PRODUCTIONS AND MODIFICATIONS OF AVOCADO AND JACKFRUIT SEED STARCHES

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MASTER OF SCIENCE
IN
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Adityo Fajar Nugroho

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ABSTRACT

This research examines the physicochemical and functional characteristics of starch derived from avocado and jackfruit seeds, which are currently underutilized in industrial applications. Starch was extracted from both seeds using wet and dry milling methods and initially characterized. To enhance their properties, citric acid esterification and hydrothermal treatments, namely annealing and heat-moisture treatment were employed. The impacts of these modifications were assessed to evaluate their industrial potential. Three experimental setups were conducted to achieve these objectives.

In Experiment 1, avocado and jackfruit seeds were processed into flour using dry and wet milling techniques, followed by starch isolation through alkaline steeping. The native flours and starches were then evaluated for proximate composition, amylose content, color, morphology (SEM), crystallinity (XRD), pasting behavior (RVA), thermal properties (DSC), and textural profiles. The results demonstrated that dry milling yielded higher levels of protein, fat, and ash, whereas wet milling combined with alkaline steeping produced starches with higher purity, lightness, and well-defined granules. Avocado starch exhibited B-type crystallinity, while jackfruit starch displayed A-type, with both showing differences in thermal behavior and paste viscosity depending on the processing method.

In Experiment 2, the isolated starches were chemically modified via citric acid esterification at three concentrations (5%, 10%, and 15%). The resulting starch citrates were examined for degree of substitution (DS), changes in color and morphology, FTIR profiles, XRD patterns, and alterations in functional performance including viscosity,

thermal transitions, and gel texture. The 5% citric acid treatment improved gel-forming capacity and increased relative crystallinity in avocado starch, whereas higher concentrations (10% and 15%) caused significant granule damage, reduced paste viscosity, and poor gel formation in both starches.

In Experiment 3, physical modifications, annealing (ANN) and heat moisture treatment (HMT) were applied individually and in combination with 5% citric acid to assess their synergistic effects. The modified starches were characterized using similar techniques. ANN and ANN with 5% citric acid treatments enhanced the structural integrity and crystallinity of starch granules, while HMT and HMT with 5% citric acid treatments led to decreased paste viscosity and greater disruption of gelatinization properties. These results suggest a strong interaction between the applied physical and chemical modifications on starch behavior.

In conclusion, both avocado and jackfruit seed starches showed promise as functional food ingredients. The most favorable modifications in terms of performance were observed in samples treated with 5% citric acid, particularly when combined with ANN. This strategy minimized chemical usage while enhancing thermal stability, gel texture, and overall functionality, supporting their potential application in some food processing systems.

Keywords: Avocado Seed, Jackfruit Seed, Wet Milling and Dry Milling, Citric Acid Treatment, Annealing, Heat Moisture Treatment

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ABBREVIATIONS AND SYMBOLS

ASF	Avocado Seed Flour
ASS	Avocado Seed Starch
ADF	Avocado Dry Milled Flour
AWF	Avocado Wet Milled Flour
ADS	Avocado Dry Milled Starch
AWS	Avocado Wet Milled Starch
JSF	Jackfruit Seed Flour
JSS	Jackfruit Seed Starch
JDF	Jackfruit Dry Milled Flour
JWF	Jackfruit Wet Milled Flour
JDS	Jackfruit Dry Milled Starch
JWS	Jackfruit Wet Milled Starch
CA	Citric Acid
ANN	Annealing
HMT	Heat Moisture Treatment
MC	Moisture Content
RVA	Rapid Visco Analyzer
TPA	Texture Profile Analyzer
DSC	Differential Scanning Calorimetry
SEM	Scanning Electron Microscopy
XRD	X-ray Diffraction
FTIR	Fourier Transform Infra-Red
DS	Degree of Substitution

CHAPTER 1

INTRODUCTION

1.1 Background

Over the past five years, there has been a rise in the worldwide production of avocados and jackfruit (FAOSTAT, 2022; Worldatlas, 2017). In addition to their consumption in fresh form, avocado and jackfruit undergo processing to create various products. The production of items derived from avocado and jackfruit results in waste generated from components like skin, pomace, and seeds. This waste possesses the risk of causing pollution, posing threats to human life, and contributing to environmental issues (Salazar-Lopez et al., 2020; Chen et al., 2016).

The seeds of avocado and jackfruit represent a valuable potential resource for the development of nutrient-rich products. These seeds are rich in essential components such as carbohydrates, lipids, proteins, and various beneficial phytochemicals. Notably, starch, serving as the primary carbohydrate store, is abundantly present in both avocado and jackfruit seeds (Chel-Guererro et al., 2016; Maryam et al., 2016; Wang et al., 2022; Jiamjariyatam, 2017; Cornelia & Christanti, 2018). Within the flour production process, milling stands out as a crucial stage. Grinding is the method typically employed, with wet and dry grinding being common techniques (Madruga et al., 2014; de Dios-Avila et al., 2022). The primary distinction lies in the utilization of water; wet grinding involves a significant water usage (Yeh, 2004), while dry grinding does not require water. Various milling approaches are recognized for yielding diverse outcomes in chemical composition and physicochemical properties (Suksomboon & Naivikul, 2006). The production of starch products from avocado and jackfruit seed flour involves an isolation or extraction procedure. This process is designed to eliminate protein and fat molecules, yielding pure starch. The chosen method is a base extraction using sodium hydroxide, known for its simplicity in equipment setup and the exceptional purity it achieves in the resulting starch (Usman et al., 2014).

Native starch properties limit its utilization in many industrial applications due to their intolerance to heat and shearing during food processing, poor gelling, and thickening properties due to acid hydrolysis (Lawton, 2004; Bashir & Aggarwal, 2019). Therefore, native starch is modified through different chemical, enzymatic, and physical processes to improve their functional properties (Cornelia & Christianti, 2018). Each method will improve the food product's functional properties, such as increasing the water-binding capacity, increasing the resistance to the heating temperature, minimizing the occurrence of retrogradation or syneresis, improving and maintaining viscosity (Abbas et al., 2010).

Chemical modification such as cross-linking, can be utilized to improve the hydrophilic and hygroscopic properties of starch. Citric acid (2-hydroxy-propane-1,2,3tricarboxylic acid) is the primary organic acid chemical found in oranges and pineapples. It has one hydroxyl and three carboxyl groups. As citric acid is a non-toxic byproduct of metabolism (Krebs cycle), it is commonly used as food ingredients (Yang et al., 2004). The reaction between the carboxyl groups in citric acid and the hydroxyl groups in starch is capable of forming strong bonds and can lower the number of free hydroxyl groups in starch, hence increasing the hydrophobicity of the starch (Thiebaud et al., 1997). Some studies have been reported regarding the use of esterification methods in starch modification. Utomo et al. (2020) reported a decrease in swelling power, solubility, and viscosity in cassava starch, while Lv et al. (2022) reported that the esterification process in tiger nut starch resulted in an increase in degree of substitution (DS), solubility, and swelling anda decrease in the degree of crystallinity. Apart from that, research conducted by Alimi and Workneh (2018) reported that there was no change in the morphological structure of Aca starch and Iburu starch.

Annealing treatment is a process that involves simple hydrothermal incubation of starch where the temperature used is higher than the glass transition temperature but lower than the starch gelatinization temperature with the aim of improving the physicochemical properties of starch (Chen et al., 2022). The starch produced with this method retains its granule structure, but experiences significant alterations in various physicochemical parameters, including crystallinity, gelatinization, swelling factor, solubility, viscosity, and hydrolysis rate. The annealing characteristics are affected not

just by the duration of heating, the temperature, and moisture level, but also by the presence of starch (Yao et al., 2018).

Heat-moisture treatment (HMT) is a physical modification method that can be employed to modify the starch structure and functional properties without destroying the starch granular structure. In HMT, starch is exposed to a high temperature, commonly above the gelatinization temperature at low moisture level, mostly less than 35% water by weight which is insufficient water to gelatinize. The starch samples are incubated for a certain amount of time at a temperature that is higher than the temperature at which glass transition occurs but lower than the temperature at which gelatinization occurs (Guneratne, 2018; Hoover, 2010). Some studies have been reported regarding the use of HMT in modifying starch properties. Piecyk and Domian (2021) reported that there was a decrease in swelling power and amylose leaching, as well as changes in the crystal structure of field bean starch. Meanwhile, Ji et al. (2015) reported that waxy maize starch treated with HMT experienced an increase in crystallinity and thermal properties such as onset temperature, peak temperature, and final temperature methods in starch modification.

In this study, the characteristics of two seed starch, avocado and jackfruit seeds were compared regarding the two milling methods, wet and dry milling. Their physicochemical properties and functional properties of flour and the corresponding starch were investigated. In addition, attempts have been made to improve functional properties of avocado and jackfruit seed starch by citric acid esterification, annealing and heat-moisture treatment. The structure, morphology and functional properties were investigated and compared among treatment conditions.

The research problem addressed in this study is the limited utilization of starch extracted from avocado and jackfruit seeds. Native starches from these underused sources typically possess inadequate physicochemical properties, highlighting the need for further exploration of their characteristics through both chemical and physical modification methods.

1.2 Objectives

1.2.1 To compare effect of wet milling and dry milling on properties of flour and corresponding starch of avocado and jackfruit seeds.

1.2.2 To investigate effect of modification methods, citric acid treatment, annealing treatment and heat-moisture treatment on properties of avocado and jackfruit seeds starch.

1.3 Research Scope

This research compared properties between the two fruit seeds, avocado seeds (AS) and jackfruit seeds (JS). Flour and starch obtained from wet and dry milling methods were compared. The obtained pure starch was further modified by chemical means, citric acid esterification (CA) and physical means, annealing (ANN) and heat-moisture treatment (HMT). The combinations of chemical and physical modifications were also investigated. The effect of these modifications on starch structure, physicochemical and functional properties were examined. The potential application of the modified ASS and JSS would be suggested.

1.4 Expected Benefits

1.4.1 Research Output

1.4.1.1 Know the properties of native flour and starch from AS and JS which were obtained from two milling methods, wet milling and dry milling.

1.4.1.2 Know the effect of modification methods, citric acid treatment, annealing treatment and heat-moisture treatment on properties of avocado and jackfruit seeds starch.

1.4.1.3 Know the potential application of the obtained modified AS and JS starch.

1.4.2 Research Outcome

1.4.2.1 Two fruit seeds, avocado and jackfruit would gain more interest as the alternative starch sources for industry.

1.4.2.2 An increase in utilization of avocado and jackfruit seed starch in industrial uses.

CHAPTER 2

LITERATURE REVIEW

2.1 Avocado Production

Avocado (*Persea americana. Mill*) is a tropical and subtropical fruit that belongs to dicotyledonous plants and is a member of the Lauraceae family (Yahia, 2012; Scaffer et al., 2013; Araujo et al., 2018). Currently, avocado fruit plants have been widely cultivated throughout the world (Alcaraz & Hormaza, 2007; Alcaraz et al., 2011). From data released by the Food and Agriculture Organization Statistics (FAOSTAT), in the past five years there has been an increase in avocado production by 5 to 13% per year. The world's leading producers are listed in Table 2.1.

Table 2.1 Top five largest production country of avocado during 2017 – 2021

No	Country	Production (tones)
1	Mexico	11.352.231,03
2	Dominican Republic	3.202.298,15
3	Colombia	3.097.026,38
4	Peru	2.992.955,79
5	Indonesia	2.513.163,46

Source FAOSTAT (2022)

The avocado (Persea americana Mill) is a berry consisting of fruit seed and the pericarp, which is the sum of the skin (exocarp), the edible flesh (mesocarp) and the inner layer that surrounds the fruit seed (endocarp) (Hurtado-Fernandez et al., 2018).

Source Hurtado-Fernandez et al. (2018)

Figure 2.1 Components of avocado fruit

Figure 2.1 illustrates the various components of an avocado fruit. Besides the flesh, the seeds and skin of avocados also contain significant amounts of protein, fiber, carbohydrates, and various bioactive compounds. Unfortunately, a substantial portion of these parts typically goes to waste and is discarded. Given the valuable constituents present in these parts, there exists potential for them to undergo further processing and be transformed into useful products (Salazar-Lopez et al., 2020).

2.1.1 Composition and Properties of Avocado Seed

The average avocado fruit weighs about 150 to 400 g, with the seed making up 15 - 16% of the total weight, although this proportion can vary among cultivars. Avocado seeds are rich sources of carbohydrates, lipids, proteins, fibers, minerals and various other bioactive compounds (Araujo et al., 2018; Salazar-Lopez et al., 2020). Table 2.2 shows a chemical composition of avocado seed of different cultivars (dry basis, % w/w).

Table 2.2 Chemical composition of avocado seed

Cultivar	Moisture (%)	Minerals (%)	Lipids (%)	Fiber (%)	Protein (%)	Carbohydrate (%)	References
Hass	14.55	2.81	3.32	3.97	0.14	-	Daiuto et al. (2014)
Hass	7.66	3.85	5.52	3.98	3.44	79.54	
Utz	9.44	2.79	6.32	4.24	3.09	78.37	
Booth 8	1.78	3.48	6.70	4.06	4.90	72.14	Bresani et al. (2009)
Panchoy	5.83	2.73	6.00	2.67	3.86	81.58	
Shupte	8.04	4.30	4.05	2.19	9.63	42.45	
Thompson Red	60.51	-	2.09	3.08	0.20	35.27	Tan et al. (2022)

Avocado fruit contains phenolic chemicals, flavonoids, carotenoids and alkaloids. Avocado seeds contain flavonoids, tannins, saponins, phenolics, antioxidants, oxalates, phytates, and alkaloids. Polyphenols are bioactive chemicals found throughout the avocado plant. According to Al-Daihan et al. (2016), the polyphenol content of avocado seeds has antibacterial, antifungal, antiviral, and wound healing effects, making it a viable traditional medicine. Cornelia and Christianti (2017) found that avocado seeds contain antiinflammatory and analgesic chemicals. In addition, avocado seeds also contain alkanols, terpenoid glycosides, derivatives with furan rings, flavonoids, and coumarins (Kosinka et al., 2012; Yasir et al., 2010).

2.2 Jackfruit Production

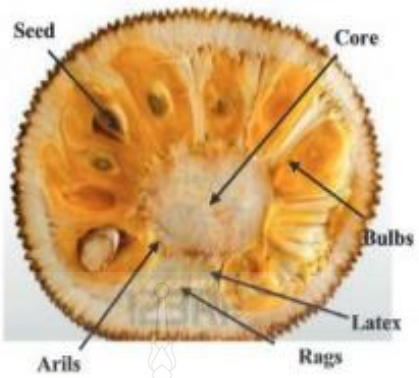
Jackfruit (Artocarpus heterophyllus) belongs to the Moraceae family, which is a native fruit from India. Apart from India, jackfruit is generally grown in tropical and subtropical countries, especially in Sri Lanka, Bangladesh, Burma, the Philippines, Indonesia, Thailand, Malaysia and Brazil as shown in Table 2.3. Jackfruit is an important tropical fruit in Thailand and other Asian countries, and contains a variety of bioactive compounds, such as carotenoids, flavonoids, total phenolic compounds, and ascorbic acid (Swami & Kalse, 2018).

Table 2.3 Top five largest production country of jackfruit

Country	Production (metric tonnes)
India	1.436
Bangladesh	926
Thailand	392
Indonesia	340
Nepal	19

Source Worldatlas (2017)

The average weight of jackfruit is around 3.5–10 kg, sometimes it can even reach 25 kg. Ripe jackfruit consists of 29% pulp, 12% seeds and 54% rind. Figure 2.2 shows the parts of the jackfruit.



Source Swami and Kalse (2018)

Figure 2.2 Components of jackfruit

Aside from the jackfruit flesh, the seeds of the jackfruit constitute approximately 10-15% of the fruit's total weight. A single jackfruit typically contains around 100-500 seeds which possess a considerable amount of carbohydrates and protein. The seeds are usually discarded or steamed and consumed as a snack or used in some local dishes. Flour of jackfruit seed can be an alternative ingredient which can be used in several food products (Swami & Kalse, 2018). This presents an opportunity to enhance the value of jackfruit seeds. Figure 2.3 displays an image of jackfruit seeds.

Source Swami and Kalse (2018)

Figure 2.3 Jackfruit seeds

2.2.1 Composition and Properties of Jackfruit Seed

Jackfruit seeds are a nutritious food option because they provide protein, fiber, and carbohydrate. In addition, they are loaded with essential nutrients like phosphorus,

potassium, calcium, magnesium, zinc, copper, etc. as shown in Table 2.4. Jackfruit seeds contain phytonutrients such as lignans, isoflavones, and saponins, which offer a range of health benefits, including anticancer, antihypertensive, anti-aging, antioxidant, and antiulcer properties. Based on these compositions of jackfruit seeds, there is significant potential for further processing to create products that can maximize the value derived from these seeds.

Table 2.4 Proximate composition, minerals, and vitamins of jackfruit seed

Proximate (per 100 g edible portion)		**Mineral and Vitamin (per 100 g edible portion)**	
Composition	**Value**	**Composition**	**Value**
Water (g)	51.0 – 64.5	Total Minerals (g)	0.9 – 1.2
Protein (g)	6.6 – 7.04	Calcium (mg)	50.0
Fat (g)	0.40 – 0.43	Magnesium (mg)	54.0
Carbohydrate (g)	25.8 – 38.4	Phosphorus (mg)	38.0 – 97.0
Fiber (g)	1.0 – 1.5	Potassium (mg)	246
Total sugars (g)	-	Sodium (mg)	63.2
		Iron (mg)	1.5
		Vitamin A (IU)	10 – 17
		Thiamine (mg)	0.25
		Riboflavin (mg)	0.11 – 0.3
		Vitamin C (mg)	11.0

Source Swami and Kalse (2018)

2.3 Utilization of Avocado and Jackfruit Seeds

Fruit seed utilization encompasses the diverse methods by which seeds from fruits are used for various applications, instead of being disposed of as waste. This approach not only reduces food waste but also optimizes the overall utility of the fruit. Currently, avocado seeds are being processed into flour, serving as a versatile ingredient in a wide range of applications, including standalone use and as a substitute for one of the primary ingredients in baked goods and various processed foods. Jackfruit

seeds have found applications in various bakery items, extruded products, and other starch-based food products. Numerous studies have explored the potential use of avocado seed flour and jackfruit seeds as substitutes for traditional flours. For instance, Hosain (2014) investigated the use of jackfruit seed flour as a flour substitute in bakery products, while David (2016) conducted research on employing jackfruit seed flour as a substitute ingredient in chocolate cake products. In addition, Meethal et al. (2017) substituted jackfruit seed flour 5 - 30% in snack bar products with acceptable sensory qualities.

2.4 Starch and its Chemical Structure

Starch is the main carbohydrate and polysaccharide in plants which chemically consists of two glucan polymers, namely amylose and amylopectin (Cornejo-Ramirez et al., 2018; Bertoft, 2017; Krithika & Ratnamala, 2019) as shown in Figure 2.4. In general, the composition of amylose in starch is in the range of 20 - 30%, while for amylopectin it is in the range of 70 - 80% (Perez & Berthof, 2010). Amylose is composed of glucose molecules with α-(1-4) glycosidic bonds forming linear chains. Meanwhile, amylopectin consists of amylose chains (α-(1-4)) bonded together to form branches with α-(1-6) glycosidic bonds (Krithika & Ratnamala, 2019). Amylopectin has a role in increasing crispness while amylose plays a role in increasing hardness (Niken & Dicky, 2013).

Starch can be found in various parts of plants, including cereal grains (such as maize, rice, wheat, barley, oat, and sorghum), roots (such as sweet potatoes, cassava, arrowroots, and yam), tubers (such as potatoes), stems (such as sago palm), and legume seeds. (Swinkels, 1985; Preiss, 2004; Krithika & Ratnamala, 2019). The amount of amylose and the structure of amylopectin have a significant impact on the physicochemical and functional properties of starch, which in turn may have implications for its use in the food or industrial sectors (Vamadevan & Bertoft, 2015; Zhang et al., 2016). The amount of amylose and the structure of amylopectin have a significant impact on the physicochemical and functional properties of starch, which in

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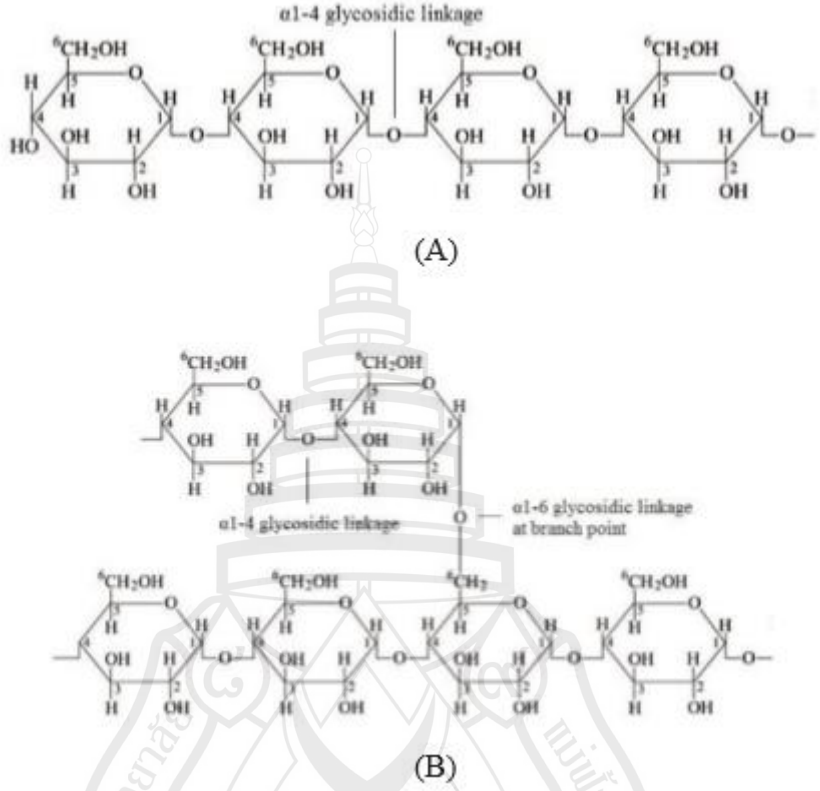


Source Nawaz et al. (2020)

Figure 2.4 Chemical structure of (A) Amylopectin and (B) Amylose

Starch's swelling power, solubility, and ability to form gels are all affected by its amylose concentration, which is responsible for several of the components that contribute to these effects. There has been a wide range of amylose content reported for different starches. This variability is attributed to the influence of botanical sources and the methods used for determination (Kaur & Singh, 2016). Table 2.5 shows amylose content in avocado seeds and jackfruit seeds starch which varied among studies.

Table 2.5 Amylose content of avocado and jackfruit seeds starch

Starch	Amylose content (%)	References
Avocado seeds	39.56	Martins et al. (2022)
	30.41	Macena et al. (2020)
	42.37	Cornelia and Christianti (2018)
	21.50	dos Santos et al. (2016)
	32.50	Builders et al. (2010)
Jackfruit seeds	38.34	Zhang et al. (2021)
	32.81	Zhang et al. (2017)
	43.96	Tran et al. (2015)
	26.57	Noor et al. (2014)
	27.12	Dutta et al. (2011)

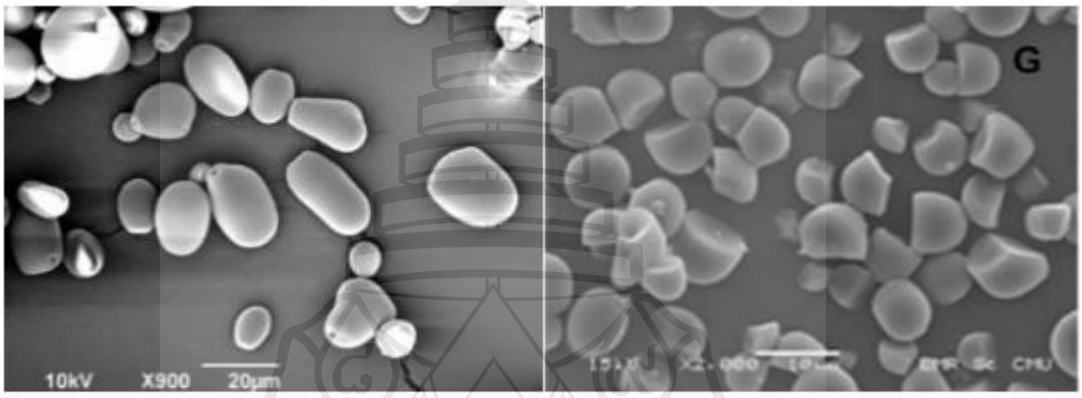
2.5 Physicochemical and functional properties of starch

2.5.1 Proximate Composition and Amylose Content

The starch content in avocado seeds is around 26% (Wang et al., 2022) while in jackfruit seeds it is around 70% (Lubis et al., 2017) so it has the potential to undergo further processing. The chemical composition contained in avocado seed starch and jackfruit seeds includes protein, fat, and carbohydrates. The chemical composition of starch products has different content when compared to flour products. The chemical composition of starch products tends to be smaller than that of flour products. Technically, starch isolation using the extraction method causes a decrease in the levels of chemical components in starch such as protein and fat. According to Harsono (2006), the presence of fat in starch or flour can inhibit the gelatinization process. This occurs because fat creates a fatty layer on the granules' surface, hindering the infiltration of water. Starch with high amylose content tends to produce hard and dense products due to the limited expansion process. Conversely, higher levels of amylopectin will stimulate the puffing process so that food products become light, porous, crisp and crunchy (Wulandari et al., 2016).

2.5.2 Starch Morphology

Scanning electron microscopy (SEM) is used to observe the granule size and shape and the presence or absence of impurities in starch after the isolation process. Granules of avocado seed starch vary in size from 10.1 to 42.5 μm, with 35.1 μm being the average. The SEM image revealed oval and ellipsoid shapes, with some elongated variants (Macena et al., 2020). SEM image of jackfruit starch shows the fine, round, and bell-shaped granules with sizes ranging from 5 to 10 μm. Figure 2.5 shows the image of avocado and jackfruit seed starch granules, respectively.



Source Macena et al. (2020) and Kittipongpatana and Kittipongpatana

Figure 2.5 Granule morphology of avocado seed starch (A) and jackfruit starch (B)

2.5.3 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR is the most common form of infrared spectroscopy. All infrared spectroscopies act on the principle that when infrared (IR) radiation passes through a sample, some of the radiation is absorbed. The radiation that passes through the sample is recorded. Because different molecules with their different structures produce different spectra, the spectra can be used to identify and distinguish between molecules. In addition, every functional group has its own frequency at which it absorbs infrared radiation. Considering the findings of earlier studies, an investigation into the various kinds of functional groups has been carried out (Sanjiwani et al. 2020).

2.5.4 X-Ray Diffraction (XRD)

The application of X-ray diffraction (XRD) is a very effective and nondestructive method used for the analysis and characterization of crystalline substances. This resource offers comprehensive data pertaining to multiple aspects of materials, including their structures, phases, preferred crystal orientations (texture), and additional structural factors such as average grain size, crystallinity, strain, and crystal defects. The XRD examination is a highly effective method for identifying faults in a specific crystal, evaluating its stress resistance, determining its texture, measuring its size and degree of crystallinity, and examining several other variables associated with the fundamental structure of the sample (Bunaciu et al., 2015).

2.5.5 Swelling and Solubility

The ability of a starch to expand when exposed to water is measured by its swelling power, which has generally been utilized to illustrate differences between the many varieties of starch. In the process of calculating the swelling volume, solubility is measured as the proportion of starch that is leached into the supernatant. The water-binding capacity of commercial starches is important for the quality and texture of certain food products because it stabilizes them against effects such as syneresis. In addition, the ability of commercial starches to bind water is crucial for enhancing the quality and texture of various food products

2.5.6 Paste Viscosity by Rapid Visco Analyzer (RVA)

The Rapid Visco Analyzer (RVA) is a widely used instrument that can evaluate the pasting qualities of flour or starch. The parameters that are displayed in the results of the RVA test are as follows: Pasting Temperature (PT), Peak Viscosity (PV), Trough Viscosity (TV) (at the point where the holding time at 95°C is to end), cold Final Paste Viscosity (FV) (at the end point where the holding time at 50°C on cooling occurs), Breakdown Viscosity (BV) and Setback Viscosity (SV) (Sasanatayart et al., 2019). Table 2.6 shows the pasting properties of avocado seed starch and jackfruit seed starch.

2.5.7 Thermal Properties by Differential Scanning Calorimetry (DSC)

When starch is heated in the presence of water, a phase transition known as gelatinization takes place. This transition involves a change from an ordered to a disordered state. The phase transition temperature can be measured with a differential scanning calorimeter as an onset transition temperature (To), peak transition

temperature (Tp), endset transition temperature (Te), and enthalpy. All these temperatures are measured in degrees Celsius (Hoover, 2010). Table 2.7 shows thermal properties of avocado seed starch and jackfruit seed starch in several studies.

2.5.8 Gel Texture

The ability of starch to form gel plays a significant role in the manufacture of food. When evaluating the performance of starch in a food system, a crucial aspect to consider is the texture of the starch in its gel form (Li et al., 2014). The texture properties of the gels depend on the starch constituents, amylose content, volume and the deformation of the granules, and the interaction between continuous and dispersed phases (Macena et al., 2020). Table 2.8 shows texture parameters according to texture profile analysis (TPA) of avocado and jackfruit seed starch.

2.5.9 Degree of Substitution

The degree of substitution (DS) refers to the amount of hydroxyl groups of starch molecules that are substituted with other groups that are susceptible to undergoing chemical modification. The maximum DS value of esterified starch might differ significantly from one another, depending on the structure of starch. DS is limited by the available total amount of hydroxyl groups. The highest possible value of DS for starch is 3 (Liu et al., 2022).

Table 2.6 Pasting properties of avocado seed starch and jackfruit seed starch

Starch	PV (cP)	TV (cP)	BV (cP)	FV (cP)	SV (cP)	PT (°C)	Pt (min)	Ref.
Avocado seeds	3418	1545	1873	6165	4620	78.20	5.13	Alemu (2022)
	284.34	-	121.00	467.42	-	80.22	5.00	Silva et al. (2017)
	-	-	-	5385.5	2880.5	88.50	5.00	Martins et al. (2022)
	4421	1239	1063.0	7403	1345.0	76.00	5.10	dos Santos et al. (2016)
Jackfruit seeds	3233	2252	981	3001	749	80.15	4.86	Zhang et al. (2016)
	2981	2281	700	3510	1229	86.80	5.27	Zhang et al. (2016)
	432.43	276.58	56.78	489.21	214.52	85.83	8.74	Mukprasirt and Sajjaanantakul (2004)
	2616	-	1383	3236	2275	81.60	-	Madruga et al. (2014)
	2483	-	824	-	2440	-	-	Dutta et al. (2011)

Table 2.7 Thermal properties of avocado seed starch and jackfruit seed starch

Source	To (°C)	Tp (°C)	Te (°C)	ΔH gel (J/g)	References
Avocado seeds	67.21	71.14	77.48	11.82	de Dios-Avila (2022)
	66.73	70.34	76.57	13.43	de Dios-Avila (2022)
	67.60	72.40	84.30	14.90	dos Santos et al. (2016)
	70.20	76.00	81.50	11.30	Bet et al. (2017)
Jackfruit seeds	72.61	76.25	88.47	16.57	Zhang et al. (2016)
	82.92	86.01	91.23	15.19	Kittipongpatana and Kittipongpatana (2011)
	87.57	88.65	92.49	7.70	Naknaen (2014)
	81.11	85.39	91.70	19.61	Chen et al. (2016)

Table 2.8 Texture properties of avocado and jackfruit seed starch

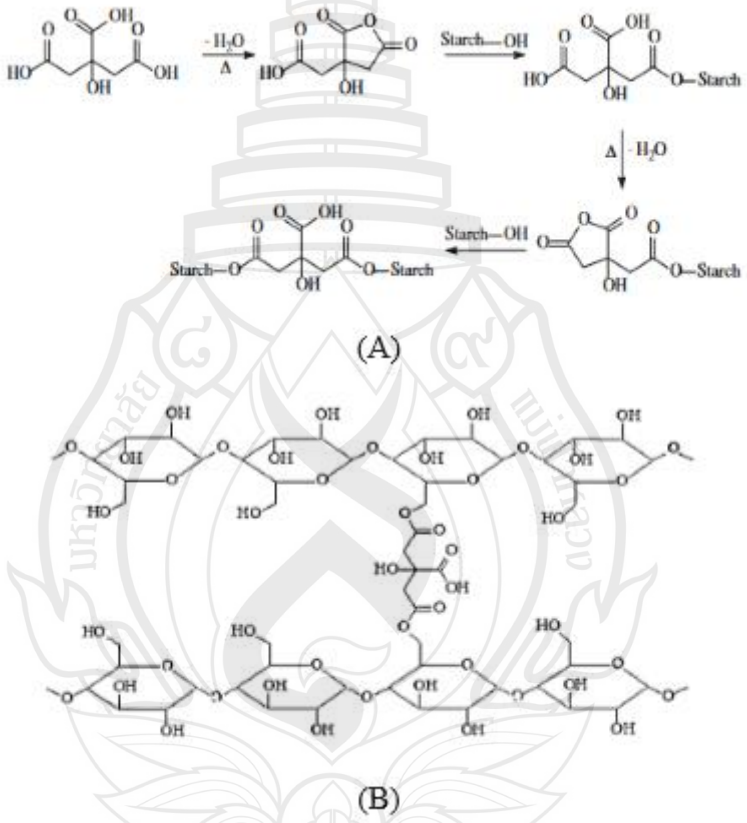
Starch	Parameter					Ref.
	Hardness (g)	**Adhesiveness (g.sec)**	**Springiness (%)**	**Cohesiveness**	**Chewiness (g)**	
Avocado seed	195.62	-	-	0.32		Macena et al. (2020)
Jackfruit seed	529	-152.63	138.26	0.23	150.96	Zhang et al. 2017)

2.6 Modification of Starch

Untreated or raw native starches are not widely used in advanced food industries. This is because they possess low thermal and shear resistance, and higher retrogradation tendency which limit the uses in food applications. To overcome these limitations, starch modification is an obligation to alter its physicochemical and functional properties based on the requirement. Starch modifications can be categorized into three main categories: physical, chemical, enzymatic, and combined modification methods (Neelam et al., 2012). These modifications aim to improve or alter certain characteristics of the starch (Syamsir et al., 2012; Fleche, 1985; Glicksman, 1969). Modified starch has advantages that can increase the functional value and quality of food products compared to natural starch so that it can be used more widely (Koswara, 2009). Some of the benefits obtained from starch modification include increasing water binding capacity, resistance to heating temperatures, viscosity, and minimizing the occurrence of syneresis processes (Cornelia & Christanti, 2018; Abbas et al., 2010). The chemical modification alters the physiochemical properties of starch by introducing new chemical or functional groups in starch without altering the shape and size of starch granules. These chemical modifications are commonly accomplished by means of derivatization techniques, such as etherification, esterification, and cross-linking. Nevertheless, these methodologies are constrained by concerns pertaining to the customers and the environment (Neelam et al., 2012). Physical modifications change the morphology and structure of starch influenced by physical factors such as moisture, temperature, pressure, pH change, radiation treatment, and ultrasonic treatment. These methodologies are widely favored due to its avoidance of chemical treatments that may pose risks to human safety (Neelam et al., 2012; Yao et al., 2018). The enzymatic modification includes designing a starch with a new structure. The molecular mass, branch chain-length distribution, and amylose/amylopectin ratio can be altered by enzymatic reactions when the enzymes react with gelatinized starch (Putri & Nisa, 2015). Native starch is subjected to various forms of modification to improve its structural and functional properties for wider applications in the food industry.

2.6.1 Citric Acid Esterification (CA)

Citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid) is the primary organic acid commonly found in fruits. It has one hydroxyl and three carboxyl groups, indicative of acidity. Citric acid is a non-toxic byproduct produced in Kreb's cycle, which is metabolic pathway (Yang et al., 2004). The crosslinking of starch with citric acid involves esterification reactions between the carboxyl groups in citric acid and the hydroxyl groups in starch (Utomo et al., 2020; Menzel et al., 2013). The mechanism of starch and citric acid interaction is shown in Figure 2.6.



Source Utomo et al. (2020)

Figure 2.6 The mechanism of crosslinking starch with citric acid (A) and the molecular structure of starch citrate (B)

In Figure 2.6A, citric acid (which has three carboxyl groups) reacts with the hydroxyl groups (-OH) present on starch molecules. This results in the formation of starch citrate adducts, where citric acid molecules are attached to starch. Upon heating,

the reaction mixture is heated, and the heat plays a crucial role in promoting further reactions. Under heat, the esterification process intensifies. The carboxyl groups of citric acid react with the hydroxyl groups of starch, leading to the formation of ester bonds between them. This esterification process forms crosslinks between starch molecules and citric acid. Figure 2.6B, crosslinking: As ester bonds continue to form, crosslinks are created between adjacent starch molecules. These crosslinks strengthen the starch structure and alter its properties. Crosslinking also reduces the number of available free hydroxyl groups in starch and increase the hydrophobicity of the starch molecule. (Thiebaud et al., 1997). The starch and citric acid esterification reaction yields mono, di, and tri esters. During the reaction between starch and citric acid, esterification begins with the creation of starch citrate adducts, and subsequent heating of the reaction medium causes a cross-linking reaction (Mei et al., 2015). At high temperatures, Hung et al. (2016) found acid hydrolysis as a parallel process to esterification and CA cross-linking, with amylopectin being more vulnerable to hydrolysis than amylose. The intermolecular esters created can be assessed by degree of substitution, indicative of the binding ability (Xie & Liu, 2004). The summary of citric acid modification on starch and their effects were presented in Table 2.9. However, systematic studies on the effect of citric acid modification on some properties such as starch digestibility and other functional properties of starch from various origins are lacking.

Table 2.9 Citric acid esterification of starch and main findings

Starch source	Citric acid esterification	Main findings	References
Corn	Acid concentration 0 –40% with temperature 140°C for 7 h	1. Solubility and water absorption were decreased. 2. Pasting temperature was increased but some. 3. Pasting temperature was increased but some parameters were decreased. 4. Higher firmness, cohesiveness, springiness, gumminess, and chewiness	Hedayati and Niakousari. (2018)
Potato	Acid concentration 10%, 20%, 40% with temperature 100°C, 130°C, 160°C for3 h	1. Swelling power and solubility were decreased. 2. Starch resistance was increased.	Kapelko - Zeberska et al. (2016)
	Acid concentration 20%, 40%, 60%, with temperature 130°C for 2 h	1. The crystalline pattern and granule morphology were not changed.	Remya et al. (2018)

Table 2.9 (continued)

Starch source	Citric acid esterification	Main findings	References
		2. Water binding capacity was increased. 3. Pasting curves were flatted. 4. The slowly digestible and resistant starch was increased.	
Cassava / Tapioca	Acid concentration 10 – 40% with temperature 130°C, for 5 h	1. The content of resistant starch was increased. 2. The swelling power and solubility of citrate starch samples were decreased. 3. A new peak appeared in all citrate starch samples. 4. Crystalline peaks were decreased or disappeared. 5. The content of resistant starch was increased.	Mei et al. (2015)

Table 2.9 (continued)

Starch source	Citric acid esterification	Main findings	References
Cassava / Tapioca		6. The swelling power and solubility of citrate starch samples were decreased. 7. A new peak appeared in all citrate starch samples. 8. Crystalline peaks were decreased or disappeared.	
	Acid concentration 10% 30%, 50% with temperature 100°C, 120°C, 140°C for5 h	1. The degree of esterification was increased. The microstructure of esterified starch was observed to have agglomerated granules with corrosions/fissures 2. Transitional enthalpy was decreased as well.	Srikaeo et al. (2019)

Table 2.9 (continued)

Starch source	Citric acid esterification	Main findings	References
Sweet Potato	Acid concentration 10 – 60% with temperature 140°C, for 4 h	1. The degree of substitution and the resistant starch were increased. 2. The shape and integrity of granules were not changed. 3. The relative crystallinity RC was decreased. 4. The gelatinization temperatures and swelling power were decreased.	Xia et al. (2016)

2.6.2 Heat Moisture Treatment (HMT)

Heat-moisture treatment of starch involves a physical modification process where starch granules are subjected to incubation at a temperature exceeding the point of glass transition but below the temperature necessary for gelatinization. This treatment is carried out for a specified duration under conditions of relative humidity where the water content is less than 35% by weight (Hoover, 2010). In HMT, starch is exposed to a high temperature, commonly above the gelatinization temperature with insufficient water to gelatinize (Gunaratne, 2018). Upon HMT, alterations in starch chain interactions and disturbances in the amorphous and crystalline regions of starch take place. These changes modify thermal characteristics, paste viscosities, gel forming properties, and digestibility of starch without causing damage to the starch granules (Sui & Kong, 2018).

2.6.2.1 Effects of HMT on granule morphology

The surface features, size, distribution, and morphology of starch granules are critical factors that have significant implications in numerous applications. The granular morphology of starch during HMT is influenced by several factors, including heating temperature, moisture content, and the type of starch used. These factors determine whether the granular morphology changes or remains relatively unchanged (Gunaratne, 2018). Earlier study showed that granule morphology of maize, wheat, potato, yam, lentil, and breadfruit starches remained unchanged after HMT (Kulp & Lorenz, 1981; Stute, 1992; Hoover & Vasanthan, 1994; Franco et al., 1995; Hoover & Manuel, 1996; Tan et al., 2017).

2.6.2.2 Effects of HMT on starch crystallinity

X-ray diffractometer (XRD) is widely used to reveal the effect of HMT on starch crystal structure (Gunaratne, 2018). HMT has been shown to change the wide-angle X-ray pattern from the B- to A-type (or A+B) for some starch like a potato starch (Sair, 1967; Hoover & Vasanthan, 1994; Gunaratne & Hoover, 2002; Vermeylen et al., 2006; Varatharajan et al., 2011; Ambigaipalan et al., 2014), yam starch (Hoover & Vasanthan 1994; Gunaratne & Hoover 2002), and breadfruit starch (Tan et al., 2017). Temperature and the moisture content of HMT appear to be affected on the changes of X-ray pattern and intensities (Hoover & Vasanthan, 1994; Vermeylen et al., 2006).

2.6.2.3 Effects of HMT on gelatinization properties

The effect of HMT on gelatinization qualities is a complex interplay of these factors. By carefully controlling and optimizing the type of starch, heating temperature, treatment duration, and moisture content, it is possible to tailor the gelatinization properties of starch for specific applications. In general, the gelatinization transition temperatures, onset (To), peak (Tp), and conclusion (Tc), as well as the gelatinization temperature range, are raised by HMT, which also widens the gelatinization temperature range (Tc-To) (Gunaratne & Hoover, 2002; Ambigaipalan et al., 2014).

2.6.2.4 Effects of HMT on pasting and gelling properties.

When starch is cooked in an excessive amount of water, it undergoes a series of transformations, with two significant processes being gelatinization and pasting. These processes occur within the same system and are often used to describe various alterations in the properties and characteristics of starch-based products (Gunaratne, 2018). According to Atwell et al. (1988), "pasting" is described as the phenomenon that occurs after the dissolution of starch and includes granular expansion, the exudation of molecular components from the granule, and, ultimately, the complete disintegration of the granules. In a wide variety of food applications, the textural qualities as well as the ability to store the product are significantly associated with the properties of starch paste. Pasting characteristics of starches can be considerably changed by HMT as HMT raises the temperature at which the starch paste is cooked and the thermal shear stability of the paste. However, it lowers the peak viscosity and the granular breakdown (Kulp & Lorenz 1981; Hoover & Vasanthan 1994; Lawal & Adebowale 2005; Gunaratne & Corke 2007; Shih et al., 2007; Varatharajan et al., 2011; Yadav et al., 2013; Sun et al., 2014). Table 2.10 summarizes the alterations on starch properties regarding HMT.

Table 2.10 Heat-moisture treatment (HMT) of starch and main findings

Starch sources	HMT	Main finding	Ref.
Jackfruit seeds	Temperature 80, 90, 100, 110 and 120°C in hot air oven for 6, 12 and 16 h Temperature 40 °C in hot air oven for 48 h, Starch moisture: 20, 25, 30 and 35	1. Higher resistant starch 2. Higher gelatinization temperature (To, Tp, Tc). 3. Lower gelatinization enthalpy.	Kittipongpatana and Kittipongpatana (2015)
Breadfruit	Temperature 120°C in thermostatically controlled oil bath for 4 h Temperature 40°C in oven to 12% moisture Starch moisture: 15, 20, 25, 30 and 35%	1. Higher gelatinization temperatures (To, Tp, Tc) and lower ΔH. 2. Higher paste temperature. 3. Peak and breakdown viscosity were not detected. 4. Lower setback viscosity.	Tan et al. (2017)

Table 2.10 (continued)

Starch sources	HMT	Main finding	Ref.
Cassava	Temperature 100°C in oven for 16 h Temperature in oven 40°C Starch moisture: 25%	1. Slight high gelatinization temperatures (To, Tp, Tc and ΔH). 2. Lower breakdown, peak and setback viscosity.	Chatpapamon et al. (2019)
Mango Kernel	Temperature 110°C in air oven for 3 h Starch moisture: 20, 25 and 30%	1. Lower swelling power. 2. Lower solubility. Higher paste temperature, peak viscosity, setback and final viscosity. 3. Lower relative crystallinities. 4. Higher paste temperature, peak, final and setback viscosity; the breakdown decreased	Bharti et al. (2019)

2.6.3 Annealing

Annealing treatment (ANN) is a process that involves a simple incubation of starch suspension at moderate temperatures where the temperature used is higher than the glass transition temperature but lower than the starch gelatinization temperature (Yao et al., 2018). It is an important physical approach to improve the physicochemical properties of starch (Chen et al., 2016). Annealing is usually carried out in excess water more than 40% w/w for a certain period (Chung et al., 2009). Annealing has been discovered primarily as a process that influences the structure and functional properties of starch materials (Jacobs et al., 1996; Jacobs et al., 1998; Su et al., 2020). This process is known to provide more stability to the starch grains during subsequent cooking and cooling and change their texture and viscosity (Malumba et al., 2022). The annealing treatment causes the molecules in the amorphous area of the granule to become hydrated, and the mobility of the amorphous phase increases (Wang et al., 2016). The slight and reversible swelling of starch granules allows some movement within crystalline domains while keeping the double helix relatively stable. This, in turn, alters the properties of starch and how enzymes break down the starch (Rocha et al., 2012).

2.6.3.1 Effects of annealing on granule morphology.

The annealing treatment allows the starch granules to maintain their morphology by controlling the temperature under the glass transition point. Several studies reported that there were no changes in the shape and size of the annealed starch granules in yam starch (Wang et al., 2017), wheat starch (Malumba et al., 2022), and waxy maize Wang et al. (2014).

2.6.3.2 Effects of annealing on starch crystallinity.

Starch is a semicrystalline material which is not interrupted by annealing. This is because AN involves insufficient energy to melt the crystalline regions and therefore, maintained starch semicrystalline structure. In general, the kind of starch used determines its level of crystallinity. According to Hsien-Chih and Sarko (1978), type A starch has higher crystallinity due to the dense network structure compared to type B starch. However, when the gelatinization process occurs, the starch granules will lose their integrity (Yao et al., 2018).

2.6.3.3 Effects of annealing on gelatinization properties.

Starch has a semicrystalline structure. When it is heated with enough water at a high temperature, this structure changes into a non-crystalline (amorphous) form. The annealing process, which also uses heat and water, can affect this structure. According to differential scanning calorimetry (DSC), annealing raises the starting (To) and peak (Tp) gelatinization temperatures, but it narrows the range between the start and end of melting (Tc–To). A higher To means the starch crystals are more stable after annealing. These changes show that the starch has a more uniform and stable crystalline structure (Stute, 1992; Jacobs & Delcour 1998; Tester & Debon 2000; Liu et al., 2009; Yadav et al., 2013; Zhang et al., 2015). The increase in gelatinization temperature is also caused by stronger bonds between amylose and amylopectin in the non-crystalline areas (Jacobs & Delcour 1998; Adebowalea et al., 2005). Annealing can also increase the gelatinization enthalpy (ΔH), which means more energy is needed to gelatinize the starch. This shows a more perfect crystal structure (Yao et al., 2018). However, ΔH can also be affected by the amount and type of amylose and amylopectin in the starch (Jayakody & Hoover, 2008).

2.6.3.4 Effects of annealing on pasting and gelling properties.

An important parameter of starch in its application in the food application is pasting properties. Pasting properties of starch changed significantly after annealing treatment. Several studies reported an increase in pasting temperature and a decrease in peak viscosity of starch (Stute, 1992; Jacobs et al., 1995). The increase in crystallinity along with the strong internal forces between the starch chains results in an increase in the gelatinization temperature, and the rearrangement of the chains contributes to a decrease in the viscosity. However, either the increase or decrease in overall paste viscosities were also reported among different types of starch under varying annealing conditions as shown in Table 2.11.

Table 2.11 Annealing (ANN) of starch and main findings

Starch source	Annealing	Main findings	References
Wheat	Temperature 30, 40, 50°C in water bath for 24 hrs.	1. Higher gelatinization temperatures. 2. Higher paste viscosities at 30 and 40°C. 3. Lower paste viscosities at 50°C.	Wang et al., (2017)
Maize	Temperature 45°C in water bath for 24 hrs and 72 hrs.	1. Little affected for morphology and crystallinity. 2. Lower paste viscosities.	Wang et al., (2014)
	Temperature 50°C in water bath for 24 hrs, 48 hrs and 72 hrs.	1. Not changed the morphology. 2. Lower peak viscosity. 3. Higher paste time.	Zheng et al., (2023)
Potato	Temperature 30, 40, 50°C in water bath for 24 hrs.	1. Higher gelatinization temperatures. 2. Higher paste viscosities at 50°C.	Wang et al., (2017)
	Temperature 50°C in water bath for 24 hrs, 48 hrs and 72 hrs.	1. Not changed the morphology. 2. Lower peak viscosity. 3. Higher paste time	Zheng et al., (2023)

Table 2.11 (continued)

Starch source	Annealing	Main findings	References
Yam	Temperature 30, 40, 50°C in water bath for 24 hrs.	1. Higher in gelatinization temperatures. 2. Higher paste viscosities at 30 and 40°C. 3. Lower paste viscosities at 50°C.	Wang et al., (2017)
Oca	Temperature 42 and 50°C in oven for 24 h.	1. Higher peak viscosity, stability ratio, gelatinization temperature, and gel firmness, but lower solubility and lower swelling power at 50°C.	Puelles-Román et al., (2021)

By precisely managing and fine-tuning the starch modification process—such as the starch type, heating temperature, treatment time, moisture level, and esterifying agent concentration—it becomes possible to customize starch functionality for targeted uses. This enables the development of starches with specialized textures, thickening properties, and other beneficial characteristics suited to both food and industrial applications.

CHAPTER 3

METHODOLOGY

3.1 Materials

There were two types of seeds used, avocado (*Persea americana Mill*) seeds and jackfruit (*Artocarpus heterophyllus*) seeds. Avocado seeds derived from avocado fruits of green mature states which were harvested in October 2022. Jackfruit seeds derived from fully ripe jackfruit which were harvested during November 2022 to January 2023. Both raw materials were collected from areas around the Chiang Rai City. Each type of seed was washed with tap water to remove dirt and impurities. Avocado and jackfruit seeds were soaked in water to soften the outer layer for easier peeling. The cleaned seeds are subsequently dried at room temperature to remove water from seed skin and stored at 18°C in the freezer till used.

3.2 Method

The study has been conducted in sequential three experiments as follows:

Experiment 1: Preparation of flour and the isolated starch of avocado and jackfruit seed.

Experiment 2: Modification of avocado and jackfruit seed starch by citric acid esterification.

Experiment 3: Modification of avocado and jackfruit seed starch by hydrothermal treatment and incorporation with citric acid esterification.

3.2.1 Experiment 1: Preparation of Flour and the Isolated Starch of Avocado and Jackfruit Seed

3.2.1.1 Dry and wet milling of avocado and jackfruit seed flour

Two milling methods, wet and dry milling were used for preparing avocado seed flour (ASF) and jackfruit seed flour (JSF) according to the method described by Rengsutthi and Charoenrein. (2011) with some modifications. For dry milling method, the peeled avocado and jackfruit seeds were sliced into thin slices with a thickness ranging between 2-3 mm and subsequently dried using hot air oven at 50°C for 24 hours to reach final moisture content around 10%. The obtained avocado and jackfruit seeds slices were ground using a hammer mill (Model CMC-20, Thailand) and sieved through a 100-mesh screen. The dry milled ASF and JSF were kept in a sealed HDPE bag and stored in refrigerator till used. For wet milling method, samples were prepared according to the method described by de Dios-Avila et al. (2022) with some modifications. The peeled seeds were soaked in water consisting of 1500 ppm potassium metabisulfite (KMS) to inactivate browning reaction. The ratio of the seed to water was 1:5 (w/v). Samples were blended and filtered through sheet cloth to obtain a fine slurry. Samples were left and stood for a total of 12 hours. After the supernatant was decanted, the starch sediment was collected and subsequently dried at 50°C for 24 hours. The dried clumps were grounded and sieved to obtain the fine powder. The wet milled ASF and ASS were kept and stored in refrigerator till used.

3.2.1.2 Isolation of avocado and jackfruit seed starch

Isolation of starch from avocado seed (ASS) and starch from jackfruit seed (JSS) was carried out using the method of de Dios-Avila et al. (2022) with some modifications. The dried and wet milled ASF and JSF from section 2.2 were mixed with 0.05M NaOH solution with a ratio of 1:5 (w/v). The samples were continuously stirred at 600 rpm by magnetic stirrer for 1 hour and allowed to stand for 1 hour. The yellow top layer of the sample was carefully removed. The white sediment or starch formed at the bottom of the container was remixed with NaOH solution, stood and collected again. This cycle was repeated for 5 times, aiming to remove as much as protein and lipids. Samples were then washed with distilled water several times, filtered to obtain residue and dried at 50°C for 24 hours to obtain final moisture content less than 10%. After drying, the starch was ground used a blender and sieved through a 100-

mesh screen. The dried starch samples were kept and stored at 10°C in a refrigerator till used.

3.2.1.3 Proximate analysis of avocado and jackfruit seed flour and starch

Proximate analysis of moisture, protein, lipid, ash, and total carbohydrate of samples were conducted regarding standard method of Association of Analytical Communities (AOAC), 2000) and apparent amylose content by American Association of Cereal Chemist (AACC), (2000) method as described below.

1.Moisture content

Empty aluminum cans and lids were pre-dried in an oven at 105°C for 20 minutes and cooled in desiccator. Exactly 2-3 g sample were weighed directly into the aluminum cans and dried in ventilated hot air oven (Memmert UF30, Germany) at temperature of 105°C for 24 hours. After drying, the cans were transferred to a desiccator to cool and weigh. Moisture content of sample was calculated using Equation 1.

$$Moisture\ (\%) = \frac{W_1 - W_2}{W_1}\ x\ 100 \qquad (1)$$

where W1 = weight (g) of sample before drying and

W2 = weight (g) of sample after drying

2.Protein content determination

One gram of sample was weighed in the weighing paper and put into the digestion tubes. The samples were mixed with 5 grams of catalyst and 12 mL of H2SO4 and put in the digestion box. The samples were connected by a scrubber machine (2001 Scrubber Unit Foss Tecator, Denmark) and heated at 420°C for 45 minutes. After 45 minutes, the samples were taken out and left at room temperature. Seventy milliliters of distilled water were added to the sample, and well mixed with vortex (Vortex-Genie 2, USA). Twenty-five milliliters of boric acid were prepared into the Erlenmeyer flask. Digestion tubes and an Erlenmeyer flask were placed into a distillation machine (2100 Kjeltec Distillation Unit Foss Tecator, Denmark) for 5 minutes. The digestion tubes and Erlenmeyer were taken. The boric acids in the Erlenmeyer were titrated by 0.1 HCl solutions. The PC of ASF and JSF was calculated using Equation 2.

$$Protein\ (\%) = \frac{(A-B)\ x\ N\ x\ 14.007\ x\ 6.25}{W} \quad (2)$$

where A = volume (ml) of 0.1 N HCl used sample titration

B = volume (ml) of 0.1 N HCl used in blank titration

N = Normality of HCl

W = weight (g) of sample

14.007 = atomic weight of nitrogen

6.25 = the protein-nitrogen conversation factor for fish and its by-products

3.Fat content determination

The fat cup was dried in a hot air oven (Memmert UF 30) at 105°C overnight. The fat cup was taken and cooled by a desiccator and weighed. Two-gram sample was weighed using a filter paper. The samples were put into thimble extraction. Seventy milliliters of petroleum eter were added to the cup fat. The thimble extraction and cup fat were put into soxlet apparatus (2055 Soxtec Manual Extraction Unit Foss Tecator, Denmark). The cup fat was dried in the hot air oven at 105°C for 2 hours. After drying, the cup fat was cooled in desiccator and weighed. The fat content of ASF and JSF was estimated using Equation 3.

$$Fat\ (\%) = \frac{Weight\ of\ fat}{Weight\ of\ sample}\ x\ 100 \quad (3)$$

4.Ash content determination

The crucible and lid were placed in the furnace at 550°C overnight. The crucible was cooled in a desiccator (30 minutes) and then weighed. Five grams of sample were weighed into the crucible and then heated over a low Bunsen fire with the lid half closed until no smoke. The crucible and lid were placed in the furnace at a temperature of 550°C overnight. The crucibles were cooled in a desiccator. The ash was weighed with a crucible and lid. The ash content of ASF and JSF was estimated using Equation 4.

$$Ash\ (\%) = \frac{Weight\ of\ Ash}{Weight\ of\ sample}\ x\ 100 \quad (4)$$

5.Total carbohydrate determination

The total carbohydrate can calculation with the Equation 5.

$$Carbohydrate\ (\%) = 100\% - \%moisture\ content - \%protein\ content - \%fat\ content - \%ash\ content \quad (5)$$

3.2.1.4 Amylose content determination

The amylose content was evaluated by iodine colorimetric method (AACC, 2000). Sample of 100 mg was suspended in a solution of ethanol (95.5% w/v) and 1 N sodium hydroxide in a 1:9 ratio and heated at 100 °C for 10 minutes. Cooked samples were allowed to stand at room temperature for one hour. Exactly 1 mL of 1 N glacial acetic acid and 2 mL iodine solution were added to the mixture, which was then incubated at 25 °C for 20 minutes. At 620 nm, the absorbance of the sample was determined using a spectrophotometer (Genesys 20, USA).

3.2.1.5 Color determination

A Hunter-Lab spectrophotometer (Konica Minolta, Japan) was used to determine the sample's color spectrum. About 10 g sample was put in a glass case on top of the instrument slit. Indicators for color evaluation, L* (light), a* (redness), b* (yellow), C* (chroma) and h (hue) with a mean value of over ten measurements (Raungrusmee et al., 2022).

3.2.1.6 Pasting properties by Rapid Visco Analyzer (RVA)

Pasting characteristics were determined using a rapid visco analyzer (Perten RVA 4500, Australia). About 2.5 g dry flour and starch samples were added with distilled water to obtain the total weight of 28 g. Then the sample was stirred using a spindle at a rotational speed of 960 rpm for the initial 10 seconds and at 160 rpm during analysis. The sample was held at 50°C for 1 minute, heated to 95°C for 3 minutes 42 seconds and held at 95°C for 2.5 minutes. On cooling, the sample was cooled to 50°C for 5 minutes 48 seconds (Martins et al., 2022). The parameters measured were peak viscosity, trough, breakdown, final viscosity, set back, peak time and pasting temperature.

3.2.1.7 Gel texture by texture analyzer

After the RVA analysis, the paddle was promptly removed from sample can. The sample was put into the refrigerator at a temperature of 4°C. The gel that formed was cut into a uniform piece with 1.5 cm in length and 2.5 cm in diameter. Gel texture was assessed by texture profile analysis (TPA) by a texture analyzer (TAXT2i, Stable Micro Systems Ltd., Surrey, UK). The modified method uses a two-cycle compression test using P/100 probe with 1.0 mm/s test speed, 3.0 g trigger force to 2.0 mm clearance. The average results of the hardness and adhesiveness test results were recorded over three repeated measurements.

3.2.1.8 Thermal properties by Differential Scanning Calorimetry (DSC)

Starch gelatinization were determined using a differential scanning calorimeter (Mettler Toledo DSC 3+, Belgium). About 3 mg samples with known moisture content was weighted into the airtight aluminum pan, added with 10 µL distilled water and tightly closed. The sample was heated at 10 °C/min from 30 °C to 130 °C under nitrogen flow at 10 mL/min. From the thermal curve, the initial temperature (To), peak temperature (Tp), end temperature (Te) and enthalpy of gelatinization were calculated (de Dios -Avila, 2022).

3.2.1.9 Morphology by scanning electron microscopy (SEM)

The morphology of the flour and starch granules was observed using SEM (Tescan MIRA 4, Czech Republic). The dried starch samples were attached to aluminum stubs with double-sided adhesive tape and then coated with gold powder using a branding machine to avoid filling under the electron beam. SEM micrographs were taken at an accelerating voltage of 10 keV at 2,000x magnification.

3.2.1.10 X-ray diffraction pattern by X-ray diffractometer (XRD)

X-ray diffractometer (PANalytical X'pert Pro MPD, Netherland) was used for examined the X-ray diffraction pattern of samples. The starch sample was poured, tapped and compressed in the grooves of the sample holder, which provides a flat surface for radiation application. The samples were scanned using Cu Ka radiation (k = 1.5405980 Å) at 40 kV and 150 mA. The spectral angle (2θ) is measured between 4° and 50° at a rate of 2°/min. Relative Crystallinity (RC) was the ratio of the area under the crystal section to the total area. The RC of starch granules was estimated using Equation 6.

$$RC(\%) = \left(\frac{A_{cp}}{A_T}\right) x\ 100 = \left(\frac{A_{cp}}{A_{cp} + A_{ap}}\right) x\ 100 \quad (6)$$

where A_{cp} is the crystalline peak area and A_{ap} is the amorphous peak area (Ali et al., 2020).

3.2.1.11 Fourier Transform Infra-Red (FTIR)

FTIR spectra of starch citrates and its corresponding control were obtained using a FTIR spectrometer (Nicolet iS50 FTIR, German). In brief, 1 mg of sample was mixed with 150 mg of potassium bromide and then tableted. The spectra were collected at a resolution of 4 cm 1 and a scanning time of 16 s in the range 4000 to 400 cm-1. The FTIR spectra were smoothed out and their baselines were corrected automatically by using Thermo Scientific software.

3.2.2 Experiment 2: Modification of Avocado and Jackfruit Seed Starch by Citric Acid Esterification

Calculated amount of citric acid (CA) was weighed and dissolved in water to obtained varying concentration of 5, 10 and 15% w/v solution. The solution was also adjusted the pH to 3.5 using 10 M NaOH. Accurately weighed 50 grams of ASS and JSS samples and put them in a 500 mL beaker. The 5, 10 and 15% citric solutions were subsequently added into samples and mixed thoroughly. The starch suspensions were poured onto a shallow aluminum tray and spread evenly. Sample was held for 16 hours at room temperature and subsequently heated for 6 hours at 60°C in a hot air oven (Memmert UF30, Germany). The sample was grounded into small particles and placed back into aluminum tray. Samples were dried for 2 hours at 130°C. The dried samples were re-wetted and washed with distilled water several times until the pH was neutral (pH 7.0). The neutral samples were filtered through No.1 Whatman filter paper using a vacuum pump (Rockker, Taiwan). The samples were again dried for 24 hours at 50°C. The resulting dry samples were grounded and sieved through a 100-mesh screen. The citric acid esterified samples were kept in HDPE zip lock plastic and store it at 10°C till used. Figure 3.1 shows the flow chart of citric acid esterification of avocado seed starch (ASS) and jackfruit seed starch (JSS).

3.2.2.1 Degree of Substitution (DS)

The amount of citric acid esterified to the starch was analyzed by the method described earlier by Srikaeo et al. (2019). Briefly, the accurate weight of 0.45 g sample was re-suspended with 2 mL of distilled water and 50 mL of 1 M KOH solution. The whole sample was heated for 10 min in a boiling water bath. After cooling, the mixture was neutralized to pH 8.5 using 5 M acetic acid. Subsequently, 25 mL of borate buffer (pH = 8.5) and 0.3 g of an indicator (a mixture of murexide and Na2SO4 at a ratio of 1:500) were added. The volume was adjusted to 300 mL using distilled water and titrated with 0.05 M CuSO4 until the red-violet color disappeared. The degree of starch esterification with the acid was expressed as the quantity of citric acid residues in 100 g sample.

3.2.2.2 Other quality measurement Samples of 5,10 and 10% citric acid esterified ASS and JSS were examined for moisture content, color, pasting characteristics by RVA, gel texture by texture analyzer, thermal properties by DSC, granule morphology by SEM, x-ray diffraction pattern by XRD, structure and chemical bonds by FTIR.

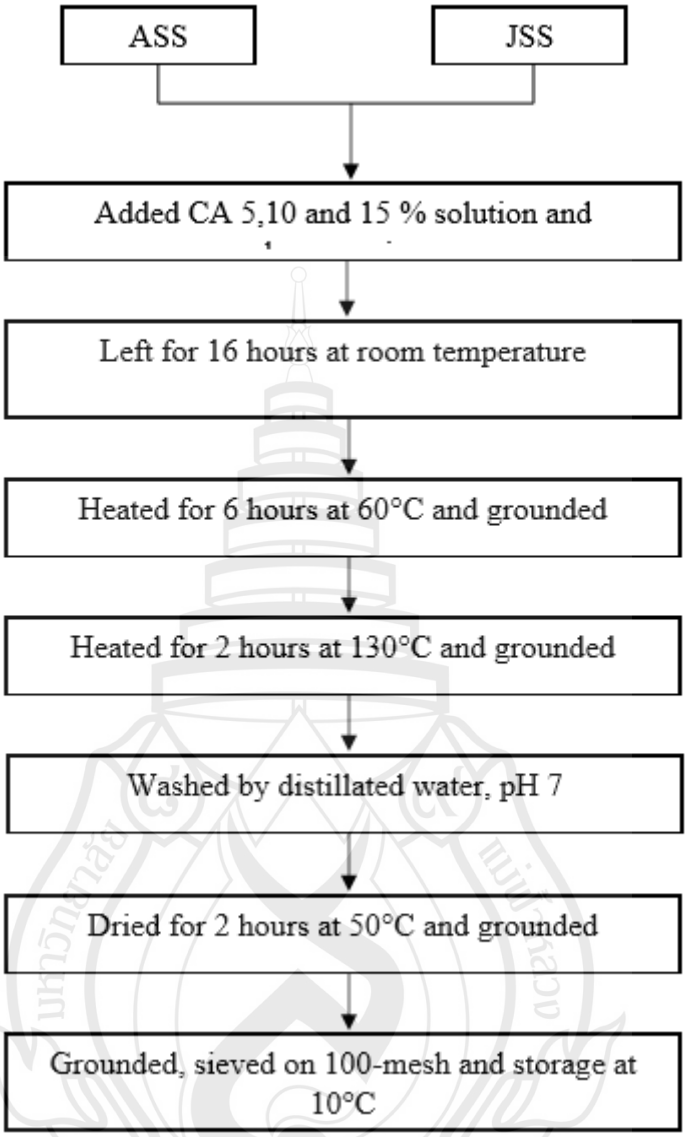


Figure 3.1 Modification of ASS and JSS by citric acid esterification

3.2.3 Experiment 3: Modification of ASS and JSS by Annealing (ANN), Annealing Combined with CA (ANN-CA), Heat Moisture Treatment (HMT) and Heat Moisture Treatment Combined with CA.

The modification of ASS and JSS samples was adopted from methods conducted by Duyen, 2020 with some modifications. According to Figure 8, for annealing, 50 grams of ASS and JSS starch was weighted into a 500 mL beaker and mixed with 100 mL distilled water containing 0.02% sodium azide as ani-microbial agent. Starch slurries were subsequently incubated at 45°C for 24 hours. For annealing

combined with CA treatment, the starch was mixed with 5% CA solution instead of water. All samples were incubated at 45°C for 24 hours, subsequently neutralized with 1 M sodium hydroxide to pH 7.0 and rinsed with distilled water. Samples were then centrifuged at 10,000 x g for 30 minutes to obtain the sediment. The samples were dried in hot air oven at 50°C for 24 hours to get a final moisture content less than 10%. For HMT, 50 grams of ASS and JSS starch was weighted and added with a calculated amount of water to obtain 25% moisture content. For HMT combining with CA treatment, the 5% CA solution was used instead of distilled water. Samples were fully equilibrated in refrigerator for 24 hours prior to transferring to glass test tubes, closed tightly with screw cap and put in a sealed plastic bag to retain their moisture content. Samples were autoclave at 110°C for 5 hours and subsequently neutralized with 1 M sodium hydroxide. The samples were then washed with distilled water and centrifuged at 10,000 x g for 30 minutes. The collected sediment was then dried in hot air oven at 50°C for 24 hours.

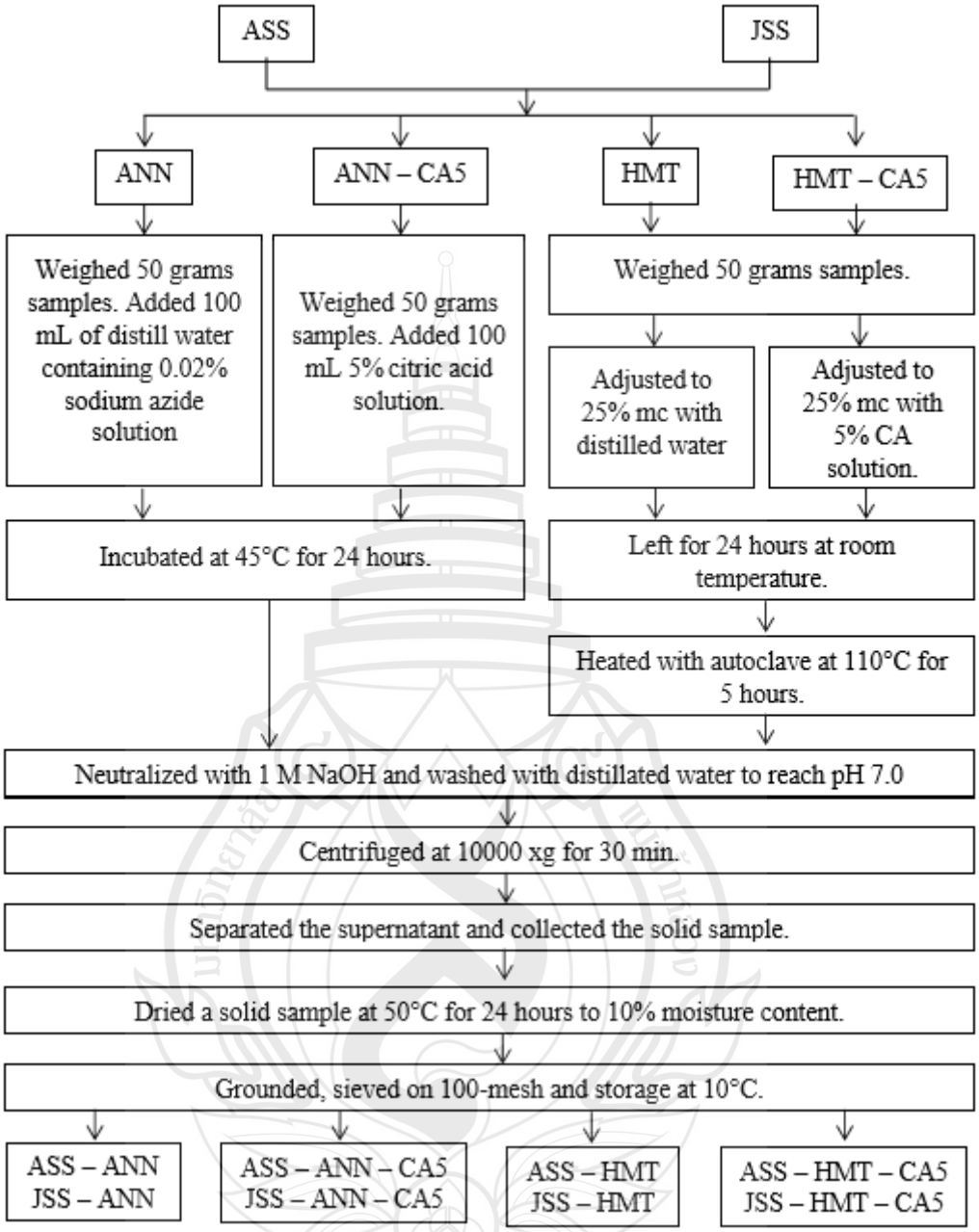


Figure 3.2 Modification of ASS and JSS by hydrothermal treatment in combining with citric acid esterification

3.2.4 Quality Measurement

Samples of ANN, ANN-CA, HMT and HMT-CA were examined for moisture content, color, pasting characteristics by RVA, gel texture by texture analyzer, thermal properties by DSC, granule morphology by SEM, x-ray diffraction pattern by XRD, structure and chemical bonds by FTIR and degree of substitution following the methods earlier described in section 3.2.2.

3.3 Statistical Analysis

Experimental data were statistical analyzed at a 95% confidence level ($p<0.05$). Significant differences among mean values were determined by Duncan's multiple range tests using IBM® SPSS® Statistics for Windows (Version 20.0, IBM Corporation, Armonk, NY, USA). All tests were performed at least in triplicate.

CHAPTER 4

RESULT AND DISCUSSION

The seeds of avocado and jackfruit were primarily prepared into flour by two different milling methods, dry and wet milling. The subsequent step involved the removing of protein and fat content from the flour samples to obtain the pure starch. Physicochemical properties in terms of food composition, amylose content, color, morphology and functional group were investigated. In addition, functional properties including pasting properties, thermal properties and crystallinity pattern were also examined and compared in experiment 1.

4.1 Experiment 1: Preparation of Flour and the Isolated Starch of Avocado and Jackfruit Seed

4.1.1 Proximate Analysis

Table 4.1 provides the results for the proximate analysis parameters of the flour and starch of avocado seeds and jackfruit seeds.

4.1.1.1 Moisture Content. The moisture content of all flour samples with dry and wet milling methods ranged between 5.50% to 9.87%. That of the starch counterparts ranged between 2.434.60%. The resulting moisture content of samples were from the drying process. Kayode et al. (2021) reported the safe moisture content which was lower than 10% for the stored dried products. The low values were also reported elsewhere de Dios-Avila et al. (2020) and Mukprasit and Sajjaanantakul (2011). Protein and fat are considered nutritive components in flour and starch-based products. In addition, ash content gave an expression of the mineral composition of the sample. Overall, dry milling process gave samples with higher proximate compositions ($p<0.05$) than the wet milling methods. The exception was for JWF which showed a greater fat content than JDF. This was possibly due to the effect of the different milling method to be discussed.

4.1.1.2 Protein and Ash Content. Generally, the dry milled samples (ADF and JDF) showed a greater protein and ash content than the wet milled samples (AWF and JWF). This was contributed to the incorporated seed coat which were blended into sample during dry milling. In wet milling, these impurities were partially removed during filtering and consequently the lower protein and ash content. Ash and protein content in the ADF samples were 1.02% and 5.56%, respectively. This result was lower than that reported by Mahawan, et al., (2015), which were 2.83% and 7.75%, respectively. After alkaline steeping process, ADS showed the lower ash (0.44%) and protein (0.37%) content ($p<0.05$). The ash and protein content in JDF samples were 1.84% and 9.29%, respectively. These were slightly differed from those reported by Kushwaha et al. (2021) in that ash content ranged between 2.55 - 3.30% while, protein content ranged from 12.15% to 15.05%. The starch counterpart, JDS showed low ash (1.26%) and protein content (0.42%), reflecting the effect of alkaline steeping in successive removing the ash and protein component from samples. The differences in ash and protein content in jackfruit seed flour and starch possibly was from the different variety and maturity level of jackfruit used in the study and this would probably be like in avocado fruit and its seeds.

4.1.1.3 Fat Content. For fat content, ADF showed the greater value than did AWF ($p<0.05$). On the contrary, JWF showed higher fat content (1.71%) than JDF (1.19%) ($p<0.05$). This was higher than the range (0.58-0.73%) reported by Kushwaha et al. (2021). In this study, the possible explanation for the higher fat content in JWF than JDF was attributed to the dissolubility of fat in water. During the wet milling process, flour slurry has been filtered several times and only water-soluble compositions has been removed and this was not included the fat. Therefore, when calculated the remaining fat content based on the total mass, % fat of the sample was apparently high ($p<0.05$). However, after the process of starch isolation, the fat content was apparently low in all starch samples ($p\geq0.05$).

4.1.1.4 Carbohydrate Content Carbohydrate content refers to starch and fiber in the sample. Flour samples in turn, showed the lower carbohydrate content than the corresponding starch in both dry and wet milled samples. In flour, carbohydrate content ranged between 78.67% to 88.17% with the highest content found in the JWF sample (88.17%). While the starch carbohydrate content ranges from 93.70% to 96.87%.

with the highest content found in the JWS sample. Based on the proximate compositions, dry milled flour, ADF and JDF with high protein, fat and minerals content could be potentially served as major food ingredient. However, other functional properties also needed to be considered.

4.1.2 Amylose Content

The amylose content of flour and starch can have significant effects on its functional properties, mainly gel forming and thickening properties. The values measured from avocado seed flour and starch were 47.93% and 48.66%, respectively and that of jackfruit seed flour and starch were 38.81% and 42.48%, respectively. Amylose content of avocado seeds starch was fall in range between 35.34% to 48.19% as earlier reported by Wang et al. (2022) and Cornelia and Christianti (2018). For jackfruit seeds flour, amylose content was like 39.23% as reported by Mukprasirt and Sajjaanantakul (2011). However, amylose content in jackfruit seed starch was higher than 24.40% as reported by Tongdang (2008). The apparent amylose content could be varied among seed sources, depending on variety, maturity, and growing conditions etc.

4.1.3 Color

Table 4.2 provides the measurement results for the color parameters of the flour and starch of avocado seeds and jackfruit seeds. Figure 4.1 shows the appearance of the ASS and JSS native flour and starch with dry and wet milling treatment. In samples of avocado seeds and jackfruit seeds, it was seen that there was an increase in the lightness value (L*) between flour and starch products. This happens both in the dry milling and wet milling processes. The L* value for starch is higher than for flour, which indicates that the impurities in the flour are removed during the starch isolation process. Apart from that, it can also be seen that the highest L* value in the two starch samples was found in the starch sample that went through the wet milling process.

Table 4.1 Proximate compositions of avocado seeds and jackfruit seeds flour and starch

Samples	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
Avocado seeds					
ADF	5.50 ± 0.13^{b}	1.02 ± 0.02^{a}	5.56 ± 0.00^{a}	0.63 ± 0.05^{a}	87.29 ± 0.01^{c}
AWF	9.87 ± 0.12^{a}	0.13 ± 0.01^{c}	2.41 ± 0.05^{b}	0.04 ± 0.01^{c}	87.56 ± 0.01^{d}
ADS	4.58 ± 0.29^{c}	0.44 ± 0.03^{b}	0.37 ± 0.05^{c}	0.40 ± 0.05^{b}	94.21 ± 0.01^{b}
AWS	3.76 ± 0.38^{d}	0.17 ± 0.05^{c}	0.16 ± 0.05^{d}	0.10 ± 0.01^{c}	95.81 ± 0.01^{a}
Jackfruit seeds					
JDF	9.01 ± 0.11^{a}	1.84 ± 0.08^{a}	9.29 ± 0.06^{a}	1.19 ± 0.02^{b}	78.67 ± 0.01^{d}
JWF	7.13 ± 0.25^{b}	0.28 ± 0.04^{c}	2.72 ± 0.05^{b}	1.71 ± 0.27^{a}	88.17 ± 0.01^{c}
JDS	4.60 ± 0.05^{c}	1.26 ± 0.02^{b}	0.42 ± 0.05^{c}	0.03 ± 0.01^{c}	93.70 ± 0.01^{b}
JWS	2.43 ± 0.13^{d}	0.33 ± 0.08^{c}	0.26 ± 0.01^{d}	0.10 ± 0.01^{c}	96.87 ± 0.01^{a}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

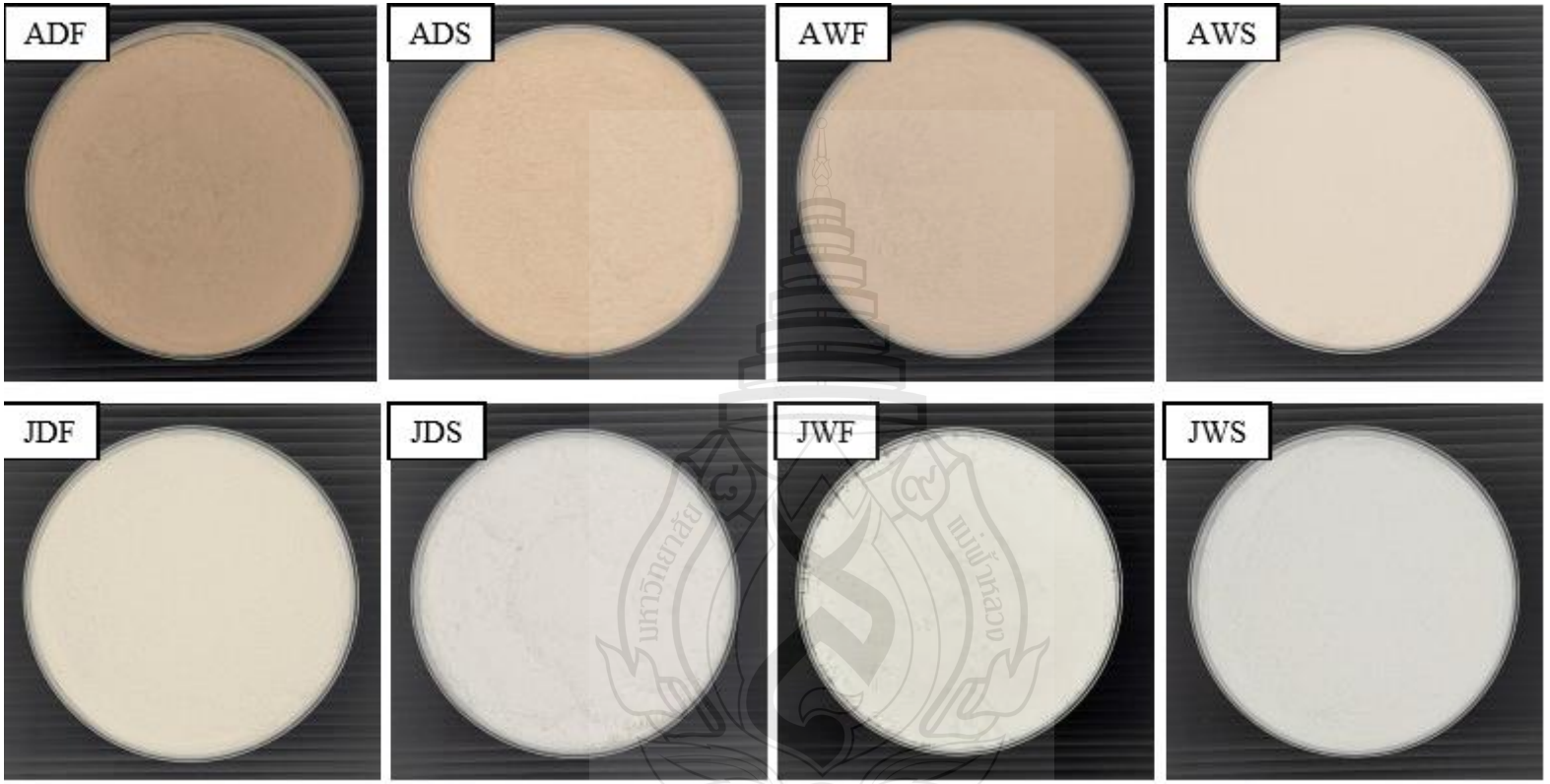


Figure 4.1 Appearance of avocado dry milled flour (ADF), avocado dry milled starch (ADS), avocado wet milled flour (AWF), avocado wet milled starch (AWS), jackfruit dry milled flour (JDF), jackfruit dry milled starch (JDS), jackfruit wet milled flour (JWF) and jackfruit wet milled starch (JWS).

The reason is that the wet milling process uses a lot of water, which allows many of the impurities contained in the flour to be wasted, thus affecting the L* value of the starch product.

Table 4.2 Color determination of avocado seeds and jackfruit seeds flour and starch

Sample	L*	a*	b*	C*	h
Avocado seeds					
ADF	78.50 ± 0.01^{d}	6.23 ± 0.01^{b}	15.14 ± 0.01^{b}	16.36 ± 0.01^{b}	67.62 ± 0.03^{c}
ADS	79.77 ± 0.06^{c}	7.42 ± 0.03^{a}	18.56 ± 0.03^{a}	19.99 ± 0.01^{a}	68.21 ± 0.01^{b}
AWF	85.61 ± 0.01^{b}	4.88 ± 0.04^{b}	11.09 ± 0.02^{c}	12.12 ± 0.04^{c}	66.23 ± 0.03^{d}
AWS	89.39 ± 0.03^{a}	3.56 ± 0.01^{a}	10.60 ± 0.01^{c}	11.18 ± 0.01^{d}	71.42 ± 0.02^{a}
Jackfruit seeds					
JDF	92.00 ± 0.02^{d}	1.03 ± 0.01^{a}	9.90 ± 0.01^{a}	9.95 ± 0.05^{a}	84.05 ± 0.01^{b}
JDS	94.90 ± 0.01^{c}	0.70 ± 0.05^{b}	4.42 ± 0.06^{c}	4.48 ± 0.01^{c}	80.95 ± 0.04^{d}
JWF	95.68 ± 0.01^{b}	0.19 ± 0.01^{d}	5.16 ± 0.01^{b}	5.17 ± 0.02^{b}	87.89 ± 0.04^{a}
JWS	97.02 ± 0.04^{a}	0.26 ± 0.01^{c}	1.91 ± 0.02^{d}	1.92 ± 0.01^{d}	82.23 ± 0.01^{c}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.1.4 Rapid Visco Analyzer

Table 4.3 shows RVA pasting properties of avocado and jackfruit seeds flour and starch, including Peak Viscosity (PV), Trough (T), Breakdown (B), Final Viscosity (FV), Set Back (SB), Peak Time (P) and Pasting Temperature (PT). There was a clear difference in pasting profiles between avocado and jackfruit seed samples. For avocado seed samples, flour samples (ADF and AWF) showed a lower viscosity ($p< 0.05$) than the starch counterparts (ADS and AWS). According to Kumar and Khatkar (2017), the viscosity curve represents the behavior of starch during heating and allows evaluation of the characteristics of the paste formed and the tendency for retrogradation during the cooling process. According to Debet and Gidley (2006) this was possibly due to the combination effect of protein and lipid onto amylose and restricted the starch granule swelling, resulting in the low paste viscosities. On heating and cooling, AS flour and starch showed a high T and consequently a very low BD, indicating high resistance to heat and shearing. On cooling, the swollen starch molecule rearranged to a more

ordered structure and gave raised FV. Pasting profiles also varied between samples with different milling methods. For avocado seeds samples, ADS showed the greatest paste viscosities (Table 4.3), indicating the potential use as gel forming agent in food products. The measured FV of ADS and AWS was 5160.50 and 4138.00 cP, respectively. These were slightly lower than that reported by Esquivel-Fajardo et al. (2022) which was around 6791 cP. Overall, paste viscosity of flour and starch varied among sources and milling methods. For jackfruit seeds samples, the typical curve with a high PV and low Trough, resulting in a high BD and the raised FV after cooling were observed. Jackfruit starch from both milling methods, JWS and JDS showed the lower paste viscosities than the flour counterparts. JWF and JDF showed PV of 1870.00 and 1736.50 cP, respectively. The possible explanation of the low protein and fat content samples in giving low paste viscosities might be due to the restricted swelling and more resistance to heat and shearing during heating and shearing. For jackfruit seeds, JWF showed the greatest FV (2581.00 cP) and could be used as thickening and gel forming agent in food application. The overall paste viscosities were considerable high compared to that reported by Mukprasirt and Sujjaananntakul (2011) in which PV, FV, B and SB values were 783.96, 697.92, 301.32 and 222.36 cP, respectively. Paste viscosities of seeds starches varied among samples and preparation methods.

Table 4.3 Pasting properties of flour and starch from avocado and jackfruit seeds

Samples	Peak Viscosity (cP)	Trough (cP)	Breakdown (cP)	Final Viscosity (cP)	Set Back (cP)	Peak Time (min)	Pasting Temperature (°C)
Avocado seeds							
ADF	702.50 ± 13.44^{d}	653.50 ± 12.02^{d}	49.00 ± 1.41^{d}	1886.50 ± 21.92^{d}	1233.00 ± 9.90^{d}	5.73 ± 0.00^{c}	89.70 ± 0.00^{a}
AWF	1659.50 ± 12.02^{c}	1567.50 ± 4.95^{b}	92.00 ± 7.07^{b}	2959.50 ± 48.79^{c}	1392.00 ± 43.84^{c}	6.04 ± 0.05^{b}	87.20 ± 0.00^{b}
ADS	2428.50 ± 20.51^{a}	1438.50 ± 27.58^{c}	68.50 ± 0.71^{c}	5160.50 ± 7.78^{a}	2800.50 ± 12.02^{a}	7.00 ± 0.00^{a}	85.15 ± 0.49^{d}
AWS	2079.50 ± 33.23^{b}	1913.00 ± 39.60^{a}	166.50 ± 6.36^{a}	4138.00 ± 149.91^{b}	2225.00 ± 110.31^{b}	7.00 ± 0.00^{a}	86.43 ± 0.04^{c}
Jackfruit seeds							
JDF	1736.50 ± 0.71^{c}	1386.00 ± 12.73^{b}	350.50 ± 13.44^{b}	2338.00 ± 16.97^{b}	952.00 ± 29.70^{b}	5.34 ± 0.09^{a}	88.45 ± 0.49^{a}
JWF	1870.00 ± 9.90^{a}	1462.00 ± 14.14^{a}	408.00 ± 4.24^{a}	2581.00 ± 36.77^{a}	1119.00 ± 50.91^{a}	4.97 ± 0.05^{d}	85.68 ± 0.04^{c}
JDS	1438.50 ± 27.58^{c}	1136.00 ± 57.98^{c}	302.50 ± 30.41^{c}	1948.00 ± 19.80^{c}	812.00 ± 38.18^{c}	5.27 ± 0.00^{b}	88.03 ± 1.17^{a}
JWS	1724.00 ± 7.07^{b}	1373.00 ± 4.24^{b}	350.50 ± 2.12^{b}	2320.50 ± 13.44^{b}	947.50 ± 9.19^{b}	5.04 ± 0.05^{c}	86.85 ± 0.57^{b}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.1.5 Texture Profile Analysis

Overall, gel hardness of avocado seed samples was greater ($p< 0.05$) than that of jackfruit seed samples. The flour and starch counterparts of the two seed samples obtained from wet milling process provided the greater gel hardness but lower adhesiveness than the dry milling process ($p< 0.05$). The differences in gel texture could be related to the RVA paste viscosities regarding the starch type and milling methods. The greater gel hardness has been attributed to the higher final viscosities (FV) as shown in Table 4.4. The difference in gel texture between flour and starch counterparts were also contributed from the protein and fat content in flour which was excluded from the starch. In term of springiness, that of flour and starch obtained from wet milling process have higher values compared to that from dry milling process. From the test results on springiness parameters, it shows that the springiness of flour and starch of avocado seeds and jackfruit seeds using dry and wet methods resulted no significant difference ($p<0.05$). It means that the use of dry and wet milling treatment did not affect the springiness properties of avocado seed flour and starches. Factors that influence springiness parameters are carbohydrate, amylose, and amylopectin content in the sample (Wulandari et al., 2019). The cohesiveness of avocado seed flour and starch using dry and wet methods showed no significant difference ($p<0.05$), while avocado seed flour and starch, jackfruit seed flour, and starch samples showed significant differences ($p<0.05$). This shows that the use of dry and wet milling methods affected the cohesiveness parameters of jackfruit seed flour and starch but not on the avocado seed flour and starch. From the data presented, it can also be seen that jackfruit seed flour and starch have higher cohesiveness values than avocado seed flour and starch. The factor that influences the cohesiveness of flour and starch is the protein content contained in the sample (Damodarand & Paraf, 1997). The protein contained in flour can make connective tissue more compact. According to Chandra and Shamasundar (2015), elasticity is closely related to the parameters of hardness, compactness, and flexibility.

Table 4.4 Texture properties of flour and starch from avocado and jackfruit seeds

Sample	Hardness g	Adhesiveness g.sec	Springiness %	Cohesiveness %	Chewiness
Avocado seeds					
ADF	142.76 ± 34.67^{d}	459.19 ± 24.72^{d}	0.527 ± 0.03^{d}	0.333 ± 0.03^{b}	24.45 ± 5.62^{d}
AWF	253.79 ± 18.25^{b}	412.41 ± 51.40^{c}	0.576 ± 0.03^{c}	0.346 ± 0.03^{a}	50.88 ± 9.81^{b}
ADS	239.76 ± 10.13^{c}	331.91 ± 18.52^{b}	0.601 ± 0.02^{b}	0.326 ± 0.01^{c}	47.16 ± 5.10^{c}
AWS	281.48 ± 19.01^{a}	202.62 ± 17.12^{a}	0.608 ± 0.01^{a}	0.321 ± 0.01^{d}	54.99 ± 5.74^{a}
Jackfruit seeds					
JDF	676.58 ± 49.24^{c}	144.18 ± 37.68^{c}	0.809 ± 0.01^{c}	0.554 ± 0.02^{c}	304.07 ± 39.25^{c}
JWF	1351.61 ± 166.14^{a}	59.86 ± 18.74^{a}	0.947 ± 0.01^{a}	0.790 ± 0.05^{a}	892.65 ± 17.93^{a}
JDS	547.59 ± 17.34^{d}	298.09 ± 26.75^{d}	0.748 ± 0.07^{d}	0.371 ± 0.08^{d}	110.96 ± 34.31^{d}
JWS	1219.58 ± 154.35^{b}	87.89 ± 61.78^{b}	0.835 ± 0.04^{b}	0.664 ± 0.02^{b}	587.23 ± 22.81^{b}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.1.6 Differential Scanning Colorimetry

Table 4.5 summarizes the thermal properties of avocado and jackfruit seeds flour and starch from dry and wet milling methods. Overall, flour samples had higher ($p<0.05$) gelatinization temperatures at onset (To), peak (Tp) and final (Te) and gelatinization endotherm than the starch counterparts. The exception was for JWF which showed lower ($p<0.05$) gelatinization temperatures than JWS. Considering the milling methods, the dry milled samples tended to show greater gelatinization temperature and endotherm than the wet milled samples.

Table 4.5 Thermal properties of flour and starch from avocado and jackfruit seeds

Samples	T_o (°C)	T_p (°C)	T_e (°C)	Entalphy (mJ/g db)
Avocado seeds				
ADF	73.85 ± 0.43^{a}	77.84 ± 0.34^{a}	82.21 ± 0.09^{a}	1914.11 ± 262.98^{b}
AWF	72.37 ± 0.01^{b}	75.83 ± 0.01^{b}	80.36 ± 0.24^{b}	3308.46 ± 334.73^{a}
ADS	71.05 ± 0.03^{d}	75.33 ± 0.01^{c}	79.87 ± 0.15^{c}	1911.47 ± 91.61^{b}
AWS	71.60 ± 0.01^{c}	75.17 ± 0.01^{c}	78.64 ± 0.01^{d}	1698.65 ± 10.00^{b}
Jackfruit seeds				
JDF	81.42 ± 0.19^{a}	85.50 ± 0.01^{a}	89.60 ± 0.08^{b}	2309.34 ± 104.85^{d}
JWF	79.80 ± 0.01^{d}	83.33 ± 0.01^{a}	87.60 ± 0.01^{d}	3271.27 ± 10.00^{b}
JDS	80.66 ± 0.06^{b}	85.17 ± 0.01^{a}	89.84 ± 0.17^{a}	2966.47 ± 44.93^{c}
JWS	80.23 ± 0.03^{c}	83.83 ± 0.01^{a}	88.93 ± 0.13^{c}	4091.25 ± 27.63^{a}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

According to de Dios-Avila et al. (2022) and Pelissari et al. (2012) these might be contributed to the non-starch components, fat, protein and fiber that were molten at higher temperature. For avocado seeds samples, ADF showed the highest gelatinization temperature between 73.85-82.21°C while AWS showed the lowest gelatinization temperature between 71.60-78.64°C. The measured values in this study were slightly higher than the gelatinization temperature range between 65.26 to 77.48°C as reported by Rivera-Gonzalez et al. (2019) and de Dios-Avila et al. (2022). Similar observation

was found in jackfruit seeds samples as JDF sample gave the highest gelatinization temperatures of 81.42- 89.60°C. However, JWF showed the lowest gelatinization temperature between 79.80- 87.60°C. These were found higher than that reported by Koy et al. (2022) and Mukprasirt and Sajjaanantakul (2011) since the gelatinization temperature were between 63.43 to 86.61°C. Gelatinization properties may vary depending on various factors including botanical source, amylose to amylopectin ratio, type crystallinity, and heating conditions.

4.1.7 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) images of avocado and jackfruit seed flour and starch from dry and wet milling methods are shown in Figure 4.2. Avocado seed starch granules were oval in shape with a smooth surface while jackfruit seeds were round-half in shape. These conformed to those earlier reported by dee Dios-Avila et al., (2022) and Tongdang (2008) in that there were some impurities adhered on the granule surface of both seed flour. The presence of impurities was observed in dry milled samples (ADF and JDF) than the wet milled samples (AWF and JWF). Results indicated that wet milling could partially remove such impurities from the original flour. The foreign particles were observed less in dry milled starch (ADS and JDS), suggesting that they were successively removed by alkaline steeping process. For AWF and AWS, clean surface granules were observed. In conclusion, wet milling process followed by alkaline steeping could give the distinct starch granules and free from any impurities from the two seeds. Based on the analysis of granule size distribution, the granule size of avocado seed starch ranged from 14.61 to 25.04 μm with an average size of 21.41 μm. From previous research, it was reported that the size of starch granules in avocado seed starch varied in range 17.8-37.0 μm (dos Santos et al., 2016; Silva et al., 2017; Bet et al., 2017; Macena et al., 2020). In jackfruit seed starch, the granule size ranged from 6.54 to 10.58 μm with an average size of 8.29 μm which were in the ranged between 6 – 12 μm as reported earlier by Naknaen (2014), Wang et al. (2021), Dutta et al. (2011) and Lubis et al. (2016).

Due to differences in starch origins and milling methods, the flour and starch derived from avocado and jackfruit seeds exhibit distinct characteristics that find practical applications in food processing. To obtain starch with high purity, the wet milling process followed by alkaline steeping were recommended for both fruit seeds.

However, some properties of avocado and jackfruit seed starch need enhancement for better performance in food applications, especially their ability to withstand temperature changes and mechanical stress during processing.

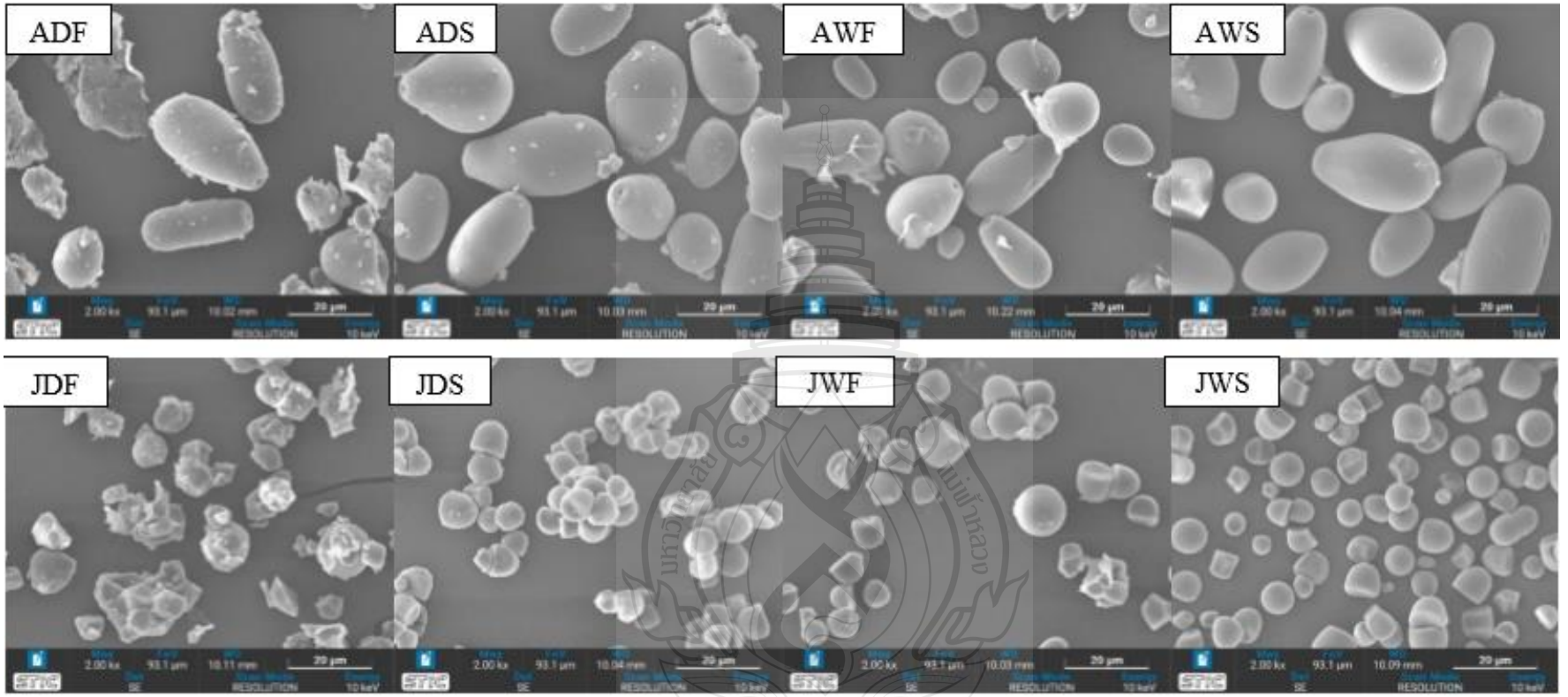


Figure 4.2 SEM images of flour and starch from avocado dry milled flour (ADF), avocado dry milled starch (ADS), avocado wet milled flour (AWF), avocado wet milled starch (AWS), jackfruit dry milled flour (JDF), jackfruit dry milled starch (JDS), jackfruit wet milled flour (JWF) and jackfruit wet milled starch (JWS)

4.2 Experiment 2: Modification of Avocado and Jackfruit Seed Starch by Citric Acid Esterification

The native form of two seed starch, ASS and JSS were subjected to the chemical modifications using citric acid esterification. Three different concentrations of citric acid, 5, 10 and 15% were applied onto the two starches following the method described in session 3.2.2. The citric acid esterified starch samples were then examined for physicochemical properties in terms of moisture content, color, morphology, and degree of substitution, XRD crystallinity patterns and microstructure under SEM. Furthermore, functional properties including RVA pasting properties, DSC thermal properties and gel texture were examined and compared among treatments.

4.2.1 Moisture Content

From the data presented in Table 4.6, moisture contents of the citric acid esterified samples were lower ($p<0.05$) than that of unmodified samples. For ASS-CA, the moisture content ranged between 3.42-3.88% which was about two folds lower than that of control ASS (6.08%). Similar results were observed in JSS-CA in which the moisture content was found in lower range 0.45-0.66% compared to that of control JSS (3.12%). The moisture content of cross-linked modified starch was partially reduced due to cross-linking in the starch molecules after citric acid esterification. The crosslinking caused the water difficult to diffuse inside the starch molecules (Rahim et al., 2021). The moisture content of modified starch is greatly influenced by process conditions, especially during drying at high temperature (Faridah & Thonthowi, 2020). Among samples esterified with varying concentration of CA, 5, 10 and 15%, some differences in moisture content were observed.

Table 4.6 Moisture content of avocado and jackfruit seed starch after citric acid esterification

Sample code	Treatments	Moisture Content (%)
Avocado seeds		
ASS – Control	Untreated avocado seed starch	6.08 ± 0.27[a]
ASS – CA5	Avocado seed starch with 5% citric acid	3.42 ± 0.17[c]
ASS – CA10	Avocado seed starch with 10% citric acid	3.72 ± 0.08[b]
ASS – CA15	Avocado seed starch with 15% citric acid	3.88 ± 0.04[b]
Jackfruit seeds		
JSS – Control	Untreated jackfruit seed starch	3.12 ± 0.10[a]
JSS – CA5	Jackfruit seed starch with 5% citric acid	0.66 ± 0.03[b]
JSS – CA10	Jackfruit seed starch with 10% citric acid	0.55 ± 0.01[b]
JSS – CA15	Jackfruit seed starch with 15% citric acid	0.45 ± 0.01[c]

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.2.2 Color

Color of starch samples were shown in Table 4.7. Results indicated that L*, b*, C and h color values increased ($p<0.05$) after citric acid treatment in both ASS-CA and JSSCA starch. The exception was for a*, indicative of redness decreased ($p<0.05$). The significant differences ($p<0.05$) in color values among samples esterified with varying CA concentration, 5, 10 and 15% were also observed. Change in color was possibly due to the effect of citric acid which hydrolyzed and partially removed the remaining impurities in the ASS and JSS, such as proteins, residual phenolic compounds and lipids, resulting in the brighter samples with an increased L* value (Alimi & Workneh, 2018). Figure 4.3 shows the appearance of the untreated and CA treated ASS and JSS between native starch and starch modified with citric acid treatment. The slight change in visual color after CA treatment was the brighter shade of color after. The colors of ASS and JSS all samples were shown in Table 3.6. The L* value for all ASS samples increased from 81.96 to 85.44 while, that of JSS increased from 96.79 to 97.61. The lightness of

all starch citrate samples (ASSCA5, ASS-CA10 and ASS-CA15) increased with increasing concentration of CA while that of JSS-CA samples (JSS-CA5, JSS-CA10 and JSS-CA15) increased slightly as compared to the control sample. This increase in whiteness was possibly due to the multiple washing steps that carried on during citric acid treatment, resulting in the less colorants retained in starch (Mukprasirt & Sajjaanantakul, 2004).

After CA treatment, the positive values of b* indicative of yellowish, increased whereas the positive a* value indicative of reddish decreased in both seed starches. Meanwhile, the value of chroma, indicative of color saturation was found in range of 15.72-16.11 for untreated and CA esterified ASS samples and in range of 2.54-3.84 for untreated and CA esterified JSS samples. The hue angle (h) in all untreated and CA esterified ASS were found in the range 66.19-73.33° while that of JSS were in range 78.22- 83.85°. These located in the red to yellow color shade (0 - 90°) (Kortei & Akonor, 2015). Overall, the observed color values of samples after CA esterification conformed to the visual color shown in Figure 4.3.

Table 4.7 Color determination of ASS and JSS after citric acid esterification.

Sample	L*	a*	b*	C*	h
Avocado seeds					
ASS-Control	81.96 ± 0.04^{d}	6.42 ± 0.01^{a}	14.54 ± 0.04^{d}	15.89 ± 0.03^{b}	66.19 ± 0.05^{d}
ASS-CA5	84.87 ± 0.07^{c}	4.70 ± 0.03^{c}	15.17 ± 0.01^{b}	15.88 ± 0.01^{c}	72.80 ± 0.10^{c}
ASS-CA10	85.01 ± 0.02^{b}	4.72 ± 0.03^{b}	15.41 ± 0.09^{a}	16.11 ± 0.10^{a}	72.98 ± 0.06^{b}
ASS-CA15	85.44 ± 0.01^{a}	4.51 ± 0.01^{d}	15.06 ± 0.01^{d}	15.72 ± 0.01^{d}	73.33 ± 0.04^{a}
Jackfruit seeds					
JSS Control	96.79 ± 0.05^{d}	0.52 ± 0.01^{a}	2.49 ± 0.02^{d}	2.54 ± 0.02^{d}	78.22 ± 0.14^{d}
JSS-CA5	97.61 ± 0.16^{b}	0.43 ± 0.02^{b}	3.39 ± 0.10^{c}	3.41 ± 0.10^{c}	82.72 ± 0.12^{c}
JSS-CA10	97.48 ± 0.43^{a}	0.43 ± 0.01^{c}	3.65 ± 0.06^{b}	3.67 ± 0.06^{b}	83.34 ± 0.04^{b}
JSS-CA15	97.59 ± 0.01^{c}	0.41 ± 0.01^{d}	3.82 ± 0.03^{a}	3.84 ± 0.03^{a}	83.85 ± 0.10^{a}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

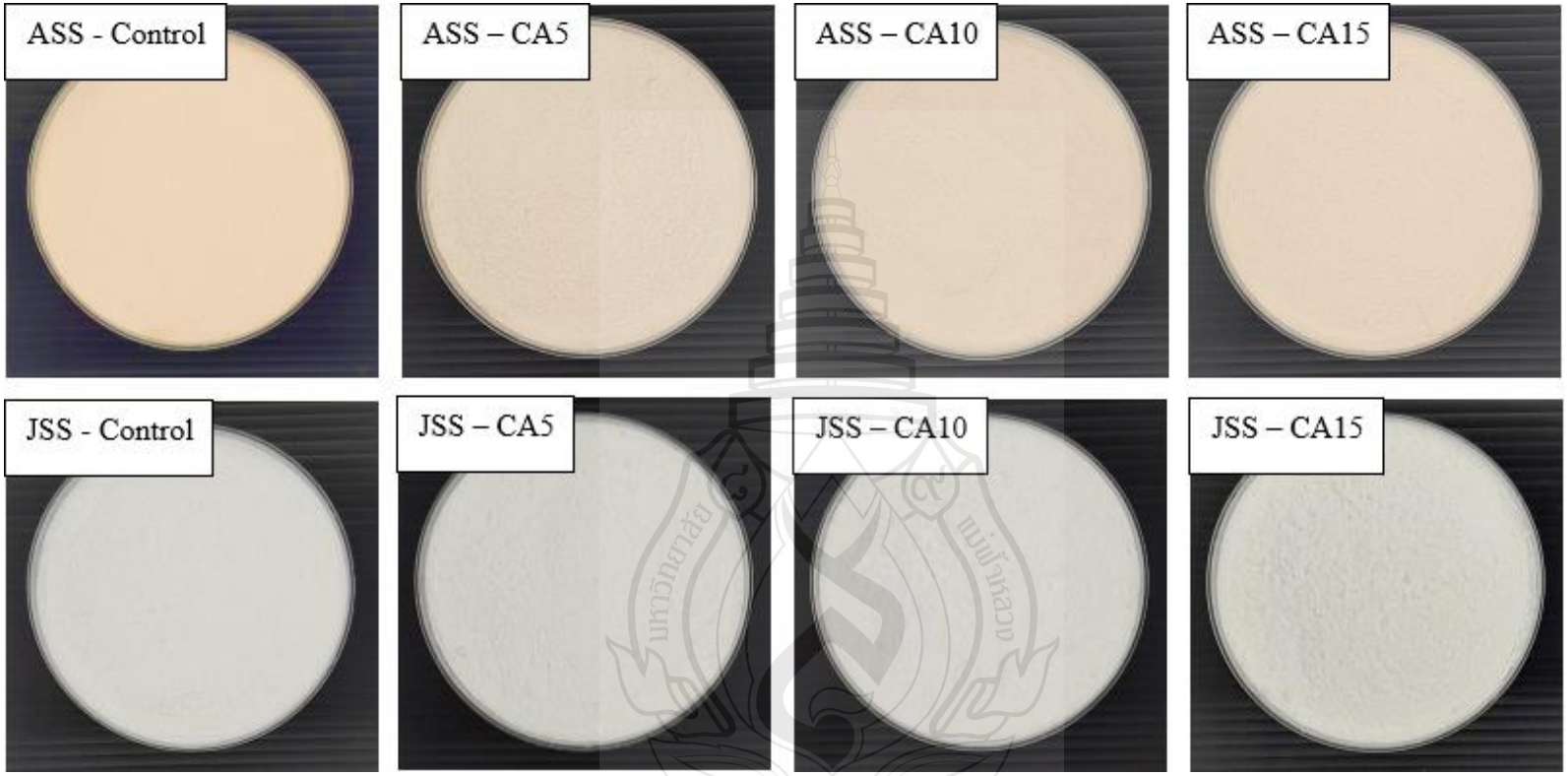


Figure 4.3 Appearance of starch from avocado seed starch-control (ASS-Control), avocado seed starch – citric acid 5% (ASSCA5), avocado seed starch – citric acid 10% (ASS-CA10), avocado seed starch – citric acid 15% (ASS-CA15), jackfruit seed starch-control (JSS-Control), jackfruit seed starch – citric acid 5% (JSS-CA5), jackfruit seed starch – citric acid 10% (JSSCA10) and jackfruit seed starch – citric acid 15% (JSS-CA15).

4.2.3 Rapid Visco Analyzer

The RVA pasting properties of native and CA esterified starch were presented in Table 4.8. Overall, paste viscosities of all ASS-CA samples significantly altered ($p<0.05$) from that of the native ASS starch. The suppressed paste viscosities of starch after CA esterification in ASS-CA5, ASS-CA10, ASS-CA15 and JSS-CA5 have been attributed to the cross-linking between anhydride group of CA and carboxyl group of starch molecules.

Overall, paste viscosities of native avocado seed starch (ASS-control) changed apparently after CA treatments. ASS-Control showed typical curve in that the peak viscosity was observed at high temperature of 89^{o}C indicative of the restricted swelling of starch granule during cooking. Trough viscosity was not decreased due to heating and shearing, resulting in the low Breakdown viscosity. Based on cooling, the raised in Final viscosity (2908.67 cP) about two folds of Peak viscosity (1471.33 cP) was observed ($p<0.05$), contributing to the high Setback value (1651.67 cP). The observed pasting curve indicated the high resistance to cooking and shearing and the good gel forming properties of the untreated ASS sample. After CA treatment, the different pasting behaviors based on heating and cooling cycle were observed among ASS treated with different concentration of CA that were 5% (ASS-CA5), 10% (ASS-CA10) and 15% (ASS-CA15). For ASS-CA5, the raised pasting curve was observed. The most increase in Final viscosity (4036.00 cP) and Setback (2261.67 cP) indicated a great improvement in ability of gel formation. For ASSCA10, a greater Peak viscosity (2097.67 cP), but the comparable Trough (1584.67 cP) and Final viscosity (2833.67 cP) to that of native starch was observed. According to Golachowski et al., (2020), changes of starch properties occurring during esterification lead to a reduced relative crystallinity, resulting in a decrease in the affinity for water, the gelatinization parameters, and the viscosity of starch citrate. The increase in paste viscosity in this study could be associated to the combined effect between the more restricted swelling and the higher resistance to heating and shearing of the swollen starch granules and therefore, resulting in a various pasting pattern. A clear suppressed pasting curve was observed in ASS-CA15 as the lowest Peak (913.00 cP), Trough (788.67 cP), Final (1147.67 cP), and Setback viscosity (359.00 cP) was observed. Starch citrate with 15%

CA did not give a stable solid gel or paste after cooling. This could be considered a limitation in some industrial applications.

Native jackfruit seed starch (JSS-Control) showed a high Peak viscosity (1789.67 cP), high Trough (1536.33 cP), and high Breakdown viscosity (253.33 cP) compared to native ASS, indicating the less heating and shearing resistance. The low Final viscosity (2382.33 cP) and low Setback value (846.00 cP) indicated the less gel forming ability than ASS-Control. In contrast to avocado seed starch, jackfruit seed starch treated with citric acid concentrations of 5% (JSS-CA5), 10% (JSS-CA10) and 15% (JSS-CA15) showed the suppressed overall pasting curves compared to the native JSS. The corresponding pasting properties showed the apparently lower Peak, Trough, Breakdown, Final and Setback viscosity ($p<0.05$). With the higher CA concentrations, the lower paste viscosities were observed. Comparing to native JSS, the apparent reduction in paste viscosities ($p<0.05$) observed in JSS-CA5 were Peak viscosity from 1789.67 cP to 270.50 cP, Trough viscosity from 1536.33 cP to 264.00 cP, Breakdown value from 253.33 cP to 6.50 cP, Final viscosity from 2382.33 to 390.00 cP, and Setback value from 846.00 cP to 126.00 cP. The time to reach peak viscosity was slightly changed from 6.90 min to 5.87min. An apparent loss in paste viscosities as resulted from CA treatment were found in JSS treated with 10% and 15% CA. The pasting temperature of these two samples, JSS-CA10 and JSS-CA15 samples was not detected.

The loss of overall paste viscosities observed in JSS-CA10 and JSS-CA15 was attributed to starch hydrolysis by CA. In general, after starch-CA esterification at low temperature 60°C for 6 h. The excess CA has been washed out and assured by the tested pH of 7.00. The esterified samples were then subjected to dry heat treatment at 130 oC, aiming to intensify the esterification process. The decreased paste viscosities indicated that all JSS-CA samples did not swell and gelatinize during heating based on RVA. The previous studies also reported a decrease in pasting properties in lentil and banana starch (Remya et al., 2018), corn starch (Xie & Liu, 2004), and sweet potato starch (Babu et al., 2015). In addition, the decrease in viscosity of acid-modified starches was attributed to the considerable breakdown of amorphous regions. Changes in the glycoside linkages of amylopectin resulted from acid treatment, possibly causing a decrease in the final viscosity of the modified starches (Han et al., 2002).

4.2.4 Texture Profile Analysis

Table 4.9 shows texture parameters based on Texture Profile Analysis of starch before and after CA treatment. A measurable solid gel was obtained only from starch esterified with 5% CA, ASS-CA5 and JSS-CA5. There was a substantial decrease ($p<0.05$) in the hardness, springiness, cohesiveness and chewiness of both ASS-CA5 and JSS-CA5, compared to the native starch counterparts. A decrease in the hardness was probably resulted from the limited swelling capability of starch granules which also limited the gel formation after heating and cooling. In this study, the springiness of ASS-CA5 and JSSCA decreased compared with both native starches. The springiness is indicated by the elasticity of samples which indicated by the ability to return to their original height after the compression force was removed (Hedayati and Niakousari et al., 2018; Hoseney & Smewing, 1999). On the other hand, the cohesiveness of ASS-CA5 slightly increased, but that of JSS-CA5 decreased compared with their native starch. The cohesiveness is indicated by the ability of the sample to withstand deformations. The cohesive gel was associated with the leaching of amylose chains out of the granules during heating and their subsequent reassociation after cooling of the CA treated starch. The chewiness of ASS-CA5 and JSSCA5 decreased compared to their corresponding native starches. The chewiness shows the amount of energy required for mastication of semi-solid foods (Farahnaky et al., 2013; Phimolsiripol et al., 2011). Overall, CA treated starch gave the soft solid gel which could be considered a limitation on some food applications.

Starch samples modified with 10% and 15% citric acid, ASS-CA10, ASS-CA15, JSS-CA10, and JSS-CA15, did not exhibit the formation of a solid gel. Consequently, these samples were not applicable for this texture measurement. The lack of gel formation in this experiment could be an unintended result, which subsequently restricts the practical use of these starches in specific applications.

Table 4.8 Pasting properties of ASS and JSS after citric acid esterification

Sample	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Set Back (cP)	Peak Time (min)	Pasting Temperature (°C)
Avocado seeds							
ASS-Control	1471.33 ± 8.08^{c}	1257.00 ± 12.49^{c}	214.33 ± 4.62^{c}	2908.67 ± 18.01^{b}	1651.67 ± 14.36^{b}	7.00 ± 0.00^{a}	89.37 ± 0.49^{a}
ASS-CA5	1975.00 ± 40.73^{b}	1774.33 ± 29.14^{a}	200.67 ± 22.14^{b}	4036.00 ± 61.02^{a}	2261.67 ± 41.30^{a}	6.18 ± 0.10^{b}	85.83 ± 0.54^{b}
ASS-CA10	2097.67 ± 40.28^{a}	1584.67 ± 55.90^{b}	506.00 ± 15.62^{a}	2833.67 ± 39.40^{c}	1249.00 ± 24.56^{c}	5.60 ± 0.12^{c}	82.58 ± 0.58^{d}
ASS-CA15	913.00 ± 3.61^{d}	788.67 ± 6.11^{d}	124.33 ± 8.62^{d}	1147.67 ± 5.86^{d}	359.00 ± 11.53^{d}	5.76 ± 0.10^{d}	84.53 ± 0.51^{c}
Jackfruit seeds							
JSS Control	1789.67 ± 16.86^{a}	1536.33 ± 30.53^{a}	253.33 ± 20.55^{a}	2382.33 ± 13.01^{a}	846.00 ± 38.74^{a}	5.33 ± 0.00^{d}	87.80 ± 0.52^{a}
JSS-CA5	270.50 ± 2.12^{b}	264.00 ± 2.83^{b}	6.50 ± 0.71^{b}	390.00 ± 4.24^{b}	126.00 ± 1.41^{b}	6.90 ± 0.14^{a}	87.20 ± 0.00^{b}
JSS-CA10	46.00 ± 1.73^{c}	44.00 ± 1.73^{c}	2.00 ± 0.00^{c}	66.67 ± 1.15^{c}	22.67 ± 0.58^{c}	5.96 ± 0.08^{c}	N.D
JSS-CA15	21.00 ± 0.00^{d}	19.00 ± 0.00^{d}	2.00 ± 0.00^{c}	26.33 ± 0.58^{d}	7.33 ± 0.58^{d}	5.87 ± 0.63^{b}	N.D

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

Table 4.9 Texture properties of ASS and JSS after citric acid esterification.

Sample	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness
	g	g.sec	%	%	
Avocado seed					
ASS-Control	177.64 ± 2.71[a]	190.43 ± 4.49[b]	0.62 ± 0.02[a]	0.33 ± 0.01[b]	0.36 ± 0.01[a]
ASS-CA5	84.34 ± 8.06[b]	46.55 ± 0.87[a]	0.57 ± 0.16[b]	0.41 ± 0.11[a]	0.20 ± 0.09[b]
ASS-CA10	N/A	N/A	N/A	N/A	N/A
ASS-CA15	N/A	N/A	N/A	N/A	N/A
Jackfruit seed					
JSS-Control	773.71 ± 134.72[b]	111.97 ± 10.93[b]	0.92 ± 0.02[a]	0.82 ± 0.01[a]	5.89 ± 1.04[a]
JSS-CA5	446.64 ± 612.85[a]	107.49 ± 5.12[a]	0.58 ± 0.08[b]	0.39 ± 0.57[b]	1.61 ±1.83[b]
JSS-CA10	N/A	N/A	N/A	N/A	N/A
JSS-CA15	N/A	N/A	N/A	N/A	N/A

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.2.5 Differential Scanning Colorimeter

DSC of all starch samples are presented in Figure 4.4. The gelatinization parameters are shown in Table 4.10. Results showed that both seed starch samples combined with citric acid (ASS-CA and JSS-CA) lowered the initial temperature (To), peak temperature (Tp), and final temperature (Te) of the untreated starch counterparts. With the increase in CA concentration from 5, 10 and 15%, the effects were more apparent ($p<0.05$). The findings suggest that JSS starch citrates displayed a reduced heat resistance, resulting in their melting at lower temperatures, in contrast to JSS, ASS samples with CA concentrations of 10% and 15% exhibited non-detection values for initial temperature (To), peak temperature (Tp), and final temperature (Te). In addition, the apparent decrease in gelatinization enthalpy values ($p<0.05$) of all CA treated samples were also observed. The findings of this study correspond with the conclusions drawn in prior research conducted by Van Hung and Morita (2005), indicating that the observed outcomes are probably due to the significant pattern of substitution inside the amorphous region. The process of starch gelatinization is widely recognized to occur when starches are heated in water. The gelatinization caused by temperature was a result of the disruption of the organized crystalline area, leading to a disordered amorphous state. The chemical groups that interact with starch can also severely disrupt the surface starch granules, particularly in the amorphous phase. This allows water to readily enter the starch granules, leading to a decrease in gelatinization peak and enthalpies (Zhu et al., 2021).

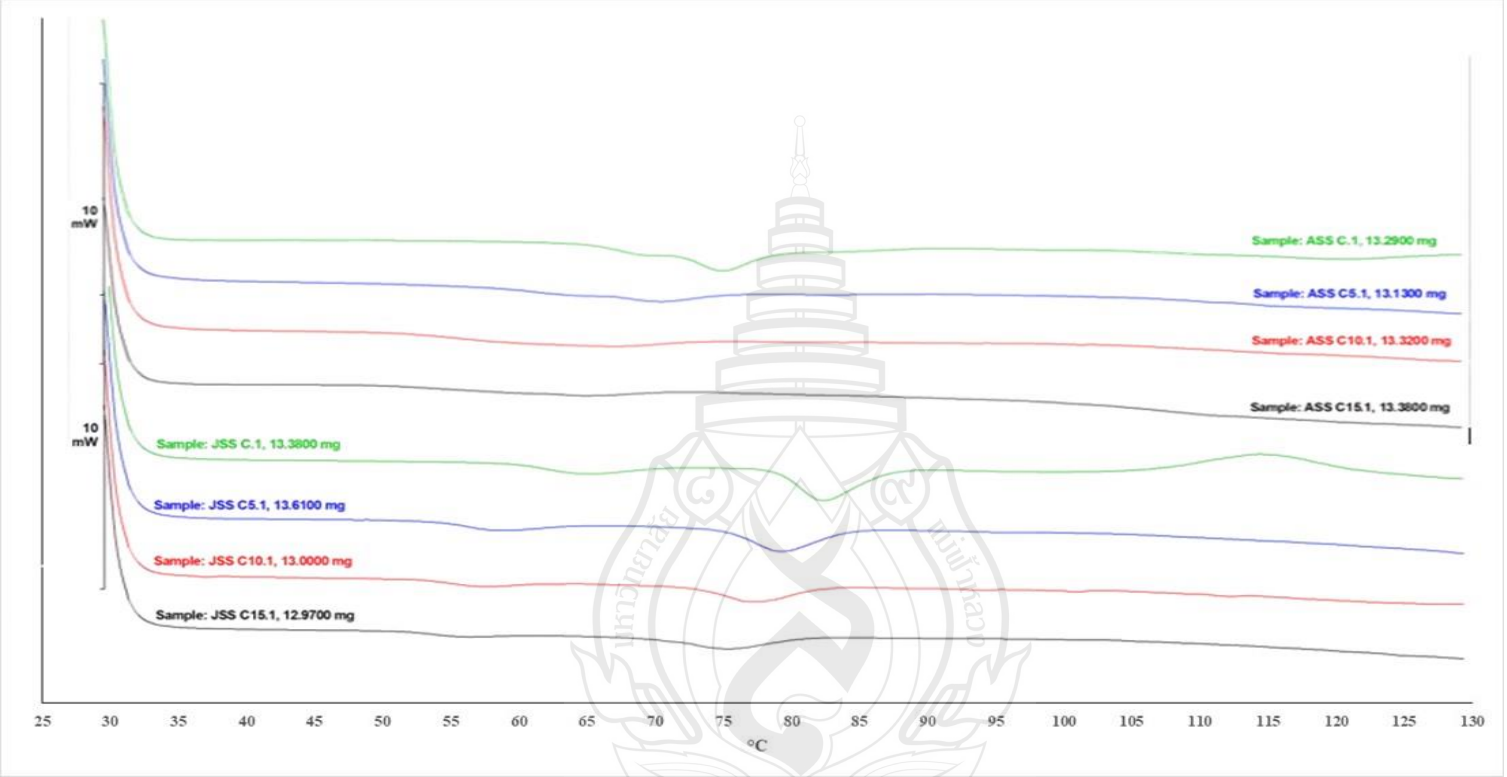


Figure 4.4 DSC of starch from avocado seed starch-control (ASS-Control), avocado seed starch – citric acid 5% (ASS-CA5), avocado seed starch – citric acid 10% (ASS-CA10), avocado seed starch – citric acid 15% (ASS-CA15), jackfruit seed starch control (JSS-Control), jackfruit seed starch – citric acid 5% (JSS-CA5), jackfruit seed starch – citric acid 10% (JSS-CA10) and jackfruit seed starch – citric acid 15% (JSS-CA15)

Table 4.10 Thermal properties of ASS and JSS after citric acid esterification

Sample	*To* (°C)	*Tp* (°C)	*Te* (°C)	Range *Te* - *To*	Enthalpy (mJ)	Enthalpy (mJ/g db)
Avocado seeds						
ASS-Control	70.86 ± 0.02^{a}	75.50 ± 0.00^{a}	80.36 ± 0.05^{a}	9.50 ± 0.03^{d}	47.18 ± 0.18^{a}	3793.83 ± 28.40^{a}
ASS-CA5	63.46 ± 0.19^{b}	70.83 ± 0.00^{b}	76.81 ± 0.29^{b}	13.35 ± 0.48^{c}	25.33 ± 2.01^{b}	1978.93 ± 139.95^{b}
ASS-CA10	N.D	N.D	N.D	N.D	N.D	N.D
ASS-CA15	N.D	N.D	N.D	N.D	N.D	N.D
Jackfruit seeds						
JSS-Control	79.51 ± 0.00^{a}	82.92 ± 0.09^{a}	87.63 ± 0.02^{a}	8.11 ± 0.02^{d}	31.46 ± 0.66^{a}	2416.43 ± 61.43^{a}
JSS-CA5	75.60 ± 0.03^{b}	79.83 ± 0.00^{b}	84.62 ± 0.15^{b}	9.02 ± 0.12^{c}	26.64 ± 0.69^{b}	1973.56 ± 47.13^{b}
JSS-CA10	73.03 ± 0.23^{c}	77.83 ± 0.00^{c}	82.99 ± 0.05^{c}	9.97 ± 0.29^{b}	19.98 ± 1.04^{c}	1516.10 ± 51.12^{c}
JSS-CA15	70.31 ± 0.37^{d}	75.92 ± 0.09^{d}	81.03 ± 0.29^{d}	10.73 ± 0.08^{a}	15.25 ± 1.31^{d}	1162.23 ± 119.56^{d}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.2.6 Scanning Electron Microscopy

Figure 4.5 shows the SEM images of all starch samples. Originally, the untreated ASS was oval in shape while, that of JSS was semi-circular. The untreated samples showed an intact and smooth granule surface without cracks or damages. After CA esterification, a slight change in granule morphology as the presence of the small breakages and damaged granules were observed. This was more apparent in starch treated with higher %CA. The occurrence of crack formation on the surface of starch treated with citric acid was observed in wheat starch (Li et al. ,2019), cassava, potato, sweet potato, lentil and banana starches (Remya et al., 2018). Alterations on granule surface morphology of the CA treated starch was probably caused by acid-hydrolysis by citric acid when it first introduced to starch suspension, although the starch particles retain their size and shape after high temperature treatment (Remya et al., 2018).

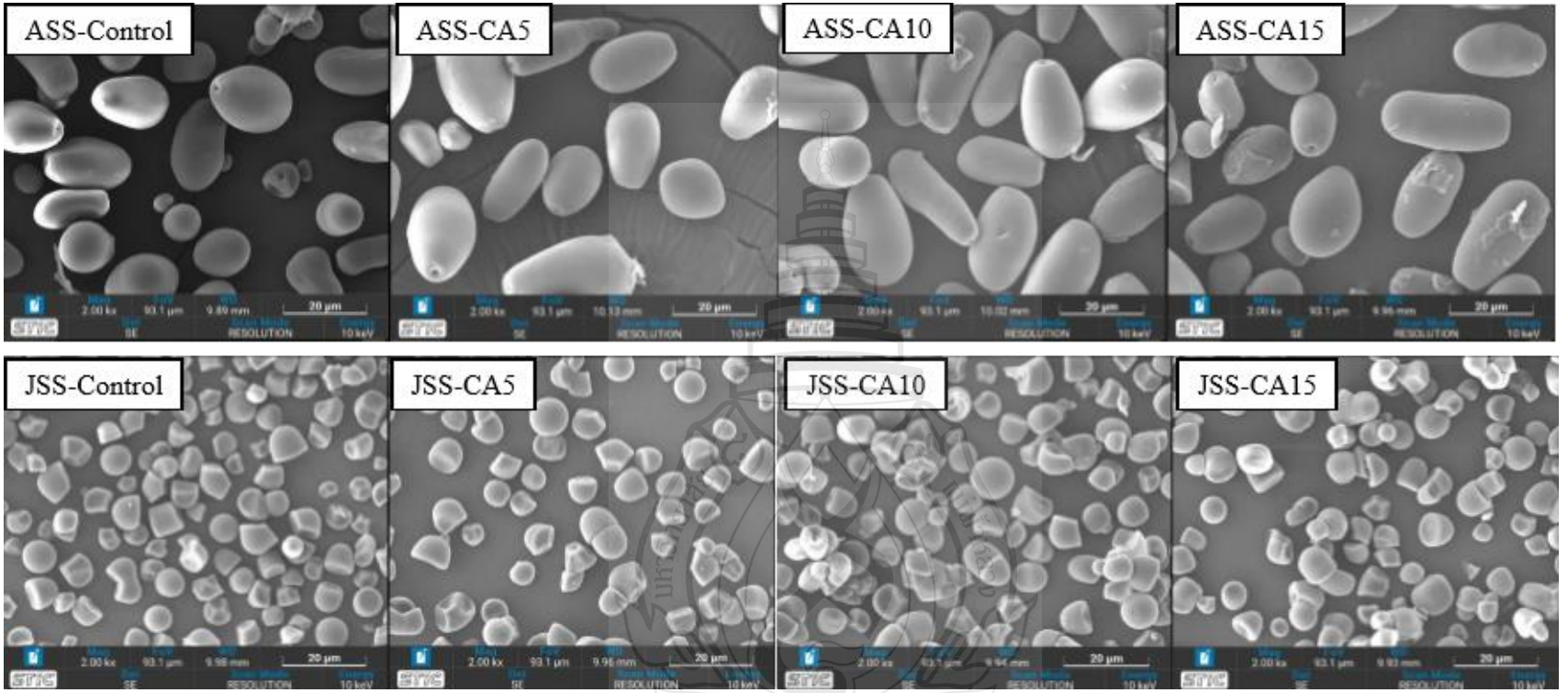


Figure 4.5 Scanning electron microscopy (x1000) of starch from avocado seed starch-control (ASS-Control), avocado seed starch – citric acid 5% (ASS-CA5), avocado seed starch – citric acid 10% (ASS-CA10), avocado seed starch – citric acid 15% (ASS-CA15), jackfruit seed starch-control (JSS-Control), jackfruit seed starch – citric acid 5% (JSS-CA5), jackfruit seed starch – citric acid 10% (JSS-CA10) and jackfruit seed starch – citric acid 15% (JSS-CA15)

4.2.7 X-Ray Diffraction

Figure 4.6 show the X-ray diffractogram of untreated and CA esterified starch of ASS and JSS, respectively. The diffraction peaks of ASS samples were observed at diffraction angles of 15°, 17°, 19.5° and 23°. These indicated the typical characteristics of the B-type pattern. Comparing to the untreated ASS, all ASS-CA did not give a distinctive pattern. The citric acid treatment to the starch did not change the crystal pattern of the avocado seed starch. The crystallization type of the original starch did not change after esterification, indicating that the esterification reaction occurred in the amorphous region of the starch (Zuo et al., 2013). The same XRD pattern was reported by Li et al. (2019) in the wheat starch and Remya et al., (2018) in the banana and lentil starch. However, based on the Remya et al. (2018), the crystallinity was showed increases after modifications. This could be explained on the basis of difference in branching patterns of amylopectin in these starches, which play a key role in the determination of the type of unit packing and X-ray diffraction pattern. The diffraction peaks of JSS samples were observed at diffraction angles of 15°, 17°, 18° and 23°. These indicated the typical characteristics of the A-type pattern. XRD diffractogram showed no different pattern from JSS native and JSSCA which indicated that citric acid treatment did not give an effect for all JSS samples. The previous study was reported by Dutta et al. (2011) shows the similar result for the JSS sample. The XRD pattern did not change in the starch with different acid–alcohol modification treatments. However, the calculation of the percentage of crystallinity JSS-CA showed a decrease value compared with that of native JSS.

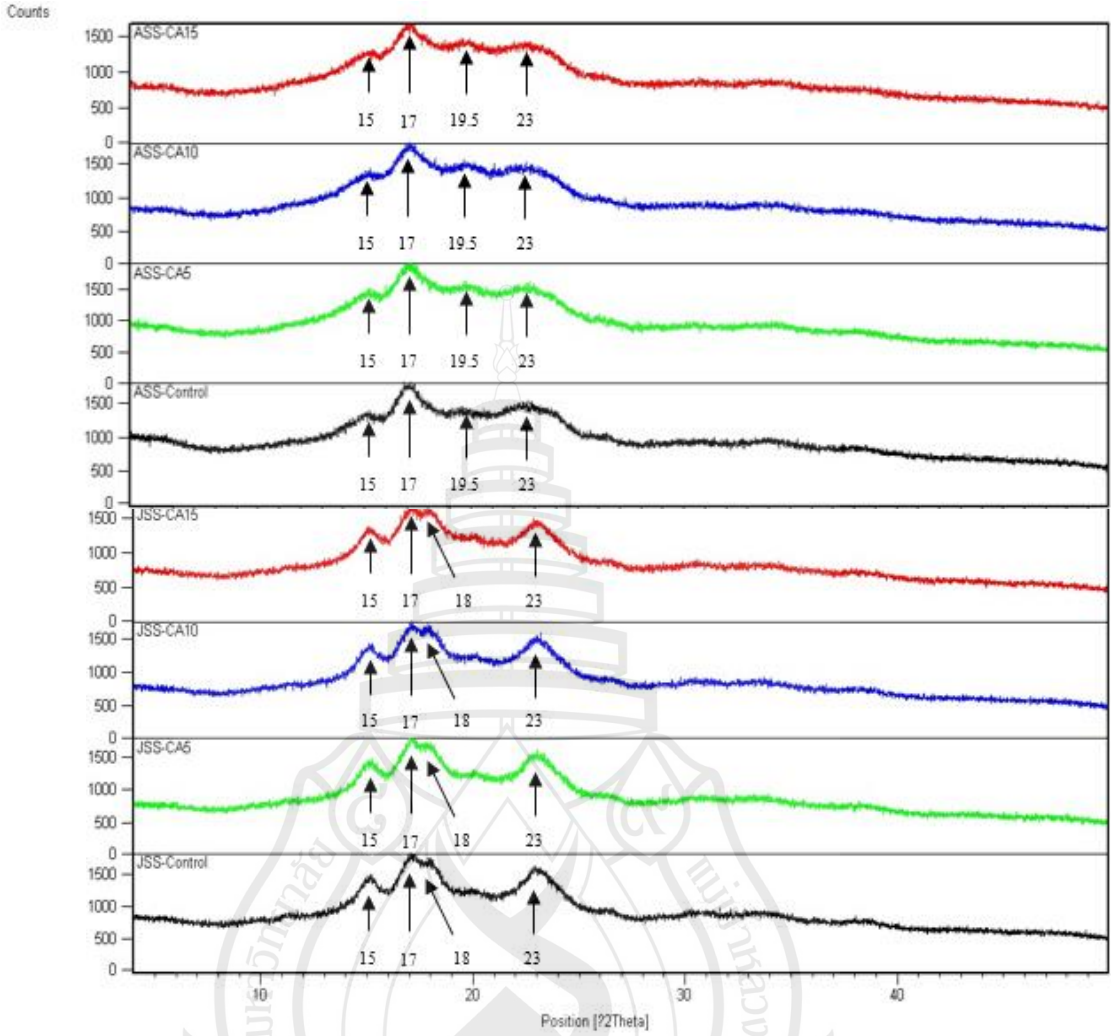


Figure 4.6 XRD of starch from avocado seed starch-control (ASS-Control), avocado seed starch – citric acid 5% (ASS-CA5), avocado seed starch – citric acid 10% (ASSCA10), avocado seed starch – citric acid 15% (ASS-CA15), jackfruit seed starch-control (JSS-Control), jackfruit seed starch – citric acid 5% (JSS-CA5), jackfruit seed starch – citric acid 10% (JSS-CA10) and jackfruit seed starch – citric acid 15% (JSS-CA15)

The percentage of crystallinity of ASS and JSS after citric acid treatment was shown in Table 4.11. The relative crystallinity was increased in all ASS-CA sample (14.27 - 14.56%) compared to that of native ASS (13.89%). Similar results were reported by Remya et al (2018) for lentil and banana starch where the relative crystallinity showed an increase after modification. Overall, the crystallinity of all ASS-CA samples significantly altered ($p<0.05$) from that of the native ASS starch. The

percentage of crystallinity was increased significantly in starch granules with the reduction in amylose concentration. Acid hydrolysis can reduce the amylose content, as the hydrolysis process facilitated by acid is more prone to cleaving amylose molecules compared to amylopectin molecules (Atichokudomchaia et al., 2000).

Table 4.11 Percentage of relative crystallinity of ASS and JSS after citric acid esterification.

Sample	%
Avocado seeds	
ASS-Control	13.89 ± 0.67^{d}
ASS-CA5	14.44 ± 0.63^{b}
ASS-CA10	14.27 ± 0.62^{c}
ASS-CA15	14.56 ± 0.67^{a}
Jackfruit seeds	
JSS-Control	25.38 ± 0.81^{a}
JSS-CA5	22.67 ± 0.35^{b}
JSS-CA10	20.46 ± 0.75^{bc}
JSS-CA15	22.19 ± 0.10^{d}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

In contrast to ASS samples, % crystallinity of JSS-CA (20.46-22.67%) significantly decreased ($p<0.05$) as compared to native JSS (25.38%). A study of JSS samples with acid modification was reported by Naknaen et al. (2014) with the similar results. The % crystallinity of JSS samples decreased during increasing CA concentration. This feature suggests that some changes may have occurred in the crystalline region of the starch. The decrease in the degree of crystallinity may be caused by the influence of the amorphous component of starch granules during esterification. This happens because the esterification process usually occurs in the amorphous area of starch (Naknaen et al., 2014).

4.2.8 Fourier Transform Infra-Red

FTIR spectra of all ASS samples with citric acid under different concentrations are shown in Figure 4.7. The spectrum pattern of the native and citrate starches gave several peaks between wavelength 4,000 and 1,000 cm-1. The FTIR spectrum of the native ASS and ASS-CA samples showed the typical peak of starch between 1,081.13 – 1,081.56 cm1 (the C-O carbohydrate bond), 1,162.02 – 1,163.34 cm-1 (the C-O-C polysaccharide bond), 1,373.32 – 1,374.81 cm-1 (the C-H and CH2 aliphatic bending group), 1,647.76 – 1,648.04 cm-1 (the C=O amide I band), 2,929.44 – 2,929.96 cm-1 (the C-H and CH2 aliphatic stretching group) and broadband around 3,404.82 – 3,420.68 cm-1 (the O-H carbohydrates proteins and polyphenols). In ASS-CA samples, the peak at 1,700 cm-1 indicative of the ester bonds was observed. In parallel, FTIR spectrums of both untreated and CA esterified JSS samples were shown in Figure 4.9. The typical bands reflecting the common chemical bonding in starch molecules were observed at 1,081.13 – 1,081.25 cm-1 (the C-O carbohydrate bond), 1,155.31 – 1,155.37 cm-1 (the C-O-C polysaccharide bond), 1,370.18 – 1,370.44 cm-1 (the C-H and CH2 aliphatic bending group), 1,639.77 – 1,643.80 cm-1 (the C=O amide I band), 2,931.69 – 2,932.29 cm-1 (the C-H and CH2 aliphatic stretching group) and broadband at 3,385.41 – 3,405.01 cm-1 (the O-H carbohydrates proteins and polyphenols), respectively. For JSS-CA samples, the peaks indicative of ester bonding was observed at 1,731.01 – 1,733.02 cm-1 which were similar to that of all ASS-CA samples. The current results were agreed to that reported in CA-esterified samples from corn starch (Gerezgiher & Szabo, 2022), tapioca starch (Utomo et al., 2020), and cassava starch (Remya et al.,2018).

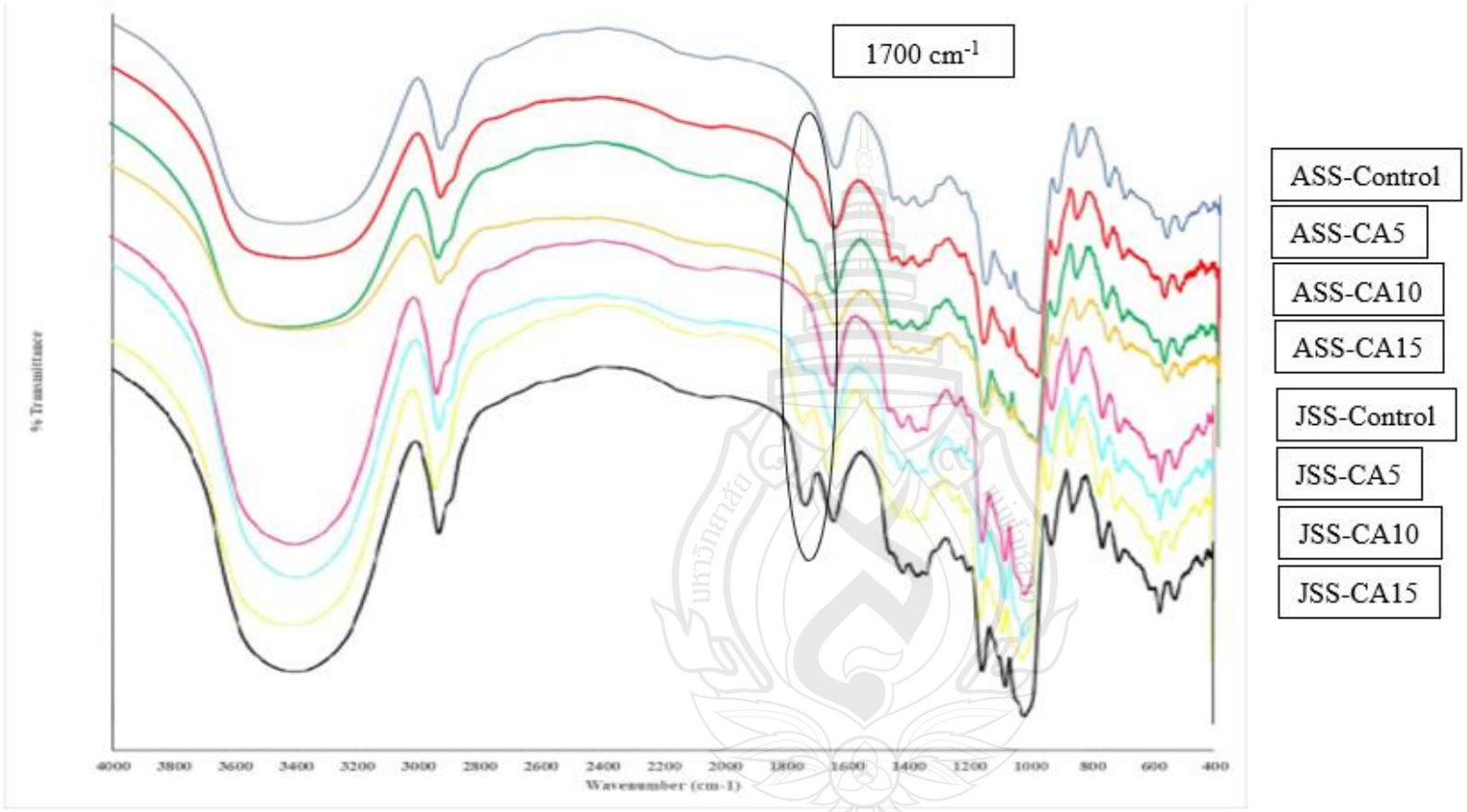


Figure 4.7 FTIR spectrums of starch from avocado seed starch-control (ASS-Control), avocado seed starch – citric acid 5% (ASS-CA5), avocado seed starch – citric acid 10% (ASS-CA10), avocado seed starch – citric acid 15% (ASS-CA15), jackfruit seed starch-control (JSS-Control), jackfruit seed starch – citric acid 5% (JSS-CA5), jackfruit seed starch – citric acid 10% (JSS-CA10) and jackfruit seed starch – citric acid 15% (JSS-CA15).

4.2.9 Degree of Substitution

The degree of substitution (DS) of CA treated ASS and JSS samples were shown in Table 4.12. DS of ASS-CA and JSS-CA increased apparently ($p<0.05$) with the increasing concentration of CA. The range of DS observed in ASS-CA samples were 0.011- 0.025 g/100g dry sample. Similar observations were found in JSS-CA samples but in a slightly higher value between 0.015 -0.046 g/100g dry sample. DS of the citrate starch was affected significantly by the concentration of citric acid. The increase in DS was caused by many hydroxyl groups of starch which reacts with anhydride compound. DS of citrated starch was significantly influenced by citric acid concentration. The increase in DS is caused by the large number of starch hydroxyl groups reacting with anhydride compounds formed due to the dehydration process when the temperature increases (Utomo et al., 2020; Wing, 1996).

Table 4.12 Degree of substitution of ASS and JSS after – citrate modification

Sample	DS (g/100g dry sample)
Avocado seed starch	
ASS-Control	-
ASS-CA5	0.01 ± 0.01^{c}
ASS-CA10	0.02 ± 0.01^{b}
ASS-CA15	0.03 ± 0.01^{a}
Jackfruit seed starch	
JSS-Control	-
JSS-CA5	0.03 ± 0.01^{c}
JSS-CA10	0.04 ± 0.01^{b}
JSS-CA15	0.05 ± 0.01^{a}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

Overall, there were differences in physical and functional properties among ASS and JSS esterified with 5, 10 and 15% citric acid. Citric acid treatment at 10 and 15% CA gave the low viscous paste. The stable solid gel after cooling could not be obtained from these treated starch samples. High concentration of CA, 10 and 15% also caused a great destruction in starch granules as reflected by the lower melting temperature and enthalpy. Overall, citric acid treatments at 10 and 15% did not give a positive effect on modifying starch properties. Therefore, 5% CA treatment was the only concentration which applicable for ASS and JSS samples. The use of low citric acid concentration was also minimized the chemicals use in the starch modification. In addition, the incorporation of other modification methods could also enhance or improve starch properties to the required level.

4.3 Experiment 3: Modification of Avocado and Jackfruit Seed Starch by Hydrothermal Treatment and Incorporation with Citric Acid Esterification

Annealing (ANN) and heat moisture treatment (HMT) were physical modification which applied onto the avocado (ASS) and jackfruit (JSS) seed starch. Chemical modification by 5% citric acid (CA) was also combined with either ANN and HMT to enhance the alteration on starch properties as resulted from a combination between physical and chemical methods. Samples that had undergone these modification techniques were then evaluated for their properties. The evaluation on physicochemical properties included moisture content, color, resistant starch content, degree of substitution, the microstructure by SEM, and crystallinity pattern by XRD. Additionally, the assessment on functional characteristics of these modified starch samples were pasting properties by RVA, thermal properties by DSC, and gel texture by texture analyzed.

4.3.1 Moisture Content

Table 4.13 presents the moisture content (MC) of all native and modified ASS and JSS samples. Results showed a slight difference in ($p<0.05$) among samples. The resulting MC between 4.18-5.64% was observed in modified ASS samples. Those of modified JSS were found between 3.78-5.26% for modified JSS samples. The low moisture content in all samples corresponded to the drying process following treatments.

Table 4.13 Moisture content of ASS and JSS modified by ANN, HMT and incorporation of CA

Samples	Treatment	Moisture Content (%)
Avocado seeds		
ASS – Control	Untreated avocado seed starch	5.70 ± 0.30^{a}
ASS – ANN	Avocado seed starch with annealing	4.60 ± 0.21^{b}
ASS – ANN – CA5	Avocado seed starch with annealing + 5% citric acid	4.18 ± 0.18^{c}
ASS – HMT	Avocado seed starch with heat moisture treatment	5.64 ± 0.09^{a}
ASS – HMT – CA5	Avocado seed starch with heat moisture treatment + 5% citric acid	5.45 ± 0.14^{a}
Jackfruit seeds		
JSS – Control	Untreated jackfruit seed starch	4.54 ± 0.17^{b}
JSS – ANN	Jackfruit seed starch with annealing	3.86 ± 0.16^{c}
JSS – ANN – CA5	Jackfruit seed starch with annealing + 5% citric acid	3.78 ± 0.30^{c}
JSS – HMT	Jackfruit seed starch with heat moisture treatment	5.26 ± 0.07^{a}
JSS – HMT – CA5	Jackfruit seed starch with heat moisture treatment + 5% citric acid	5.14 ± 0.16^{a}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.3.2 Color

Figure 4.8 shows the color appearance of ASS and JSS samples after ANN, HMT and a combined treatments with 5% CA. The corresponding color values of samples were shown in Table 4.14. The measured L*, a*, b*, C*, and h values were significantly altered ($p<0.05$) from that of the native ASS and JSS starch. For native ASS, the observed pale-yellow color was reflected by a lower L* but greater a* and b* than those found in native JSS which showed the bright white color. After ANN, L* slightly increased in both ASS and JSS samples. With the combined effect of CA esterification, L* increased to the greatest value. During ANN, samples had been soaked in warm water for some period before rewashing and drying. These soaking and washing processes possibly removed the colorants presented in starch samples. With the addition of 5% citric acid, a brighter color was observed. In contrast with the L* and h values, after ANN with and without CA, the a* b* and C values decreased. After HMT, similar results in apparent reduction of L* but increase in h value were clearly observed in the two starch. Upon HMT alone, there was no washing process to remove any colorants. In addition, the effect of high temperature heating could possibly enhance the dark color. In HMT combined with CA treatment, the addition citric acid slightly brightened the color of starch. The a* and b* values decreased in ASS samples but increased in JSS samples. The positive value of a* and b* indicated that ASS and JSS sample was reddish and yellowish in color. The observed C values between 0 - 90° indicated that sample were in between red to yellow shade (Kortei & Akonor, 2015).

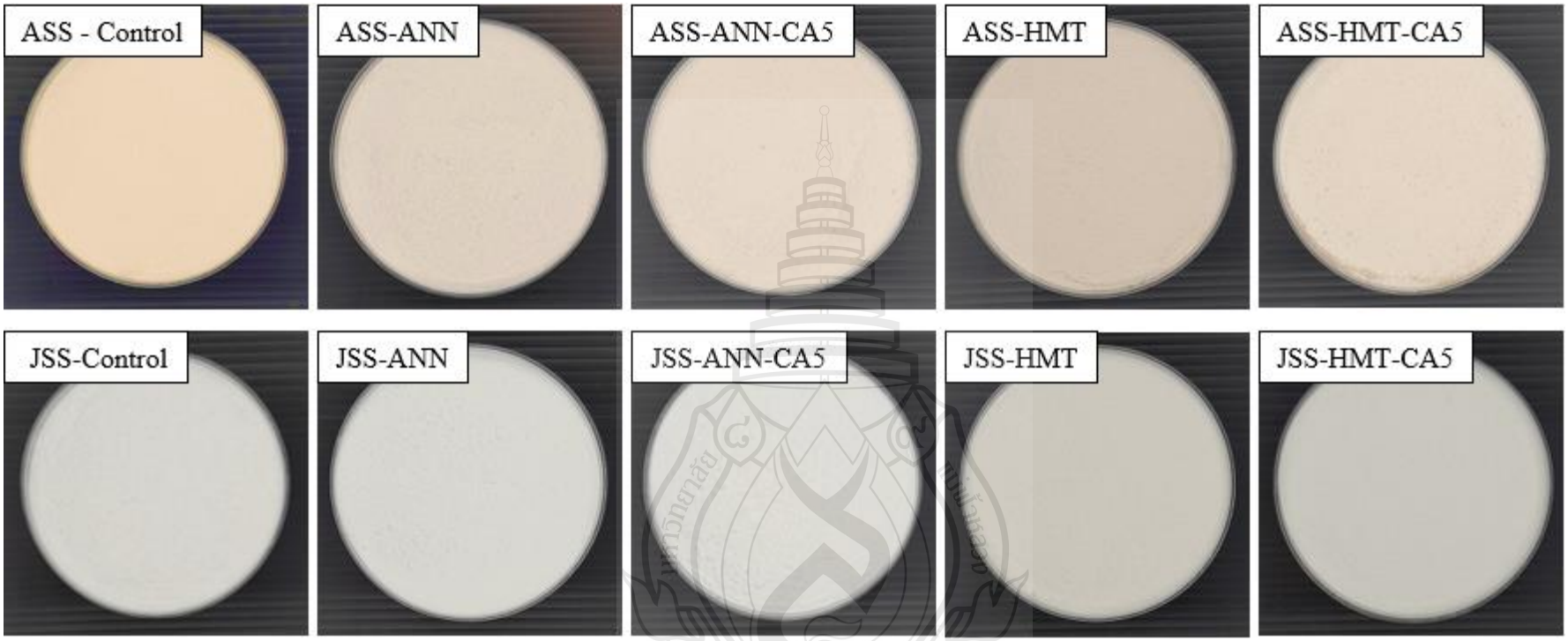


Figure 4.8 Appearance of avocado seed starch – control (ASS-Control), avocado seed starch – annealing (ASS-ANN), avocado seed starch – annealing – citric acid 5% (ASS-ANN-CA5), avocado seed starch – heat moisture treatment (ASS-HMT), avocado seed starch – heat moisture treatment – citric acid 5% (ASS-HMT-CA5), jackfruit seed starch – control (JSS-Control), jackfruit seed starch – annealing (JSS-ANN), jackfruit seed starch – annealing – citric acid 5% (JSS-ANN-CA5), jackfruit seed starch – heat moisture treatment (JSS-HMT) and jackfruit seed starch – heat moisture treatment – citric acid 5% (JSS-HMT-CA5)

Table 4.14 Color determination of ASS and JSS modified by ANN, HMT and incorporation of CA

Sample	L*	a*	b*	C*	h
Avocado seeds					
ASS – Control	86.20 ± 0.07^{c}	5.21 ± 0.01^{a}	12.15 ± 0.04^{b}	13.22 ± 0.04^{a}	66.78 ± 0.03^{e}
ASS – ANN	87.01 ± 0.05^{b}	4.26 ± 0.02^{b}	11.56 ± 0.04^{c}	12.32 ± 0.04^{c}	69.77 ± 0.05^{d}
ASS – ANN – CA5	87.47 ± 0.06^{a}	4.31 ± 0.03^{b}	12.26 ± 0.06^{b}	12.99 ± 0.07^{b}	70.63 ± 0.02^{c}
ASS – HMT	84.21 ± 0.05^{e}	4.14 ± 0.02^{c}	12.20 ± 0.07^{b}	12.88 ± 0.07^{b}	71.27 ± 0.06^{b}
ASS – HMT – CA5	85.37 ± 0.21^{d}	4.02 ± 0.08^{d}	12.55 ± 0.18^{a}	13.18 ± 0.20^{a}	72.24 ± 0.08^{a}
Jackfruit seeds					
JSS – Control	97.69 ± 0.03^{c}	0.43 ± 0.01^{c}	1.91 ± 0.01^{e}	1.96 ± 0.01^{e}	77.43 ± 0.06^{e}
JSS – ANN	97.78 ± 0.01^{b}	0.38 ± 0.00^{d}	2.25 ± 0.01^{c}	2.28 ± 0.01^{c}	80.36 ± 0.07^{c}
JSS – ANN – CA	98.11 ± 0.03^{a}	0.39 ± 0.01^{d}	2.14 ± 0.01^{d}	2.17 ± 0.02^{d}	79.58 ± 0.13^{d}
JSS – HMT	95.14 ± 0.04^{e}	0.65 ± 0.01^{a}	4.89 ± 0.02^{a}	4.93 ± 0.02^{a}	82.42 ± 0.04^{a}
JSS – HMT – CA5	96.48 ± 0.05^{d}	0.61 ± 0.02^{b}	3.76 ± 0.05^{b}	3.81 ± 0.05^{b}	80.86 ± 0.16^{b}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.3.3 Rapid Visco Analyzer

The corresponding measured values including peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), setback (SB), peak time (Pt), and pasting temperature (PT) were shown in Table 4.15. The apparent suppressed paste viscosities after treatments were observed in both ASS and JSS samples. In addition, effects obtained from HMT were greater than those from ANN.

Overall, the decrease in PV, TV, BV, FV, and SB values in relation to the native starch were observed in both ASS and JSS samples ($p<0.05$). Following ANN and ANNCA treatments, the effect of ANN on starch structure restricts the swelling of starch granule on cooking and therefore, the decreased paste viscosities. The effect was more apparent when incorporating 5% CA since it created the cross-linking which restricted the granule's swelling. In ASS samples subjected to ANN and ANN-CA5 treatment, a consistent reduction in almost all pasting parameter was observed. ASS-ANN-CA5 exhibited the lowest values of PV (1599.33 cP), TV (1505.33 cP), Breakdown (94.00 cP), and Final Viscosity (4534.33 cP). The resulting BV (94.00 cP) and SB (3029.00 cP) which were lower than that of ASSControl and ASS-ANN samples. Pasting temperature of ASS were conversely, increased with ANN (about 2^{o}C) and ANN-CA5 (about 3oC). Similar observations were also found in JSS samples. The pasting curves of starch after AAN and ANN-CA5 apparently declined compared to JSS-Control. It was expected for the lowest paste viscosities of JSS after combined modification of annealing and 5% CA treatment. However, that effects were obtained from JSS-ANN. The observed values were PV (1396.67 cP), TV (1149.67 cP), Breakdown (247.00 cP), and Final Viscosity (1837.33 cP) with the resulting BV (247.00 cP) and SB (687.67 cP), respectively. The greater increase in pasting temperature was also observed in JSS-ANN samples (about 1.2^{o}C) than JSS-ANN-CA5 (about 0.5^{o}C).

Annealing treatment improves the thermal stability of starch and delays the progress of gelatinization during the heating process. The increase in gelatinization temperature can delay the expansion of starch granules and affect the formation of pasting (Biduski et al., 2022). The rise in pasting temperature results from the decreased solubility of starch and an elevated crystal melting temperature induced by the annealing treatment. This effect is likely attributed to the increased stability of the

partial microcrystalline structure formed during the treatment. (Zhang et al., 2015; Hu et al., 2020; Liu et al., 2016). In addition, annealing treatment possibly increases the interaction between starch molecular chains and improves the crystalline order, resulting in the limited hydrogen bonding force between starch molecules and water molecules, which reduces the swelling power and peak viscosity of starch granules (Xu et al., 2018).

The application of HMT had a significant impact on the pasting characteristics of both ASS and JSS samples. The apparent low paste viscosities, PV, BV, TV, FV, and SB after treatments were observed. The ASS samples treated with ANN and ANN-CA5 exhibited similar effects in that a whole pasting curve was apparently suppressed and there was no pasting temperature observed. For JSS sample, the pasting curve could be observed from JSS-ANN as the paste viscosities were apparently low, PV (1396.67 cP), TV (1149.67 cP), Breakdown (247.00 cP), and Final Viscosity (1837.33 cP) with the resulting BV (247.00 cP) and SB (687.67 cP), respectively. The pasting temperature of sample was apparently increased from that of JSS-Control for about 5.7 ^{o}C. It was expected that the lowest paste viscosities would be observed in JSS-ANN-CA5 samples due to the dual effects. However, the effect was not observed. Earlier reports demonstrated varying trends in pasting behavior of starch after HMT. HMT was found to elevate the pasting temperature and improve the thermal shear stability of starch paste while concurrently reducing the peak viscosity and granular breakdown (Hoover & Vasanthan, 1994; Lawal & Adebowale, 2005; Gunaratne & Corke, 2007; Shih et al., 2007; Yadav et al., 2013; Sun et al., 2014). The extent of setback, which reflects the degree of retrogradation, was reported to exhibit both increases and decreases (Sun et al., 2013; Shih et al., 2007; Varatharajan et al., 2011; Gunaratne & Corke, 2007). Notably, the magnitude of these changes appeared to depend on factors such as the starch source and treatment conditions (Hoover, 2010). HMT was found to enhance the intermolecular association of starch chains, reinforcing granular bonding. Consequently, a higher level of heat energy was necessary to disrupt the HMT granules during the paste formation (Gunaratne, 2018).

Table 4.15 Pasting properties of avocado and jackfruit seeds starch modified by ANN, HMT and incorporation of CA

Sample	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final Viscosity (cP)	Set Back (cP)	Peak Time (min)	Pasting Temperature (°C)
Avocado seeds							
ASS – Control	2149.67 ± 14.15^{a}	2014.33 ± 25.81^{a}	135.33 ± 11.72^{a}	5600.00 ± 98.81^{a}	3585.67 ± 77.84^{a}	7.00 ± 0.00^{a}	86.68 ± 0.49^{c}
ASS – ANN	1813.00 ± 15.10^{b}	1630.00 ± 17.00^{b}	183.00 ± 2.65^{b}	5316.67 ± 168.94^{b}	3686.67 ± 170.90^{a}	7.00 ± 0.00^{a}	88.80 ± 0.10^{b}
ASS – ANN – CA5	1599.33 ± 11.24^{c}	1505.33 ± 10.97^{c}	94.00 ± 7.94^{c}	4534.33 ± 53.97^{c}	3029.00 ± 46.94^{b}	7.00 ± 0.00^{a}	89.12 ± 0.51^{a}
ASS – HMT	19.33 ± 1.53^{d}	14.33 ± 1.53^{d}	5.00 ± 0.00^{d}	16.00 ± 1.00^{d}	1.67 ± 0.58^{c}	5.80 ± 0.40^{c}	ND
ASS – HMT – CA5	21.33 ± 0.58^{d}	15.00 ± 1.00^{d}	6.33 ± 0.58^{d}	18.33 ± 0.00^{d}	3.33 ± 0.58^{c}	6.04 ± 0.70^{b}	ND
Jackfruit seeds							
JSS – Control	1642.33 ± 13.65^{a}	1257.33 ± 23.12^{a}	385.00 ± 29.00^{a}	2149.67 ± 13.61^{a}	892.33 ± 10.60^{a}	5.29 ± 0.03^{d}	88.83 ± 0.03^{d}
JSS – ANN	1396.67 ± 17.56^{b}	1149.67 ± 48.17^{b}	247.00 ± 45.13^{b}	1837.33 ± 85.41^{b}	687.67 ± 50.21^{b}	5.44 ± 0.14^{c}	90.00 ± 0.39^{b}
JSS – ANN – CA5	1326.33 ± 20.40^{c}	1248.33 ± 23.29^{a}	78.00 ± 13.08^{c}	1937.33 ± 40.27^{c}	689.00 ± 19.00^{b}	5.44 ± 0.10^{c}	89.50 ± 0.57^{c}
JSS – HMT	368.67 ± 5.13^{d}	311.00 ± 5.20^{c}	57.67 ± 1.53^{c}	611.67 ± 4.04^{d}	300.67 ± 1.15^{c}	7.00 ± 0.00^{a}	94.53 ± 0.03^{a}
JSS – HMT – CA5	27.67 ± 1.15^{e}	24.67 ± 0.58^{d}	3.00 ± 1.00^{d}	34.67 ± 0.58^{e}	10.00 ± 1.00^{d}	6.78 ± 0.24^{b}	ND

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.3.4 Texture Profile Analysis

The gel characteristics of ASS and JSS samples both before and after treatment were presented in Table 4.16. In summary, the samples exhibited significant alterations in gel texture ($p<0.05$) when compared to the native ASS and JSS starches. The hardness parameter quantifies the firmness of the starch gel, while adhesion characterizes the starch gel's ability to adhere to external surfaces. This adhesion effect arises from the binding of more densely packed, water-soluble amylopectin groups to the probes after the initial compression, as explained by Iftikhar and Dutta (2019). In addition, cohesiveness reflects the extent to which the structure of the starch gel is disrupted by the initial compression applied during testing, as discussed by Zhang et al. (2017).

Based on TPA analysis results presented in Table 4.16, both ASS and JSS after ANN and HMT gave a soft and sticky gel texture compared to untreated samples as hardness decreased ($p<0.05$) but adhesiveness increased ($p<0.05$). keep the number The springiness, cohesiveness and chewiness were also varied ($p<0.05$). With the combination of 5% CA, the hardness was less changed by the adhesiveness was increased for both ASS and JSS. Earlier reports showed varying effects of ANN and HMT on starch gel texture changes in gel texture of starch after annealing and HMT.

Majzoobi et al. (2012) observed an increase in starch gel hardness which could can be attributed to the reorganization of starch molecules induced by ANN. This reorganization results in a reduction in starch's swelling power and solubility, leading to a decrease in the proportion of gel volume and an increase in gel hardness (Zavareze & Dias, 2011; Majzoobi et al., 2012; Singh et al., 2011; Wang et al., 2018). A decrease in gel adhesion could be attributed to ANN which enhanced the intermolecular interactions among starch chains, and limited the leaching of amylose and swelling of starch granules. These led to a reduction in the viscosity of amaranth and taro starch gel, resulting in a reduced adhesion (Biduski et al., 2022). However, these effects were not observed in this study. Following HMT modification, data (Table 4.16) revealed a decrease in hardness and an increase in adhesiveness ($p<0.05$) of JSS. Gel texture measurement could not be applicable for JSS-HMT-CA5 since the solid starch gel could not be formed.

Table 4.16 Texture properties of ASS and JSS modified by ANN, HMT and incorporation of CA

Sample	Hardness	Adhesiveness	Springiness	Cohesiveness	Chewiness
	g	g.sec	%	%	
Avocado seed					
ASS – Control	194.15 ± 13.39^{a}	166.50 ± 88.05^{a}	0.74 ± 0.06^{b}	0.40 ± 0.07^{a}	55.13 ± 4.18^{a}
ASS – ANN	167.89 ± 4.98^{c}	231.80 ± 3.28^{b}	0.79 ± 0.04^{a}	0.37 ± 0.01^{b}	49.10 ± 2.04^{c}
ASS – ANN – CA5	193.75 ± 7.55^{b}	273.57 ± 45.89^{c}	0.74 ± 0.07^{c}	0.35 ± 0.03^{c}	50.49 ± 7.26^{b}
ASS – HMT	N/A	N/A	N/A	N/A	N/A
ASS – HMT – CA5	N/A	N/A	N/A	N/A	N/A
Jackfruit seed					
JSS – Control	747.90 ± 17.34^{a}	121.42± 26.28^{b}	0.67 ± 0.32^{d}	0.67 ± 0.26^{a}	2.38 ± 1.56^{a}
JSS – ANN	563.50 ± 77.21^{c}	100.24 ± 30.31^{a}	0.79 ± 0.80^{a}	0.48 ± 0.12^{b}	1.91 ± 0.26^{b}
JSS – ANN – CA5	608.08 ± 37.34^{b}	187.86 ± 4.55^{d}	0.72 ± 0.08^{c}	0.25 ± 0.03^{d}	1.05 ± 0.20^{c}
JSS – HMT	220.43 ± 22.01^{d}	181.90 ± 26.42^{c}	0.74 ± 0.04^{b}	0.29 ± 0.02^{c}	0.45 ± 0.06^{d}
JSS – HMT – CA5	N/A	N/A	N/A	N/A	N/A

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

This corresponded to the paste characteristics of samples as the starch did not develop the viscous paste upon heating and cooling by RVA (Section 4.1.3). Likewise, ASS-HMT and ASS-HMT-CA5 did not gave the measurable starch paste and the solid gel. Starch gel hardness is often contingent on its starch structural composition. Apart from amylose and amylopectin structure (Yu et al., 2009), hardness of starch gel is influenced by the hydrogen bonding that occurs between starch molecules and water (Aaliya et al., 2021). To this study, the strong formation of cross-links between intermolecular and intramolecular bonds induced by the physical modification, HMT limited starch swelling, paste viscosity development and the formation of solid gel structure of the two starch, ASS and JSS. The effects were more pronounced when incorporating citric acid treatment.

4.3.5 Differential Scanning Colorimeter

Annealing treatments led to a significant increase in the gelatinization temperatures in all samples of avocado seed starch (ASS) and jackfruit seed starch (JSS) compared to that of native starch counterparts. Table 4.17 displays the melting temperatures (To, Tp, and Te), gelatinization temperature range (Te–To), and the gelatinization enthalpy (ΔH). DSC thermograms of all ASS samples were presented in Figure 4.9 and 4.10, while those of JSS sample were presented in Figure 4.14 and 4.15, respectively. Overall, the treated samples were significantly altered ($p<0.05$) in their gelatinization properties compared to that of the native ones.

The melting temperature of weak, general, and strong crystallites is denoted by To, Tp, and Te, respectively (Song et al., 2014). The significant increases ($p<0.05$) were observed in the values of To, Tp, and Te across all treated samples. The observed elevation in To, Tp, and Te may potentially be attributed to the retrogradation of starch, as the annealing procedure induced a partial gelatinization of starch (Alcazar-Alay & Meireles, 2015). In contrast, the process of annealing demonstrated a notable rise in the temperatures To, Tp, and Te of starch. This implied that the starch subjected to annealing required a greater amount of external heat energy absorption in order to achieve complete gelatinization, as compared to the native starch samples. Elevated values of To, Tp, and Te 100 were indicative of enhanced thermal stability exhibited by annealed starch granules during the heating process. The smaller range of gelatinization (Te–To) observed from all ANN samples indicated the more

homogenous starch crystallites. The gelatinization enthalpy change (ΔH) is associated with the level of disorder in double helices and the extent to which the double helix structure unravels throughout the process of gelatinization (Liu et al., 2016). ANN showed a significant increase in ΔH ($p<0.05$), suggesting the presence of the formation of novel double helices during annealing.

Following HMT, for ASS samples, ASS-HMT and ASS-HMT-CA5 showed no gelatinization peak. These corresponded to the RVA results (section 4.1.3) in that all HMT treated samples completely not swell and gelatinized by heat and therefore, did not give a cooked starch paste with the raised viscosity. For JSS, JSS-HMT showed the increased To, Tp, Te, and ΔT, suggested that the treated starch was more resistant to heat and therefore, melt at high temperature than the native starch. However, a decreased ΔH than that of untreated one indicated a partial melting of some starch crystallites. This finding aligned with previous research indicating a considerable increase in the levels of To, Te, and Tp following HMT treatment (Huang et al., 2016; Sui et al., 2015; Xia et al., 2016). In their study, Huang et al. (2016) investigated the phenomenon of starch forming more stable crystals during the process of HMT. Alterations in gelatinization process of HMT starch in comparison to native starch could be attributed to several factors. According to Schafranski et al. (2021), the phenomena could be explained as initially, during the heat treatment, a new surface is formed on the starch, which acts as a barrier, impeding the penetration of water into the starch granules. Consequently, this delays the swelling process and the destruction of the more organized crystalline structure which occurred at the elevated temperatures (To, Tp, and Te). In addition, partial gelatinization which may occur during high-temperature processing as a result of vapor pressure during heating, can possibly induces phase separation of amylose and amylopectin, as well as shrinkage reactions of the starch particles.

For HMT starch with a combination of 5% CA treatment, JSS-HMT-CA5 was completely not melted. Results reflected the strong intermolecular bonds between starch chain because of crosslinking and esterification by CA.

The findings of the study indicated that as the HMT treatment progressed, there was a direct correlation between the bonding strength within particles and the interconnection of starch chains. Consequently, this led to a continuous increase in the

temperatures at which the onset (To), conclusion (Te), and peak (Tp) of the starch crystallization occurred. The thermal stability of treated starch exhibited a notable enhancement. Furthermore, it was discovered that the type of starch played a crucial role in defining the extent and nature of the observed changes as seen between ASS and JSS samples.

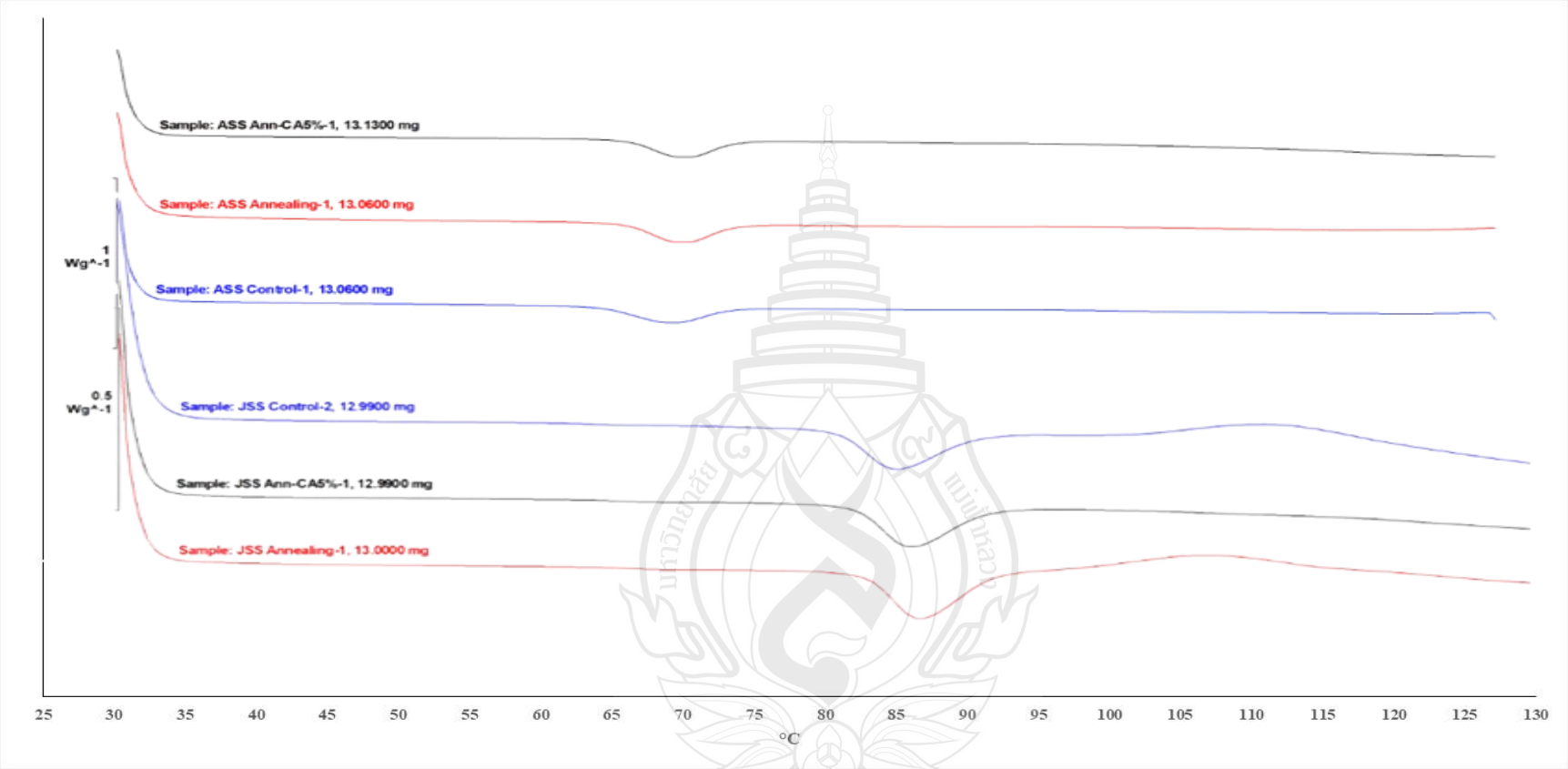


Figure 4.9 DSC thermograms of avocado seed starch – control (ASS-Control) avocado seed starch – annealing (ASS-ANN), avocado seed starch – annealing – citric acid 5% (ASS-ANN-CA5), jackfruit seed starch – control (JSS-Control), jackfruit seed starch – annealing (JSS-ANN), and jackfruit seed starch – annealing – citric acid 5% (JSS-ANN-CA5)

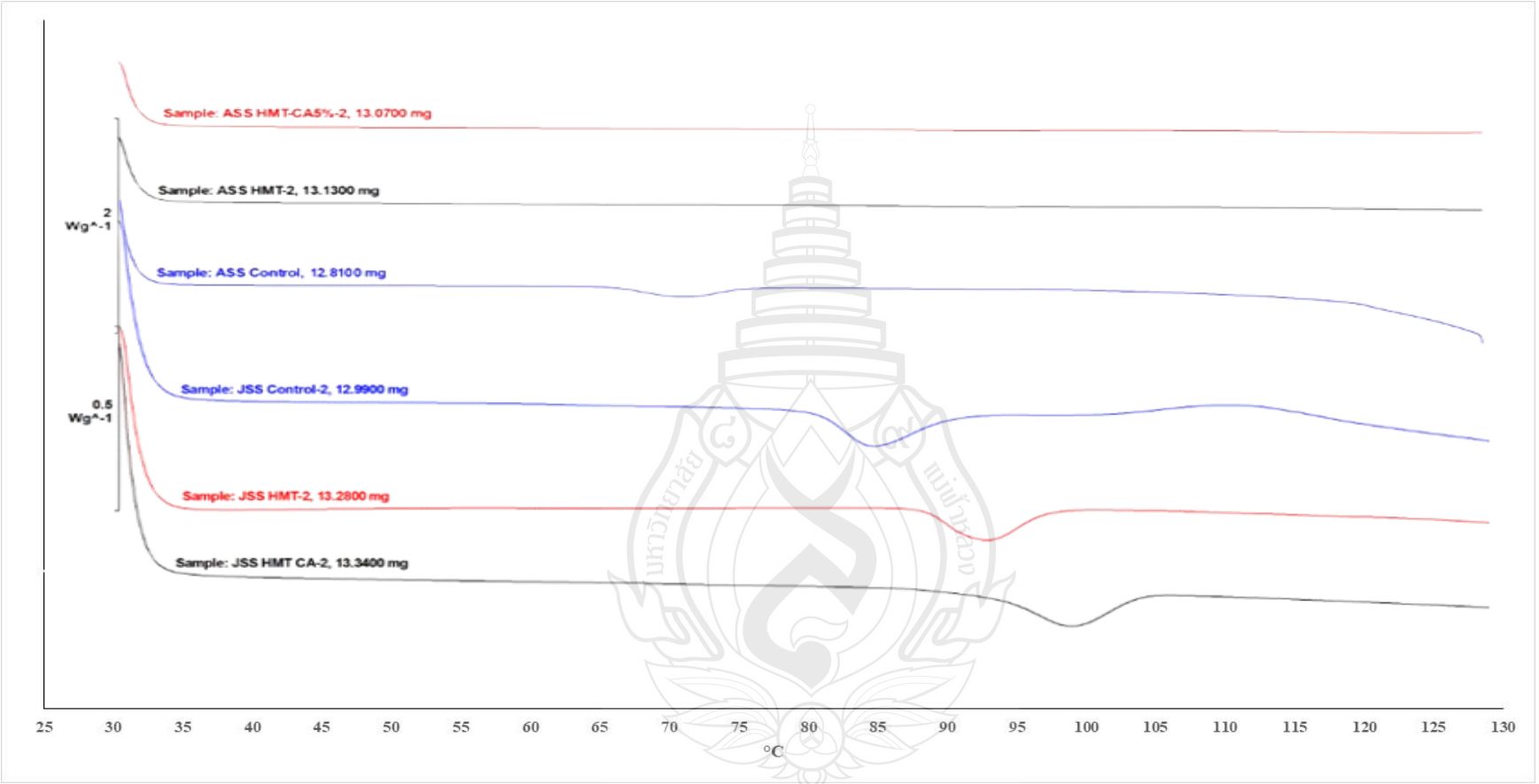


Figure 4.10 DSC thermograms of avocado seed starch – control (ASS-Control) avocado seed starch – heat moisture treatment (ASS-HMT), avocado seed starch – heat moisture treatment – citric acid 5% (ASS-HMT-CA5), jackfruit seed starch – control (JSS-Control), jackfruit seed starch – heat moisture treatment (JSS-HMT), and jackfruit seed starch – heat moisture treatment – citric acid 5% (JSS-HMT-CA5)

Table 4.17 Thermal properties of ASS and JSS modified by ANN, HMT and incorporation of CA

Sample	T_o (°C)	T_p (°C)	T_e (°C)	Range T_e - T_o	Enthalphy (mJ)	Enthalphy (mJ/g db)
Avocado seeds						
ASS-Control	64.27 ± 0.06^{c}	69.00 ± 0.14^{b}	73.05 ± 0.07^{c}	8.78 ± 0.13^{a}	35.21 ± 1.75^{c}	2879.50 ± 138.58^{c}
ASS-ANN	66.00 ± 0.16^{b}	66.00 ± 0.16^{c}	73.51 ± 0.06^{b}	7.51 ± 0.10^{b}	41.23 ± 0.30^{a}	3266.09 ± 63.15^{a}
ASS-ANN-CA	66.72 ± 0.95^{a}	70.00 ± 0.00^{a}	73.85 ± 0.27^{a}	7.12 ± 0.68^{c}	40.61 ± 1.42^{b}	3221.13 ± 107.51^{b}
ASS-HMT	N.D	N.D	N.D	N.D	N.D	N.D
ASS-HMT-CA	N.D	N.D	N.D	N.D	N.D	N.D
Jackfruit seeds						
JSS-Control	80.88 ± 0.15^{e}	84.83 ± 0.13^{e}	90.25 ± 0.16^{e}	9.37 ± 0.16^{c}	43.19 ± 3.40^{c}	3465.54 ± 269.70^{c}
JSS-ANN	82.81 ± 0.06^{c}	86.67 ± 0.00^{c}	91.83 ± 0.28^{c}	9.02 ± 0.13^{d}	48.97 ± 2.78^{a}	3831.67 ± 263.20^{a}
JSS-ANN-CA	82.28 ± 0.04^{d}	86.17 ± 0.00^{d}	91.73 ± 0.28^{d}	9.45 ± 0.26^{b}	46.93 ± 0.91^{b}	3786.91 ± 131.32^{b}
JSS-HMT	88.77 ± 0.03^{b}	93.75 ± 0.03^{b}	97.73 ± 0.06^{b}	8.96 ± 0.07^{e}	35.71 ± 2.78^{e}	2816.22 ± 211.99^{e}
JSS-HMT-CA	93.69 ± 0.17^{a}	99.48 ± 0.11^{a}	104.62 ± 0.38^{a}	10.93 ± 0.55^{a}	41.84 ± 5.11^{d}	3297.49 ± 387.44^{d}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

4.3.6 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) images of native ASS and JSS were shown in Figure 4.11. The ASS starch granules exhibited an oval morphology with a smooth surface. JSS displayed a round-half shape with a smaller size compared to ASS. There were no revealed granule damages found, reflective the adequacy of the earlier starch extraction process.

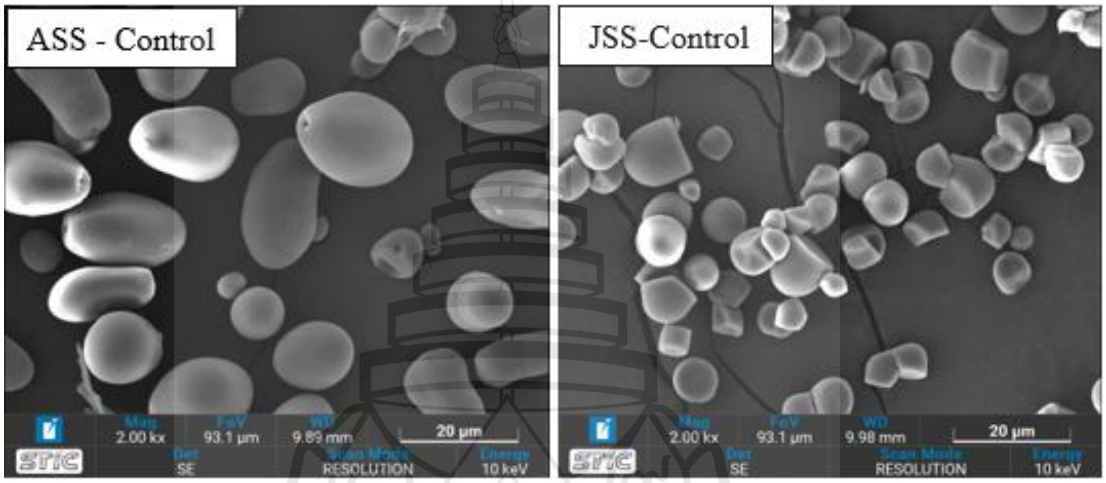


Figure 4.11 SEM images of avocado seed starch – control (ASS-Control) and jackfruit seed starch – control (JSS-Control)

Figure 4.12 displayed ASS and JSS samples subjected to ANN and ANN-CA treatments. The morphology of all samples remained unchanged after treatments, indicative of the rigid structure. Results was aligned with prior studies on rice starch (Dias et al., 2010), jackfruit seed starch (Bhattacharjya et al., 2015), and barley starch (Waduge et al., 2006). Figure 4.13 illustrated starch granules morphology of ASS and JSS samples following HMT and HMT-CA treatments. In contrast to those obtained from ANN, both ASS and JSS samples displayed some changes in their shapes. ASS-HMT and JSS-HMT samples showed the course granule surface and collapses which were more apparent in samples combined treated with 5%CA. Results indicated partial damage or disruption to the granules from high temperature treatment. With the incorporation of 5% CA, the effect was more apparent. Previous research showed no changes in starch granule shape and size following HMT (Piecyk & Domian, 2021; Hung et al., 2020; Gunaratne & Hoover, 2002). Damage to starch granules upon HMT was highly associated with the moisture content of the starch. The granule shape

remained unchanged when the moisture content was under 20%, while moisture content above 20% resulted in extensive collapses and structural disruptions (Li et al., 2020).

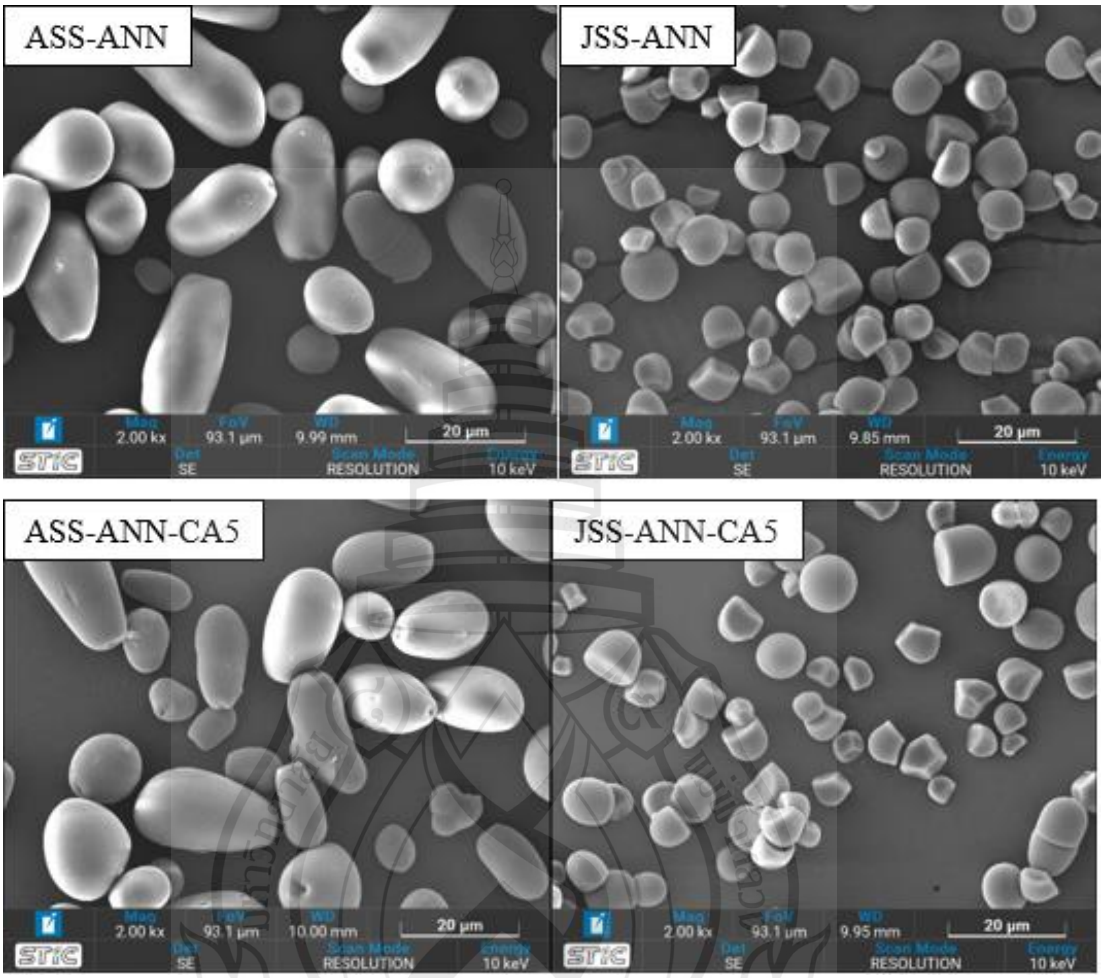


Figure 4.12 SEM images of avocado seed starch – annealing (ASS-ANN) jackfruit seed starch – annealing (JSS-ANN), avocado seed starch – annealing – citric acid 5% (ASSANN-CA5) and jackfruit seed starch – annealing – citric acid 5% (JSS-ANN-CA5)

These effects could be attributed to the disruption of the native starch granular structure due to heating, along with granule aggregation and a distorted granular shape (Xia et al., 2016; Lee et al., 2011). The partial disruption of granular starch was also conformed to the reduction in gelatinization enthalpy observed from DSC (Section 4.1.5).

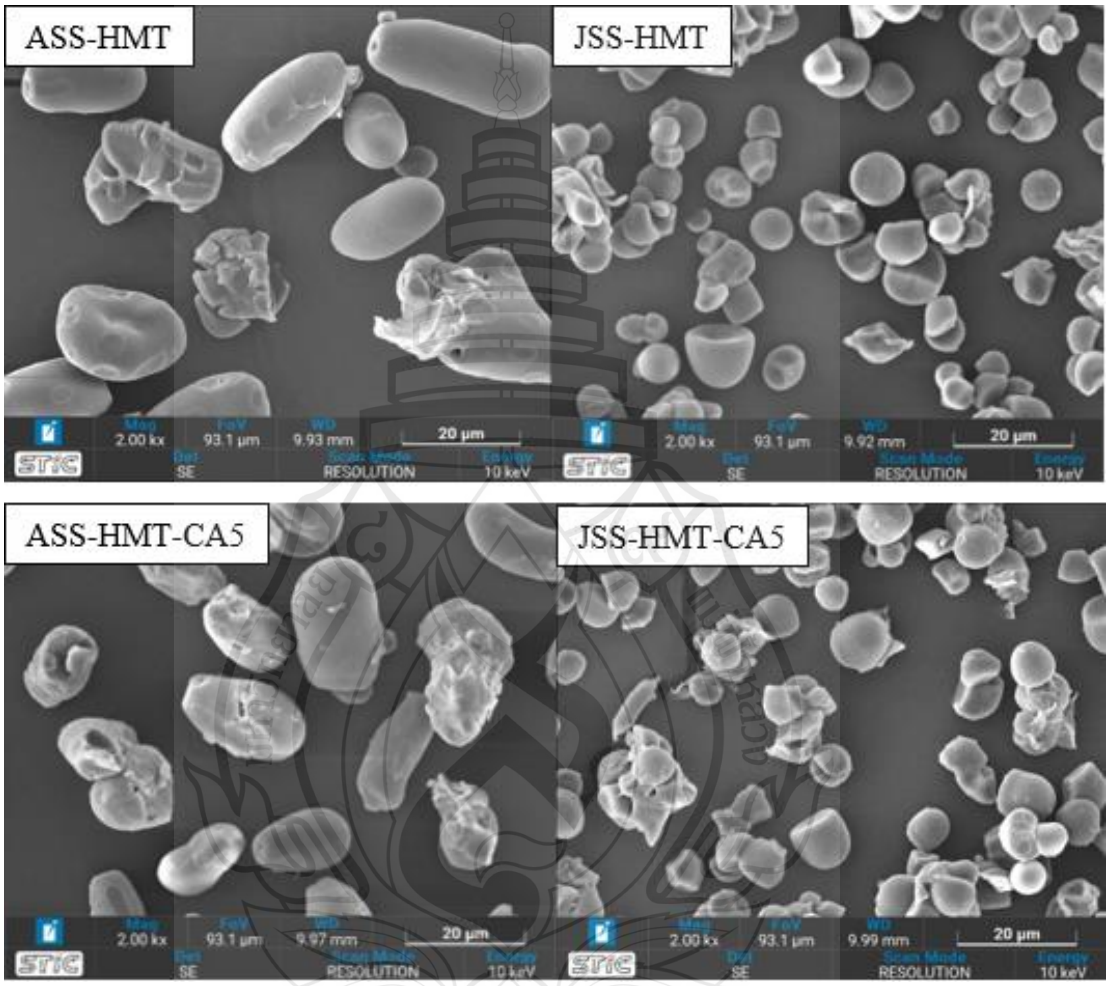


Figure 4.13 SEM images of avocado seed starch – heat moisture treatment (ASS-HMT), jackfruit seed starch – heat moisture treatment (JSS-HMT), avocado seed starch – heat moisture treatment – citric acid 5% (ASS-HMT-CA5) and jackfruit seed starch – heat moisture treatment – citric acid 5% (JSS-HMT-CA5)

4.3.7 X-Ray Diffraction

XRD diffractograms of all ASS starch after ANN and HMT was provided in Figure 4.14 and 4.15, respectively. The interaction between the incident beam's atoms and the electrons of the selected starch allows for the differentiation of starch into many types, namely A, B, C, and V, as supported by previous studies (Purohit et al., 2019; Schafranski et al., 2021).

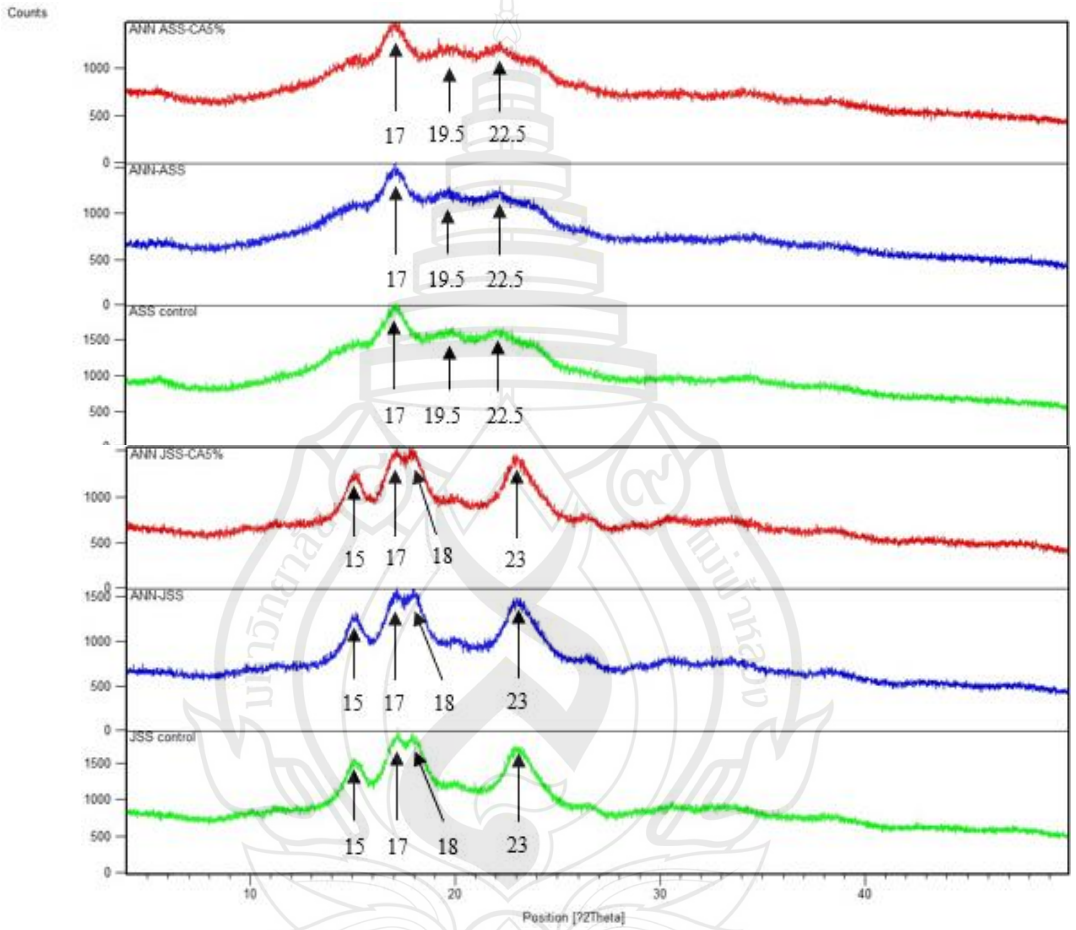


Figure 4.14 Diffractogram of avocado seed starch – control (ASS-Control) avocado seed starch – annealing (ASS-ANN), avocado seed starch – annealing – citric acid 5% (ASSANN-CA5), jackfruit seed starch – control (JSS-Control), jackfruit seed starch – annealing (JSS-ANN), and jackfruit seed starch – annealing – citric acid 5% (JSS-ANNCA5)

For ASS, there were no changes observed in diffraction pattern following ANN either by ANN alone or ANN combined with 5%CA. However, alterations were observed in samples after HMT. Alterations in the diffraction angle range, specifically

at 15°, 17°, 18°, and 23° (2θ), indicated a transition from the typical B-type diffraction pattern of native ASS to an A-type diffraction pattern after HMT. In line with this observation, earlier research reported the transformation from a B-type diffraction pattern into a combination of B-type and A-type diffraction patterns in natural waxy potato starch (Lee & Moon, 2015) and in common potato and yam starch (Hoover & Vasanthan, 1994) following heat-moisture treatment (HMT). The presence of peak at diffraction angle 13° was also observed in ASS after HMT-CA5. This alteration in the X-ray diffraction pattern of natural starch due to HMT can be attributed to the dehydration of water molecules within the starch matrix. This dehydration process leads to the displacement of the double helix structure, subsequently influencing the formation of starch microcrystals and altering crystal orientation (Gunaratne, 2018; Schafranski et al., 2021).

XRD diffractograms of all JSS starch after ANN and HMT were presented in Figure 4.14 and 4.15, respectively. The JSS displayed typical type A crystalline pattern which characterized by the presence of peaks at specific diffraction angles, 15.3°, 17.1°, 18.2°, and 23.5°, as reported by Schafranski et al. (2021). The diffraction patterns of all JSS samples exhibited prominent peaks at 15°, 17°, 18°, and 23°, indicating that HMT did not induce any alterations in the crystal structure of JSS. In other words, all treated JSS remained unchanged in terms of starch crystallinity pattern. Similar observations were earlier reported in HMT sweet potato starch by Trung et al. (2017) and in mango seed starch by Bharti et al. (2019).

Table 4.18 presents the percentage relative crystallinity of ASS and JSS samples modified by ANN, HMT, and combined with citrate esterification. An increase in percentage relative crystallinity was obtained from all treated ASS and HMT starch with the exception for annealing treatment alone.

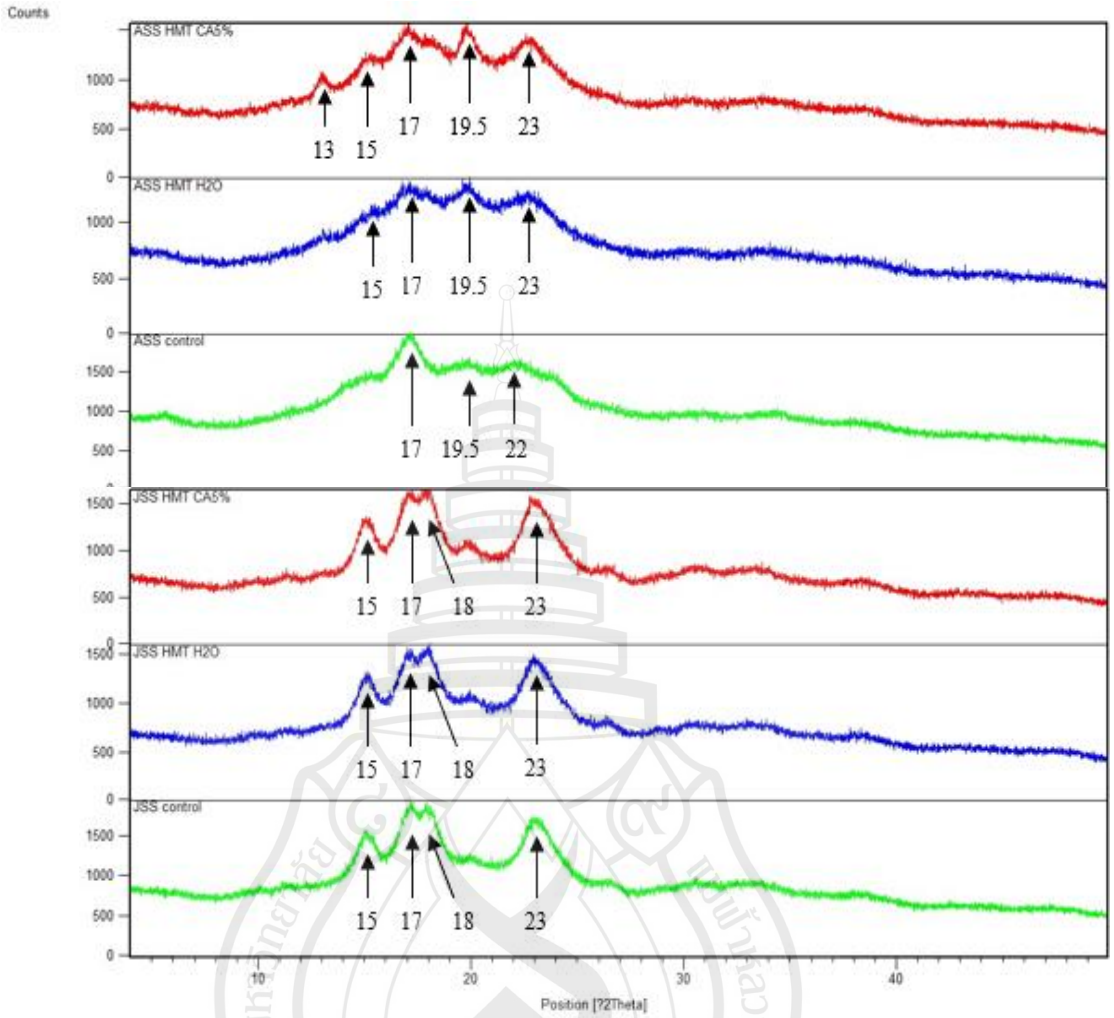


Figure 4.15 Diffractogram of avocado seed starch – control (ASS-Control) avocado seed starch – heat moisture treatment (ASS-HMT), avocado seed starch – heat moisture treatment – citric acid 5% (ASS-HMT-CA5), jackfruit seed starch – control (JSSControl), jackfruit seed starch – heat moisture treatment (JSS-HMT), and jackfruit seed starch – heat moisture treatment – citric acid 5% (JSS-HMT-CA5)

For ANN-CA5, the increase in crystallinity was attributed to the effect of citric acid esterification. This was also associated to the additional peaks observed at 13º. For HMT and HMT-CA5, the effect was more apparent in both starch, ASS and JSS. The rearrangement of the double helix chain induced by HMT can lead to an increase in starch crystallinity (Syamsir et al., 2012). Several factors can contribute to an increase in starch crystallinity after hydrothermal modification. One such factor is the

application of heat, which can enhance starch crystallinity. When starch is heated, the intermolecular bonds within the starch molecules weaken, allowing the molecules to arrange themselves in a more regular crystal structure upon cooling.

Table 4.18 Percentage relative crystallinity (%) of ASS and JSS modified by ANN, HMT and incorporation of CA

Samples	%
Avocado seed starch	
ASS – Control	13.41 ± 0.12^{d}
ASS – ANN	13.11 ± 2.47^{e}
ASS – ANN – CA5	18.36 ± 1.01^{a}
ASS – HMT	15.61 ± 0.83^{c}
ASS – HMT – CA5	17.53 ± 0.93^{b}
Avocado seed starch	
JSS – Control	25.96 ± 0.23^{d}
JSS – ANN	25.83 ± 2.51^{e}
JSS – ANN – CA5	26.80 ± 1.02^{b}
JSS – HMT	26.71 ± 2.26^{c}
JSS – HMT – CA5	29.17 ± 0.18^{a}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

Additionally, the moisture content in starch plays a role in its crystallinity, with low moisture content in starches generally displaying higher crystallinity due to the less disruptive effects of water on the arrangement of starch molecules. This could possibly explain the greater increase ($p<0.05$) in % relative crystallinity in HMT and HMT-CA5 with lower moisture content (25% w/w) as compared to ANN-CA5 with high moisture content (more than 50% w/w). The type of starch utilized can also influence crystallinity, as certain starch types, such as amylose, have a greater propensity to form crystalline structures than others.

4.3.8 Fourier Transform Infra-Red

Figure 4.16 show FTIR spectra of all ASS samples. The FTIR spectrum of the native ASS, ASS-ANN and ASS-ANN-CA5 samples showed the typical peaks, indicative of specific functional group at 1016.81 – 1017.25 cm-1 (the Si-CH=CH2 Trans C-H wagging vibration bond), 1081.40 – 1082.04 cm-1 (the C-O carbohydrate bond), 1158.75 – 1162.02 cm-1 (the C-O-C polysaccharide bond), 1373.32 - 1374.81 cm-1 (the C-H and CH2 aliphatic bending group), 1645.03 - 1647.76 cm-1 (the C=O amide I band), 2928.80 - 2929.44 cm-1 (the C-H and CH2 aliphatic stretching group) and broadband at 3402.95 - 3420.68 cm-1 (the O-H carbohydrates proteins and polyphenols). Results showed that the additional peaks at 2043 - 2045 cm-1 indicative of the thiocyanate (2000 cm-1) was found in ASS-ANN and ASS-ANN-CA5 (Moosvi et al., 2016). The formation of ketone groups during annealing may occur if there was a chemical reaction involving the removal or change of functional groups present in the molecule. Comparing among FTIR spectrum of the native ASS, ASS-HMT and ASS-HMTCA5 in the figure 4.17, the the typical peaks were observed at 1015.19 – 1016.82 cm-1 (the Si-CH=CH2 Trans C-H wagging vibration bond), 1081.40 – 1081.95 cm-1 (the C-O carbohydrate bond), 1159.16 – 1162.02 cm-1 (the C-O-C polysaccharide bond), 1369.71 - 1373.32 cm-1 (the C-H and CH2 aliphatic bending group), 1646.98 – 1648.05 cm-1 (the C=O amide I band), 2929.44 - 2929.80 cm-1 (the C-H and CH2 aliphatic stretching group) and broadband at 3331.97 - 3420.68 cm-1 (the O-H carbohydrates proteins and polyphenols). Results indicated that there was no alteration in functional groups of ASS starch after HMT and HMT-CA5.

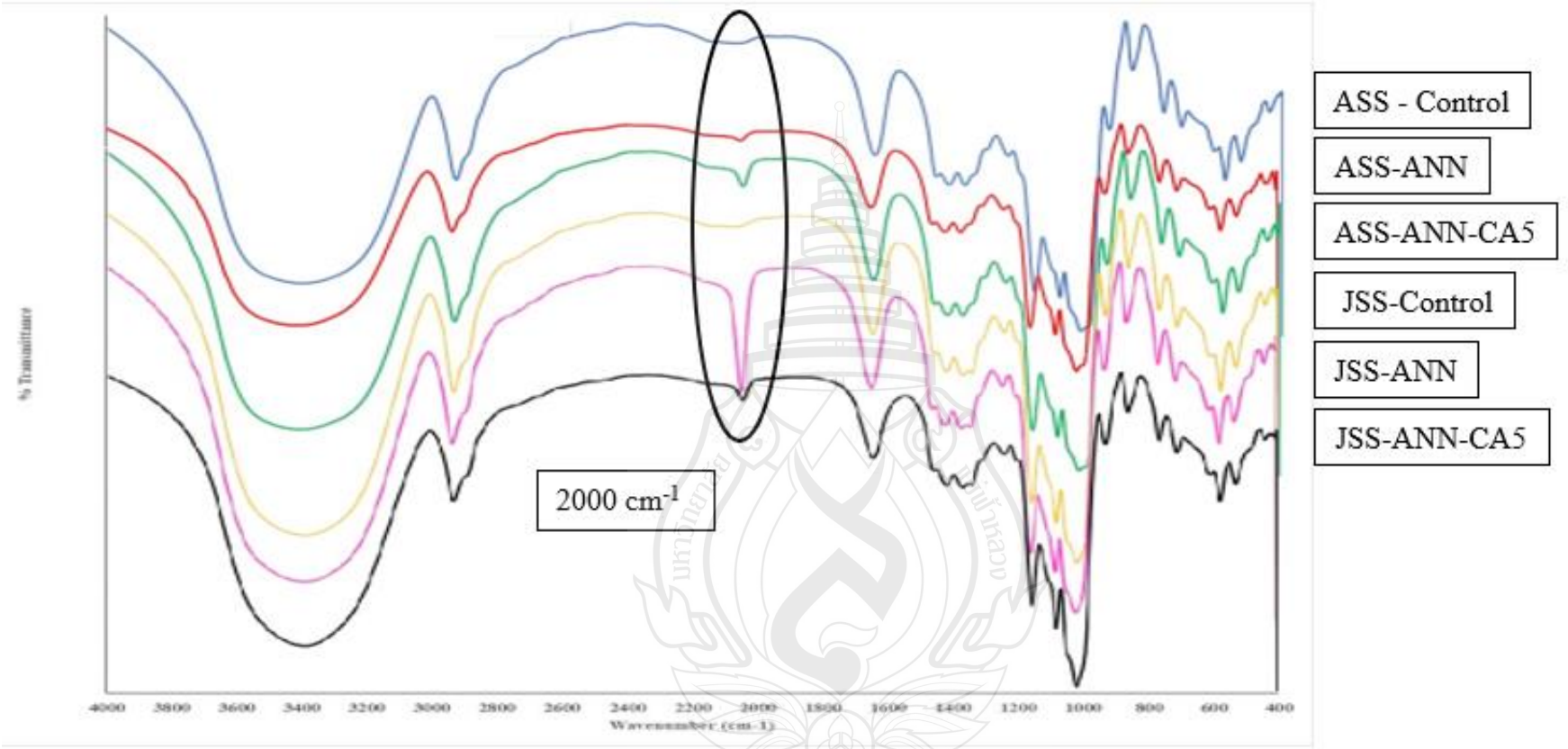


Figure 4.16 FTIR spectrum of avocado seed starch – control (ASS-Control) avocado seed starch – annealing (ASS-ANN), avocado seed starch – annealing – citric acid 5% (ASS-ANN-CA5), jackfruit seed starch – control (JSS-Control), jackfruit seed starch – annealing (JSS-ANN), and jackfruit seed starch – annealing – citric acid 5% (JSS-ANN-CA5)

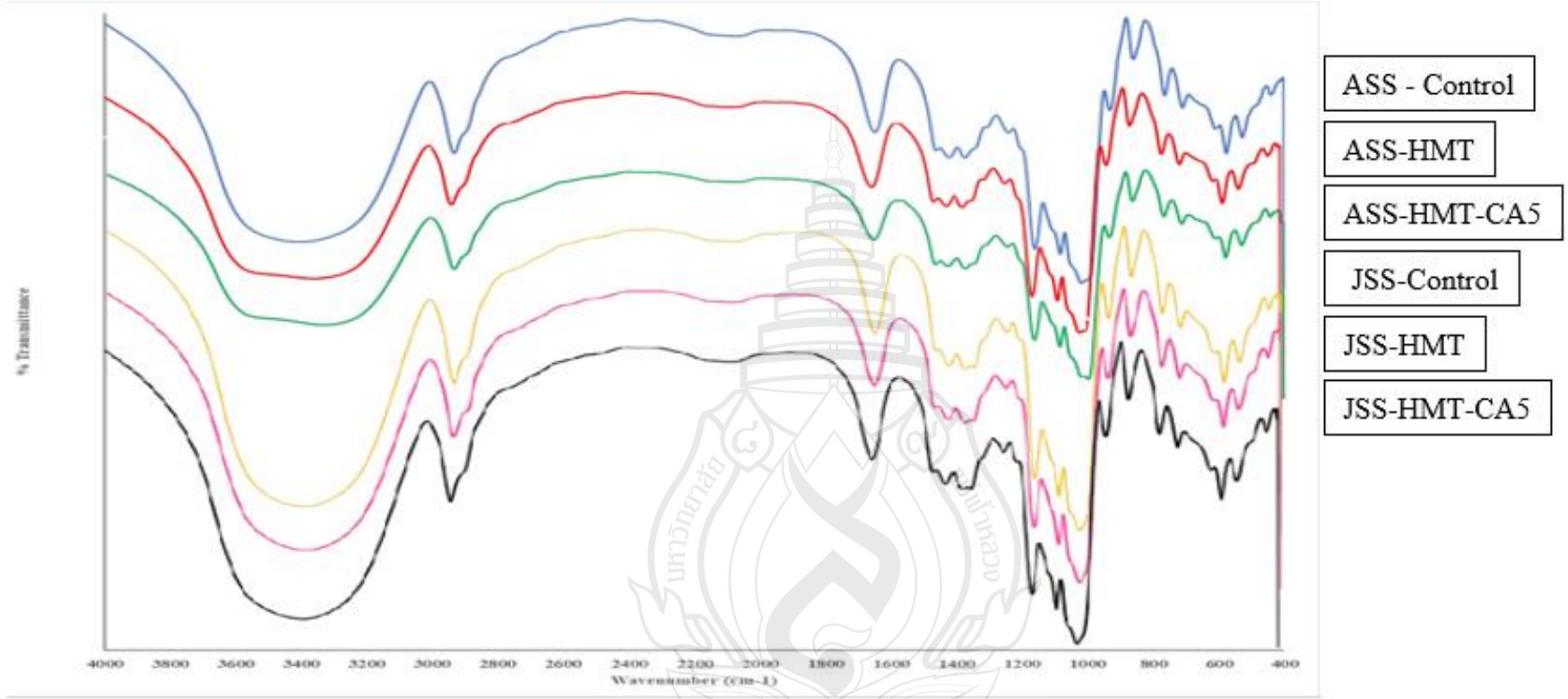


Figure 4.17 FTIR spectrum of avocado seed starch – control (ASS-Control) avocado seed starch – heat moisture treatment (ASS-HMT), avocado seed starch – heat moisture treatment – citric acid 5% (ASS-HMT-CA5), jackfruit seed starch – control (JSS-Control), jackfruit seed starch – heat moisture treatment (JSS-HMT), and jackfruit seed starch – heat moisture treatment – citric acid 5% (JSS-HMT-CA5)

The FTIR spectrum of the native JSS, JSS-ANN and JSS-ANN-CA5 showed the typical peaks at 1017.80 – 1017.93 cm-1 (the Si-CH=CH2 Trans C-H wagging vibration bond), 1080.92 – 1080.95 cm-1 (the C-O carbohydrate bond), 1155.73 – 1155.96 cm-1 (the C-O-C polysaccharide bond), 1367.43 – 1368.25 cm-1 (the C-H and CH2 aliphatic bending group), 1643.51 – 1644.26 cm-1 (the C=O amide I band), 2931.73 – 2932.09 cm-1 (the CH and CH2 aliphatic stretching group) and broadband at 3382.79 – 3386.89 cm-1 (the O-H carbohydrates proteins and polyphenols). The apparent alterations in functional groups of JSS starch were obtained only after ANN but not from HMT. JSS-ANN and JSS-ANNCA5 showed the peaks appeared between 2043 - 2045 cm-1 indicative of the thiocyanate group (2000 cm-1) (Moosvi et al., 2016).

The FTIR spectrum of the native JSS, JSS-HMT and JSS-HMT-CA5 samples showed the typical peaks at 1017.16 – 1017.93 cm-1 (the Si-CH=CH2 Trans C-H wagging vibration bond), 1080.93 – 1081.25 cm-1 (the C-O carbohydrate bond), 1155.77 – 1156.10 cm-1 (the C-O-C polysaccharide bond), 1365.61 – 1367.52 cm-1 (the C-H and CH2 aliphatic bending group), 1643.57 – 1644.25 cm-1 (the C=O amide I band), 2931.73 – 2932.32 cm-1 (the C-H and CH2 aliphatic stretching group) and broadband at 3382.34 – 3385.65 cm-1 (the O-H carbohydrates proteins and polyphenols). However, no variation in those peaks observed among native JSS and JSS after HMT or HMT-CA5.

4.3.9 Degree of Substitution

The degree of substitution (DS) was displayed in Table 4.19. Results revealed an overall increase in DS values of both ASS and JSS samples after CA treatments, hydrothermal treatments (ANN and HMT), and hydrothermal treatment combined with CA treatment. Among samples, it was found that the ASS-CA5 and JSS-CA5 samples exhibited the highest DS values among all treated samples. The increase in DS values was attributed to the substitution of hydroxyl (OH) groups within starch molecules due to citric acid esterification. The starch substitution primarily takes place at the hydroxyl groups situated on the carbon atoms along the starch chain. The greater DS values of either ANN or HMT combined with CA treatment were expected. Results however, showed the lower DS than CA treatment alone.

Table 4.19 Degree of substitution of ASS and JSS modified by ANN, HMT and incorporation of CA

Sample	DS
Avocado seed starch	
ASS-Control	0.000 ± 0.00^{d}
ASS-CA5	0.011 ± 0.01^{a}
ASS-ANN-CA5	0.004 ± 0.01^{c}
ASS-HMT-CA5	0.005 ± 0.01^{b}
Jackfruit seed starch	
JSS-Control	0.000 ± 0.00^{d}
JSS-CA5	0.020 ± 0.01^{a}
JSS-ANN-CA5	0.002 ± 0.01^{b}
JSS-HMT-CA5	0.001 ± 0.01^{c}

Note Mean values with different superscripts within the same column are significantly different according to the Duncan test ($p < 0.05$)

Overall, physical modification of ASS and JSS by hydrothermal treatments, ANN and HMT methods caused a significant alteration in physicochemical and functional properties of the two starch. Major changes in pasting characteristics and thermal properties were discovered. The combination of CA treatment could also induce these changes than hydrothermal treatment alone. The enhancement in starch crystalline structure and intermolecular bonding between starch chains were evident from XRD and FTIR results. The extreme heat stable starch was obtained from the combination of hydrothermal treatment and CA treatment. However, the possible applications in food industry needed to be considered.

CHAPTER 5

CONCLUSION

This study evaluated the physicochemical and functional properties of avocado seed starch (ASS) and jackfruit seed starch (JSS) through comparative analysis. It examined the effects of different milling techniques—both dry and wet—on these properties. Additionally, various starch modification methods were explored, including chemical modification using citric acid, physical treatments like annealing and heat-moisture treatment, and combined physical-chemical methods such as annealing with citric acid and heat-moisture treatment with citric acid.

For chemical modification, starches were mixed with citric acid at 5%, 10%, and 15%, then dried. A chemical reaction happened between the acid and the starch, shown by changes in the FTIR results. But the starch shape and crystal structure stayed mostly the same. At 10% and 15% acid, the starch became watery and formed weak gels. This means the starch granules were damaged. Only 5% acid made thick paste and strong gel, so it worked best.

For physical modification, starches were treated with heat using annealing (ANN) or heat-moisture treatment (HMT). HMT changed the starch shape more than ANN. It caused the starch to become less thick, form weak gels, and show changes in heating properties. Both methods made the starch more stable when heated. When chemical and physical methods were combined (5% citric acid + ANN or HMT), the starches did not form thick paste or stable gel. This showed stronger damage to the starch structure.

Overall, native starch of the two seeds, avocado and jackfruit seeds could be served as functional ingredients in food applications since they gave a viscous paste with a strong gel. Chemical, physical and a combination of physical-chemical modification of the two starches provided the less viscous paste with poor gel texture.

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