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Triple modification onto arrowroot starch production and its functional properties alterations

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Abstract. The aim of this research was to obtain information about resistant starch (RS) content on modified arrowroot starch (AS) and its functional properties as a consequence of modification to meet suitable application in the food industry. Two treatments were used to increase RS content in the AS i.e. through branched chain cutting of AS using *pullulanase* enzyme with certain concentration (1.3 U/g or 10.4 U/g of AS) followed by autoclaving (heating)-cooling (AC) process, and combination of these treatments with acid hydrolysis (AH). Results show that the modification process significantly ($p<0.05$) influenced solubility, swelling power, water holding capacity (WHC), freeze-thaw stability, and color of AS pasta, but not RS content. Nevertheless, the modification process can change the gel strength and viscosity of the arrowroot RS. It had lower viscosity than the control model, making it may be applied to food products that have low viscosity.

1 Introduction

In industrial applications, modified starch is often used to overcome several problems in the use of native starch [1]. For that purpose, recent studies have been undertaken to study starch modification from tuber crops such as cassava [2], corn [3], sweet potato [4,5], potato and taro [6], to gain better characteristics of the starch when applied as food ingredients. From all research carried out studying tuber and root crops and their starch modification, only a little research had been undertaken on arrowroot. It is understandable since this crop is scarce and available limitedly in many areas across the globe. Despite its low production, optimizing its properties to be better absorbed in the food industries needs to be conducted.

Arrowroot (*Marantha arundinacea* L.) is one of root crops which have potential to be used as carbohydrate source [7–9], particularly in the countries where wheat is not its primary crop [10]. Furthermore, it possesses a low glycemic index, making it suitable for consumption of certain people [8]. This root has high carbohydrate content (98.74%), particularly in the form of starch [11] and a lower glycemic index (GI = 14) compared other tubers [12].

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Therefore, arrowroot is suitable to consume by diabetics. However, utilization of arrowroot as functional food is still very limited [11].

Arrowroot has good quality of starch, one of which is that it has very delicate starch therefore it is easy to digest and can be used in the baby foods and food for special dietary use, for instance old-ages and ill peoples [13]. Due to its slightly low protein content (0.4%) [11], other ingredients are usually added, for example wheat flour in the preparation of bread or biscuits [14], red bean in snack bar [15], soybean in wet noodle [16], and others.

Increasing resistant starch (RS) content in arrowroot starch (AS) can help increase its utilization, especially in improving the nutritional functioning and health benefits of the final food product. This method works similarly to enhancing dietary fiber. The human upper digestive system's enzymes are unable to break down RS, but they can ferment in the colon yielding short-chain fatty acids [17].

Resistant starch (RS) is a low molecular weight linear polymer derived from α-1,4-D-glucan, originating from the retrograded fraction of amylose [18]. Resistant starch (RS) is categorized into five groups: 1) physically entrapped starch within plant cells and food matrices (RS1), 2) naturally occurring starch resistant to α-amylase enzyme (RS2), 3) physically modified starch (RS3), 4) chemically modified starch (RS4), and 5) starch derived from amylose and lipid gelatinized at elevated temperatures (RS5) [19,20]. Among all RS types, RS3 has undergone significantly greater development, as it may preserve the sensory attributes of a food system when starch is transformed into food products [21].

This study aimed to obtain information about RS3 content from AS that undergone modification by several processes i.e. acid hydrolysis, a cycle in which a process of autoclaving (heating) was followed by cooling, and the process of AS branched chain cutting using *Pullulanase* enzyme. Functional properties testing aimed to know more about the produced AS as a consequence of modification, therefore suitable application in the food industry can be chosen based on the research results.

2 Materials and Methods

Arrowroot (*Marantha arundinacea* L.) aged 10-12 months old were obtained from the farmer in Ciampea district, Bogor regency, West Java, Indonesia. Enzymes used were pullulanase (Sigma Aldrich E2412-50 mL, USA), amyloglucosidase (AMG) (Sigma Aldrich D7095-50 mL, Denmark), pancreatin enzyme (Sigma Aldrich P7545-100G, USA), reaction agent 3,5-DNS (Sigma Aldrich D0550-25G, India), 2.2 N of HCl, 1.2 M of NaOH, 0.1 M acetate buffer (pH 5.2), NaSO3, KNAC4H4O6H2O, C2H5OH, aquades, deionized water, glucose, and palm oil.

2.1 Starch Isolation [17]

The arrowroot was cleaned, peeled, and then cleaned once more for an hour before being submerged in water. Next, arrowroot was steeped and grated, adding water (1:3.5). The grated arrowroot was then filtered and deposited to isolate the starch. The starch was further purified and desiccated in an oven at 50 °C until entirely dry. After that, grinding was employed to the AS prior to sieving through a 100-mesh size-sieve. Subsequently, the starch was bottled and stored in a refrigerator at temperatures ranging from 4 °C and 10 °C for further use.

2.2 Modification process (enzymatic and physical modification of AS)

2.2.1 Branched chain cutting and autoclaving (heating)-cooling (AC) processes [22]

Up to 20% (b/v) of AS was solubilized with acetate buffer (pH 5.2), sterilized in an autoclave, and subsequently chilled at 4 °C for 24 hours. AS that had undergone one cycle of sterilizing-cooling was then heated at 50 °C, then *Pullulanase enzyme* with the concentration of 1.3 U/g or 10.4 U/g of starch was added at 50 °C, pH 5.2 for 24 h. Starch was then subsequently objected to the second cycle of heating-cooling then was dried using oven at 50 °C. Modified starch was then stored at 4 °C–10 °C.

2.2.2 Combination of acid hydrolysis (AH), branched chain cutting, and autoclaving (heating)-cooling (AC) [22]

2.2 N of HCl was used to hydrolyze 150 g of AS (2 h, 35 °C), neutralized using NaOH until reached pH 6.0, and dried at 50 °C. After that, 20% of hydrolyzed AS was then solubilized using buffer acetate at pH 5.2 and continued undergoing the similar protocol as it has been written above.

2.3 Determination of moisture and RS content

Moisture content of modified AS was analyzed using AOAC method (2006). RS content was analyzed according to the protocol that has been carried out previously [24].

2.4 Pasting properties [25]

The pasting properties of modified AS was analyzed using protocol of Charles et al., [25] to measure peak, holding, breakdown, final, and setback viscosities.

2.5 Functional properties

2.5.1 Gel hardness feature [26,27]

Gel hardness was analyzed using Wang et al., [27] and Astuti et al., [26] procedures, with slight of modifications.

2.5.2 Solubility and swelling power [28]

The solubility and swelling power were examined using a method developed by Ikegwu et al. [28].

2.5.3 Freeze-thaw stability [25]

Technique of Charles et al. [25] was used to determine the freeze-thaw stability.

2.5.4 Water holding capacity (WHC) and oil holding capacity (OHC) [29]

The modified AS's WHC and OHC were measured using Chau & Cheung's [29] technique.

2.5.5 Color of the starch powder and its pasta [30]

The Chromameter apparatus was used to analyze color using Milotta et al., [30] approach.

2.6 Statistical analysis

Statistical analysis was conducted using SPSS version 20.0. Four replications were used to complete each experiment. ANOVA was performed, followed by Duncan test to examine the significance at level of 5%.

3 Results and Discussion

3.1 Effect of physical and enzymatic modification on resistant starch yield

In this study, the modified AS samples had lower RS levels than the control starch (Table 1). Among the four types of modified samples, the treatment of AH-AC 1.3 U/g had the highest level of RS3 (96.25%), yet based on statistical tests, the level of RS in each sample was not significantly different. This indicated that the modification process had no significant effect on the RS3 starch content. Meanwhile, research that has been done by Faridah et al., [31] showed that the modification could increase fraction II in the form of amylose and short chain amylose, thereby the RS3 levels increased. This might be caused by the effect of the combination of 2 hours acid hydrolysis + autoclaving-cooling + 1.3 U/g branched chain cutting (using *Pullulanase* enzyme) conferring the highest percentage increase in fraction II. However, they reported that the RS3 levels were not increased when only acid hydrolysis treatment was employed.

Table 1. Composition of resistant starch (RS3) from modified AS in comparison with control

AS samples and the treatments	RS3 (%)
Control	97.54±0.18^{a}
AC 1.3 U *Pullulanase*/g of AS	95.89±2.33^{a}
AC 10.4 U *Pullulanase*/g of AS	95.71±2.24^{a}
AH-AC 1.3 U *Pullulanase*/g of AS	96.25±1.76^{a}
AH-AC 10.4 U *Pullulanase*/g of AS	95.93±2.03^{a}

^{a,b,c} Superscript differences within the same column are statistically significant (P<0.05)
AC = Autoclaving (Heating)-Cooling, AH = Acid Hydrolysis

Previous studies have been reporting that even though some of the researchers found if the starch modification can increase the level of RS3 [32,33] some of others found the contrary [26,34], particularly when the modification were only employing moderate temperature for heating (below 100 °C) and heating (autoclaving)-cooling cycles were not plenty enough to recrystallize the amylose chain. Consequently, this study concludes that the amalgamation of these treatments (chemical, physical, and enzymatic) does not inherently result in a change of RS3 content of AS.

Furthermore, the RS3 yield might have been caused by the plant botanical sources such as origin, soil and the climate condition [35], plant ages [36], as well as the harvesting season [37]. In this study, the RS3 level of the modified AS, which did not significantly differ from the control starch, was likely attributed to the insufficient degree of polymerization (DP) of amylose resulting from the modification process. The optimum amylose DP value for RS formation is around 10-40 [38]. Meanwhile, amylose DP below 10 might inhibit the formation of RS3 because it inhibit amylose recrystallization [39].

3.1.1 Pasting properties

Testing of pasting properties was undertaken once, on all samples on the same replication. Based on the test results, information about the viscosity was obtained under several conditions (Fig. 1). According to Tan et. al., [40], peak viscosity refers to the highest viscosity that starch can achieve as it undergoes autoclaving and holding at a temperature of 95 °C. Hold viscosity is the lowest viscosity that remains after autoclaving at the same temperature. Final viscosity is the viscosity that forms after pasta cools to 50 °C. Breakdown viscosity is the numerical value obtained by subtracting the hold viscosities from the peak viscosities, whereas setback viscosity is the numerical value obtained by subtracting final viscosity with the hold viscosity. The hold viscosity of a starch quantifies its ability to maintain stability at elevated temperatures, while the setback viscosity quantifies its capacity to undergo retrogradation [15]. Table 2 shows the gelatinization properties using Rapid Visco Analyzer (RVA) for each sample.

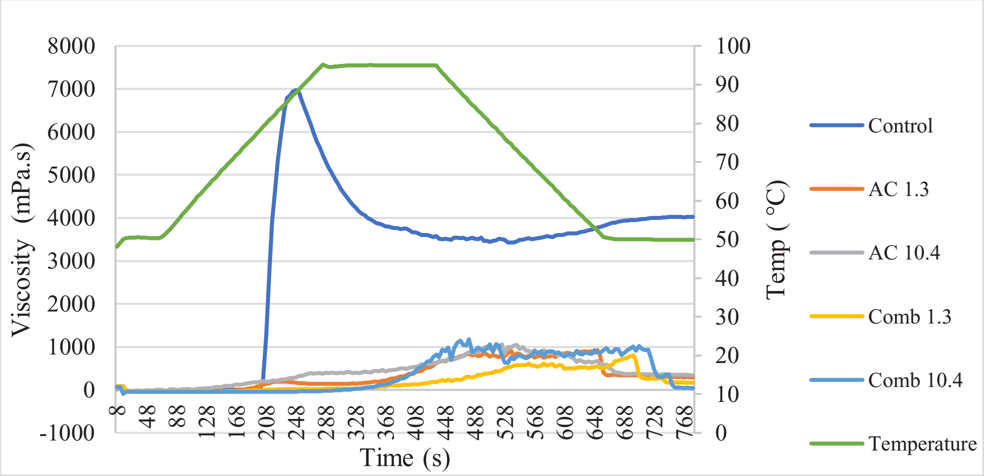

Fig. 1. Pasting properties of dual and triple modified AS

Table 2. Pasting properties of modified AS in comparison with control.

AS samples	Parameters				
	Peak viscosity (cP)	Hold viscosity 95°C (cP)	Final viscosity 50°C (cP)	Breakdown viscosity (cP)	Setback viscosity (cP)
Control	6973^{d}	3445^{d}	4024^{d}	3528^{e}	579^{d}
AC 1.3	864^{b}	368^{b}	294^{b}	469^{b}	74^{a}
AC 10.4	1077^{c}	525^{c}	345^{c}	552^{c}	180^{b}
AH-AC 1.3	469^{a}	124^{a}	164^{b}	372^{a}	40^{a}
AH-AC 10.4	1178^{c}	392^{b}	45^{a}	786^{d}	347^{c}

^{a,b,c} Superscript differences within the same column are statistically significant. (P<0.05)

Modified AS had the characteristic of lower viscosity, in comparison with the control. This reducing viscosity was likely caused by depolymerization of amylose and amylopectin particles decreasing the swelling power and ability of starch to form a gel [41]. Based on the RVA graph and its viscosity value (Fig.1), control starch and modified AS are classified as non-Newtonian fluids. On one hand, control starch is included in the dilatation group, which will experience an increase in viscosity along with stirring. On the other hand, modified AS is a type of pseudoplastic which will experience a decrease in viscosity with stirring [42].

In the control starch, the peak viscosity (PV) was reached in the autoclaving phase. PV of AH-AC 1.3, AC 10.4, and AH-AC 10.4 were achieved when the holding temperature was 95 °C. Meanwhile, the highest viscosity in the starch sample AH-AC 1.3 was achieved in the cooling phase, which was at 8.73 minutes. This shows that the achievement of peak viscosity is not only caused by an increase in temperature, but also by the stirring process. In addition, based on this result, the sample of AH-AC 1.3 has a low extensibility suggesting that the sample would be suitable for application to low-viscosity food products, for instance in soy milk and chili sauce.

3.1.2 Gel hardness feature

The test of gel hardness feature is intended to see the ability of starch to form and maintain gels [43]. The parameter in this test is the gel strength level, expressed as hardness, which is determined by using a texture analyzer equipped with a cylinder probe measuring 0.5 cm in diameter and 0.6 mm in penetration depth.

The measurement of the gel hardness after 6–168 hours of storage is presented in Fig. 2. The power to increase the gel hardness each starch sample is different. In general, the modified AS had a higher gel hardness level than the control starch, especially at 168 hours. Gel formation correlates with high amylose presence [43]. These linear polymers have the ability to form junction zones, recombine, and form fast hydrogen bonds [44]. In addition, the increase in gel hardness after the cold storage process was also influenced by amylopectin crystallization in starch pasta, which never formed junction zones [45]. This can be seen in Fig. 2, gel formation at 6-72 hours (control starch, AH-AC 1.3 and AC 10.4) and 6-24 hours (starch AH-AC 1.3 and AH-AC 10.4) may be influenced by amylose content in the sample. There was an increase in gel hardness after 72 hours (control starch, AC 1.3 and AC 10.4) and 24 hours (starch AH-AC 1.3 and AH-AC 10.4) apart from being influenced by amylose content, possibly caused by the formation of amylopectin crystals. In samples AH-AC 1.3 and AH-AC 10.4, the gel harness after 72 hours was no longer increased, this was probably due to the low amylopectin content in the samples due to the modification process.

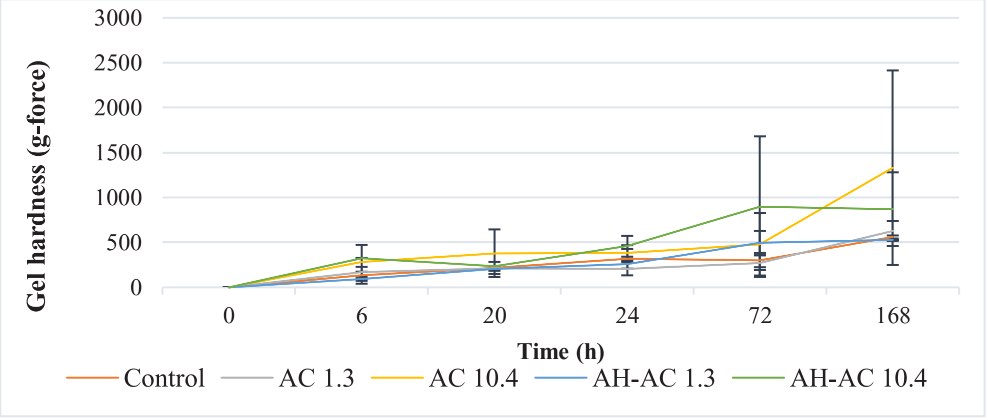

Fig. 2. Gel hardness properties of dual and triple modified AS

3.1.3 Solubility and swelling power

The levels of amylose and amylopectin not only affect the level of RS3 in the sample, but also its physical properties, including ability to dissolve (solubility) and expansion (swelling power). Research by Zi-Ni et. al., [46] showed that samples containing higher amylopectin had higher swelling power. This is because amylopectin has bulk properties and can expand more freely than amylose. From Table 3 it can be seen that the modified starch with lower levels of RS (AH-AC 1.3 and AC 10.4) had a greater swelling power than samples with higher levels of RS3 (AH-AC 1.3 and AH-AC 10.4). However, control starch with the highest levels of RS3 (97.54%) had the highest swelling power (868.72%). This is probably because the control starch naturally has a high amylopectin content. Based on statistical analysis, the modification process had a significant effect on the swelling power ($p<0.05$).

Table 3. Relationship between RS3 level towards solubility and swelling power of modified AS in comparison with control

AS Samples	RS3 Content (%)	Solubility at 95ºC (%)	Swelling power at 95ºC (%)
Control	97.54 ± 0.18^{a}	1.27 ± 0.13^{b}	868.72 ± 80.09^{c}
AC 1,3	95.89 ± 2.33^{a}	0.92 ± 0.04^{a}	581.81 ± 37.99^{b}
AC 10,4	95.71 ± 2.24^{a}	1.02 ± 0.06^{a}	566.92 ± 59.44^{b}
AH-AC 1,3	96.25 ± 1.76^{a}	$1.38\pm0.06^{b,c}$	432.16 ± 77.10^{a}
AH-AC 10,4	95.93 ± 2.03^{a}	1.45 ± 0.07^{c}	409.57 ± 39.91^{a}
^{a,b,c} Differences in superscripts within the same column are statistically significant ($P<0.05$)			

In general, solubility is negatively correlated with the level of RS3 [47]. Nevertheless, Ozturk et. al., [48] showed that the higher the RS level, the higher the solubility of the sample. The main factor that contributes to this is the processing conditions [46], one of which is the use of enzymes in the starch modification process [47]. The higher the level of RS3 prepared by involving the enzyme, the higher the solubility. This is because the *Pullulanase* enzyme works to cut the α-1,6 branched chain in amylopectin to produce short chain amylose and amylose which are more soluble in water [49]. Here, the results of statistical analysis showed that the modification process significantly affected the solubility ($p<0.05$).

Based on the graph of the pasting properties (Fig. 1) and the swelling power (Table 3), the relationship between the swelling power and the viscosity in each sample can be seen. The peak viscosity (PV) of the control starch was achieved when the autoclaving phase indicated that the starch had high swelling power. Samples AH-AC 1.3, AC 10.4, and AH-AC 10.4 reached PV when the holding temperature was 95 °C indicating the high swelling power of the samples. The sample AH-AC 1.3 reached PV during the cooling phase indicating the low swelling power of the samples. Table 4 shows that even though the expansion (swelling) power of AH-AC 10.4 is lower than AH-AC 1.3, but the value of the two is not statistically significant ($p>0.05$).

Table 4. Relationship between swelling power towards freeze-thaw stability (syneresis) of modified AS in comparison with control

Samples	Swelling power at 95ºC (%)	Syneresis (%)
Control	868.72 ± 80.09^{c}	0.00 ± 0.00^{a}
AC 1.3	581.81 ± 37.99^{b}	130.59 ± 29.88^{b}
AC 10.4	566.92 ± 59.44^{b}	164.84 ± 3.84^{c}
AH-AC 1.3	432.16 ± 77.10^{a}	161.37 ± 12.25^{c}
AH-AC 10.4	409.57 ± 39.91^{a}	193.19 ± 2.68^{d}
^{a,b,c} Differences in superscripts within the same column are statistically significant ($P<0.05$)		

3.1.4 Freeze-thaw stability

During frozen storage, starch molecules undergo reorganization resulting in syneresis, as a form of the release of water from the starch network [50]. Under freezing conditions, the water present in the starch pasta will solidify, and when kept at normal temperature, it will liquefy and detach from the starch structure (a process known as thawing). The higher the syneresis percentage of a sample, the lower the freeze-thaw stability. From Table 4 it can be seen that starch which has a higher extensibility has a lower percentage of syneresis. Although sample AH-AC 1.3 had a lower percentage of syneresis (161.37%) compared to sample AC 10.4 (164.84%), the value of the two was not significantly different (p>0.05).

The current investigation found that the control starch exhibited excellent freeze-thaw stability, as evidenced by its absence of syneresis (Table 5). Yet, the use of AS may also enhance the freeze-thaw stability in the food chain. Such is demonstrated by the study conducted by Charles et. al.,[25] on the composite flour made from arrowroot and tapioca (cassava) starch. Their findings indicated that the addition of AS in composite flour resulted in superior freeze-thaw stability compared to its absence.

Table 5. Water holding capacity (WHC) and oil holding capacity (OHC) of modified AS in comparison with control

Samples	RS3 content (%)	WHC (%)	OHC (%)
Control	97.54±0.18	83.44±6.56^{a}	103.80±27.76^{a}
AC 1,3	95.89±2.33	176.80±13.94^{c}	99.00±1.81^{a}
AC 10,4	95.71±2.24	168.47±21.85^{b,c}	103.94±19.83^{a}
K 1,3	96.25±1.76	159.14±24.39^{b,c}	100.54±5.12 ^{a}
K 10,4	95.93±2.03	137.73±10.93^{b}	98.91±4.33^{a}
^{a,b,c} Superscripts which differ within the same column are significantly different (P<0.05)			

3.1.5 Water holding capacity (WHC) and oil holding capacity (OHC)

Water holding capacity (WHC) quantifies the capacity of starch granules to engage with water molecules. RS3 generally consists of retrograded amylose formed by strong hydrogen bonds, which forms a water-insoluble crystalline structure [46]. The formation of this structure makes it difficult for RS3 to form bonds with water molecules so that the WHC samples have a negative correlation with RS3 levels [46]. This can be seen in **Table 5**. Control starch with the highest RS3 content (97.54%) had the lowest WHC value (83.44%). In general, the modification process can significantly increase the WHC value of the sample (p<0.05).

Samples with high levels of RS3 have high oil holding capacity (OHC) values [46]. Control starch with the highest RS3 content (97.54%) had the second highest OHC value (103.80%). Nevertheless, the statistical analysis revealed that the conversion procedure did not have a substantial impact on the OHC value of the sample.

3.1.6 Color

The brightness (L value) of the modified AS powder was lower than control (Table 6). These findings suggest that the alteration has the potential to decrease the luminosity of starch powder. This is corroborated by the study conducted by Faridah et. al.,[31] demonstrating that starch maintained protein content, albeit in a minimal quantity ranging from 0.34% to 0.84%. Nevertheless, the modified starch pasta exhibited a greater level of brightness compared to the control starch pasta. Quantitative study revealed that the colour brightness of starch powder did not exhibit a statistically significant difference. Conversely, the magnitude of the starch pasta color's brightness varied significantly ($p<0.05$).

Table 6. Values of L (Brightness) values of starch powder and starch pasta from modified AS in comparison with control.

Samples	AS powder (%)	AS pasta (%)
Control	102.30 ± 0.85^{b}	47.31 ± 1.58^{a}
AC 1,3	87.48 ± 6.17^{a}	58.29 ± 5.45^{b}
AC 10,4	89.25 ± 1.98^{a}	$65.09 \pm 2.35^{b,c}$
AH-AC 1.3	$92.49 \pm 5.53^{a,b}$	$61.98 \pm 6.35^{b,c}$
AH-AC 10.4	$91.37 \pm 9.29^{a,b}$	70.71 ± 7.81^{c}

^{a,b,c} Superscripts which differ within the same column are significantly different ($P<0.05$)

3.1.7 Compilation of data results

Statistical analysis of the data revealed variations in the concentrations of RS3 and the functional characteristics of the control starch and modified starch for each sample (Table 7). Each sample's quality depends upon the intended use of the food product. Although several functional characteristics of modified starch are inferior to those of control starch, modified starch can nevertheless be used in specific appropriate food products. Sample AC 10.4, AH-AC 1.3, and AH-AC 10.4 are suitable for utilisation in food items with low viscosity, such as soymilk products. Equally, the AH-AC 1.3 sample is suitable for use in food products with a moderate viscosity, such as chilli sauce products. Table 7 demonstrates that modified AS (AC 1.3 and AC 10.4) exhibits low solubility but possess a high-water holding capacity (WHC), which is an indicator of their dietary fiber function. This characteristic is consistent with other modified AS, indicating their potential for further development as sources of digestive fiber.

Table 7. Modified AS properties from modified AS in comparison control.

Samples	Moisture	RS3	Viscosity	Solubility	Swelling power	Freeze-thaw stability	WHC	OHC	Gel properties	AS color	Arrow-root pasta color
Control	Sub optimal	Elevated	Elevated	Medium	Elevated	Very stable	Sub optimal	Sub optimal	Good	White ++	Opaque
AC 1.3	Sub optimal	Elevated	Medium	Sub optimal	Medium	Less stable	Elevated	Sub optimal	Good	White+	White
AC 10.4	Sub optimal	Elevated	Sub optimal	Sub optimal	Medium	Less stable	Elevated	Sub optimal	Good	White+	White
AH-AC 1.3	Sub optimal	Elevated	Sub optimal	Medium	Sub optimal	Less stable	Elevated	Sub optimal	Good	White+	White+
AH-AC 10.4	Sub optimal	Elevated	Sub optimal	Medium	Sub optimal	Less stable	Elevated	Sub optimal	Good	White+	White +

4 Conclusion

The modification process did not significantly affect the moisture content, RS3 content and functional properties of modified starch, namely OHC, and starch powder color. Nonetheless, the modification process significantly ($p<0.05$) changed the functional properties of starch, namely solubility, swelling power, WHC, freeze-thaw stability, and starch pasta color. In addition, the modification process can change the gel strength and viscosity of the modified starch. Modified starch has a lower viscosity than control starch, therefore it has the bigger opportunity to be applied to food products that have low viscosity, for example chili sauce (AC 1.3) and soy milk (AC 10.4; AH-AC 1.3; and AH-AC 10.4).

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