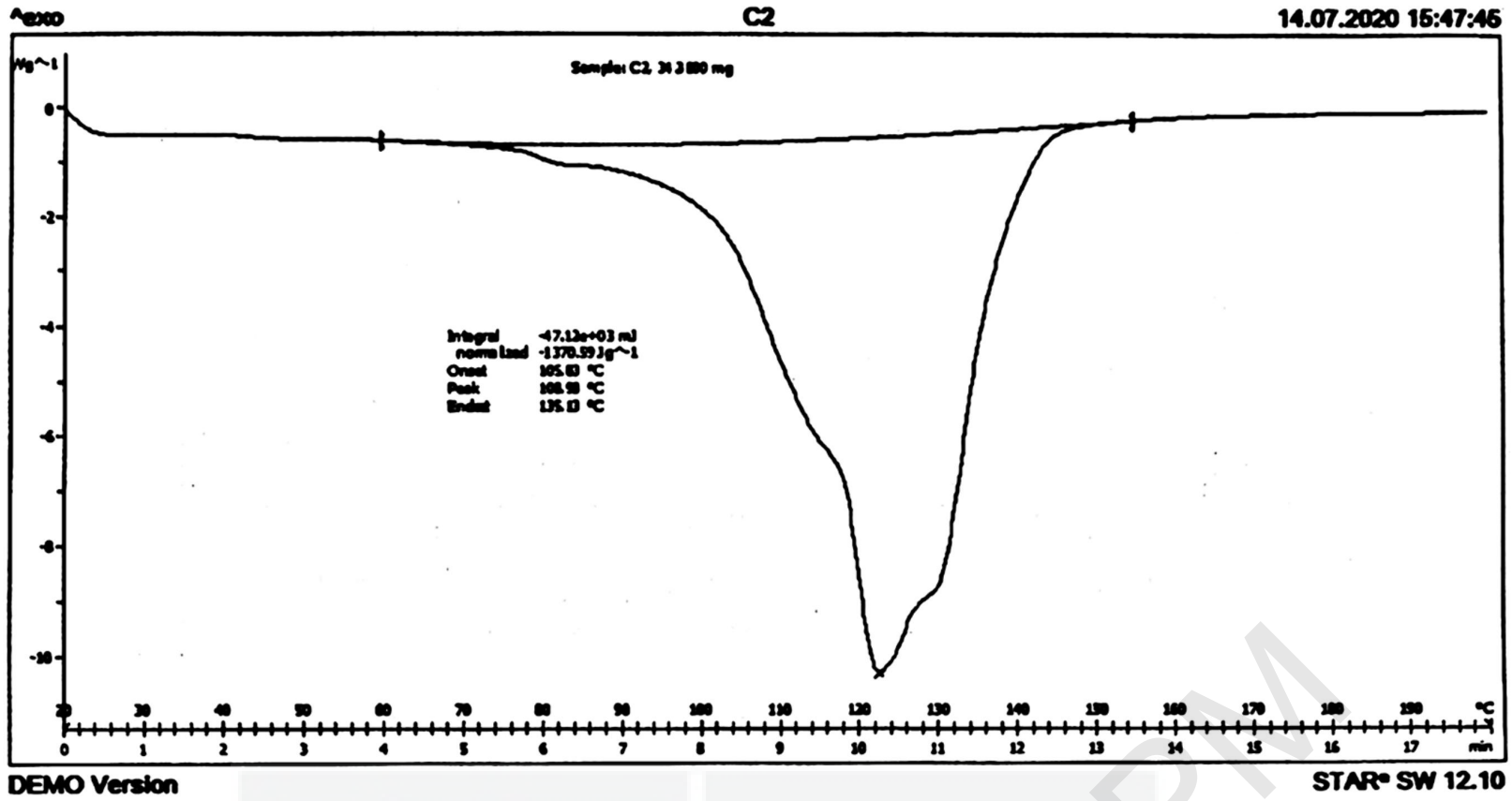


Figure 3: Starch gelatinization profiles of Sample C

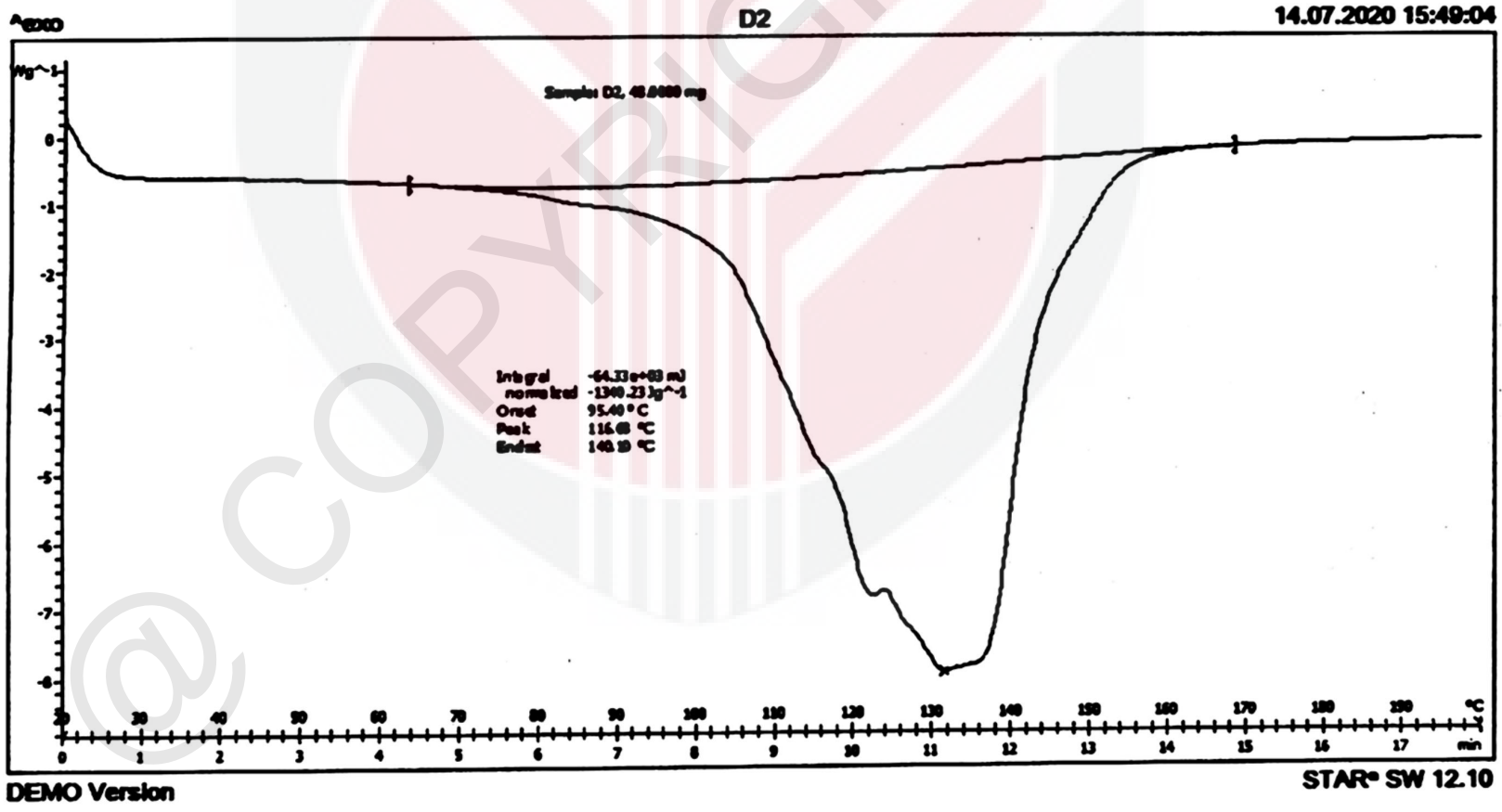


Figure 4: Starch gelatinization profiles of Sample D

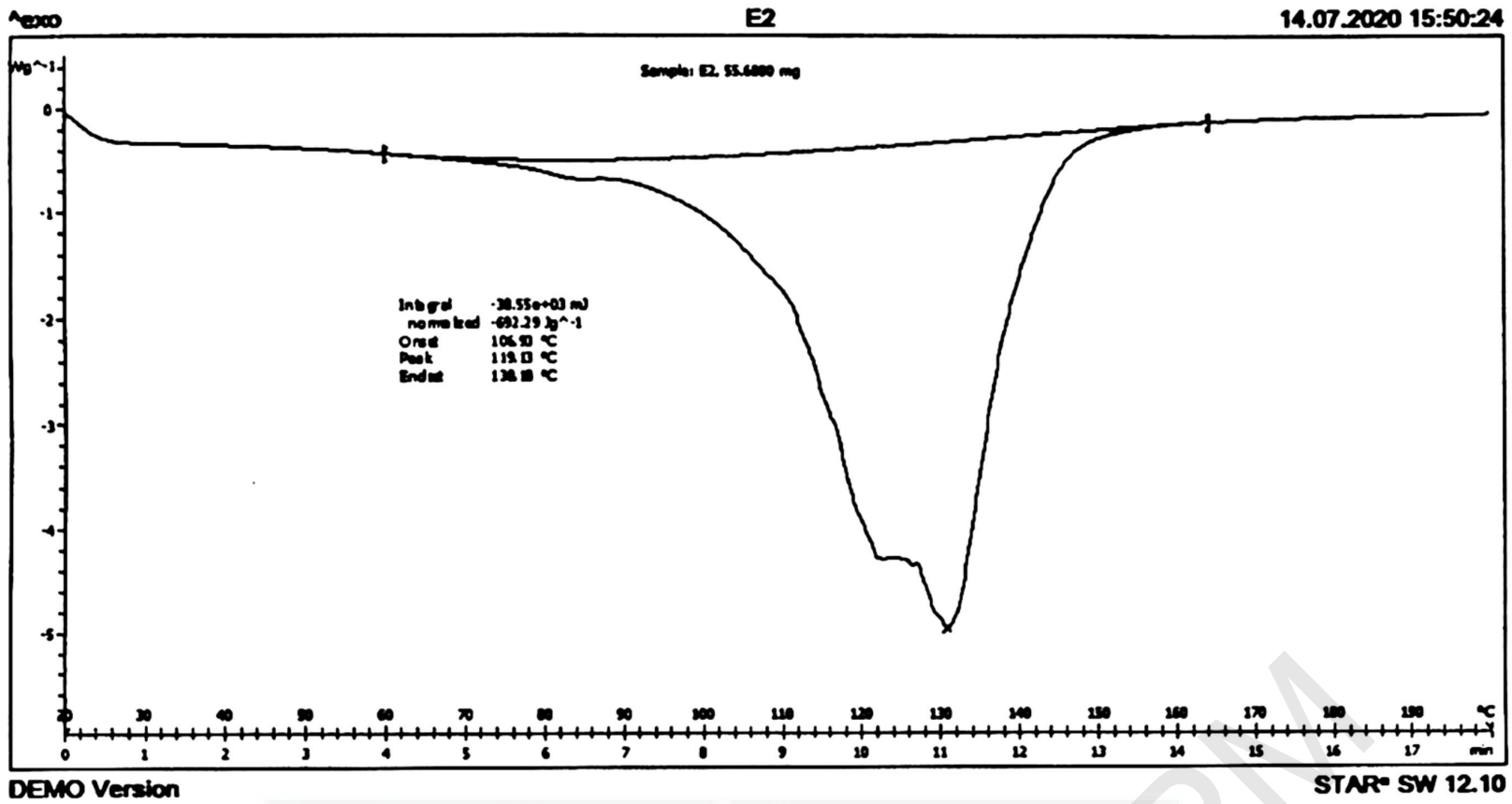


Figure 5: Starch gelatinization profiles of Sample E

Figure 6: Experimental set-up for sonication

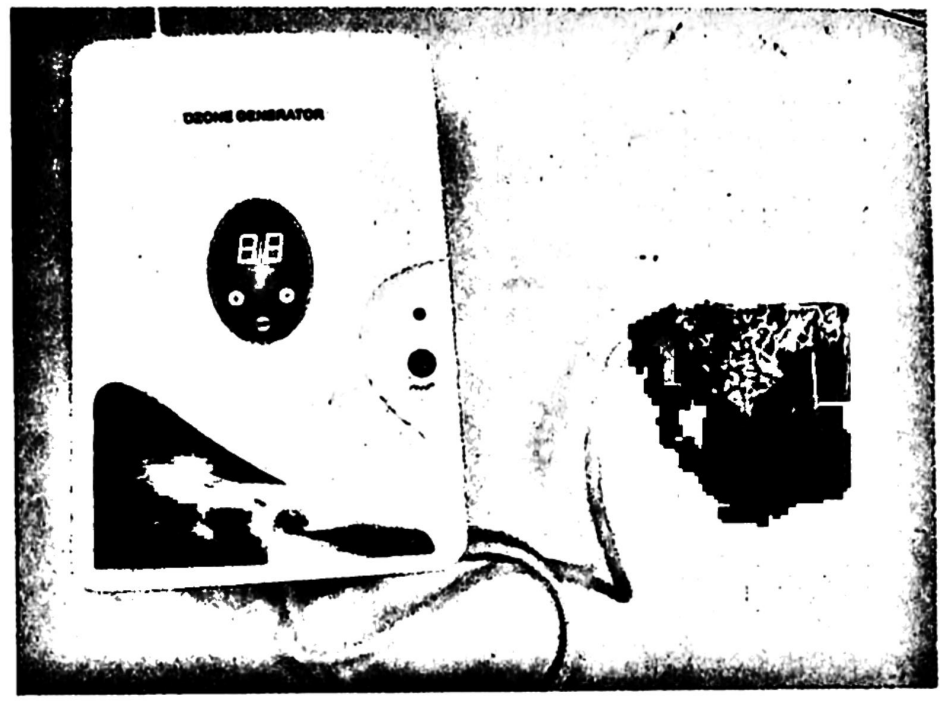


**Figure 7: Experimental set-up for ozone-oxidation**