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FULL PAPER

Physical and chemical characteristics of enzymatically modified *Batu* banana (*Musa balbisiana* Colla) and *Kepok* banana (*Musa paradisiaca formatypica*) flours

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Abstract

Batu (*Musa balbisiana* Colla) and *Kepok* (*Musa paradisiaca formatypica*) bananas are two food commodities with high resistant starch content. The later has been suggested to prevent colorectal cancer. Modifications of these, into flour via the addition of pullulanase enzymes and autoclaving-cooling method are expected to improve the quality. The physical characteristics, levels of resistant starch, amylose, amylopectin and *in vitro* digestibility of enzymatically modified banana starches were determined. Therefore, a descriptive study was performed with untreated *Batu* banana flour (TP), autoclaving-cooling (AC), autoclaving-cooling + pullulanase enzyme (AC + E), and autoclaving-cooling + pullulanase enzyme + autoclaving-cooling (AC + E + AC). The granular morphology, water absorption, and swelling ability were evaluated as physical characteristics. Amylose, amylopectin content, and starch digestibility were evaluated as chemical characteristics. Based on SEM, the analysis of granular morphology showed structural modification in both treatments, although *Batu* banana flour showed greater water absorption and swelling ability, with and without AC + E + AC. However, both samples demonstrated better outcomes, in terms of high levels of starch resistance, low amylose, high amylopectin, and low *in vitro* digestibility after treatment with AC + E + AC.

1. Introduction

Colorectal cancer is one of the most common cancer events, and the third-largest cause of cancer-related death in men and women worldwide, with 130,000 new cases diagnosed each year. This incidence is expected to increase by 60% in the year 2030 (Birt and Philips, 2014), especially in developing countries. The incidence in Indonesia was 12.8 out of 100,000 adult populations, with a mortality of 9.5% total cancer cases. In 2013, the estimate of 70,000 new cases was reported, with around 25,000 related deaths (Rojas-Puentes *et al.*, 2014). Cumulatively, the risk of colorectal cancer was projected at 1 in 20 people (5%) (Kemenkes, 2017).

Colorectal cancer is an abnormality of cells of the large intestinal tissue, comprising the colon and rectum. In addition, data from the American Cancer Society reported the existence of two risk factors, encompassing the modified, including obesity, unbalanced diet, low physical activity, smoking, as well as long-term alcohol consumption, and the non-modifiable, comprising age, race, family history, and a history of chronic inflammatory diseases of the intestine (American Cancer Society, 2017). The study hypothesis by O'Keefe *et al*. (2015) used to explain these risks is determined by the possible interaction between diet and microbiota. Moreover, the high incidence amongst the group resulted from the low intake of fiber and resistant starch, alongside high saturated fat consumption (O'Keefe *et al*., 2015).

Resistant Starch (RS) is defined as a starch fraction

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or degradation product that resist digestion in the small intestine and are also resistant to hydrolysis by digestive enzymes (Shin *et al.,* 2004; Fuentes-Zaragoza *et al*., 2010). However, starch has also been grouped as a form of dietary fiber (Musita, 2009; Fuentes-Zaragoza *et al*., 2010). Starch also has the advantage to serve as a prebiotic, compared to Fructo Oligosaccharide (FOS) and inulin, resulting from the ability to bind and maintain fecal water level. This material, therefore, is unable to cause constipation and flatulence after consuming large quantities (Nugent, 2005; Setiarto *et al*., 2015). The numerous health benefits of RS including glycemic response reduction, ability to lowers blood cholesterol, serve as a functional probiotic, and also cause an increase in the production of short-chain fatty acid (SCFA) (Nugent, 2005). This is particularly generated through fermentation in the intestine and is characterized by the ability to inhibit cancer cell growth (Yafeng *et al*., 2016), as seen with butyric acid (Nugent, 2005; Ahmad *et al*., 2014), which hinders proliferation in the affected colon. Also, there have been reports on the ability to induce apoptosis *in vitro* and *in vivo* by inhibiting Histone Deacetylase (HDAC) enzyme activity (Purwani *et al*., 2012). Moreover, starch resistance in food elevates intrinsic physiological effects, including the tendency to be fermented by commensal intestinal bacteria (Musita, 2009).

This starch staple is identified in rice, potatoes, corn, beans, and bananas. Of interest, bananas were selected as a raw material in this study, due to one of the horticultural commodities that are very likely be used in food diversification and food security in Indonesia (BPTP Lampung, 2011). Banana flour is prepared from unripe banana into a semi-processed product by using the drying method to possibly overcome the perishable characteristics. This is an alternative intermediate form recommended due to the long shelf-life, ease of mixing (made composite), fortification, formation, and cooking, according to the demands of modern practical life (Radiena, 2016). In addition, in this investigation, we used the bananas *Batu* (*Musa balbisiana* Colla) and *Kepok* (*Musa paradisiaca formatypica*) varieties. These two varieties of bananas have high resistant starch content of 39.35% and 27.70%, respectively (Musita, 2009). Furthermore, yellow *Kepok* bananas are able to yield brighter and whiter flour compared to others (Putri *et al*., 2015).

The resistant starch level is influenced by the amylopectin to amylose ratio, enzyme pullulanase and starch concentration, temperature, heating and cooling cycle (autoclaving-cooling), storage conditions, and the presence of lipids or substances with low molecular, e.g., sugar (Fuentes-Zaragoza *et al*., 2010). The resistant property is influenced through the use of autoclaving-cooling and pullulanase, an enzyme adopted to disrupt the branching bonds of amylopectin (debranching) molecule, to produce raw materials in the form of short-chain amylose (Reddy *et al*., 2008). This enzyme also helps in the innovation of food production (Reddy *et al*., 2015). In addition, autoclaving-cooling treatment is adopted because of the ability to reduce starch digestibility and increase type III resistance (Sugiyono *et al*., 2009). Hence, a combination of both techniques is expected to yield methylated amylose, and consequently elevate the level of RS (Nurhayati *et al*., 2014).

There have been limited applications of pullulanase enzymatic modifications together with autoclaving-cooling. This study, therefore, aimed to evaluate the effect of enzymatic treatment on *Batu* and *Kepok* banana flours, based on physical characteristics (microstructure and morphology, water absorption, and swelling ability), resistance, amylose and amylopectin content, and *in vitro* digestibility. Based on the literature review, the modifications are expected to improve product characteristics.

2. Materials and methods

2.1 Preparation making banana flours

Furthermore, *Batu* banana was obtained from Rasamala Market, Semarang city of Central Java Indonesia, while the *Kepok* variety was procured from Jangli Market, Semarang city of Central Java Indonesia. Both samples were peeled and sliced with a thickness of±1 cm before processed into flour by drying in a microwave for 20 mins, followed by sieved with 80 mesh sieves become the flour. The samples were exposed to four treatments, including blank, without treatment (TP), use of autoclaving-cooling (AC) methods, autoclaving-cooling + pullulanase enzyme (AC + E), and autoclaving-cooling + pullulanase enzyme + autoclaving-cooling (AC + E + AC).

2.2 Determination of physical and chemical characteristics

The data collected include physical characteristics in forms of Scanning Electron Microscopy (SEM) sightings, with 1000-5000x magnification, while absorption and swelling ability were evaluated using the method by Saragih (2013). In addition, the resistant starch test was performed three times, using the approach of Kim *et al*. Iodine Colorimetry and method of difference was used to evaluate amylose, and amylopectin levels, respectively, while starch digestibility was assessed *in vitro* with the Association of

Official Analytical Chemists (AOAC) method.

3. Results

Table 1 shows the physical characteristics, in terms of water absorption and swelling ability in each treatment of *Batu* and *Kepok* banana flour. Based on Table 1, there was an increase in physical characterization (i.e. the forms of absorbency) of *Batu* banana flour between treatments, and the highest value of 69±1.41% was observed with AC + E + AC treatment. However, the untreated samples produced the lowest water absorption of 33.0±1.41% and least swelling ability at 0.30±0.14 cm/g. This parameter was particularly high (0.67±0.25 cm/g) in the flour samples exposed to AC + E method, while AC + E + AC produced a lower value at 0.50±0.14 cm/g.

In contrast, the *Kepok* banana flour showed a proportional decline in absorption and swelling ability during the treatment provided. However, the highest values were recorded in untreated samples at 37.67±4.04% and 1.60±0.28 cm/g, respectively. Conversely, the lowest records, 31.33±4.93% and 0.85±0.70 cm/g were found in the combination of *Kepok* banana flour with AC + E + AC.

The modification process changes the microstructure of compounds contained in starch and was confirmed using SEM micrograph analysis. Figure 1 shows the result of *Batu* banana flour exposed to various treatments while the *Kepok* variety was represented in Figure 2. Figure 1 shows SEM results (3000x magnification) of (a) *Batu* banana flour samples with untreated treatment (TP), (b) autoclaving-cooling (AC), (c) autoclaving-cooling + pullulanase enzyme (AC + E), and (d) autoclaving-cooling + pullulanase + autoclaving-cooling enzyme (AC + E + AC) while Figure 3 shows SEM results (1000x magnification) of *Kepok* banana flour samples with untreated treatment (a), autoclaving-cooling (b), autoclaving-cooling + pullulanase enzyme (c), and autoclaving-cooling + pullulanase + autoclaving-cooling enzyme (d).

Table 2 shows the sample resistant starch levels between treatments, and the value for *Batu* banana flour increased by 52.77±7.43%, after using the AC method. However, a decline was observed after exposing the sample to AC + E at 46.71±0.41%, compared to the control. Between treatments, the highest outcome at 52.95±6.32% was reported in the AC + E + AC treatment. Moreover, the amylose content was highest at 17.77±0.81% in *Batu* banana flour treated with AC + E +

Table 1. Characteristics of banana flour in various treatments: swelling ability of banana flour and water absorption of banana flour

Treatment Variation	Swelling ability (cm/g)		Water absorption (%)	
	Batu Banana Flour	*Kepok* Banana Flour	*Batu* Banana Flour	*Kepok* Banana Flour
TP	0.30±0.14	1.60±0.28	33.00±1.41	37.67±4.04
AC	0.45±0.07	1.25±0.70	37.50±3.53	33.33±4.93
AC + E	0.67±0.25	0.95±0.70	43.00±4.24	32.33±4.93
AC + E + AC	0.50±0.14	0.85±0.70	69.00±1.41	31.33±4.93
P-value	p=0.287**	p=0.027*	p=0.001*	p=0.182**

*One Way ANOVA Test **Kruskal Wallis Test

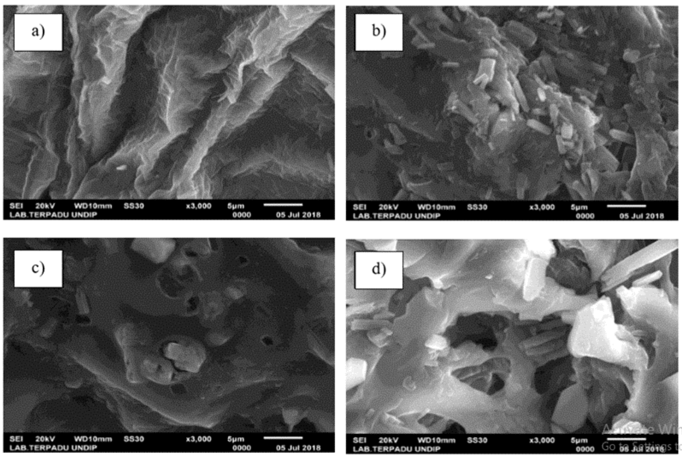

Figure 1. SEM results (3000x magnification) of *Batu* banana flour samples with untreated treatment (a), autoclaving-cooling (b), autoclaving-cooling + pullulanase enzyme (c), and autoclaving-cooling + pullulanase + autoclaving-cooling enzyme (d).

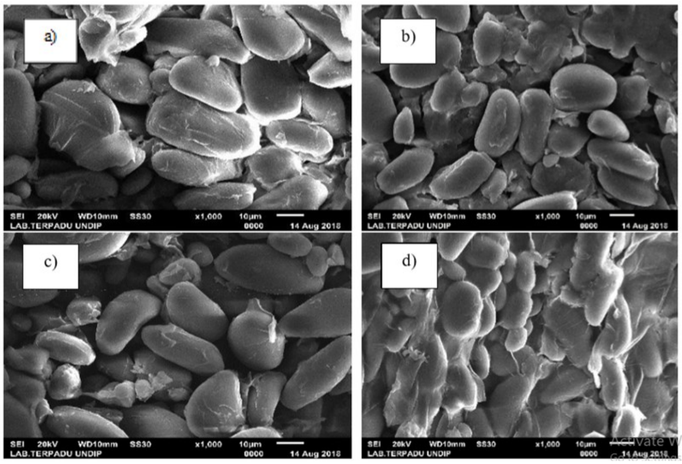

Figure 2. SEM results (1000x magnification) of *Kepok* banana flour samples with untreated treatment (a), autoclaving-cooling (b), autoclaving-cooling + pullulanase enzyme (c), and autoclaving-cooling + pullulanase + autoclaving-cooling enzyme (d)

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Table 2. Resistant starch level, amylose, amylopectin and *in vitro* starch digestibility in various treatment toward *Batu* banana flour

Treatment Variation	Resistant Starch Level (%)	Amylose Level (%)	Amylopectin Level (%)	*In Vitro* Starch Digestibility (%)
TP	50.47±9.16	0.97±0.9	99.03±0.092	62.54±0.28
AC	52.77±7.43	3.42±0.41	96.58±0.42	42.49±0.64
AC + E	46.71±0.41	4.75±1.62	95.25±1.63	48.62±0.37
AC + E + AC	52.95±6.32	17.77±0.81	82.22±0.81	41.79±0.08
P-value	p=0.775*	p≤0.001*	p≤0.001*	p≤0.001*

*One Way ANOVA Test **Kruskal Wallis Test

Table 3. Resistant starch level, amylose, amylopectin and *in vitro* starch digestibility in various treatment toward *Kepok* banana flour

Treatment Variation	Resistant Starch Level (%)	Amylose Level (%)	Amylopectin Level (%)	*In Vitro* Starch Digestibility (%)
TP	39.00±0.00	4.92±0.70	95.08±0.70	13.60±0.05
AC	46.50±3.25	10.38± 0.42	89.62± 0.42	12.33 ±0.07
AC + E	49.20±2.26	13.53± 0.41	86.47±0.41	10.64±0.10
AC + E + AC	55.80±0.84	15.47±0.42	84.53±0.42	10.91±0.30
P-value	p=0.005*	p=0.018**	p=0.018**	p≤0.001*

*One Way ANOVA Test **Kruskal Wallis Test

AC, while untreated samples had the least value of 0.97±0.90%. Conversely, amylopectin level reduced between treatments, as samples without treatment demonstrated the highest levels of 99.03±0.10%, and a decline was observed in treatments with AC + E + AC, showing the least outcome of 82.22±0.81%.

The values of *in vitro* starch digestibility also fluctuated between treatments, as AC method led to a reduced value, at 42.49±0.64%, compared to non-treatment (62.54±0.28%). Moreover, samples exposed to AC + E demonstrated an increase, at 48.62±0.37%, compared to the AC alone, while AC + E + AC generated the lowest outcome of 41.79±0.08%.

Based on Table 3, TP samples produced the lowest starch level at 39±0.00%, while the highest was found in the *Kepok* variety treated with AC + E + AC (55.8±0.84%). The TP amylose level was lower than others, at 4.92±0.70%, while treatments with AC + E + AC generated the most significant levels up to 15.47±0.42%.

The TP samples had relatively higher amylopectin levels compared to other treatments, at 95.08±0.70%, while the least value (84.53±0.42%) was obtained in AC + E + AC. Also, the TP demonstrated higher digestibility *in vitro* with a value of 13.60±0.05%, while the AC + E sample (10.64±0.10%) was lower compared to treatments with AC (12.33±0.07%) or AC + E + AC (10.91±0.30%).

4. Discussion

The water absorption and swelling ability parameters are intended to characterize the resistant starch component of banana (Musita, 2009). Furthermore, water absorption is a trait with the ability to influence the outcome of food produced from flour, by facilitating the formation of gluten during mixing. This is because samples with little water-binding capacity yield inelastic and stiff dough, while those with good binding promotes elasticity and ease of expansion (Saragih, 2013).

Based on Figure 1, there was an increase in the absorption of *Batu* banana flour between treatments, with the highest value of 69% reported in samples exposed to autoclaving-cooling + enzyme pullulanase + autoclaving -cooling, while untreated samples were least, at 33%. Previous studies attributed high water absorbency to the water content in ingredient can affect the flour absorption ability, hence low moisture content of flour indicates high absorption power (Saragih, 2013). This study may suggest the positive influence of autoclaving-cooling, assumed to facilitate absorbency, induced high pressure and temperature, and consequently reducing water levels. Contrary to *Batu* banana flour, the untreated *Kepok* variety demonstrated the highest water absorption capacity. This phenomenon is suspected to occur because of the material water level of *Kepok* affects flour absorption (Saragih, 2013). The ability for starch granules to absorb large amounts of water is caused by the presence of hydroxyl groups. This characteristic was also associated with the ease of flour dough homogenization while mixing with water (Syahputri and Wardani, 2015).

The highest swelling ability was reported in *Batu* banana flour samples modified with autoclaving-cooling + enzyme pullulanase method, at 0.67 cm/g. This was attributed to an increase in total sugar, as the fermentation process utilizes instant yeast, which functions by breaking down starch into simple sugars, as observed with pullulanase enzyme.

The results show the ability for modified treatments with autoclaving-cooling to reduce *Kepok* banana flour starch development (0.31 cm/g). This is in line with the research conducted on taro starch. In principle, the method requires the use of heat and limited moisture content, therefore increasing gelatinization temperature, paste viscosity, limits swelling, and improves paste stability. Also, increase starch retrogradation, consequently affecting crystallinity and granule development potential (Wiadnyani, 2017). Moreover, the autoclaving-cooling process results in carbohydrate interactions with food ingredients, including protein and fat, where the respective amounts play a major role in calculating the carbohydrate level (Nurhayati *et al*., 2014). In addition, there are several enzymes (e.g., proteases) implicated in the breakdown of proteins present in flour, into nitrogen compounds absorbed by yeast to form new cells. This is evidenced by the impact of protein level on the decline in enzyme ability, consequently affecting flour development.

There were significant changes in the results of the SEM analysis for *Batu* (Figure 2) and *Kepok* banana flour (Figure 3). This is evidenced by the structure and intact surface of untreated samples, while those exposed to autoclaving-cooling demonstrated a fragmented structure. Furthermore, samples treated with autoclaving-cooling + pullulanase enzyme display a slightly damaged erosion surface, and those exposed to autoclaving-cooling + pullulanase + autoclaving-cooling showed similar structure in smaller fractions compared to the samples with autoclaving-cooling alone. The variations observed between treatments confirmed the effect of modifications on the banana flour surface structure. Previous studies affiliated these changes with the intrinsic gelatinization temperature, roughness, and the presence of cavities, indicating modification (Reddy *et al*., 2015).

The highest levels of resistant starch were recognized in *Batu* banana flour samples treated with autoclaving-cooling + pullulanase + autoclaving-cooling, at 52.95±6.32%. This outcome is almost the same as the outcome from the *Kepok* variety, at 55.80±0.84%. These results indicate a relatively higher level compared with previous studies, estimated at 39.35% and 27.70%, respectively (Musita, 2009) which is the finding is similar to ours. In contrast with Ratnasari *et al*. (2018) reports on the preparation of cookies with autoclaving-cooling and fermentation method, using 75% substituted *Batu* banana flour. This yielded a resistance level of 15.79%, and the two-fold increase in autoclaving-cooling was confirmed in previous studies. Furthermore, repeating the treatment increases the formation of amylose-amylose, amylose-amylopectin, and also the formation of more perfect crystalline products. The heating and cooling cycle (autoclaving - cooling), accompanied by enzymatic debranching results in higher levels of resistant starch, compared to cycles without debranching. This discrepancy is due to the excision of amylopectin molecule branches by the enzyme, leading to more straight-chain polysaccharide starch polymers, with a smaller degree of polymerization. Moreover, the formation of more short-chained molecules is affiliated with an increase in the level of resistant starch, possibly generated through retrogradation (Setiarto *et al*., 2015; Ardhiyanti *et al*., 2017).

The level of resistant starch in food is increased through the retrogradation processes of both amylose and amylopectin. This elevates the type 3 resistant starch content by physical processes, including the use of autoclaving-cooling method, and also pullulanase enzymes. Also, this product feature is influenced by the amylose and amylopectin ratios, pullulanase enzyme and starch concentration, temperature, heating and cooling cycle, storage conditions, and the presence of lipids

The results showed an increase in amylose level between treatments, and the highest value was reported in samples exposed to autoclaving-cooling + pullulanase + autoclaving-cooling, at 17.77±0.81% and 15.47±0.42% in *Batu* and *Kepok* banana flour, respectively. This indicates the effectiveness of two-autoclaving-cooling cycle methods in increasing amylose concentration, and in line with a study by Nurhayati *et al*. (2014), after the highest amylose level was generated following the fermentation of banana flour with two cycles of autoclaving-cooling. Furthermore, the treatment was assumed to cause amylopectin debranching, thus producing oligomers with shorter degrees of polymerization, and also increases the formation of methylated or crystallized amylose fractions (Nurhayati *et al*., 2014). These further bind to other amylose fractions through hydrogen bonds to form a double helix structure, which subsequently binds to the others, thus generating crystals. Hence, there is recrystallization of the amylose fractions, known as the RS 3 formation process, in linear polymer to the short-chain amylose fractions in food, formed during the autoclaving process (Ardhiyanti *et al*., 2017). Furthermore, amylose formation is directly proportional to resistant starch levels (Tharanathan and Mahadevamma, 2003), which is congruent with the results of previous research.

Amylose is part of a linear polymer with α-bonds (1->4) glucose units, characterized by a straight chain, with source-dependent degree of polymerization ranging from 500-6000 glucose units. In addition, pullulanase enzyme has the capacity to hydrolyze starch at the amylopectin branching point α- (1,6), facilitating the generation of short-chain amylose components.

The various treatments yield different levels of amylopectin reduction, and the lowest values (82.22±0.81% and 84.53±0.42%) were respectively found in *Batu* and *Kepok* banana flour exposed to autoclaving-cooling + pullulanase + autoclaving-cooling. This is in line with previous research, where the ratio of total carbohydrate amylopectin fraction obtained from arrowroot starch decreased after treating with up to three cycles of heat-cooling (Faridah *et al*., 2010). In addition, exposure to high temperatures promotes the dissociation of hydrogen bonds from amylopectin double helix structure, while the pullulanase enzymes break down the inherent glycosidic α-1,6 branching bond (Setiarto *et al*., 2015). The debranching process yields straight short-chain glucose polysaccharide polymer with a smaller degree of polymerization (DP 19-29), affiliated with the increase in resistant starch levels (Setiarto *et al*., 2015). A study by Faridah *et al*. (2010) reported on the association between arrowroot starch modification with pullulanase enzymes and a decline in percentage amylopectin content, followed by higher amylose fraction (degradation of amylopectin and amylose into short-chain amylose fractions).

Amylopectin is a polymer with α- (1-> 4) glucose units and α- (1-> 6) side or branching chain (Musita, 2009; Yongliang *et al*., 2014). In addition, pullulanase enzymes are used to break the α-1-6 glycosides, thus increasing the amount of amylose, and subsequently promoting resistant starch yield (Syahputri and Wardani, 2015). Amylopectin plays a major role in the intrinsic properties and possible development of starch dough, which is greater in samples with a longer chain, although the amylose content inhibits potential progression. In addition, the water absorption and power augmentation of starch is directly proportional to the amylopectin level, and the branch characteristics contribute to the development value, resulting from the ease to trap water. The results show similarly low amylose (<20%) and high amylopectin levels in all samples of banana flour, facilitating the manufacture of bread products with light and soft texture. Moreover, Samples with the least swelling ability to yield products with the minimal requirement in terms of further development, including noodles (Ramadhani and Murtini, 2017).

Based on the results, at least *in vitro* digestibility was reported in *Batu* banana flour treated with autoclaving-cooling + pullulanase enzyme + autoclaving-cooling (41.79±0.08%), followed by the *Kepok* variety exposed to autoclaving-cooling treatment + pullulanase enzyme (10.64±0.10%). Previous studies associate the incidence of starch digestibility with the see, amylose-amylopectin, and inter amylopectin methods. These have been linked with the strengthening of bonds, leading to greater difficulties in the digestion process (Ratnasari *et al*., 2018). The *in vitro* decline in this parameter is associated with a high level of resistant starch present in the food (Ratnasari *et al*., 2018). This outcome is in accordance with the results of the current investigation, where samples with the least digestibility were observed in samples with high resistance, including *Batu* banana flour treated with autoclaving-cooling + pullulanase + autoclaving-cooling enzyme.

Furthermore, the outcome is achieved through the mechanism of starch molecules rearrangement between amylose - amylose, amylose - amylopectin and amylopectin – amylopectin, resulting in stronger bonds, characterized by difficulties in digestion (Sugiyono *et al*., 2009). The study conducted on cassava flour, showed the significant effect of higher numbers of pressurized-cooling heating cycles on digestibility, after production (Setiarto *et al*., 2018).

5. Conclusion

The SEM analysis of granular morphology showed the structural modification in *Batu* and *Kepok* banana flour between treatments. However, the *Batu* variety treated with autoclaving-cooling + enzyme pullulanase + autoclaving-cooling (AC + E + AC) and *Kepok* flour with untreated (TP) demonstrated the best absorption and swelling ability. Furthermore, both samples showed the best results, in terms of high levels of resistant starch, low amylose, high amylopectin, and low *in vitro* digestibility after subjection to autoclaving-cooling + pullulanase + autoclaving-cooling.

The results of physical characteristics ascertain the tendency to select both flour varieties in the manufacture of products that require minimal swelling ability.

Conflict of interest

The authors declare no conflict of interest.

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