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Production of Indonesian *Canna edulis* type IV resistant starch through acetylation modification

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Abstract

Edible canna *(Canna edulis)* is a potential forest product that can be used for food diversification. The objective of this study was to obtain Type IV resistant starch which has been proven beneficial because of its physiological effect. In this study, the production of resistant starch was done by chemical modification using different concentration of acetic anhydride, namely 3, 4, and 5%, in 300 g of canna. Two types of canna used in this study were red and white canna. The degree of substitution gained through acetylation was 0.082, 0.168, and 0.257 for red canna and 0.083, 0.168 and 0.256 for white canna. Through proximate analysis, modification of canna starch resulted in increase of water content and decrease of ash content, while protein content, lipid, and carbohydrate content showed no significant difference. Total starch content (amylose and amylopectin), dietary fiber, resistant starch content, and digestibility of starch were measured for native and the selected modified canna starch (4% acetic anhydride concentration). The result also showed that modification through acetylation increased the resistant starch content. It increased about 3.54% in red canna, while for the white one was about 3.80%. This influenced the increasing of total dietary fiber with value of 2.45% for red canna and 1.95% for white one. The increasing of dietary fiber also resulted in the decreasing of starch digestibility with value of 1.58% for red canna and 1.98% for white one.

Introduction

Indonesia has a great potential in non-wood forest product, for example *Canna edulis* or edible canna that has several health benefits. Canna tubers contain high amount of carbohydrates. It can be consumed as a source of energy supply for the body. Converting the tubers into flour and starch can be highly prospective in terms of functional properties and chemical composition.

Chemical modification of starch is performed to improve its properties. Extracted starch from canna tubers can be chemically treated using acetic anhydride to produce type IV resistant starch. Resistant starch has characteristics similar to dietary fiber, which resists to hydrolysis and digestive enzymes. It cannot be digested in the small intestine but can be fermented in the colon, thus it can be classified into the food fiber (Nugent, 2005). The process of chemical modification by acetylation of the starch has been reported to decrease the digestibility of starch and increase the levels of resistant starch. According to Sajilata *et al.* (2006), resistant starch has good physiological functions for the body, which can lower the glycemic index, cholesterol and reduce the risk of colon cancer. This type of starch has better properties, so it is widely applied in the food industry as an adjuvant for certain food products.

This study was aimed to produce and analyze chemically modified starches derived from edible canna. Analyses were conducted on the proximate analysis and the degree of substitution followed by analyses of total starch (amylose and amylopectin), dietary fiber, resistant starch content and digestibility of the native and modified canna starches.

Materials and Methods

Extraction of canna starch

Canna tubers aged of 5-10 months was obtained from Bogor, Indonesia. Starch extraction was carried out referred to Lingga *et al.* (1986). Canna starch was made through stages of stripping, washing, soaking, extraction, precipitation, drying, milling and screening. After being washed and peeled, tubers were soaked for one hour in order to soften the tissue because canna bulbs have high fibers. The grate was aimed to destroy tissues and cells so that the starch can easily come out. The process of extraction was done by separating the water and starch from the pulp. The suspension was formed after 12 hours. The precipitation of the starch was formed at the bottom

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and water at the top layer. Drying of precipitation was done with an oven with temperature of 50°C for six hours. Chunks of milled starch formed were blended and filtered through 60 mesh sieve. It was ready to be used in the modification process.

Preparation of chemically modified starch

Chemical modification of starch was conducted following to Patent EP 1629728 B1. The red and white canna starches were reacted with different concentration of acetic anhydride (3%, 4% and 5%, on the starch dry weight).

Proximate analysis

Proximate analysis was referred to the established standards, including moisture content (AOAC, 1995), ash content (AOAC, 1995), protein content by Kjeldahl method (SNI 01-2891-1992), fat content by Soxhlet method (AOAC, 1995) and carbohydrate content by difference.

The degree of substitution

This analysis was conducted to determine the amount of acetyl groups which have been substituted. It was referred to Chen and Voregen (2004).

Total starch content

Total starch content was determined by hydrolysis of starch with acid and the determination of reducing sugars was determined by the Anthrone method. It was referred to the method developed by Apriyantono *et al.* (1989).

Amylose content

Amylose content was determined by the standard curve equation obtained. It was referred to Apriyantono *et al*. (1989).

Dietary fiber content

Dietary fiber content was determined as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). It was referred to AOAC (1995).

Resistant starch content

Resistant starch content was determined enzymatically, referred to AOAC (1995).

Starch digestibility

Starch digestibility was enzymatically determined referred to the method developed by Muchtadi *et al.* (1992).

Results and Discussion

Extraction of canna starch

The varieties of red and white canna were used in this study. The starch yield was calculated from the weight ratio of the starch and tubers dry weights that have been peeled. It can be influenced by the age of the tubers. White canna starch yield was higher than the red one. The yield of starch from white canna was 10.39%, while from the red one was 9.76%. The white canna is more common to be extracted to produce starch because it has a higher yield. The red one is more common to be processed immediately. However, farmers prefer to plant the red type in Bogor, Indonesia.

Water content measurements were performed to determine the water contained in the starch. The low water levels causing microbes difficult to live, and therefore contribute to the storage period. This was in accordance with Winarno (2004), the water content in food ingredients will determine the acceptability, freshness, and durability of the material. Based on the results, it can be seen that the water content ranged from 5.94 – 9.05%. The shelf life of the flour with moisture content below 14% is one year (Rahayu *et al*., 2003). Canna starch with lower water content can be kept longer at room temperature.

Modification of Type IV resistant starch

The goal of starch modification is in order to produce better properties, especially the physicochemical and functional properties, or to change other properties (Saguilan, *et al*., 2005). The development in starch chemical modifications has been introduced to the food, pharmaceuticals, and textiles industries (Abbas *et al.*, 2010).

In this study, type IV resistant starch from canna bulbs was made by reacting starch with acetic anhydride. The concentrations of acetic anhydride were 3, 4, and 5%, on the starch dry weight. Acetylation method was done commercially to produce acetylated starch with a low degree of substitution by using acetic anhydride in alkaline pH (Xie *et al*., 2005). The reaction between starch and acetic anhydride will break the hydrogen bonds and the hydroxyl group will be replaced by an acetyl group. Acetic anhydride was chosen as a modifier because it was more reactive. This was in accordance with Hart *et al.* (2003) that the anhydride is more nucleophile than an ester, but less reactive than acyl halides. Sodium hydroxide acted as catalyst in modification reaction. It increased the initial reaction velocity (Villalobos and Feria, 2008). Introduction of these acetyl groups reduces the bond strength

Table 1. Chemical composition of native and modified canna starch

Chemical Composition (% dw)	Sample							
	Red				White			
	Red Canna	MRCS 3% AA	MRCS 4% AA	MRCS 5% AA	White Canna	MWCS 3% AA	MWCS 4% AA	MWCS 5% AA
Water content	8.81	16.83	16.81	16.95	6.49	16.51	17.07	14.69
Ash Content	0.41	0.30	0.23	0.27	0.37	0.36	0.36	0.31
Protein	0.89	0.86	0.98	1.16	0.45	0.50	0.69	0.58
Fat	0.79	0.74	0.62	0.75	0.67	0.63	0.71	0.86
Carbohydrate	97.88	98.10	98.17	97.82	98.53	98.51	98.25	98.25

Note: MRCS: modified red canna starch; MWCS: modified white canna starch; AA: acetic anhydride; dw: dry weight

between starch molecules and thereby alters the properties (Rutenberg and Solarek, 1984).

Acetic anhydride is widely used in industry to produce acetate cellulose, acetate fibers, plastic fibers and layers of fabric (Celanese, 2010). Acetylated corn starches with various degree of substitution (DS) were achieved by multiple treatments with acetic anhydride under alkaline conditions (Ayucitra, 2012). It was observed that the changes in some physical starch properties were proportional to the DS achieved by acetylation (Ayucitra, 2012).

Proximate analysis of native and acetylated canna starch

Proximate analysis was done to test raw materials to be processed further into finished goods in the industry (Mulyono, 2000). The result was showed in Table 1.

Water content

The water content in food affects the durability of the food against the microbe, expressed as water activity (Aw). Water content refers to the amount of free water that can be used by microorganisms for growth (Winarn, 2004). The greater the water content, the greater the value of Aw. This means that the weaker the grocery durability against invading microorganisms, thus the shelf life was shorter. Table 2 showed that the water content of red and white canna starch was low enough. It was below 14%. It can be stored for a period of one year storage. Meanwhile, the water content of modified starches was much higher than that of native starches. After modification, it was difficult to achieve lower water content only by air drying.

Ash content

Ash content indicates the amount of mineral content in the material. The results of this study showed a reduction in ash content after modification. This value meets Indonesia National Standard (SNI 01-6057-1999) with the maximum limit is 0.5%. However, the ash content was still higher compared to other studies. The ash content of canna starch ranged from 0.19 to 12.23% (Damayanti, 2002), and 0.2% (Richana and Sunarti, 2004). The difference may be caused by differences in the canna varieties, fertilizer application, and the soil of growth place. It also can be affected by the processing technology of the starch. Canna starch was obtained by extraction and repeated washing with water. The washing can cause minerals dissolve. The slight difference between native canna starches with its modified forms was also caused by leaching and filtering back when the modification process took place.

Protein and fat content

Protein and fat are minor components in the starch. Otherwise, the protein determines the properties of the starch. In this study the protein content of starch ranged from 0.45 - 1.16% (dry weight). The range was quite large when compared with Damayanti (2002), canna starch protein content ranged from 0.44 - 0.54% (dry weight), and quite high when compared with the results Chansri *et al.* (2005) about of 0.21-12.33% (dry weight).

The fat content ranged from 0.6 - 0.8% (dry weight). Richana and Sunarti (2004) produced starch with fat content of 0.75% (dry weight), while Damayanti (2002) produced about of 0.37-0.72% (dry weight). However, when compared between the two types of cultivars, red canna had the higher protein and fat. Although the high fat and protein content can complement the nutritional content in starch but its existence is not expected due to lower levels of resistant starch. Several components in food interact with starch and ultimately affect the formation of resistant starch include: protein, dietary fiber, enzyme inhibitors, ions, and lipids (Sajilata *et al.*, 2006).

Table 2. Composition of amylose and amylopectin

Sample	Total Starch (%dw)	Amylose (%dw)	Amylopectin (%dw)
Red Canna Starch	87.33	24.06	63.27
White Canna Starch	86.59	25.54	61.05
Modified Red Canna Starch	86.60	24.76	61.84
Modified White Canna Starch	86.07	26.24	59.83

Note: dw: dry weight

Carbohydrate content

Determination of carbohydrates by difference yielded an estimate of the overall amount of carbohydrates, both simple and complex carbohydrates. The study showed that the carbohydrate content of white canna was higher than the red one. Therefore, white canna is more suitable used as a raw material for resistant starch. It gave a higher yield, had a lower protein and fat content and the higher carbohydrate content. However, the differences were not seen between the carbohydrate content of native and modified starch. This was because of all carbohydrates were calculated, either complex or simple.

Analysis of degree of substitution (DS)

The substituted acetyl group assay was conducted to evaluate the modified canna starch by acetylation. The results showed that the higher the acetic anhydride concentration was given, the more the -OH group was substituted by an acetyl group. The treatment of 3%, 4% and 5% acetic anhydride to the canna starch gave the degree of substitutions about 0.082, 0.168 and 0.257 for red canna and 0.083, 0.168 and 0.256 for white canna. According Wurzbug (1995) in Stephen (1995) acetyl group can be linked to the degree of substitution. Each one glucose molecule contains three hydroxyl groups which can be substituted on the atom C No. 2, 3 and 6. This result suspected that in the red and white canna contain the same number of the hydroxyl group which can be substituted. High levels of acetyl provide a high degree of substitution. It was increased as the concentration of acetic anhydride increased. It is not only led to a high degree of molecular collisions but also the availability of acetic anhydride molecules around the starch (Xu *et al*., 2012).

According to US Food Drug Administration (FDA), the starch acetate is allowed to use for improving binding, thickening, stability, and texturizing of food in the value of DS from 0.01 - 0.2. The low degree of acetylation can increase the strength of starch such as viscosity, stability, and texture of the starch (Saputro *et al*., 2012). According to this study the treatment of 5% acetic anhydride was not allowed to apply as food material.

Based on the value of the degree of substitution obtained, a sample with 4% acetic anhydride treatment was then selected to detect resistant starch, dietary fiber content, and the digestibility. The value of the degree of substitution is proportional to the increasing resistant starch and the fiber content of the food. It lowers the starch digestibility. The resistance properties depends on the degree of substitution with carbon atoms located in close proximity to the hydrolyzed glucosidic α-1,4-bond (Zieba *et al*., 2011).

Amylose and amylopectin content

Starch is an important form of polysaccharides in plant tissue. It is stored in the form of granules in the chloroplasts of leaves and in the seeds and tubers (Sajilata *et al*., 2006). Starch is composed by amylose and amylopectin. The total starch content of four types of samples did not significantly differ, but there was a slight decrease in total starch content in the modified starch (Table 2).

According to Aliawati (2003), amylose content in starchy foods are classified into four groups: very low amylose content with level of less than 10%, low amylose content about of 10-20%, medium amylose content about of 20-24%, and high amylose content with level higher than 25%. Based on the classification, the results of this present study showed that red and white canna starches were classified as high amylose content starches. Amylose content in the modified white canna starch was higher than the red one. Amylose has a long straight chain, making it more difficult to be degraded by enzymes compare to amylopectin, which has more branches. Amylose has a 1,4-glycoside bond of α-unbranched amylose led it harder and harder to digest (Parker, 2003). Therefore, the amylose is more widely used as raw material for resistant starch. The high resistant starch content correlated to high amylose content (Shu *et al*., 2007).

Generally, amylopectin content is higher than amylose. The result showed that after modification there was an increase in amylose content and a reduction in amylopectin content, but only in a few amount. However, the modification did not alter amylose and amylopectin content, but only change its structure. The modification changed the structure of amylose became more resistant. The hydroxyl group was replaced by an acetyl group. Therefore, the substitution did not increase the total content of amylose and amylopectin. Anggraini (2007) also mentioned that the process of type III and type IV

Table 3. Dietary fiber content in native and modified starch

Sample	IDF (% dw)	SDF (% dw)	TDF (% dw)
Red Canna Starch	3.21	4.98	8.19
White Canna Starch	3.60	4.98	8.59
Modified Red Canna Starch	3.82	6.82	10.64
Modified White Canna Starch	4.54	5.99	10.54

Note: dw; dry weight; IDF : insoluble dietary fiber; SDF: soluble dietary fiber; TDF : total dietary fiber

resistant starch only alters the structure of amylose and not its content.

Resistant starch content

Resistant starch has a smaller size than conventional particle size of dietary fiber. Resistant starch showed lower binding capacity (water holding capacity). It can be used to improve the texture, appearance and mouth feel of food products (Sajilata *et al*., 2006). Therefore, resistant starch has more advantages, giving functional effects such as dietary fiber while maintaining the natural acceptability.

This study indicated that the resistant starch content increased with the presence of modification process. White canna had resistant starch bigger than the red one. It was about of 1.76% (dry weight) for red canna and 3.50% (dry weight) for the white one. After modification, there was an increase in the resistant starch content. Modified red canna had resistant starch about 5.30% (dry weight), whereas the white one had about 6.37% (dry weight).

Ratio of amylose and amylopectin influence the resistant starch content (Sajilata *et al*., 2006). White canna starch has higher amylose content, and therefore has higher levels of resistant starch after modification. Each type of starch has different amylose and amylopectin content ratio. The different characteristics of each type of starch is affected by the botanical source, the shape and size of the starch granules, amylose and amylopectin ratio, the content of non-starch components, as well as crystalline and amorphous structures (Mali *et al*., 2005). High content of resistant starch on canna starch is related to the crystalline structure of type -B in the original native starch, such as raw potatoes, bananas and high-amylose corn starch (Hung and Morita, 2005). Crystalline region is formed by amylose molecules, whereas amorphous region is formed by amylopectin.

Dietary fiber content

Physiologically, dietary fiber is defined as component of plants that are not degraded enzymatically into sub-units, which can be absorbed by the stomach and small intestine (Winarno, 2004). The modification produce resistant starch which have physiological effects that are beneficial to health (Sajilata *et al*., 2006). Nugent (2005) stated that resistant starch has characteristics similar to dietary fiber. It resists to hydrolysis and digestive enzymes. The starch cannot be digested in the small intestine but can be fermented in the colon. Therefore, resistant starch is also classified as dietary fiber.

Total dietary fiber is the sum of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). SDF can be dissolves in water, including pectin and gum. IDF is composed by cellulose, lignin and hemicellulose (Andarwulan *et al*., 2011). The study showed that the total dietary fiber of modified starch was higher than the native (Table 3). SDF and IDF also contributed to the increase of total dietary fiber. Haralampu (2000) stated that resistant starch will be detected as insoluble dietary fiber, but its physiological function acts as soluble fiber. The main property that distinguishes SDF and IDF is its ability to absorb water. SDF can absorb water and form a gel. Therefore it will inhibit stomach emptying. On the other hand, the IDF is not soluble in water and does not form a gel, thus the fiber may pass through the digestive tract relatively intact.

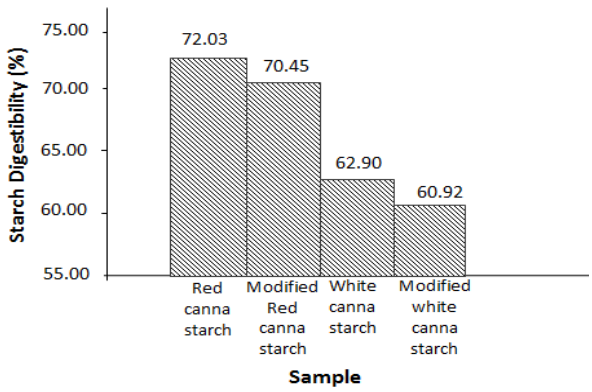

Figure 1. Native and modified canna starch digestibility

Starch digestibility

Starch digestibility is a degree of starch access. It measures the ease of starch to be hydrolyzed by the breaking enzymes into smaller molecules that can be absorbed by the body. Figure 1 showed that modified white canna starch had the lowest digestibility. The increasing of resistance against of hydrolysis is associated with increased levels of resistant starch. The modification increased the resistant starch content by replace the hydroxyl groups on the starch with an acetyl group. Therefore, the α-amylase enzyme cannot recognize the modified starch substrate.

Conclusion

The treatment of modification with acetic anhydride on red and white canna starches increased the resistant starch content. Therefore, total dietary fiber of canna starch also increased. The increase in dietary fiber resulted in the decrease of starch digestibility of modified canna starch. White canna is potential to be used as raw material of resistant starch because it had starch yield, carbohydrate content, and amylose content higher than that of red canna.

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