UNIVERSITI PUTRA MALAYSIA

IMPACT OF HURDLE METHODS ON THE NUTRITIONAL COMPOSITION, 5-HMF AND MICROSTRUCTURES OF OXIDIZED ORANGE SWEET POTATO (VITATO TM) STARCH

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ABSTRACT

Orange sweet potato, VitAto™ is known for its high content of β-carotene, vitamin C, dietary fiber content and low glycemic index. However, industrial utilization is extremely limited where orange sweet potato is not commonly used in advanced technologies to produce starch, flour and powder. Thus, the main objective of this study is to investigate the effects of maceration mediums (sodium metabisulfite and distilled water) and hurdle processing treatments (ultrasound and ozone) on vitamin A, E and C content, 5-Hydroxymethylfurfural (HMF), microstructures, starch yield, and color of oxidized orange sweet potato starch. Starch oxidation via ultrasound (24W, 20kHz, 30 mins and 40% amplitude) and ozonation (1 ppm for 4 hours) treatments are proposed. Comparison study between sodium metabisulfite and distilled water were chosen as maceration medium to lower the risk of health effects from the chemical. The result revealed that the ozonation degraded vitamin A content while ultrasound degraded vitamin E content. Meanwhile, the ultrasound and ozonation increased vitamin C content. The presence of heat generated from ultrasound increased the content of 5-HMF through the Maillard reaction. However, sodium metabisulfite was shown to have preserved the carotene content and produced lower content of 5-HMF. Besides, ultrasound disrupted the starch granule and enhanced the starch yield of oxidized orange sweet potato starch. The relation between vitamin A and 5-HMF with lightness of color were inversely proportional where when the lightness increased, the content of vitamin A and 5-HMF decreased. Finally, the optimum method to produce oxidized orange sweet potato starch in this study was obtained from the combined treatment of ultrasound and ozone with sodium metabisulfite as a maceration medium.

ABSTRAK

Ubi keledek oren, VitAto^{TM} terkenal dengan kandungan β-karoten, vitamin C, serat nutrisi dan index glisemik yang rendah. Walau bagaimanapun, penggunaan industri sangat terhad di mana ubi keledek jarang digunakan dalam teknologi canggih untuk menghasilkan kanji, tepung dan serbuk. Oleh itu, objektif kajian ini adalah untuk mengkaji kesan pelbagai medium maserasi (natrium metabisulfit dan air suling) dan rawatan pemprosesan rintangan (ultrasound dan ozon) pada kandungan vitamin A, E dan C, 5-Hydroxymethylfurfural (HMF), struktur mikro, hasil kanji, dan warna kanji ubi keledek oren teroksida. Pengoksidaan kanji melalui ultrasound (24W, 20kHz, 30 min dan amplitud 40%) dan rawatan ozon (1 ppm selama 4 jam) dicadangkan. Kajian perbandingan antara natrium metabisulfit dan air suling dipilih sebagai medium maserasi untuk menurunkan risiko kesan kesihatan dari bahan kimia tersebut. Hasil kajian menunjukkan bahawa ozon menurunkan kandungan vitamin A sementara ultrasound menurunkan kandungan vitamin E. Sementara itu, ultrasound dan ozon meningkatkan kandungan vitamin C. Kehadiran haba yang dihasilkan dari ultrasound meningkatkan kandungan 5-HMF melalui reaksi Maillard. Walau bagaimanapun, natrium metabisulfit terbukti dapat mengekalkan kandungan karoten dan menghasilkan kandungan 5-HMF yang lebih rendah. Seterunya, ultrasound mengganggu butiran kanji dan meningkatkan hasil kaji ubi keledek oren teroksida. Hubungan antara vitamin A dan 5-HMF dengan kecerahan adalah berkadar songsang bermaksud apabila kecerahan meningkat, vitamin A dan 5-HMF menurun. Justeru itu, kaedah optimum untuk menghasilkan kanji ubi keledek oren teroksida dalam kajian ini diperoleh daripada rawatan gabungan ultrasound dan ozon dengan natrium metabisulfit sebagai medium maserasi.

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LIST OF ABBREVIATIONS

5-HMF	5-Hydroxymethylfurfural
BRIS	Beach Ridges Interspersed with Swales
CAGR	Compound annual growth rate
DU	Ultrasound with Distilled Water as a Maceration Medium
DUO	Combined treatment of Ultrasound and Ozone with Sodium Distilled Water as a Maceration Medium
ECER	East Coast Economic Region
FAO	United Nation's Food Agriculture Organization
FDA	Food and Drug Administration
GRAS	Generally recognized as safe
MARDI	Malaysia Agricultural Research and Development Institute
O_2	Oxygen
O_3	Ozone
SO	Ozone with Sodium Metabisulfite as a Maceration Medium
SPCSV	Sweet potato chlorotic stunt virus
SPFMV	Sweet potato feathery mottle virus
SUO	Combined treatment of Ultrasound and Ozone with Sodium Metabisulfite as a Maceration Medium
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background

Malaysia is one of the producers of sweet potatoes other than Vietnam and Indonesia in Southeast Asia. It is reported by Hanizah Rasad (2017), there are about 2726.59 hectares of land are used for planting sweet potatoes, indicating that demand of sweet potatoes is stable throughout the year. Sweet potato (Ipomoea batatas) belongs to the Convolvulaceae family which originates from America with tropical regions. Sweet potatoes grow underground and are considered root vegetables which the root is described as tuberous root. The growing period of sweet potato is 110 days with a temperature of at least 27°C to 30°C, which is ideal for Malaysia's climate.

Malaysian Agricultural Research and Development Institute (MARDI) introduced a new variety of sweet potato called VitAto™, a 10 years breeding program established in June 2007. VitAto™ variety is also listed as one of the recommended crops under the East Coast Economic Region (ECER). Based on research by Roslan Ismail (2012), VitAto™ is suitable to be planted on BRIS (Beach Ridges Interspersed with Swales) soil which is a type of soil commonly found in Kelantan and Terengganu. Furthermore, the cultivation of VitAto™ has excellent properties such as high yield performance, rich in beta carotene

(2066 mg/100 g), vitamin C (1.33 mg/100 g), dietary fiber content (2.49 g/100 g) and has a low glycemic index of 54 (Mahmood et al., 2007). Since sweet potato is gluten-free, a person with gluten intolerance or celiac disease can consume sweet potato (Watson, 2020). To a large extent, the excellent properties of VitAto™ should be fully utilized in the local food industry.

Over the years, starch is widely used in the food industry as a thickening agent, food additive and snack as it has the capability to retain the nutritional value of the raw materials, available in abundance, low cost and consumable. As stated by Gina (2017), the shelf-life of raw sweet potato which is 3 to 5 weeks at room temperature while the starch has an extended period of shelf-life up to one year at temperature range of 12-15°C with 85-90% relative humidity (Issah et al., 2017). For this reason, starch has been extensively used in both food and non-food application by the industry. Truong and Ramesh (2010) stated that sweet potato puree and powders might be the major components in processed foods, providing the functionality necessary. Starch is commonly used in the processing food industry to produce natural and healthy products such as baby puree, baked products, desserts, bread, and biscuits (Amy, 2020). Based on Sweet Potatoes Market – Forecast (2021 – 2026) by analyst of IndustryARC, sweet potatoes market size was around $42.0 billion to $43.5 billion in 2018 and the compound annual growth rate (CAGR) is expected to be 1.1% to 1.6% during the forecast period of 2019-2025. The market growth is driven mainly by the high demand for natural and balanced food ingredients. Hence, starch is a suitable replacement for synthetic ingredients added in food to enhance the color, flavor and sweetness of the product.

Because of the increasing utilization of sweet potato starch for various purposes in the food industry, the functional properties of sweet potato starch have the greatest significance. Oxidized starch is one of the modifications of native starch to establish the highest quality of products that have a longer shelf-life with lower production costs (Pandiselvam et al., 2019). As reported by Syahariza & Yong (2017) the modified starch has outstanding heat stability and improves solubility. This is in agreement with a study by Shah et al. (unpublished work) where oxidized sweet potato starch produced with the combination of gaseous ozone and ultrasound treatment have increased the water uptake, solubility, and swelling ability significantly. In response to the rising demand for starch, modified starch gained the most attention to be used in food processing.

5-Hydroxymethylfurfural (HMF) is a food contaminant with harmful properties such as cytotoxic that can cause damage to healthy cell. 5-HMF developed from glucose and fructose that were available in sweet potato react with acids at high temperature. Honey is commonly being studied for 5-HMF content due to its high in sugar content. In contrast to starch, there have only been a few studies on 5-HMF. Besides, most studies of oxidized sweet potato starch have been on the proximate composition (Zulkifli et al., 2021), granular structure (Lima et al., 2021), swelling power and solubility (Wang et al., 2020), gelatinization (Paixão et al., 2021) and pasting properties (Paixão et al., 2021). Even so, lack of study approach on the nutritional value of oxidized starch after the treatments. Due to the good performance of oxidized sweet potato starch from the previous study of Shah et al. (unpublished work), further study on the nutritional composition treated with ultrasound and ozone will be investigated to prolong the shelf-life of the oxidized starch while preserving the nutritional value and carotenoid content.

1.2 Problem Statement

In Malaysia, sweet potato is an under-exploited plant but available in abundance. Usually, sweet potato is used to prepare meals and desserts such as mashed sweet potato, fried sweet potato and '*kuih keria*'. Industrial utilization is extremely limited where sweet potato is not commonly used in advanced technologies to produce starch, flour and powder. This may be seen by observing at the market, where cassava flour, maize flour, and rice flour are widely available and purchased by customers. The observation disclosed that food industries are unaware of the advantages and outstanding characteristics of sweet potato to produce a wide range of products such as crispy chips, French fries, sauce, puree and frozen traditional cakes.

When local sweet potatoes are used as raw material, it will prosper the earnings of the agriculture sector, farmers, manufacturers and the country. For instance, imported wheat flour and Irish potato for making fries and snacks were about US$220 million and US$16.5 million, respectively, each year (Tan & Zaharah, 2006). Thus, instead of using imported food, we should make use of our local sweet potato starch to reduce the high food import bill. To add, sweet potato has excellent properties such as rich in energy, nutrients, and minerals, as well as able to meet daily dietary requirements for β-carotene, thiamin, iron, vitamin C, and protein (Yu et al., 2020).

Besides, there are only few studies on nutritional composition of sweet potato that undergo advanced oxidation technologies method such as ultrasound and ozone treatment. The conventional processing treatments will have adverse effects on the nutritional quality of starch where the amount of nutrients decreased and also have significant impact on the bioaccessibility of beta-carotene supported by previous studies of (Wei et al., 2017; Dhuique et al., 2018; Kourouma et al., 2019; Kolawole et al., 2020). Thus, in order to produce high quality oxidized sweet potato starch that fulfills industrial purposes, the optimal processing treatments need to be investigated.

The use of advanced technologies for starch modification such as ultrasound and gaseous ozone are relatively minimal. There have been few studies on starch and flour which have been researched by (László et al., 2008; Brodowska et al., 2018; Pandiselvam et al., 2019; Lima et al., 2021a; Lima et al., 2021b). Based on previous researches, gaseous ozone treatment improves the rheological, thermal and structural properties of samples, extends the shelf life of products, eliminates undesirable odors and eliminates the presence of microorganisms in food. On the other hand, ultrasound applied to food have higher yields, shorter processing periods, excellent quality characteristics, and reduced microbial populations (Zhu, 2015). However, ultrasound generates heat during the treatment. A study by Castenmiller (1998) showed that presence of heat disrupts the plant cell walls leading to the release of fat-soluble compounds also known as carotenoid content from the protein complexes. This significantly will reduce the amount of nutrients in the sweet potato.

Lastly, to the best of our knowledge, there are limited studies on the impact of ultrasound and ozone treatment on the nutritional composition of sweet potato have been published which means the effects of these treatments has yet to be studied.

1.3 Objectives

The objectives of the study are:

a) To investigate the effects of maceration mediums (sodium metabisulfite and distilled water) and hurdle processing treatments (ultrasound and ozone) on the nutritional composition, 5-Hydroxymethylfurfural (HMF), starch yield and color of oxidized sweet potato starch.
b) To study the effects of maceration mediums (sodium metabisulfite and distilled water) and hurdle processing treatments (ultrasound and ozone) on the microstructures of oxidized sweet potato starch.
c) To determine the optimum parameters to produce oxidized orange sweet potato starch yield.

1.4 Description of Thesis

1.4.1 Scope of Limitations

The scope of this study is limited to VitAto™, a variety of sweet potato obtained from MARDI Bachok, Kelantan. 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.

A standard laboratory test is required to validate the effectiveness of the oxidized orange sweet potato starch produced. However, there will be several limitations throughout conducting this study such as:

i. Heat generated from ultrasound treatment will influence the analysis.
ii. Fluctuation temperature will impact instable humidity and air velocity throughout the drying process.
iii. Able to achieve homogenization during ultrasound and ozone treatment.

1.4.2 Contribution of Thesis

This study contributes to the field of food engineering by proposing the use of hurdle methods to produce oxidized starch. Compared to the conventional method, which took a day to produce starch, hurdle methods reduced the duration to only 8 hours. Other than excellent physicochemical qualities, this study revealed public concern on food safety by looking at the effect of hurdle methods on the nutritional properties, food contaminants, and color of oxidized orange sweet potato starch.

1.4.3 Thesis Structure

The introduction chapter (Chapter 1) introduced the overview of the topic being studied. The main aspects are background information, problem statements, and objectives of the thesis. Next, the literature review (Chapter 2) showed previous research on the topics and presented them in a structured manner. Here, the gaps between the research are known. The methodology (Chapter 3) revealed the method used in this study to obtain the result analysis of the experiment. After that, the result (Chapter 4) is to analyzes the result analysis data and justifies the results. In this chapter, graphs, figures and tables are provided to visualize the results. Finally, the conclusion chapter (Chapter 5) includes a summarized analysis and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Sweet Potato

Sweet potato (Ipomoea batatas) originated in Central and South America (Doucleff, 2013). Sweet potatoes grow well in tropical or temperate climates with temperatures ranging from 24 to 35°C. Furthermore, it can be cultivated in a wide variety of altitudes, from sea level to 2500 meters (Sweet Potato Facts and Figures, n.d). Sweet potato harvesting can begin as early as 120 days or about 4 months. Normally, sweet potato harvested twice within a year.

The sweet potato grows best in BRIS soil, which is well-drained, fine sandy loam soil with a slightly acidic pH 5 to 7.5. In Peninsular Malaysia, this type of BRIS soil covered 165,000 ha and 3,041 hectares are planted with this crop (Tan, 2015). BRIS soil allows the sweet potato to grow easily but not remain in a moist environment that promotes rot and disease such as sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV) (Nome et al., 2007).

Sweet potato can be a supplementary staple to rice as sweet potato is high in protein, dietary fiber, minerals and vitamins. The list of nutrients can be referred to Table 2.1.

Table 2.1 Nutrient composition of sweet potato compared with cassava, potato and white rice (100 g edible portion) *source from Utar Agriculture Science Journal

Nutrient	Unit	Sweetpotato[1]	Cassava[1]	Potato[1]	White rice[2]
		per 100 g edible portion			
Proximates					
Water	g	12.0	12.0	12.0	12.89
Energy	kcal	333	349	328	360
Protein	g	6.08	2.97	8.60	6.61
Total fat	g	0.19	0.61	0.38	0.58
Ash	g	3.83	1.35	3.41	0.58
Carbohydrate (by difference)	g	77.93	83.07	74.41	79.34
Total dietary fibre	g	11.6	3.9	9.4	N
Minerals					
Calcium	mg	116	35	51	9
Iron	mg	2.36	0.59	3.32	0.80
Magnesium	mg	97	46	98	35
Phosphorus	mg	182	59	243	108
Potassium	mg	1305	592	1793	86
Sodium	mg	213	31	26	1
Zinc	mg	1.16	0.74	1.24	1.16
Copper	mg	0.585	0.218	0.404	0.110
Manganese	mg	0.999	0.838	0.767	1.100
Selenium	mcg	2.3	1.5	1.4	N

2.1.1 VitAto^{TM}, a Variety of Sweet Potato

The flours and starches from different sweet potato cultivars differ in their amylose, amylopectin, ash and phosphorus contents, starch granule size, water absorption and solubility, retrogradation, crystallinity, and their thermal and pasting properties (Paixão et al., 2021). In Malaysia, there are several studies on varieties of sweet potato conducted by MARDI such as Gendut, Telong, Jalomas and VitAto^{TM}. Each type of variety has different benefits and functions. The Gendut variety suitable for snacks, Telong and Jalomas has excellent properties for flour while VitAto^{TM} suitable for food products and beverages. This can be referred to Table 2.2.

Table 2.2 MARDI sweet potato varieties *source from Utar Agriculture Science Journal

Variety	Root yield (t/ha)	Special characteristics	Suitability
Gendut	20-25	Yellow flesh; Fragrant and highly palatable	Roots: table use (boiled or steamed); making fries Shoots: vegetable
Telong	20-25	High dry matter content (30%) in roots; High lutein content in leaves	Roots: flour (possibly for biofuel production) Leaves: vegetable with high lutein
Jalomas	15-20	Orangey flesh; High dry matter content (25%) in roots	Roots: flour
VitAto™	25-40	Orange flesh, high in carotenoids (source of vitamin A)	Roots: carotene-rich flour; processing into various food products; juice

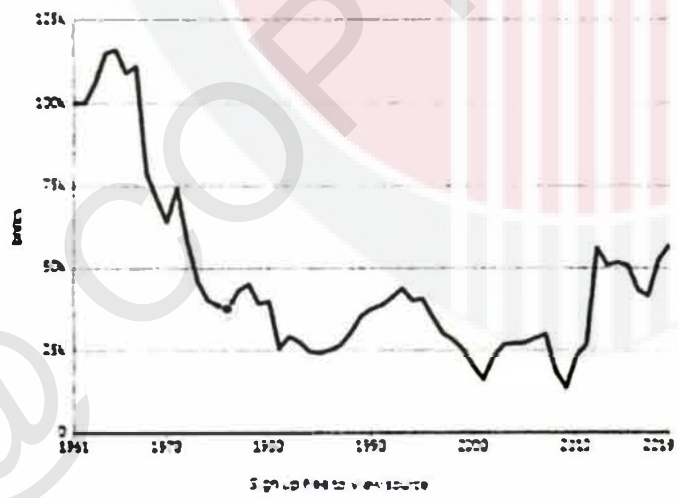
The VitAto^{TM} variety is the popular varieties of sweet potato used in Malaysia due to its outstanding properties and widespread purposes. Table 2.3 shows several studies on VitAto^{TM} variety.

Table 2.3 Several studies on VitAto^{TM} variety.

Information	Studies	References
The VitAto^{TM} has highest protein (5.7%), dietary fiber (14.8%), energy of 399.6 kcal/100g.	Physicochemical and flowability characteristics of a new variety of Malaysian sweet potato, VitAto^{TM} Flour	(Mohd Hanim et al., 2014)
The VitAto^{TM} is rich in beta carotene (2066 mg/100 g) and vitamin C (1.33 mg/100 g), dietary fiber content (2.49 g/100 g) and has low glycemic index of 54.	Varieti ubi keledek baru (The variety of new sweet potato)	(Mahmood et al., 2007)
The findings indicated that VitAto^{TM} exhibited the highest starch (16.95% – 17.17%) and crude protein (0.86% – 1.15%) contents in all parts as compared to Anggun 1, Anggun 2, Anggun 3 and White. The skin contained the highest crude protein, crude fat, ash and crude fiber among all the parts.	Proximate composition of Malaysian local sweet potatoes	(Zulkifli et al., 2020)

2.1.2 Market Analysis of Sweet Potato

With a global yearly production of approximately 133 million tonnes, sweet potato is the world's seventh most significant food crop, following wheat, rice, maize, potato, barley, and cassava (Ali et al., 2017). To add, Asia is the largest sweet potato producing region with 125 million tons of annual production (Arogundade et al., 2012). Besides, sweet potato production increased from 2.516 million metric tonnes in 2006 to 3.4 million metric tonnes in 2007, with China accounting for over 90% of global sweet potato production at 117 million tonnes per year (Srinivas, 2006). According to an IndustryARC analyst's business intelligence study, Asia-Pacific (APAC) controls 80-83 percent of sweet potato market share and expected to grow in the forecast period of 2019-2025. Furthermore, in Malaysia, sweet potato production increase from 2010 to 2019 (Figure 2.1). These increased were due to improve of technological inputs, international and national initiative in researching and potential of sweet potato in starch industry.



DATE	VALUE	CHANGE, %
2019	56,323.00	785 %
2018	52,225.00	26.62 %
2017	41,245.00	-4.55 %
2016	43,212.00	-14.61 %
2015	50,60700	-1.69 %
2014	51,476.00	1.43 %
2013	50,748.00	-9.12 %
2012	55,838.00	11006 %
2011	26,582.00	15.30 %
2010	23,054.00	7083 %
2009	13,495.00	-25.97 %
2008	18,228.00	

Figure 2.1 Graph of sweet potato production through 1970-2019 in Malaysia *source from Knoema

2.1.3 Application of Sweet Potato

Sweet potato marketing is a profitable business with high net return on investment. According to the food and beverage market analyst of IndustryARC, sweet potatoes market size was around $42.0 billion to $43.5 billion in 2018. The rise in market size verifies that sweet potato is functional crop which can be utilized in various type of field.

Sweet potato can be health-promoting foods as it rich with nutrients. For instance, sweet potatoes contain anthocyanins, a kind of antioxidant that inhibits the development of cancer cells. Thus, suggested sweet potato has protective effect that may reduce the risk of cancer. Furthermore, people nowadays become more health-conscious and seek vitamin supplements in their diet. Therefore, sweet potato can be developed as a dietary supplement.

Besides, wheat flour can be replaced by using sweet potato flour where annual imports of wheat are 4.5 million tonnes, worth RM101.3 million. Flour made from sweet potato can be used to prepare cakes, muffins, and biscuits, snacks and sweet buns. Nuggets and breaded sweet potato also can be made directly from raw sweet potato. In addition, sweet potato can replace imported 'Irish' potato for fries production that worth RM229 million per year. As a consequence, sweet potatoes cultivated in Malaysia may be utilized as useful ingredients in food product while also contributing to the country's economic development.

2.2 Starch

2.2.1 Native Starch

In general, starch is a type of carbohydrate obtained from plants and each plant varied in starch distribution based on its species (Wang et al., 2015). According to Lai et al. (2016), sweet potato contains 50% to 80% starch. Starch is composed of α-glucose polymers which are amylose and amylopectin, the two most important components found in starch compounds. Normally in starch harvested products, amylose was found to be 20– 30%, while amylopectin 70 – 80%. Amylose to amylopectin ratio are important to determine physicochemical properties of starch (Schwall et al., 2000). As reported by McMaugh et al. (2014), amylose to amylopectin ratio affects cooking, processing, organoleptic characteristics, and starchy food digestion. In addition, starch properties also influence by the average chain lengths of amylose and amylopectin and the way that amylopectin is packaged within the semi-crystalline domains of starch granules (Vanier et al., 2017).

Amylose has a low molecular weight and numerous long branches, whereas amylopectin has a large number of small branches and highly branched polymers (Syahariza et al., 2013). Amylose is a linear polymer with a-1,4 linked glucopyranosyl units(Figure 2.2(a)) while amylopectin is a highly branched molecule with (1-4)-linked a-D-glucopyranosyl units in chains joined by a-1,6 linkages(Figure 2.2(b)). These two major polysaccharides present in the form of water-soluble lamellae and a semi-crystalline structure within the starch granule (Ahmed et al., 2016). According to Bento et al. (2019), the amylose content effect the functional characteristics of the starch, since it contributes to an increase in the resistant starch content due to retrogradation that favoring low glycemic values. As stated by Qiu et al. (2017), during heating of starch with abundance of water, gelatinization occurred where starch takes up water and swelling inside the granule. In this condition, amylose and amylopectin disappear. When there is no presence of heat, amylose and amylopectin begins to develop again, the process is known as retrogradation.

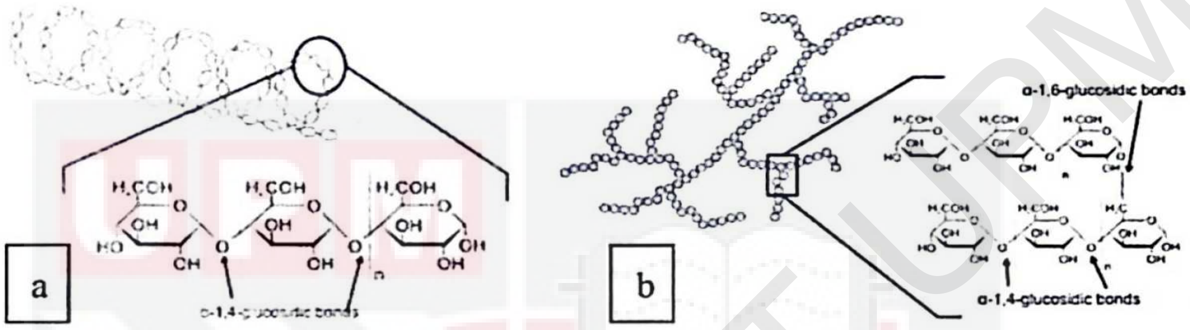


Figure 2.2 Chemical structure of amylose(a) and amylopectin(b) *source from The Science of Nutrition Blog

For many years, starch is widely utilized as an industrial component in huge amounts across a wide range of industries, including food, feed, paper, textile, laundry finishing, chemical, petrochemical, pharmaceutical, bioethanol, building materials, and biodegradable goods (Vanier et al., 2017). In order to improve the quality of starch-based products, numerous starch sources have different degrees of gelatinization, allowing them to be used as functional food ingredients (Xing et al., 2017). Therefore, starch is gaining biggest attention as compared to other carbohydrate polymers due to its diverse application of food products (Santoso, 2018). Because of its widespread availability and inexpensive cost, starch also has been modified to fit industrial needs. Therefore, a development of native starch into modified starch established to meet the industries requirements.

2.2.2 Modified Starch

Starch will be less functional if it is in its native conditions despite of its sources and origins. This is supported by a previous research, which found a number of drawbacks of native starch, such as sensitive to pH, poor starch paste clarity, high retrogradation, low syneresis, and decomposition (Bismark et al., 2018). In addition, Hu et al. (2013) stated that native starch cannot be utilized in food preparation due to several adverse natural properties, such as low gelatinization point and instability.

To meet the needs of industrial manufacturing, many techniques such as chemical, biological, and physical changes have been used in the past (Figure 2.3). Based on research, modified starch had been proven to improve viscosity, shelf life, textures, solubility, appearance, and emulsification of food products (Falade & Ayetigbo, 2015). Three most common function of modified starch in food industry work as agent of gelling, stabilising and thickening (Pietrzyk et al., 2018).

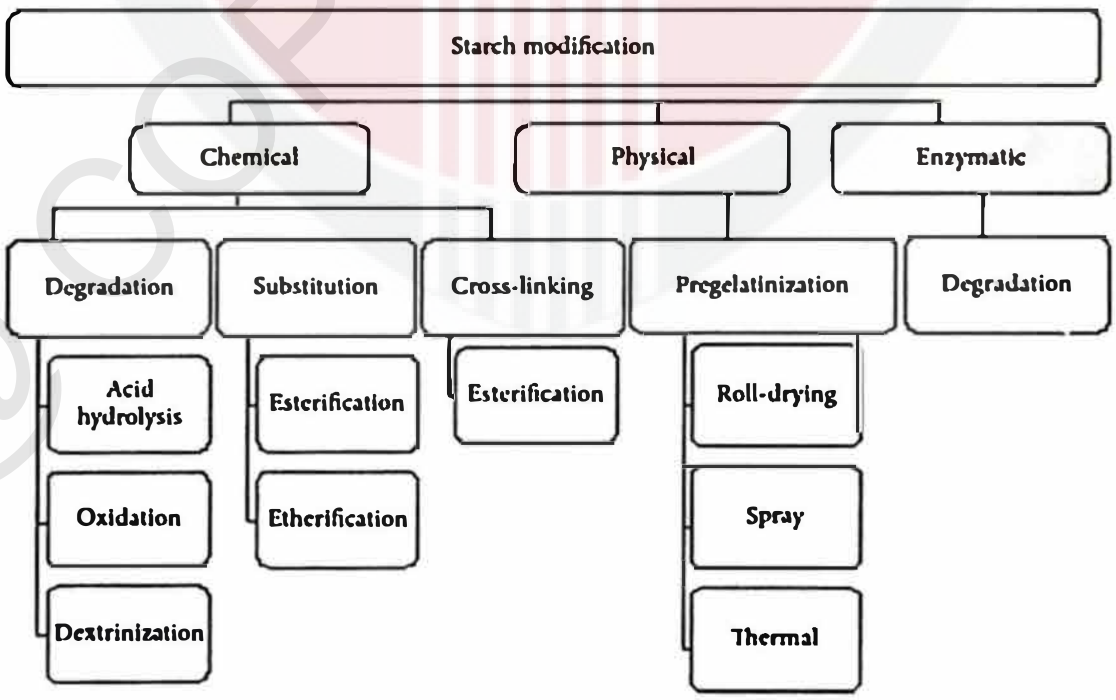


Figure 2.3 Starch modification techniques *source from (Lewicka et al., 2015)

2.2.3 Oxidized Starch

Chemical modification methods are the most widely used in industry because of their low cost and ease of use. Oxidized starch is one of the modified starch that undergoes treatment for better starch characterization (United Nation's Food Agriculture Organization, 2016). Besides, the structural and physicochemical properties of oxidized starch can be modified depends on molecular structure of the native starch, the packing of the crystalline lamellae, the size of the amorphous lamellae as well as the type of oxidizing agent used along with the reaction conditions (Sangseethong et al., 2010; Wang & Wang, 2003).

The oxidized starch claimed to have better water solubility, lower viscosity, and retrogradation tendency compared to the native starch. Futhermore, Zaidul et al. (2007) also reported that oxidized starch has low gelatinization temperature, high water-binding capacity, high paste consistency, and good paste clarity. A study by Biduski et al. (2017), oxidized starch has stable tensile strength. Previous study also suggested that oxidation improves the solubility and paste clarity of white sorghum starch, making it a suitable choice for food products needing high solid content without over-thickening (Ali & Hasnain, 2014).

2.2.4 Application of Sweet Potato Starch

Different starches source fulfilling various industrial demands. Food products based on starch provide 50–55% of a person's total daily energy (Mohamad Yazid et al., 2018). The common starch-based products available in Malaysia are sauce production, puddings, foods for baby processed, meats, snacks, bread, fermented food, pasta, pastries, soups and noodles. This is because, food industry used starch as a thickening, bulking agent, gelling and for water holding capacity making it an excellent ingredient for all types of food (Afolabi et al., 2018). On the other hand, starch viscosity properties, retrogradation, and pasting factors that can affect the appearance, grade, and shape of foods, making starch the important functional component in food products (Ahmad et al., 2012). According to Syahariza et al. (2017) and Zhou et al. (2018), food industry used starch as food additive due to its gel-forming ability, thickening agent and stabilizing food products. Table 2.4 listed some of application of sweet potato starch in food industry.

Table 2.4 List of sweet potato application

Application	References
Starch Noodles and Vermicelli	(Chen et al., 2003; Huang et al., 2008; Zhao et al.,2009)
Tomato sauce	(Wu et al., 2009)
Thickening agent	(Zou et al., 1996)
sauces, snacks, instant soups, dairy desserts, yogurts, and bread.	(Morais et al., 2021)
Treating diabetic patients	(Li et al., 2017)
Food additive	(Syahariza et al., 2017; Zhou et al., 2018)
Soup mixture	(Senanayake et al., 2014)

2.3 Ultrasound Treatment on Oxidized Starch

In recent years, physical modification method has been paid a lot of attentions. Ultrasound is one of physical modification method that is unique and offers a wide range of applications in the production of modified starch. It is called physical modification because of electrical energy is used to power transducers (ceramic materials), which transform the energy into mechanical vibrations and create high-intensity ultrasound (Kentish and Feng, 2014). According to a recent study, ultrasound is gaining popularity due to its several advantages such as cheap cost, high efficiency, and eco-friendliness because free from chemicals usage (Li et al., 2018).

Ultrasound is composed of mechanical sound waves that develop from oscillation of molecular movements in a propagation medium. The ultrasonic waves are classified into two categories which are low intensity and high intensity. The low intensity involved in the noninvasive analysis such as ultrasonography used in medical diagnosis (Mulet et al., 2002). In contrast, the high intensity wave can modify physicochemical properties of food and disrupt the crystalline area in starch granules (Ortega, 2012).

Ultrasound can generate cavitation and mechanical action in liquid. Cavitation is the fundamental mechanism of ultrasound that allows chemical reactions to occur. According to Wang et al. (2020), ultrasound means the mechanical waves with a frequency exceeding 16 kHz, which can be transformed to "micro-jets", "high shearing force and turbulence" and "high pressure" through the cavitation effect, causing the molecular degradation and inherently structural transformations of starch granules. Therefore, the cavitation effect of ultrasound can produce three kinds of mechanical forces, including local ultra-high pressure, high speed jet, and high frequency vibration (Nguyen et al., 2017). The starch is attacked by active radicals such as hydroxide (OH), oxygen (O_2), and hydroperoxyl radical (HO_2) produced by the transient collapse of cavitation bubbles. Figure 2.4 show example of rice fissure mechanism caused by ultrasonic irradiation by a study from (Bonto et al., 2021). To add, cavitation impact is highly dependent on the operation conditions, especially the frequency. According to Fan et al., (2017) lower frequency of ultrasound will produced larger bubbles and the cavitation process causing both covalent and non-covalent bond inside starch to be broken down.

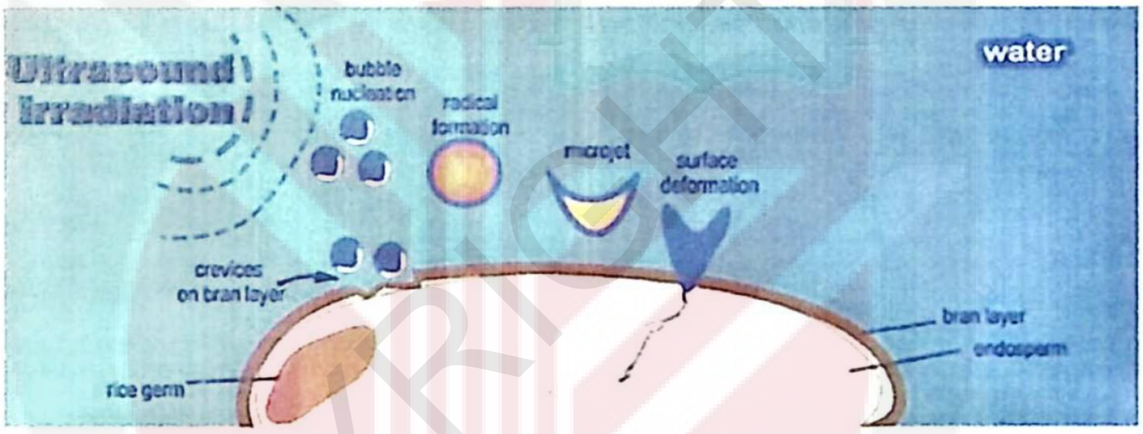


Figure 2.4 Rice fissure mechanism caused by ultrasonic irradiation * source from (Bonto et al., 2021)

Based on Figure 2.5, the treatment can cause visible cracks and pores on the granule surface that may increase in swelling power and solubility of starch granules due to increase in surface area of starch (Zheng et al., 2013). Table 2.5 showed several studies of ultrasound treatment on sweet potato starch. Mainly, the ultrasound treatment is purposed to enhance the quality of oxidized starch, safety of the products and sustain the shelf-life of the product. Furthermore, ultrasonic treatment has grown more interesting to both researchers and users as a physical modification technique since it offers numerous advantages in terms of green processing and manufacturing, environmental friendliness, effectiveness, and energy savings (Zhu et al., 2015).

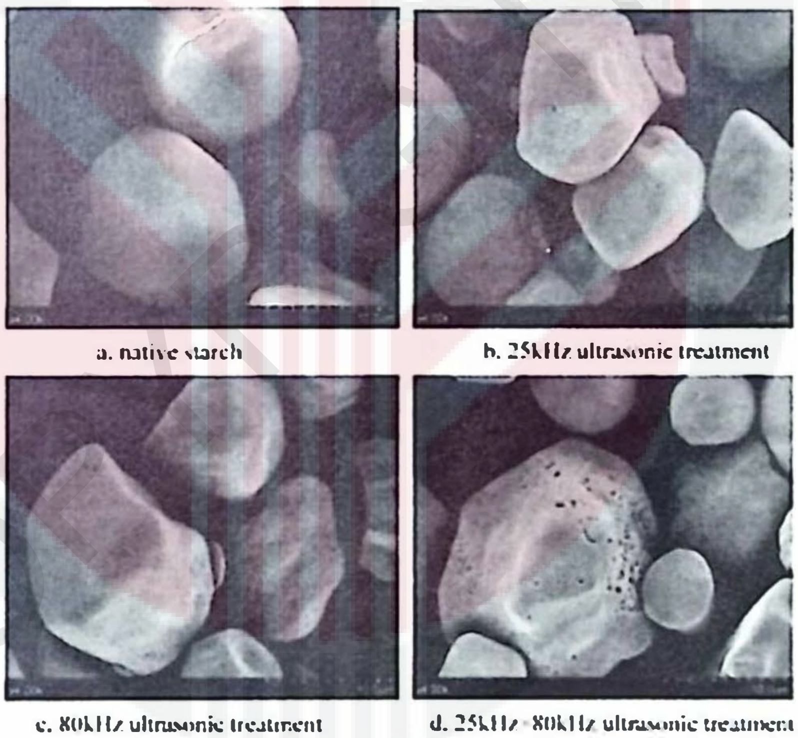


Figure 2.5 SEM photos (4000x) of starch samples treated by different frequency ultrasound for 60 min *source from (Zheng et al., 2013)

Table 2.5 Several studies of ultrasound treatment on sweet potato starch

Findings	Studies	References
Increase the irradiation time will increase the amylose content, particle size and weaken the pasting temperature, peak and breakdown viscosity.	Impact of ultrasonication on the aggregation structure and physicochemical characteristics of sweet potato starch	(Wang et al., 2020).
Ultrasound effects that decreased the pasting and thermal properties, reduced the relative crystallinity of sweet potato starch and caused damage to the surface of starch granules and to the linkage between starch granules.	Effects of high-power ultrasound on the enzymolysis and structures of sweet potato starch	(Jin et al., 2020)
Ultrasound produced a significant mechanochemical effect on starch and went through the stress stage, aggregation stage, and agglomeration stage. The quality of modified starch was significantly improved in the stress stage and agglomeration stage.	Mechanochemical effect of ultrasound on sweet potato starch and its influence mechanism on the quality of octenyl succinic anhydride modified starch	(Zhang et al., 2019)
No significant changes occur in the size of granules when treated with ultrasound, but many dents and holes are found on the surface of starch granules after ultrasonic treatment. Lowering of the viscosity and the peak viscosity of starch. Starch–iodine complex determination shows that ultrasound destroys amylopectin and starch chains, and amylose content increases.	Dual-frequency ultrasound effect on structure and properties of sweet potato starch	(Zheng et al., 2013)

2.3.1 Limitation of Ultrasound Treatment

The effects of ultrasound technology on a product depend on several factors such as the characteristics of the system, characteristics of the product and the ultrasound equipment. The main ultrasound drawback in this case is due to the cavitation effect. The cavitation phenomenon happened when ultrasound waves circulate through a product, the medium undergoes compression-expansion cycles (induced by the pressure differential generated by the acoustic waves), resulting in the creation and implosion of vapour bubbles (Anese et al., 2013). This will cause increased in temperature and pressure due to a great amount of energy and shear is dissipated in a small area. Therefore, while being classified as a "non-thermal" process, ultrasound accidentally creates heat on a microscale, which must be cooled if the study's goal is to examine just the impacts of the ultrasound. In addition, the cavitation on metal surface could cause equipment erosion. Usually, the equipment surface is neglected during processing which may contaminate the sample with metal particles (Anese et al., 2013). These metal micro or nanoparticles can change the properties of measured starch or/and act as bullets during processing which potentially destroying starch granules.

2.4 Ozone Treatment on Oxidized Starch

The industry widely uses chemical alterations such as acetylation, oxidation, cross-linking, hydroxypropylation, and etherification to change native starches. Ozone is an advanced oxidation technology that is regarded as a "environmentally friendly" technology because ozone decomposes into oxygen without leaving harmful by-products (Çatal et al., 2014; Pandiselvam et al., 2019; Maniglia et al., 2021). Recently, consumer and industry preferences have shifted to "environmentally friendly" modification technologies. Ozone treatment is also one of several available techniques that aid in the enhancement of food product safety and quality (Brodowska et al., 2018). To add, ozone can have the GRAS (generally recognized as safe) status for food processing depending on the application (Alexandre et al., 2012; Gaou et al., 2005).

The US Food and Drug Administration (FDA) legally approved the use of ozone as an antimicrobial agent for the treatment, storage, and processing of foods in gas and aqueous phases in response to a Food Additive Petition submitted in August 2000, and stated concentration limit exposure of ozone is 0.05 ppm for 8 hours. Ozone can be applied to starch in aqueous solution or in gaseous phase (Maniglia et al., 2020). According to Brodowska et al. (2018), ozone can be produced in several ways which are exposing air or another gas mixture containing oxygen to an energy source like a high-energy electrical field (corona discharge technique), UV light (phytochemical method), or the conversion of oxygen molecules (O_2) to ozone (O_3) (chemical method). Ozone generators produce ozone by breaking apart oxygen (O_2) molecules into single atoms, which then attach to other oxygen molecules in the air to form ozone (O_3). Table 2.6 show several studies on starch by ozone treatment.

Table 2.6 Several studies of ozone treatment on starch

Findings	Studies	References
Ozone processing modified the structure of starch by reducing the size of amylose and amylopectin, as well as replacing hydroxyl groups with carbonyl and carboxyl groups (via oxidation)	Functional, thermal, and molecular properties of ozonated starches	(Handarini et al., 2020).
Cassava starch showed higher oxidation levels (increase of carboxyl and carbonyl content) and reduction of large and branched molecules with consequent increase of smaller sized molecules. Hydrogels based on cassava starch treated by ozone maintained their consistency even at acidic conditions. Treated starch showed greater water solubility index, lower water absorption index and greater paste clarity than the native starch.	Ozone processing of cassava starch	(Lima et al., 2021)
Ozone treatment resulted in starches oxidized (formation of carbonyl and carboxyl groups) and with lower molecular size. It was also observed better oven expansion, increase of paste clarity, and softer texture.	Ozone technology as an alternative to fermentative processes to improve the oven-expansion properties of cassava starch	(Matta Junior et al., 2019)

This study shows that ozone treatment affects the functional, thermal and molecular properties of the various starches when treated under the same conditions. The treated starches showed properties similar of starches oxidized using conventional alkaline methods.	Functional, thermal and molecular behaviours of ozone-oxidised cocoyam and yam starches	(Oladebeye et al., 2013)
The use of ozone enhanced the colour of the flour. Wheat flour became brighter and less yellow as the ozonation period increased, compared to control flour.	Comparison Between Potassium Bromate and Ozone as Flour Oxidants in Breadmaking	(Sandhu et al., 2011)

2.5 Combined Treatment of Ultrasound and Ozone

The majority of study has focused on the effects of ozonation and ultrasonication performed separately (Castanha et al., 2017; Lee et al., 2017; Vanier et al., 2017; Zhu, 2015). Ozone is desirably in modifying starches from various sources such as corn, cassava, wheat, potato and rice. Whereas the ultrasound treatment improves the starch structure that enhance ozone activity (Castanha et al., 2019). Moreover, both ozonation and ultrasound operate without the need of heat energy, resulting in energy savings. In the starch processing industry, the approach of combining ultrasound and ozonation might be a viable alternative to traditional methods. Table 2.7 showed several studies of combining ozone and ultrasound treatment on starch. The starch industry may benefit from the combination of ozonation and ultrasound because it lowers operational costs, reduces environmental hazards, and leaves no residue on the final products (Ibanoğlu et al., 2018). However, the combined effect of ultrasound and aqueous ozonation processes has only been studied in a few raw materials such as wheat, potato and maize.

Table 2.7 Several studies of combined ozonation and ultrasound treatment on starch

Findings	Studies	References
The combined treatment ozonation and ultrasound generated the most significant colour change, resulted the flour gradually becomes paler. The combined treatment resulted in a significant reduction in bacteria count.	Comparison of the Effects of Ozone, UV and Combined Ozone/UV Treatment on the Color and Microbial Counts of Wheat Flour	(László et al., 2008)
Ultrasonication before ozonation resulted decrease in onset gelatinization temperature and enthalpy of gelatinization. The flow properties changed to have time-dependent, non-Newtonian behavior when ultrasonication was combined with ozonation. Improved starch properties such clarity and binding properties as ozonated and ultrasonicated starches exhibit lower viscosity at high solid content.	Effects of Ultrasonication and Aqueous Ozonation on Gelatinization and Flow Properties of Potato Starch	(Ibanoğlu et al., 2018)
When a combination of treatments was applied, paste clarity was significantly improved, especially when the ultrasound was used before the ozone treatment, demonstrating the hypothesis that the ultrasound modified the starch structure which enhancing ozone activity.	Combining ozone and ultrasound technologies to modify maize starch	(Castanha et al., 2019)

2.6 Analysis of Different Treatments on Nutritional Compositions, Color, 5-HMF and Microstructures

Treatment/Method	Analysis	Findings	Reference
Ultrasound	• Vitamin C • Structure • Carotenoid	• Sample treated with ultrasound and macerated in distilled water did not affect the structure because sample gained water and caused the internal cells to be turgid. Therefore, ultrasound cannot breakdown the cell structure. • The more the water loss and solid gain, the more collapse the cell structure. • This might be due to the fact that ultrasound creates microbubbles that collide with the sweet potato's surface, causing the carotenoid content to degrade in the presence of oxygen, resulting in a loss of carotenoid content. • Low carotenoid retention also due to leaching when soluble solid loss to the solution. • Short pretreatment did not cause degradation of vitamin C content. • Ultrasound disrupt the cell wall and caused loosened of vitamin C bound and released to the medium.	(Oladejo et al., 2017)
Ozonation	• Vitamin C • Color	• Ozonation preserve the vitamin C content by not penetrate into the fruit. • Free radicals generated from ozonation degrades the vitamin C.	(Sachadyn-Król & Agriopoulou, 2020)

		• The preservation of vitamin C content produced by activating the natural defense mechanisms explains the beneficial effect of ozonation on vitamin C content. • Browning potential increased when ozone concentration and storage time increased. This is caused by the byproducts of decomposition of ozone leading to the browning on the surface of product.	
Boiling Baking Microwave oven	• Vitamin C • Beta-carotene	• Carotene content is greater in orange fleshed sweet potato cultivars than in white fleshed variants because carotenoid responsible to orange-colored pigments. • The flesh color associated with beta-carotene content and vitamin A.	(Rayamajhi & Mishra, 2020)
Different temperature UV Light	• Vitamin E	• Alpha-Tocopherol able to prevent the destruction of lipids by capturing free radicals. • The degradation of alpha-Tocopherol increased when high solubility of oxygen.	(Sabliov et al., 2009)
Ultrasound	• Vitamin E	• Ultrasound produced hydrogen peroxide that can reduce vitamin E content or tocopherol in avocado. • Degradation of vitamin E also due to cavitation bubbles produced during ultrasound treatment. • Addition of tocopherol acetate improved vitamin E content.	(Femandes et al., 2016)
Ozone	• 5-HMF	• Ozone reduce enzyme activity by lowering respiration rate of vegetables.	(Rico et al., 2006)

2.7 Summary

Sweet potato can be rice substitute as it contains high protein, dietary fiber, minerals, and vitamins that are necessary for food consumption. Sweet potato available in abundance and highly requested for starch industry. Starch has long been used commercially as main ingredient by variety of industries, including food industries, paper industries and textile industries. Native starch has several drawbacks which are sensitive to pH, poor starch paste clarity, high retrogradation, low syneresis, and decomposition. Therefore, native starch undergo modification to satisfy industrial needs such as low viscosity, longer shelf life, improved textures and appearance and higher solubility of starch. Oxidized starch is one of the modifications of starch that involved with chemical and addition of oxygen. The ultrasound treatment is believed to enhance the quality of oxidized starch, as well as the product's safety and shelf life. While ozonation is environmentally friendly technology that leaving no harmful residue in treated food product. Thus, combined treatment of ultrasound and ozone may benefit the production of starch by lowering the operational costs, reduces environmental hazards, and enhanced food safety and quality of the oxidized starch.

CHAPTER 3

METHODOLOGY

3.1 Materials

Sweet potato (VitAto^{TM}) were purchased from Malaysian Agricultural Research and Development Institute (MARDI) Bachok, Kelantan. The VitAto^{TM} were stored in a cool and dark environment. Sodium metabisulfite was used as a solvent purchased from Eva Chem Company.

3.2 Research Design

The starch content from sweet potato (VitAto^{TM}) extracted by using following method in Figure 3.1. To enhance yield of starch, ultrasound treatment and maceration with sodium metabisulfite were involved in the processing process. Next, extracted starch undergo ozone treatment and oven drying to obtain the final product. Figure 3.1 show the flowchart of research design to produce oxidized starch.

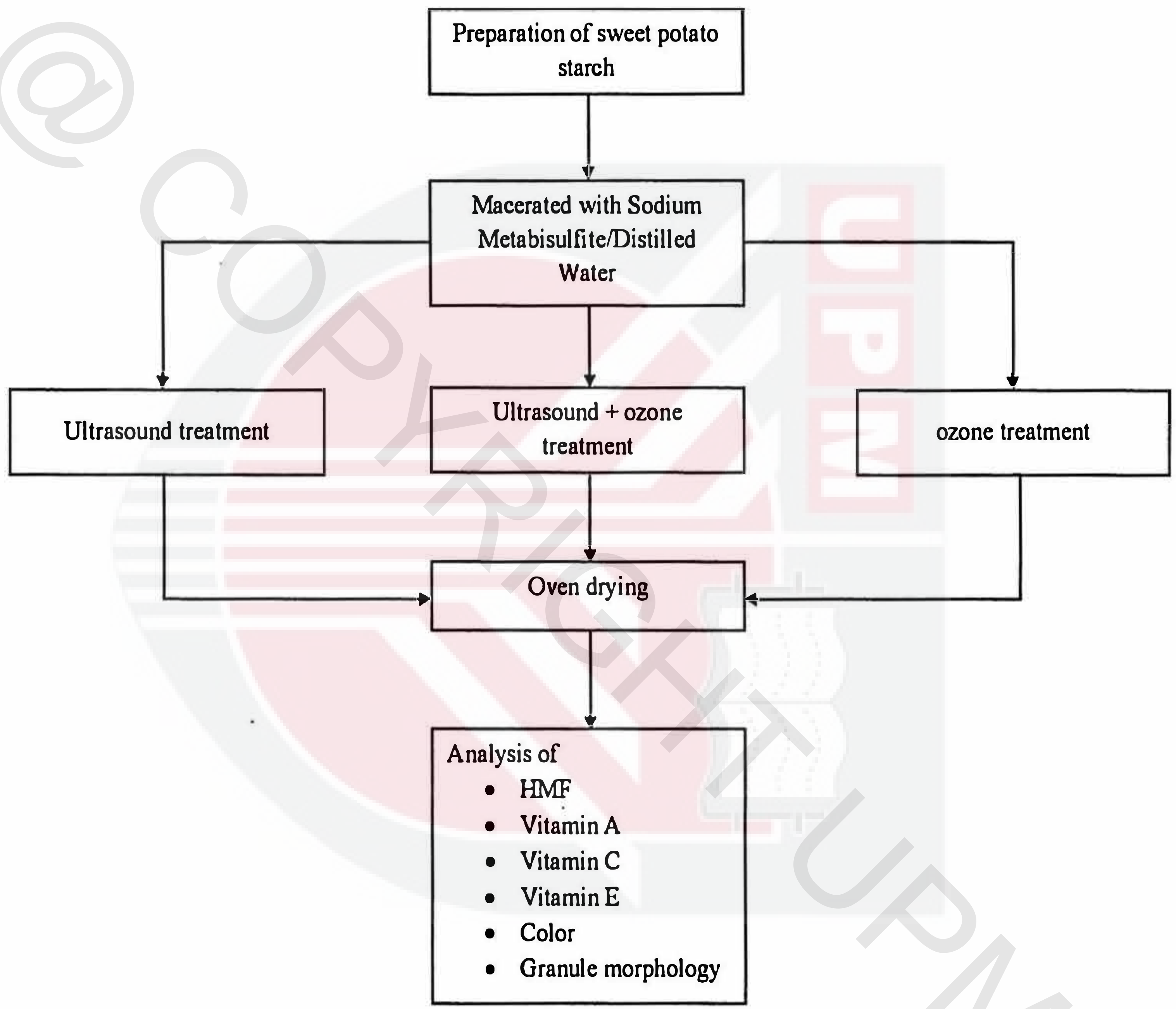


Figure 3.1 Method to produce oxidized sweet potato starch

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. Table 3.1 summarized method to produce oxidative starch.

Table 3.1 Method for each sample

Sample	Method
Control (control)	Maceration (Sodium Metabisulfite) + Oven dried 2 hours
SUO	Maceration (Sodium Metabisulfite) + Ultrasound 30 mins + Ozone 4 hours + Oven dried 2 hours
SO	Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 2 hours
DUO	Maceration (Distilled Water) + Ultrasound 30 mins + Ozone 4 hours + Oven dried 2 hours
DU	Maceration (Distilled water) + Ultrasound 30 mins + Oven dried 2 hours

3.4 Preparation of Oxidized Orange Sweet Potato Starch

3.4.1 Control Sample

The sweet potato (VitAto^{TM}) were peeled, washed properly and free from mud, infestation and injuries. They were cut into dices. 2400 g of sweet potato dices were weighted by using METTLER TOLEDO Weighing Balances (ABT 120-4NM, Germany) and poured into a blender (MX-900M, Malaysia). Then, filled a 500ml beaker with 0.01M sodium metabisulfite ($Na_2S_2O_5$), pH=4.5 and pour into a blender (MX-900M, Malaysia) that filled with sweet potato dices. The mixture was blend until homogenized at low speed in a blender (MX-900M, Malaysia). Then, heated on a hotplate (MSH-20D, Korea) for 10 minutes. Next, the mixture was incubated (WIS-20R, Korea) at 40°C for 2 hours. After that, the slurry was sieved through filtering cloth for 3 times and washed with distilled water. The suspension was stood for 15 minutes for starch sedimentation and then centrifuged (1401, Germany) at 9000 rpm for 3 minutes. The white starch was collected and dried in the Memmert oven (100-800, Germany) at 50°C for 2 hours. Finally, the dried starch was ground into powder and stored at 25°C in a glass bottle.

3.4.2 Combined Treatment of Ultrasound and Ozone with Sodium Metabisulfite as a Maceration Medium (SUO)

The sweet potato (VitAto^{TM}) were peeled, washed properly and free from mud, infestation and injuries. They were cut into dices. 2400 g of sweet potato dices were weighted by using METTLER TOLEDO Weighing Balances (ABT 120-4NM, Germany) and poured into a blender (MX-900M, Malaysia). Then, filled a 500ml beaker with 0.01M sodium metabisulfite ($Na_2S_2O_5$), pH=4.5 and pour into a blender (MX-900M, Malaysia) that filled with sweet potato dices. The mixture was blend until homogenized at low speed in a blender (MX-900M, Malaysia). Then, heated on a hotplate (MSH-20D, Korea) for 10 minutes. 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 slurry 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 (WIS-20R, Korea) at 40°C for 2 hours. After that, the slurry was sieved through filtering cloth for 3 times and washed with distilled water. The suspension was stood for 15 minutes for starch sedimentation and then centrifuged (1401, Germany) at 9000 rpm for 3 minutes. The white starch was collected and undergo ozonation (GL-3189, China) for 4 hours (Pudjihastuti et al., 2018) before being dried in the Memmert oven (100-800, Germany) at 50°C for 2 hours. Finally, the dried starch was ground into powder and stored at 25°C in a glass bottle.

3.4.3 Ozone with Sodium Metabisulfite as a Maceration Medium (SO)

The sweet potato (VitAto™) were peeled, washed properly and free from mud, infestation and injuries. They were cut into dices. 2400 g of sweet potato dices were weighted by using METTLER TOLEDO Weighing Balances (ABT 120-4NM, Germany) and poured into a blender (MX-900M, Malaysia). Then, filled a 500ml beaker with 0.01M sodium metabisulfite ($Na_2S_2O_5$), pH=4.5 and pour into a blender (MX-900M, Malaysia) that filled with sweet potato dices. The mixture was blend until homogenized at low speed in a blender (MX-900M, Malaysia). Then, heated on a hotplate (MSH-20D, Korea) for 10 minutes. Next, the mixture was incubated (WIS-20R, Korea) at 40°C for 2 hours. After that, the slurry was sieved through filtering cloth for 3 times and washed with distilled water. The suspension was stood for 15 minutes for starch sedimentation and then centrifuged (1401, Germany) at 9000 rpm for 3 minutes. The white starch was collected and undergo ozonation (GL-3189, China) for 4 hours (Pudjihastuti et al., 2018) before being dried in the Memmert oven (100-800, Germany) at 50°C for 2 hours. Finally, the dried starch was ground into powder and stored at 25°C in a glass bottle.

3.4.4 Combined Treatment of Ultrasound and Ozone with Distilled Water as a Maceration Medium (DUO)

The sweet potato (VitAto™) were peeled, washed properly and free from mud, infestation and injuries. They were cut into dices. 2400 g of sweet potato dices were weighted by using METTLER TOLEDO Weighing Balances (ABT 120-4NM, Germany) and poured into a blender (MX-900M, Malaysia). Then, filled a 500ml beaker with distilled water pour into a blender (MX-900M, Malaysia) that filled with sweet potato dices. The mixture was blend until homogenized at low speed in a blender (MX-900M, Malaysia). Then, heated on a hotplate (MSH-20D, Korea) for 10 minutes. 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 slurry 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 (WIS-20R, Korea) at 40°C for 2 hours. After that, the slurry was sieved through filtering cloth for 3 times and washed with distilled water. The suspension was stood for 15 minutes for starch sedimentation and then centrifuged (1401, Germany) at 9000 rpm for 3 minutes. The white starch was collected and undergo ozonation (GL-3189, China) for 4 hours (Pudjihastuti et al., 2018) before being dried in the Memmert oven (100-800, Germany) at 50°C for 2 hours. Finally, the dried starch was ground into powder and stored at 25°C in a glass bottle.

3.4.5 Ultrasound with Distilled Water as a Maceration Medium (DU)

The sweet potato (VitAto™) were peeled, washed properly and free from mud, infestation and injuries. They were cut into dices. 2400 g of sweet potato dices were weighted by using METTLER TOLEDO Weighing Balances (ABT 120-4NM, Germany) and poured into a blender (MX-900M, Malaysia). Then, filled a 500ml beaker with distilled water and pour into a blender (MX-900M, Malaysia) that filled with sweet potato dices. The mixture was blend until homogenized at low speed in a blender (MX-900M, Malaysia). Then, heated on a hotplate (MSH-20D, Korea) for 10 minutes. 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 slurry 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 (WIS-20R, Korea) at 40°C for 2 hours. After that, the slurry was sieved through filtering cloth for 3 times and washed with distilled water. The suspension was stood for 15 minutes for starch sedimentation and then centrifuged (1401, Germany) at 9000 rpm for 3 minutes. The white starch was collected and dried in the Memmert oven (100-800, Germany) at 50°C for 2 hours. Finally, the dried starch was ground into powder and stored at 25°C in a glass bottle.

3.5 Nutritional Composition

3.5.1 Vitamin A

Vitamin A was determined by AOAC 20th edition. 960.45

A 1.25g of premix was weighed twice. In a 100 ml beaker, the material was dissolved in 60-80ml of distilled hot water (80°C). A watch glass was placed on top of each beaker. The solution was incubated in a water bath at 50-60°C for 15 minutes (WIS-20R, Korea). The solution was then transferred to a volumetric flask with a capacity of 100 mL. The beaker was rinsed with distilled water and the washings were transferred to the volumetric flask, mixed thoroughly, and distilled water was added to make up to 100 mL.

Next, diluted and extracted vitamin A from the premix solution. 2 mL of the solution was transferred to a 20 mL tube, along with 8 mL of 2-propanol (to achieve a 2:10 dilution). In a Vortex mixer, the ingredients were mixed. Then, into 20 mL tubes, 1 mL of the previous step's solution was added, along with 9 mL of 2-propanol (to create a 1:10 dilution). Mixed using a Vortex mixer for 5 seconds. Transferred in duplicate 3 mL of the solution from previous step and placed in a 20 mL tube. 3 mL 0.1-N hydrochloric acid and 4 mL hexane were added to the mixture. Mixed in a Vortex mixer for 30 seconds.

Spectrophotometer adjusted to zero with hexane. Then, transferred the organic phase, using a Pasteur pipette to a 1 cm light path spectrophotometer cuvette and read the absorbance at 326 nm.

3.5.2 Vitamin C

For Vitamin C, introduced by (Eitenmiller et al. 1998) to determine the Vitamin C (ascorbic acid) by using the 2,6-dichlorophenol indophenol titration method. The samples (2 g) were ground with mortar and homogenized with 10 mL of metaphosphoric/ acetic acid solution. Then the sample was extracted by using Buchner funnel. The residue was extracted two times. The third time residue was poured on the Buchner funnel and rinsed with 15 mL of the extraction solution, until the filtrate was clear. Following the extraction, the filtrates were pooled into a 50 mL volumetric flask and extraction solution filled to the mark of 50 mL volumetric flask. After that, three aliquots (10 mL) of the extract were pipetted into Erlenmeyer flasks and titrated with the indophenol dye to a faint pink end point. Vitamin C content was determined by comparing the volume of the dye used to titrate a sample to the volume of the dye used to titrate ascorbic acid standard of known concentration.

3.5.3 Vitamin E

Vitamin E was determined by using HPLC according to Gimeno et al. (2000).

To make the stock solution of 0.1 mg/ml, DL-tocopherol (10 mg) was dissolved in 96% ethanol and built up to 100 mL in a volumetric flask. Wrapped in aluminium foil, it was protected from light. Following that, various concentrations (1.0 g/ml, 2.5 g/ml, 5 g/ml, 10 g/ml, and 20 g/ml) were produced using various dilutions. Before being injected into the HPLC (Agilent 1100 HPLC series, USA), each standard solution was filtered. Internal standard was tocopheryl acetate solution (300 g/ml). Each run was 12 minutes long.

Each sample (1 g) was weighed into a 10-mL volumetric flask and filled up to volume with hexane. In an ultracentrifuge container, 200 litres of water were combined with 940 litres of methanol and 60 litres of internal standard (300 g/ml stock solution). It was centrifuged (1401, Germany) at 4000 rpm for 5 minutes. Tocopherol analysis was carried out by RP-HPLC (Agilent 1100 HPLC series, USA) using the method of Gimeno et al. (2000) with major modifications to column temperature, flow rate and dilution factor. 20 litres of each prepared sample were injected into the HPLC as previously stated, following the chromatographic processes.

3.6 5-Hydroxymethylfurfural (HMF)

The absorbance A of the prepared standard solution is measured using a UV spectrophotometer at 285 nm in 1 cm quartz cells with water in the blank cell, according to Homogenized Methods of The International Honey Commission 1.5.1 / HPLC. The concentration of the standard solutions can be calculated from the literature values for molar absorptivity, $\varepsilon = 16830$ or absorptivity, $a_{1\%}^{1cm} = 133.57$ where A is the absorbance of the standard solution. 10 g of prepared sample were weighed and pour into 50 ml beaker. 25 ml of water was used to dissolve the sample and transferred to 50 ml of volumetric flask. Then, diluted with 50 ml of water. The sample filtered through a 0.45 µm membrane filter to provide solution for chromatography. The 5-HMF content of the sample is calculated by comparing the corresponding peak areas of the sample and those of the standard solutions, considering the dilution. There is a linear relationship between the concentration and the area of the HMF peak. Results are expressed in mg/kg.

3.7 Yield and Color of Starch

The weight of dried starch based on fresh root weight was used to calculate the percent yield of the extracted starch by using Eq.3-1.

$$yield\ (\%) = \frac{weight\ of dried\ starch}{fresh\ root\ weight\ of\ sweet\ potato} x\ 100 \qquad \text{Eq. 3-1}$$

The color values in CIELAB system (L*, a*, b*, ΔE and hue angle) were determined by spectrophotometer (HunterLab UltraScan® PRO D65/10 Hunter Associates Laboratory Inc., Virginia, USA). The device was adjusted with white and black tiles as reference. The values of color were expressed as lightness, L*, is a measure of total solar spectrum reflectance relative to a pure white surface where it varies from 100 for perfect white to zero for black. a* is a measure of the degree of red-green where redness when positive and greenness when negative. b* is a measure of yellow-blue where yellowness when positive and blueness when negative.

Hue angle were calculated by using Eq.3-2 Hue angle able to determine the color such as orange, yellow, red, etc. Color differences between the treated samples and the control samples (ΔE) were calculated on the basis of the CIE L*, a* and b* values via Eq.3-3.

$$Hue\ angle = tan^{-1}(b^*/a^*) \qquad \text{Eq. 3-2}$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad \text{Eq. 3-3}$$

Where,

$\Delta L = L^* - L_0$

$\Delta a = a^* - a_0$

$\Delta b = b^* - b_0$

$L^{*} - L_0$, $a^{*} - a_0$, and $b^{*} - b_0$ are the differences calculated between treated sample (SUO, SO, DUO and DU) and the control sample (Control) from the average coordinates. Measurements were performed 3 times and the results were then averaged.

3.8 Starch Granule Morphology

The morphology of the starch granules was examined using a scanning electron microscope (Shidmadzu,SSX-550). A small quantity of each sample was spread directly onto the stub surface and dried for 1 h in an Memmert oven (100-800, Germany) at 32°C. Subsequently, all the samples will be gold-coated and examined under a 15kV acceleration voltage and 5000x magnifications in the scanning electron microscope (Klein et al., 2014).

3.9 Summary

Sweet potato (VitAto^{TM}) was prepared and 5 type of methods were proposed to produce oxidized orange sweet potato starch. Control sample was prepared by macerated in sodium metabisulfite. Sample that undergo combined treatment of ultrasound and ozone with sodium metabisulfite as a maceration medium denoted as SUO. Sample that undergo ozone with sodium metabisulfite as a maceration medium denoted as SO. Sample that undergo combined treatment of ultrasound and ozone with distilled water as a maceration medium denoted as DUO. Lastly, sample that undergo ultrasound with distilled water as a maceration medium denoted as DU. Next, the content of vitamin A was determined by following method suggested by AOAC 20th edition. 960.45. The content of vitamin C was determined by titration method introduced by (Eitenmiller et al., 1998). The content of vitamin E was determined by using HPLC according to Gimeno et al. (2000). The content of 5-HMF was determined by using HPLC according to Homogenized Methods of The International Honey Commission 1.5.1. The color of oxidized orange sweet potato starch was examined by using spectrophotometer while the starch granule morphology was examined by using a scanning electron microscope. The starch yield and color values such as color difference (ΔE) and hue angle was calculated according to Eq.3-1, Eq.3-2 and Eq.3-3, respectively.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Starch Yield

The extracted starch yield was obtained from carbohydrate content in fresh root of sweet potato (Soison et al., 2015). Based on Figure 4.1, the extracted starch yield for control, combined treatment of ultrasound and ozone with sodium metabisulfite as a maceration medium (SUO), ozone with sodium metabisulfite as a maceration medium (SO), combined treatment of ultrasound and ozone with distilled water as maceration medium (DUO) and ultrasound with distilled water as maceration medium (DU) were 6.7, 6.3, 5.3, 6.5, 7.1% respectively. DU had the highest starch yield whereas the lowest starch yield was obtained from SO. This yield was similar to the study of Soison et al. (2015) reported that starch yield of sweet potato ranged from 6 to 13%. The result showed ultrasound treatment and distilled water enhanced the starch yield in sweet potato based on sample SUO, DUO and DU. This is because ultrasound treatment enhanced the starch yield in sweet potato when more granule breakdown and starch molecules released into the medium. This is in line with a research by Carchiochi et al. (2017), found that the separation process aids in enriching the yield extracts in the molecules when the solvent extraction is not satisfactory. Therefore, the ultrasound and distilled water enhanced the starch yield obtained for this study. To add, starch exposure during cutting process could also contribute to the loss of starch yield. According to Allonsy (2018), more starch was loss from sliced potato compared to whole peeled potato due to the greater surface area exposed on sliced potato. Hence, further studies should consider using whole peeled sweet potato during the preparation to increase the starch yield.

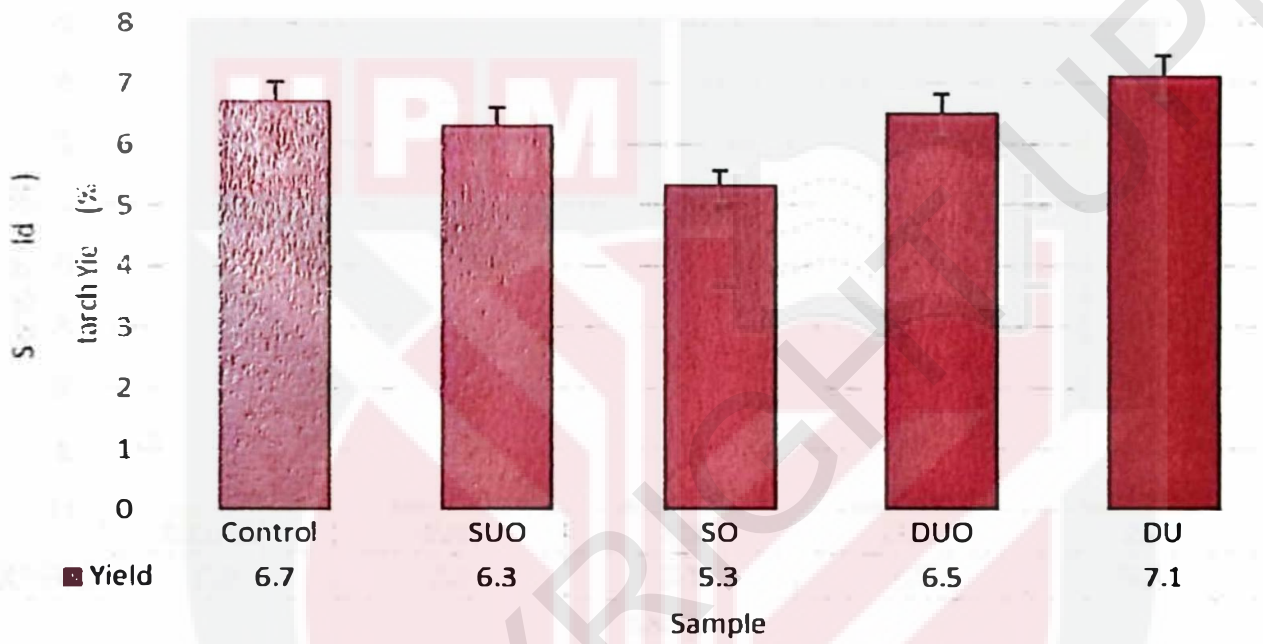


Figure 4.1 Starch yield of oxidized orange sweet potato starch with different methods

4.2 Color of Oxidized Orange Sweet Potato Starch

The CIE Lab color system was used to determine the color of the starch, where the L* value describes the lightness which correlates with pigments in sweet potato flesh, a* is the degree of redness while b* is the degree of yellowness, which correlates the carotenoid content of the starch specifically beta-carotene.

Based on Figure 4.2, the results revealed that starch from SO had the highest lightness which was 82.06, followed by Control sample, SUO, DU and DUO which were 80.05, 78.87, 77.94 and 68.11, respectively. A study by László et al. (2008) reported that ozone treatment combined with UV treatment led to a whiter product. This is because ozone is a powerful bleaching agent that produced oxygen and nascent oxygen. This nascent oxygen bleaches the carotenoid pigments permanently. However, despite being treated with ozone, DUO showed the opposite outcome which was darker oxidized starch. This might be due to the development of light brown pigments happened in the presence of 5-HMF produced by the Maillard reaction (Pourmohammadi et al., 2019).

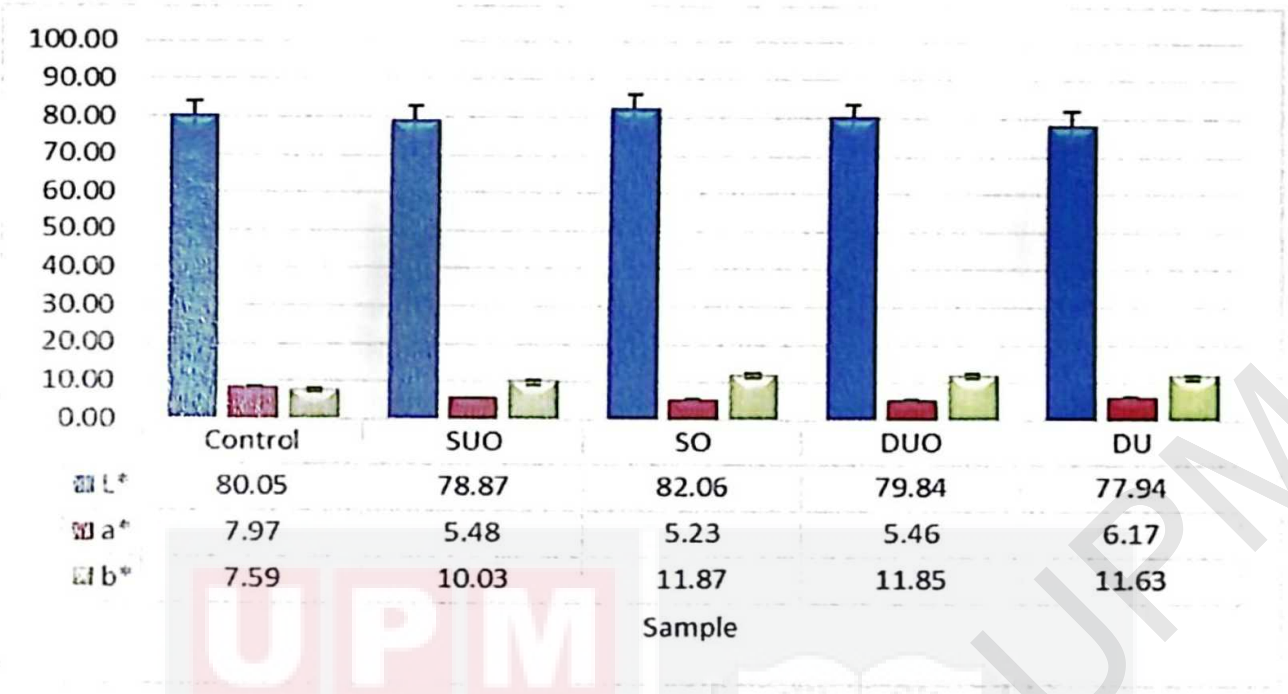


Figure 4.2 L*, a* and b* values of oxidized orange sweet potato starch different method

More specific results can be obtained by calculating the total color difference (ΔE*). Based on Table 4.1, the most significant changed was caused by SO followed by DUO, DU, and SUO were 5.46, 4.95, 4.90 and 3.68, respectively. A study by Adekunte et al. (2010), color of tomato juice after sonication changed which the researchers linked to isomerization of carotenoid pigments. Hence, ultrasound and ozone affecting the carotene content of sweet potato and caused changed of color. According to Huang et al. (1999), different processing technique influences the carotene content of orange-fleshed sweet potato. In this study, the ultrasound and ozone influence the degradation of carotene content.

Table 4.1 The L*, a*, b* , ΔE* and hue angle of oxidized orange sweet potato starch from different methods

Sample	Color values				
	L*	a*	b*	ΔE*	Hue angle
Control	80.05	7.97	7.59	-	43.61
SUO	78.87	5.48	10.03	3.68	61.35
SO	82.06	5.23	11.87	5.46	66.22
DUO	79.84	5.46	11.85	4.95	65.27
DU	77.94	6.17	11.63	4.90	62.06

Regarding the Hue angle, the results presented on Figure 4.3 show changes and tonality different for all samples. SUO was the smallest hue angle at 61.35° after undergo ultrasound and ozone. The small hue angle indicates high content of beta-carotene. This is in an agreement with a study by Ornelas-Paz et al. (2007) reported that small hue angle values in mango corresponded with high beta-carotene results in mango flesh.

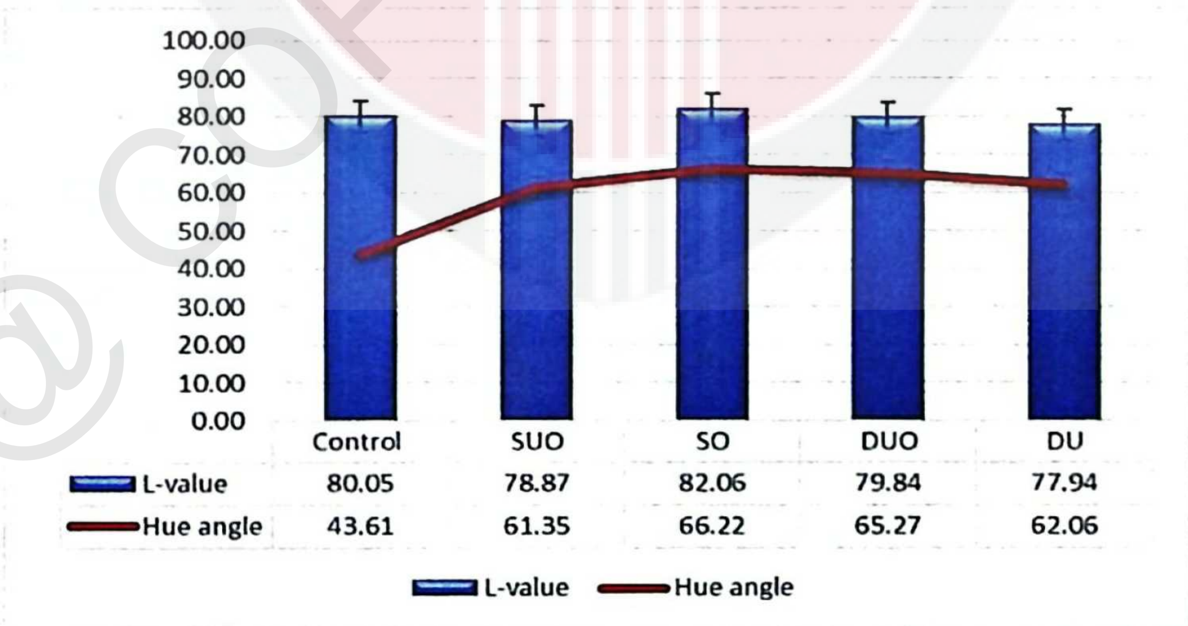


Figure 4.3 Hue angle and Lightness of oxidized orange sweet potato starch with different methods

4.3 Vitamin A (Beta-carotene)

Vitamin A in sweet potato is directly associated with the carotenoid content specifically beta-carotene. Carotenoids are pigments in plant, which responsible for orange-colored pigments for sweet potato. Therefore, beta-carotene and vitamin A can be related as the higher the amount of beta-carotene, the higher the amount of vitamin A in sweet potato.

Based on Figure 4.4, the result obtained for control sample was 77.7 mg/100g. However, result after treatments decreased where 14.17 mg/100g, 6.34 mg/100g, 15.4 mg/100g for SUO, DUO, and DU, respectively. The degradation of vitamin A is due to ozonation process. According to Pandiselvam et al. (2019), ozone has bleaching agent that caused discoloration in food. The bleaching affected the carotenoid pigment in sweet potato, thus correlated with the loss of vitamin A.

Next, SUO and DUO were compared to determine the effect of different maceration medium. Both samples undergo the same method which were ultrasound and ozone, but different in maceration where SUO macerated in sodium metabisulfite while DUO macerated in distilled water. From Figure 4.4, sodium metabisulfite macerated for SUO was able to retain higher vitamin A than DUO which was in distilled water. This can be shown on Figure 4.4 where the amount of vitamin A in SUO was 14.17 mg/100g higher than vitamin A in DUO was 6.34 mg/100g. According to Andri Kumoro and Jefri Hidayat (2018), sodium metabisulfite improved the nutrition and functional properties of durian seed flour by inhibit enzymatic browning. Therefore, sodium metabisulfite was able to preserve the carotenoid content as well as the orange pigment that serves as a precursor to vitamin A.

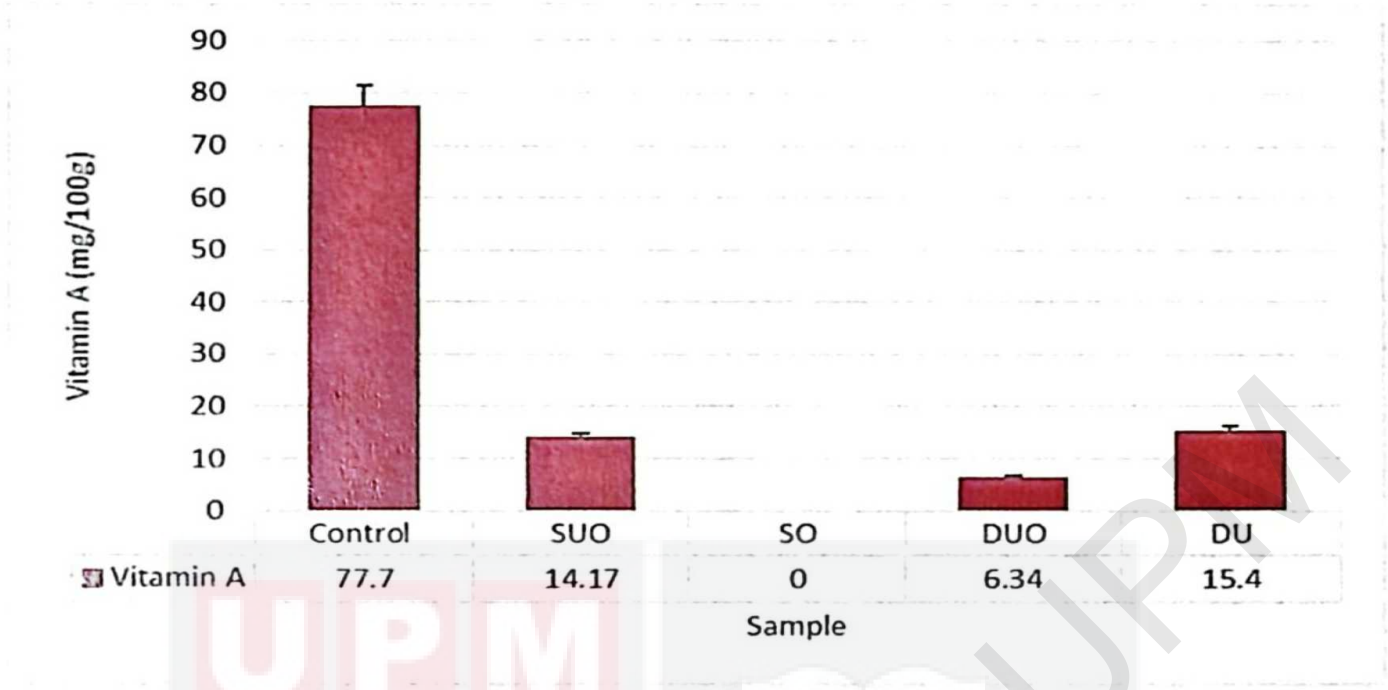


Figure 4.4 Vitamin A content of oxidized orange sweet potato starch with different methods

Next, DUO and DU were compared to determine the effect of hurdle processing treatment of ultrasound and ozone. Both samples were macerated in the same medium which were distilled water. However, based on Table 4.2, vitamin A in DU was able to retain higher which was 19.82% compared to DUO which was 8.16%. As reported by Oladejo et al. (2017), carotenoids degrade in the presence of oxygen, this resulted in a decrease of beta-carotene and reduction of vitamin A content. This clearly showed that ozone treatment had a significant effect to the degradation of vitamin A content. Besides, vitamin A in SO which undergo ozonation and macerated in sodium metabisulfite cannot be detected probably due to high loss of vitamin A during ozonation.

Table 4.2 Retention of vitamin A of oxidized orange sweet potato with different method

Method	Retention (%)
Control sample	100
SUO (Sodium metabisulfite + Ultrasound + Ozone)	18.24
SO (Sodium metabisulfite + Ozone)	ND
DUO (Distilled water + Ultrasound + Ozone)	8.16
DU (Distilled water + Ultrasound	19.82

Footnote: ND is not detected

4.4 Vitamin C

Based on Figure 4.5, vitamin C for all samples increased after treatment where SUO, SO, DUO and DU were 4.4 mg/100g, 4.89 mg/100g, 4.88 mg/100g and 4.4 mg/100g, respectively. The reason for this is the bound of vitamin C weakened during ultrasound process which disrupts the cell wall of starch granule of sweet potato. Hence, the amount of vitamin C released to the medium increased (Abid et al. 2013). Similar to previous study by Oladejo et al. (2017), the vitamin C content of sweet potato higher than the control sample due to ultrasound treatment. On the other hand, ozonation help to preserve the vitamin C content in sweet potato by stimulating the natural defense mechanisms to withstand free radical generated by ozone (Sachadyn-Król et al., 2020). This can be demonstrated by SO that only been treated by ozone showed the highest retention of vitamin C which was 125.06%. Hence, combined treatment of ultrasound and ozone showed improvement of vitamin C content in oxidized orange sweet potato starch.

Next, DUO and DU were compared to show the effect of ozone on vitamin C content. Both samples were macerated in distilled water, but DUO was treated by ultrasound and ozone while sample DU was treated by ultrasound only. The result on Figure 4.5 showed that vitamin C content of DUO had 10% higher than DU indicates the presence of ozone influences the increasing of vitamin C content in oxidized orange sweet potato starch.

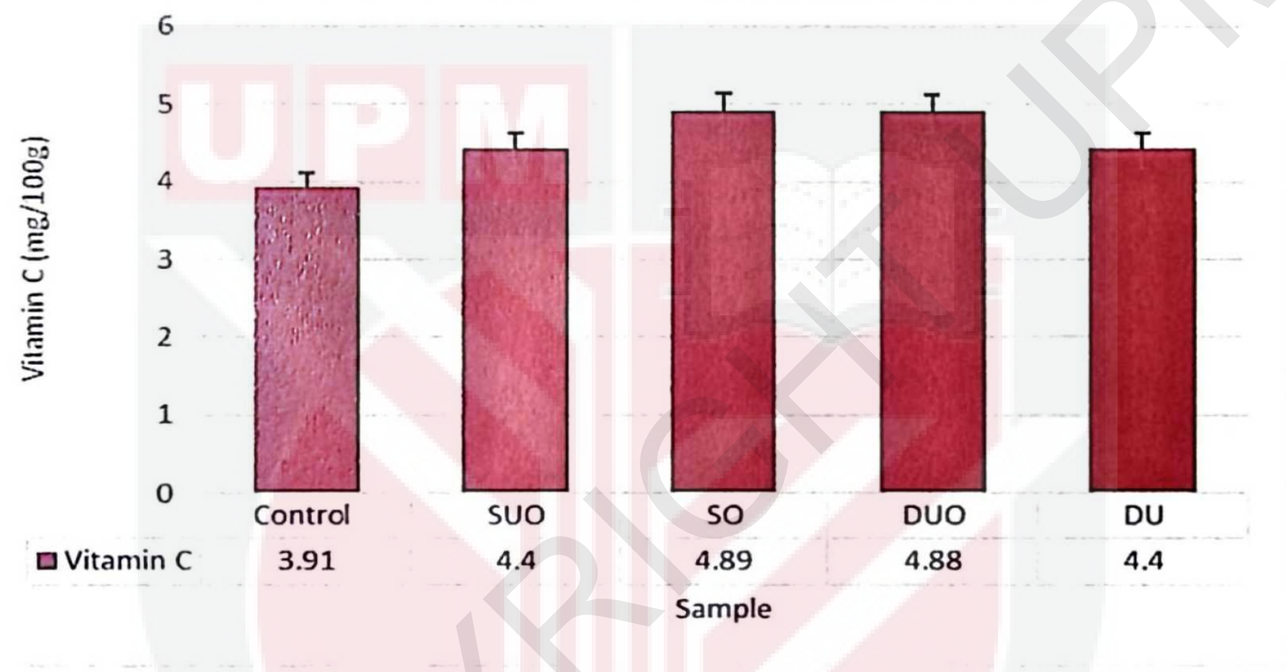


Figure 4.5 Vitamin C content of oxidized orange sweet potato with different methods

4.5 Vitamin E

Based on Figure 4.6, the content of Vitamin E in oxidized orange sweet potato starch degraded for all samples which were SUO and DU for 0.06 mg/100g and 0.07 mg/100g, respectively. A study by Tan (2015), the content of vitamin E in sweet potato was 1.01 mg/100g. In this study, the content of vitamin E in control sample was 0.13 mg/100g, which was lower than reported by Tan (2015). Moreover, vitamin E was the lowest vitamin obtained from this study. This was probably due to experimental error during drying process. The temperature of convection oven was not stable during the process, thus affecting the degradation of vitamin E. Since the vitamin E content was low, the fluctuating temperature also might have a significant effect to the content of vitamin E in oxidized orange sweet potato starch. According to Abushita et al. (2000), thermal processing enhances the loss of vitamin E. Along with a study by Wang et al. (2009) claimed that ultrasound treatment destroyed the cell wall caused releasing of tocopherol from the sweet potato. The loss of tocopherol influenced the degradation of vitamin E content. Despite the degradation of vitamin E, SUO and DU were able to retain content of vitamin E for 46.15% and 53.85%, respectively.

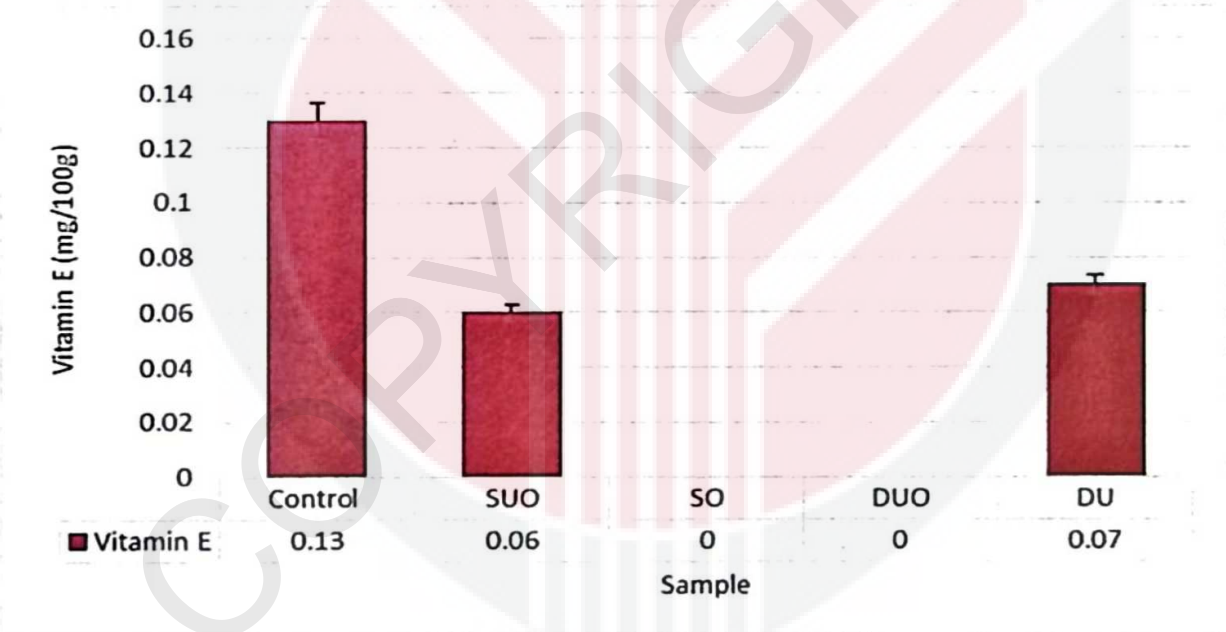


Figure 4.6 Vitamin E content of oxidized orange sweet potato with different methods

4.6 5-Hydroxymethylfurfural (HMF)

Based on Figure 4.7, Control, SUO and SO which were macerated into sodium metabisulfite showed low content of 5-HMF compared to DUO and DU which were macerated into distilled water. The content of 5-HMF in sodium metabisulfite below 1.0 mg/Kg which were 0.76 mg/Kg, 0.82 mg/Kg, and 0.36 mg/Kg for samples control, SUO and SO, respectively. On the other hand, formation of 5-HMF in maceration medium of distilled water above 1.0 mg/Kg were DUO for 1.11 mg/Kg and DU for 1.21 mg/Kg. This showed that, the use of sodium metabisulfite reduce the content of 5-HMF in oxidized orange sweet potato starch. This is because sodium metabisulfite reduced the pH value that caused low activity for Maillard Reaction. Cortez et al. (2008) stated that sodium metabisulfite used for processed apple was able to inhibit enzymatic and non-enzymatic browning. Moreover, a study by Nascimento et al. (2020) claimed that sodium metabisulfite able to control browning and prolonged the shelf life of potatoes for 8 months. This clearly showed the good effect of using sodium metabisulfite as a maceration medium.

Next, SUO and SO were compared to determine the effect of ultrasound to the oxidized orange sweet potato starch. Based on Figure 4.7, the content of 5-HMF in SUO which undergo ultrasound and ozone macerated in sodium metabisulfite was higher compared to SO which undergo ozone macerated in sodium metabisulfite. This is because ultrasound generates heat during the process. Presence of heat increase the chance for development of 5-HMF through Maillard reaction. Therefore, sample SUO, DUO and DU that undergo ultrasound had a higher content of 5-HMF compared to a method that undergo ozone only (SO). In addition, a study by Cavka et al. (2015) revealed that the formation of 5-HMF decreased by using ozonation. This can be demonstrated by SO that only been treated by ozone showed the lowest content of 5-HMF which was 0.36 mg/Kg. Thus, ozonation and sodium metabisulfite as a maceration medium were able to reduce the content of 5-HMF in oxidized orange sweet potato starch.

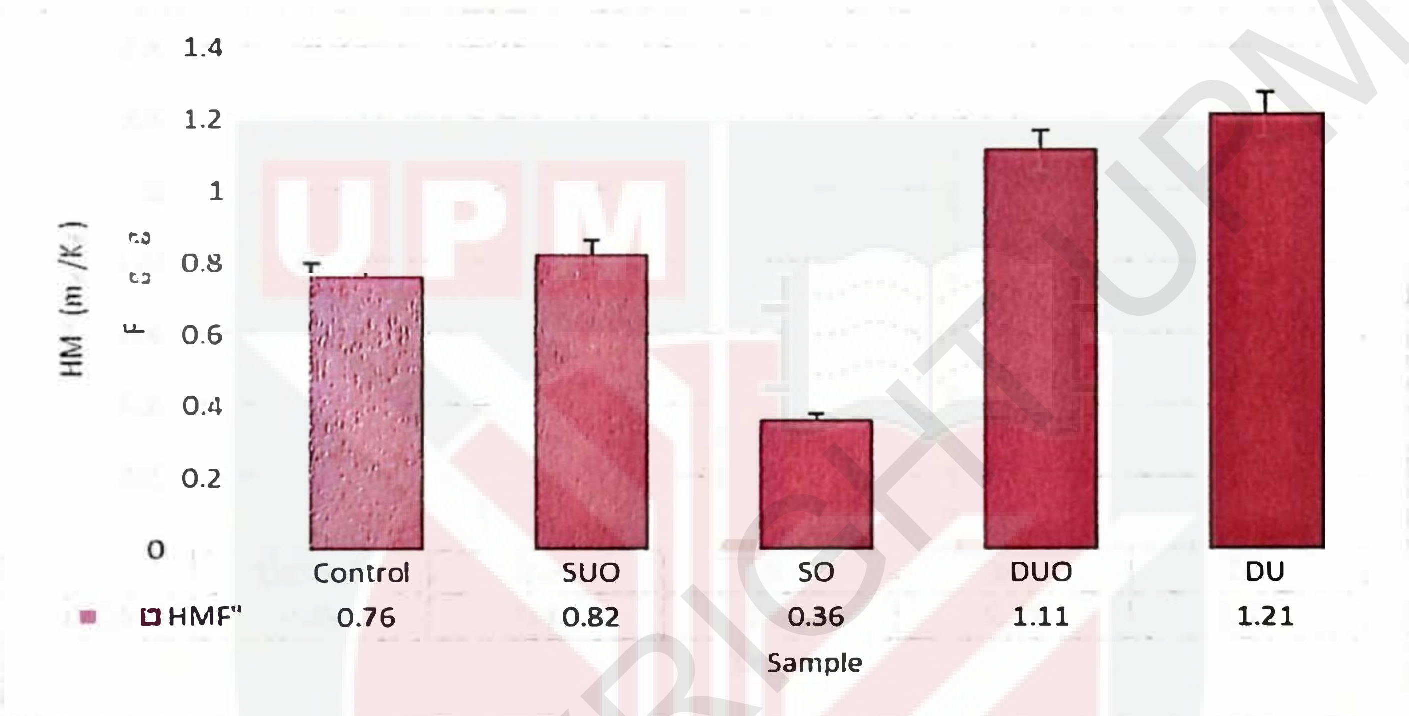


Figure 4.7 Formation of 5-HMF of oxidized orange sweet potato with different maceration medium and methods

4.7 Relation between Vitamin A and 5-HMF with color (L-value)

Figure 4.8 showed relation between Vitamin A and Lightness. The interaction between vitamin A and lightness was inversely proportional where vitamin A decreased when lightness increased. This is because higher lightness indicates low amount of carotenoid pigments which responsible for orange-colored sweet potato. The ozonation that has bleaching effect removed the carotenoid pigments and the product become whiter. Furthermore, the reduction of carotenoid pigments related to the vitamin A content. According to Ping Che et al. (2016), beta-carotene degrades due to oxidative degradation induced by ozonation. Therefore, ozonation gave a significant effect on the loss of vitamin A. Besides, ultrasound also enhances the loss of carotenoid pigments when beta-carotene degraded because sensitive to high temperature.

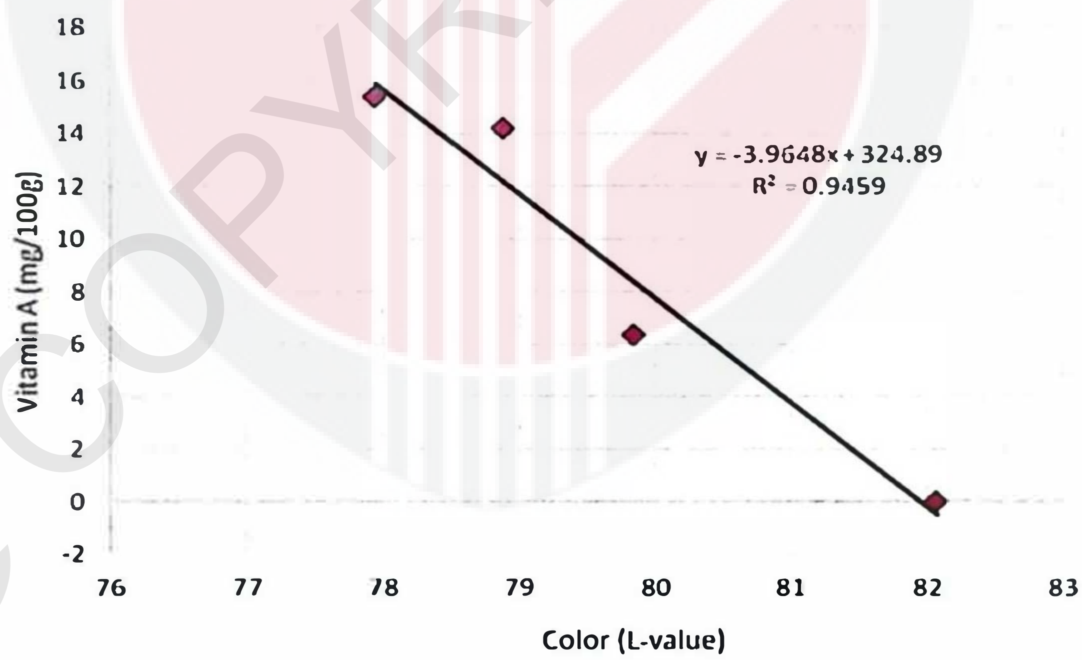


Figure 4.8 Relation between Vitamin A content with color (L-value)

Next, Figure 4.9 demonstrated relation between 5-HMF and Lightness. The relationship between 5-HMF content and lightness was inversely proportional where 5-HMF content decreased when lightness increased. The ultrasound which produced heat due to cavitation effect influence the development of 5-HMF. The 5-HMF developed when reducing sugar in sweet potatoes such as fructose and glucose react with protein and acid with presence of heat. Therefore, the reaction produced brown pigment on the oxidized starch through Maillard reaction during the process. However, sodium metabisulfite able to inhibit the Maillard reaction and reduce the formation of 5-HMF in oxidized orange sweet potato starch. Despite the fact that ultrasound induced the formation of 5-HMF, ozonation and sodium metabisulfite as a maceration medium were able to decrease the chances for 5-HMF to develop in oxidized orange sweet potato starch.

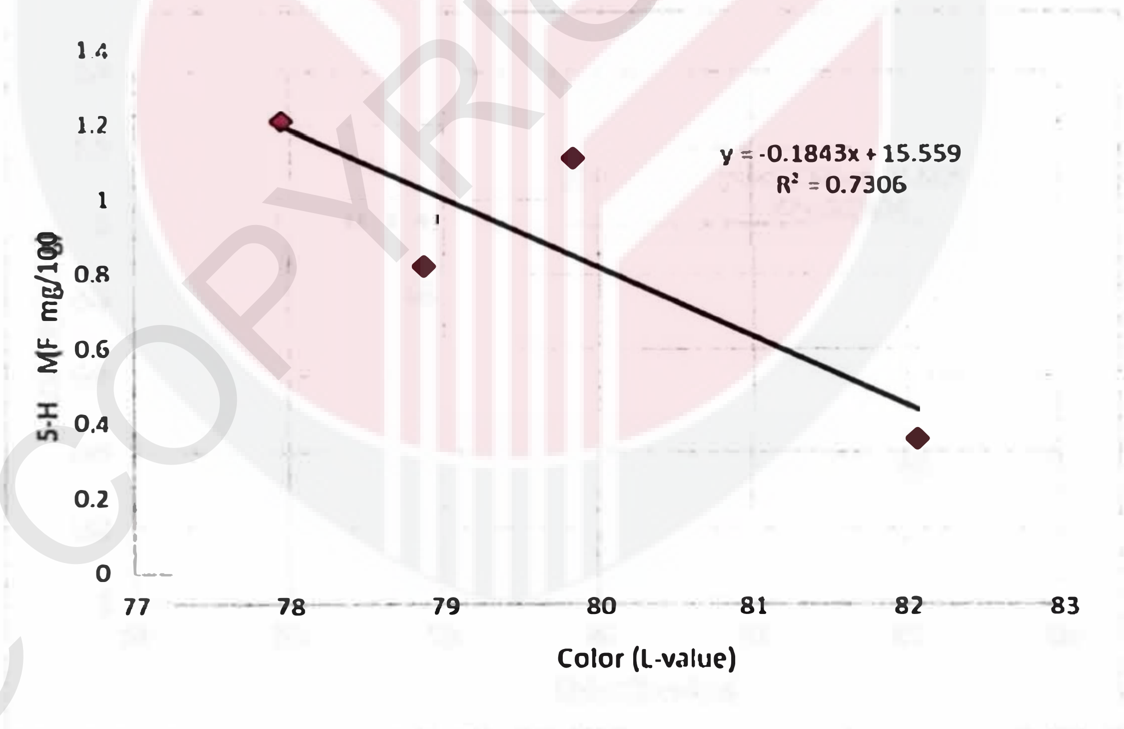


Figure 4.9 Relation between 5-HMF content with color (L-value)

4.8 Starch Granule Morphology

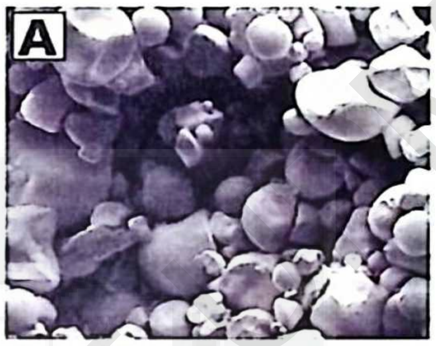
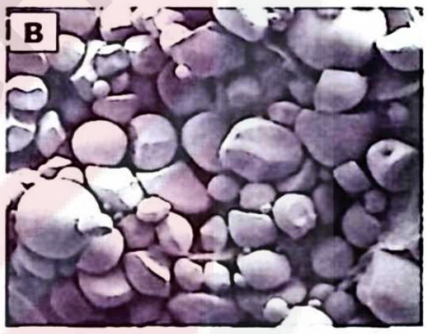
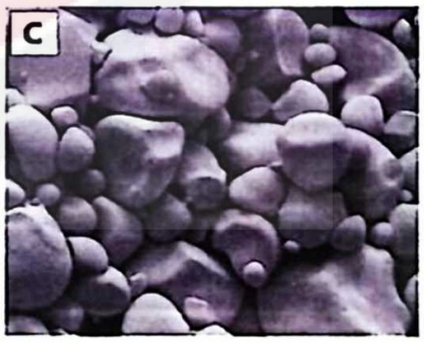
Scanning electronic microscopy (SEM) showed morphology of oxidized sweet potato starch. Table 4.3 showed the result of oxidized orange sweet potato starch after treatments applied on them. Control sample presented irregular shape with relatively smooth surface and contained no pores and cracks, as expected for native sweet potato starch. The result agreed with the previous report described by (Wang et al., 2020).

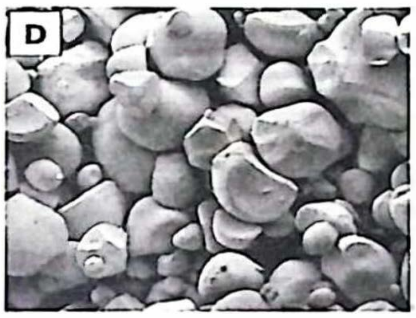
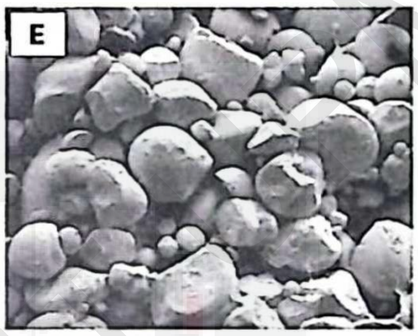
Overall, the findings revealed that all samples had same outcomes where the surface was smooth, free of fissures and cracks. This might be due to the low parameters used during the process. A study by Wang et al. (2004) and Wang et al. (2009) also showed the same result where no damage on the starch granule for ultrasound frequency of 20 kHz at 750W, suggesting that ultrasound treatment affecting only the non-covalent bonding between the starch and protein. As reported by Castanha et al. (2019), insufficient parameters such as frequency, power, temperature and time taken during the ultrasound treatment may not alter the starch granules' morphology. On the other hand, ultrasound treatment can cause alteration in amorphous region and crystalline region (Zheng et al., 2013). Hence, further study on the effect of ultrasound treatment power, frequency, temperature and time taken on amorphous region and crystalline region need to be investigated.

In contrast with the result of granule's surface, the size of granule for SUO, DUO and DU smaller than Control and SO. This showed that ultrasound treatments disrupted the starch granule during the process. As reported by Lu et al. (2018), Yang et al. (2019), and Falsafi et al. (2019), free radicals released during ultrasound treatment may potentially aid in the breakdown of starch structure. Based on Table 4.3, DU had many small starch granules indicates more shear force that can break the starch chain.

Next, SUO which macerated in sodium metabisulfite and DUO which macerated in distilled water were compared to determine the effect of maceration based on the same applied treatment which were combined treatment of ultrasound and ozone. Based on Table 4.3, DUO which macerated in distilled water had high amount of small starch granules compared to SUO which macerated in sodium metabisulfite. This is because starch granules were expanded and collapsed freely in distilled water resulting in higher starch granule breaking.

Table 4.3 Scanning electron micrographs of oxidized orange sweet potato starch at 5000x magnification

Sample	Vitato^{TM}
Control Maceration (Sodium Metabisulfite) + Oven dried 2 hours	
SUO Maceration (Sodium Metabisulfite) + Ultrasound 30 mins + Ozone 4 hours + Oven dried 2 hours	
SO Maceration (Sodium Metabisulfite) + Ozone 4 hours + Oven dried 2 hours	

DUO Maceration (Distilled Water) + Ultrasound 30 mins + Ozone 4 hours + Oven dried 2 hours	
DU Maceration (Distilled water) + Ultrasound 30 mins + Oven dried 2 hours	

4.9 Optimization

The purpose of optimization is to find a suitable set of conditions that helps to determine the best method for this study. Design expert was used to determine the optimum conditions to produced oxidized orange sweet potato starch. Based on Table 4.4, the data was duplicate for each sample of oxidized sweet potato starch.

Table 4.4 Design Expert Software duplicate data and responses

Std	Run	Method	Vitamin A (mg/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)	5-HMF (mg/Kg)
1	1	Control	77.7	3.91	0.13	0.76
9	2	DUO	6.34	4.88	0	1.11
2	3	SUO	14.17	4.4	0.06	0.82
8	4	SO	0	4.89	0	0.36
5	5	DU	15.4	4.4	0.07	1.21
10	6	DU	14.99	4.4	0.07	1.2
4	7	DUO	6.32	4.88	0	1.09
3	8	SO	0	4.89	0	0.35
7	9	SUO	14.18	4.39	0.06	0.8
6	10	Control	77	3.91	0.12	0.78

Figure 4.10 presented Vitamin A, Vitamin C, Vitamin E and 5-HMF as responses to determine the optimum conditions to produce the highest yield of oxidized orange sweet potato starch. The blue circle represents the optimum condition for each response. The maximum value of nutritional composition which was Vitamin A, Vitamin E and Vitamin C and minimum value of 5-HMF were the conditions to produce optimum oxidized orange

sweet potato starch where vitamin A for 14.175 mg/100g, vitamin C for 4.395 mg/100g, vitamin E for 0.06 mg/100g and 5-HMF for 0.81 mg/kg.

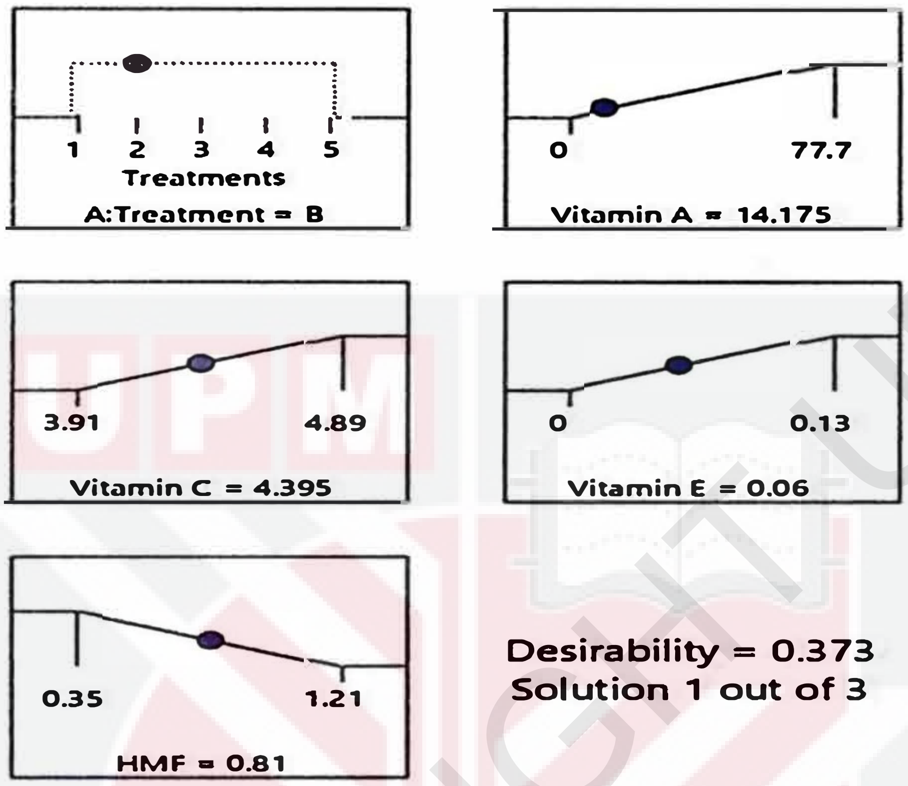


Figure 4.10 Optimization of oxidized orange sweet potato starch

In this study, SUO showed the most optimum condition to produce the highest yield of oxidized starch with a desirability of 0.373. SUO was treated with maceration medium of sodium metabisulfite (0.001M) undergo ultrasound treatment for 30 minutes, power of 24W, frequency of 20kHz with amplitude of 40% and ozonation for 1 ppm for 4 hours. Therefore, further study should achieve these optimum conditions in order to produce the best oxidized orange sweet potato starch.

4.10 Summary

Based on the research had been done can be seen that the ultrasound and ozone had significant impact to the content of vitamin A, E and C, 5-HMF content, microstructures, starch yield and color of oxidized orange sweet potato starch. While sodium metabisulfite as a maceration medium had no significant effect on the starch yield, and the content of vitamin C and E. The combined treatment of ultrasound and ozone was highly significant to the degradation of vitamin A, color of starch and development of 5-HMF. The beta-carotene which the precursor to vitamin A and responsible to the orange-colored of sweet potato starch degraded due to ozonation. Whereas ultrasound increased the content of 5-HMF through Maillard reaction. Nonetheless, sodium metabisulfite was shown to have preserved the carotene content and produced lower content of 5-HMF. The ultrasound treatment had significant effect to the content of vitamin E, starch yield and microstructures. The ultrasound disrupted the cell wall of starch granule which enhancing the starch molecules released to the medium but also influence the loss of tocopherol from the sweet potato. The relation between the content of vitamin A and 5-HMF with lightness of oxidized orange sweet potato starch were inversely proportional. The higher the lightness, the lower the content of vitamin A and 5-HMF. In this study, sample that undergo combined treatment of ultrasound and ozone with sodium metabisulfite as a maceration medium (SUO) had the optimum treatments to produce oxidized orange sweet potato starch.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, oxidized sweet potato starch was successfully developed using ultrasound treatment and gaseous ozone. The maceration medium of sodium metabisulfite and distilled water was found to influence the content of vitamin A, 5-HMF content and color. Samples macerated with sodium metabisulfite were able to retain the carotenoid pigment and reduced formation of 5-HMF. In addition to that, the hurdle treatments of ultrasound and ozone had significant impact to the content of vitamin A, E, C, 5-HMF, microstructure, starch yield and color of oxidized orange sweet potato starch. The result also revealed ozonation had no significant effect to the degradation of vitamin E and starch yield obtained. Furthermore, the starch granule was found to have fissures in sample macerated in distilled water and ultrasound was observed to affect the microstructure of oxidized orange sweet potato starch. In this study, SUO was the most optimum treatment to produce the highest yield of oxidized starch by using sodium metabisulfite as a maceration medium, combined with the treatment of ultrasound and ozone to further oxidize the starch. The amount of 5-HMF found and sodium metabisulfite used in this study are safe for consumption as the amount presence are not exceeding the general standard limit which are 40 mg/Kg and 200 mg/Kg, respectively. The characteristics of oxidized orange sweet potato starch produced can be further utilized in the manufacture of noodles and thickening agents. It is with high hopes that the outcomes of this study will provide practical insights for manufacture oxidized orange sweet potato starch optimally in order to lessen the cost and energy usage and further perusing the local production and lessening the dependency on imported starch.

5.2 Recommendations

Further study should be done to determine the optimum parameters such as temperature, concentration of ozone treatment and ultrasound on the factors that influence the degradation content of vitamin A and vitamin E. Addition of tocopherol acetate for further study could increase the vitamin E content. Besides, the suitable equipment of ozonation and ultrasound need to be considered for better homogenization during the treatment. Apart from that, further study on the effect of hurdle processing treatment such as ultrasound and ozone should be utilized in food technology to produce high quality of food that have high nutritional composition as well as excellent physicochemical and functional properties.

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APPENDICES

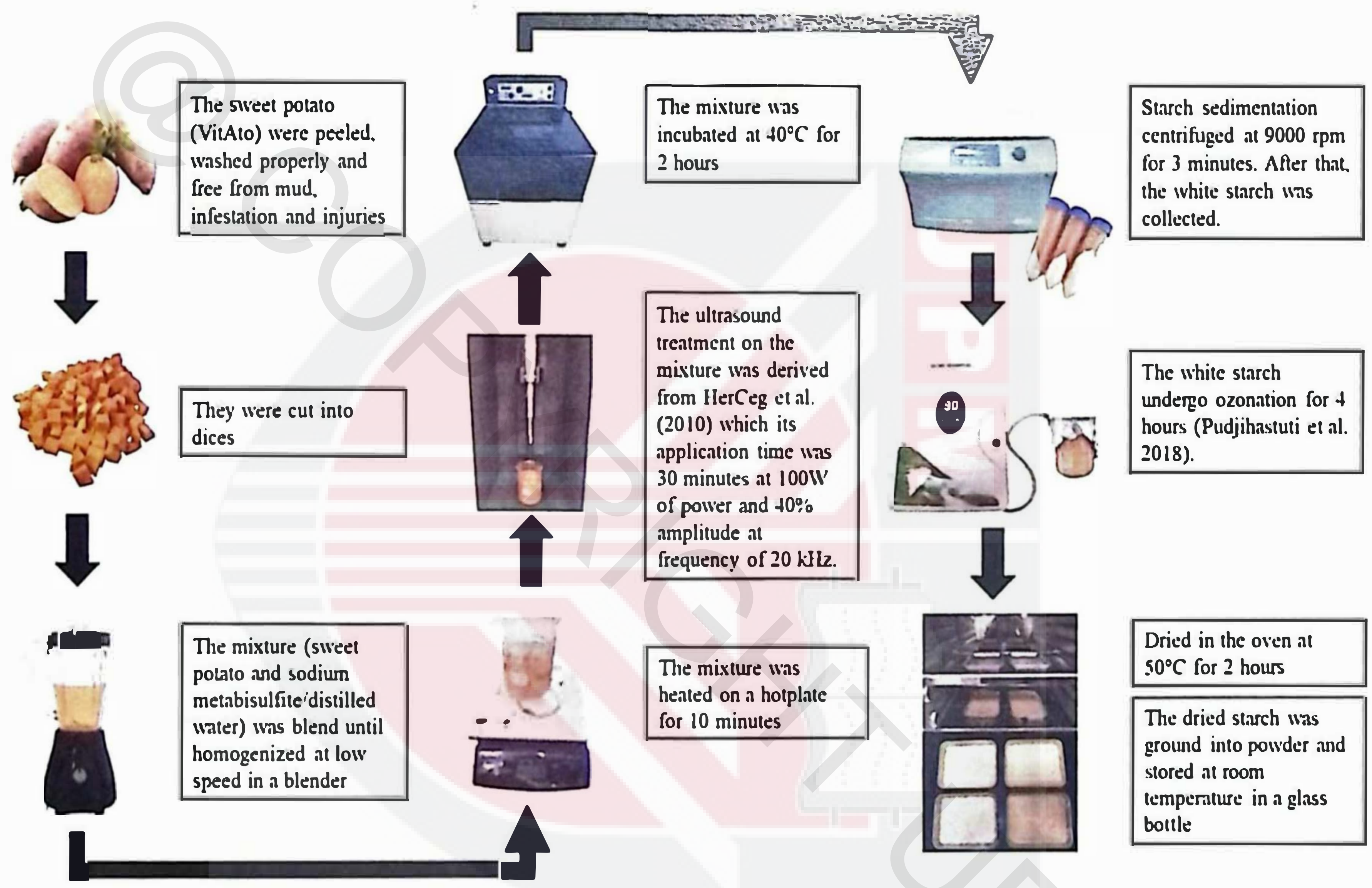


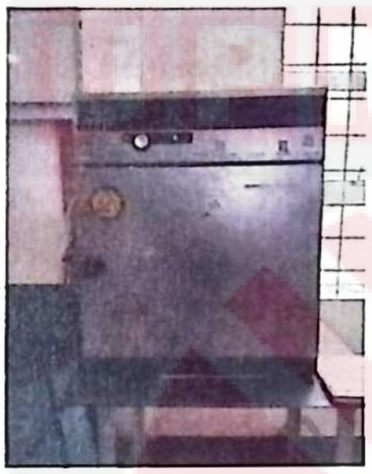
Figure above show methods to produce oxidized orange sweet potato starch

Table below show analysis of oxidized orange sweet potato starch

Sample	Analysis				
	Starch Yield (%)	Vitamin A (mg/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)	5-HMF (mg/Kg)
Control sample	6.7	77.7	3.91	0.13	0.76
SUO (Sodium metabisulfite + Ultrasound + Ozone)	6.3	14.17	4.4	0.06	0.82
SO (Sodium metabisulfite + Ozone)	5.3	ND	4.89	ND	0.36
DUO (Distilled water + Ultrasound + Ozone)	6.5	6.34	4.88	ND	1.11
DU (Distilled water + Ultrasound)	7.1	15.4	4.4	0.07	1.21

Table below show equipment used to produce oxidized orange sweet potato starch

Equipment	Information
	Model: Digital Sonifier (450) Voltage: 200V-245V Brand: BRANSON ULTRASONICS CORPORATION Made in Mexico
	Model: GL-3189 Voltage: 220V/50Hz Power: 12W Dimensions: 270 x 190 x 75 mm Net weight: 0.8 Kg
	Model: Precise Shaking Incubator (WIS-20R) Voltage: 230V/60HZ Power: 1KW Current: 5A Brand: DAIHAN Scientific Co. Ltd

	Model: Centrifuge (1401) Voltage: 200-240V/50-60Hz Current: 2.0A Max. speed: 15000 rpm
	Model: Convection Oven (Memmert 100-800) Temperature: range up to +300 °C Voltage: 230V, 50-60Hz Current: 2.7A Power: 600W
	Model: Spectrophotometer (HunterLab UltraScan® PRO D65/10) Power: 100 - 240 VAC/ 47 to 63 Hz Hunter Associates Laboratory Inc., Virginia, USA