# Physicochemical Properties and Starch Digestibility of Autoclaved-Cooled Water Yam (Dioscorea alata L.) Flour

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# Physicochemical Properties and Starch Digestibility of Autoclaved-Cooled Water Yam (*Dioscorea alata* L.) Flour

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Water yam (*Dioscorea alata* L.) is suitable for resistant starch high flour because of its high amylose content. The purple, yellow, and white water yams were treated by autoclaving and cooling with one, two, and three cycles to obtain resistant starch. Water yam tubers were cooked in an autoclave for 15 min, and then cooled prior to drying. Autoclaving–cooling of water yams decreased protein, fat, dietary fiber, water soluble polysaccharide, dioscorin, and diosgenin but increased amylose and sugar. The treatments significantly raised resistant starch and reduced *in vitro* starch digestibility that depended on water yam types, and affected starch granule morphology.

*Keywords*: Autoclaving–cooling, Physicochemical properties, Resistant starch, Starch digestibility, Water yam.

## INTRODUCTION

*Dioscorea alata* L., in English, is known as water yam, greater yam, and 10-month yam. In other cultures and languages, it is known variously as *ratalu* or *violet yam* in India, *rasa valli kilangu* in Tamil, *kondfal* in Marathi, *kachil* in Malayalam, and *khoai mõ* in Vietnam. For the Igbo people of southern Nigeria, it is called *ji* or *ji abana*; while for the Yoruba people of the southwestern Nigeria, it is called *isu ewura*or. It is a creep and shrub plant that generally grows under forest plants. Water yam tuber is limitedly used as a major food product, although this species has high productivity and good tuber storability.[1] *D. alata* tubers have variability in shapes with the majority is cylindrical. The flesh of the tuber ranges in color from white to purplish. Differences in growing environment, maturity stage, method of

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storage, and species may also affect variation in the tuber composition.[2] Water yam tuber has not been studied as extensively, especially in Indonesia, as other roots or tuber crops.

Water yam has a potential prospect to develop as a resistant starch (RS) high flour because it has a high amylose content. Other researchers reported that the starch content of *D. alata* flour was 60.42–77.56%, with 21.69–31.56% being amylose.[3,4] It is reported that *D. alata* contains several bioactive compounds such as dioscorin, diosgenin, and water soluble polysaccharides (WSPs).[1] Dioscorin is the major storage protein in yams and its function is to inhibit angiotensin converting enzyme, which converts angiotensin I to angiotensin II that is responsible for hypertension.[5] Diosgenin is a steroidal sapogenin compound that is absorbed through the gut and plays an important role in the control of cholesterol metabolism.[6] WSP of the yam is a viscous mucilage containing glycoprotein.[7] Our previous study showed that WSP had hypoglycemic effect because of its viscous properties and it easily generates gel structure, hence hindering macronutrient and glucose absorption.[8]

Roots and tubers are staple foods in both developed and developing countries. All contain starch, but the starch digestibility is greatly influenced by processing and plant type, and also depends on physicochemical characteristics of the starch. Most starch-related foods are cooked before consumption and consequently starch gelatinization and retrogradation play important roles on the quality and digestibility of the food products. The cooling of starchy foods after heating in excess water leads to the formation of crystals which hinder starch digestion.[9]

RS is generally defined as the fraction of dietary starch that is not digested in the small intestine of healthy individuals.[10] There are many physiological effects which have been ascribed to RS that have proved its health benefits. RS as a component of dietary fiber has hypoglycemic and hypocholesterolemic, acts as a prebiotic, reduces gall stone formation, and prevents colonic cancer.[11]

RSs have got much attention for both its prospective health benefits and useful properties.[10] A recent study has proven that RS physiologically behaves like fiber which exhibits a low level of digestibility and can be used as a vehicle for slowing down the glucose release. The RS is like soluble fiber and has a more positive impact on colonic health by increasing the crypt cell production rate and decreasing the colonic epithelial atrophy in comparison to no-fiber diets.[9]

RS has been divided into four categories which are: type-1, type-2, type-3, and type-4 for physically inaccessible starch, raw crystalline starch, retrograded starch, and chemically modified starch, respectively.[12] Among the four types of RS, the type-3 is of a great interest due to its thermal stability during food processing.[13] It was previously reported that RS type-3 could be produced by physical treatments including autoclaving.[14] The cooling of starchy foods after heating in excess water leads to the formation of crystals which hinder the digestion process.[9]

Processing techniques may affect both the gelatinization and retrogradation that influence RS formation. This fact is of a great importance for the food industry since it offers the possibility of increasing the RS content of processed foods and foodstuffs. Baking, pasta production, extrusion cooking, autoclaving, and so forth are known to influence the yield of RS in foods.[15] In general, starch can be modified chemically, enzymatically, and physically. The previous research showed that modified starch can increase the resistance of starch digestibility in human digestion process.-[16] Physical modification of banana starch by heating and subsequent cooling increases RS, hence it can potentially be used as an ingredient for functional foods.[17]

Starch retrogradation is mainly caused by amylose molecule interaction, since the formation of hydrogen bound between amylose is easily formed. Increasing leached amylose fractions from the granule during gelatinization makes more retrograded starch formation during cooling.[18] The water yam has high starch (60.42–77.56%) and amylose contents (21.69–31.56%),[3] therefore, it is supposed to be suitable for high RS flour. In this study, purple, yellow, and white water yam was subjected to repeated autoclaving–cooling treatment. Then the content of RS and starch digestibility (*in vitro*) were examined and the physicochemical properties were also characterized.

## MATERIALS AND METHODS

### Materials

Three types of water yams (*Dioscorea alata*), purple, yellow, and white, were obtained from a local farmer in Tuban, East Java, Indonesia. Analytical grade reagents used in this study were ethanol 96% (pa), distilled water, Na-phosphate buffer (pH 7.0), α-amylase (EEC 232-560-9), amyloglucosidase (EEC 232-877-2), and pullulanase (EC 3.2.1.41; by Sigma Chemical Co.).

### Methods

#### *Autoclaved–cooled flour preparation*

Ninety to 100 g of whole yam tubers (all part of the tuber, without peeling and cutting) were weighed in a 500 mL beaker and autoclaved for 15 min at 121°C and 1.1 kgf/cm$^2$ using an autoclave (ALP Co., Model KT-30T 3-3-10). After autoclaving, the samples were allowed to cool and stored for 24 h in a refrigerator (4 ± 2°C) which was termed as one cycle. This autoclaving–cooling cycle was repeated up to one, two, or three times. The treated samples were peeled, sliced, and dried in a cabinet drier at 60 ± 5°C for 12 h. The dried sample was milled into fine flour (80 mesh size) and stored in a polyethylene bag at 0°C for analysis.

*Chemical analysis*. The control (without an autoclaving–cooling cycle) was analyzed for proximate composition, starch,[19] and dietary fiber content by Association of Official Analytical Chemists (AOAC) method 985.29 and treated water yam flours were analyzed for protein by AOAC method 2.057,[18] fat by AOAC method 155.7 056,[20] amylose by the colorimetric method of Juliano,[21] and sugar content by Nelson-Somogyi method, RS content,[19] and *in vitro* starch digestibility.[22]

#### *Bioactive compound analysis*

Bioactive compounds were analyzed for control and three cycle autoclaved–cooled water yam flours. Water-soluble polysaccharide was determined by a modified method of Ohashi et al.[23] The brief procedure is as followed: Distilled water (300 mL) of was heated to about 75–80°C and then added to aluminium sulfate (10% w/w based on flour) and 6 g of yam flour. The mixture was stirred for 60 min, and then centrifuged. The filtrate was coagulated in twice the volume of 96% ethanol for 12 h.

In dioscorin analysis, 3 g of flour was added to 9 mL aquadest. Water soluble protein was separated from the flour suspension by precipitation using 10% tricholoroacetic acid. Subsequently, the solution was centrifuged for 15 min at 3000 rpm. Precipitate was crude water soluble protein extract and further analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to confirm the presence of dioscorin[24] and quantified by gel documentation (Chemidoc XRS, Bio rad Laboratories).

Diosgenin was determined by spectrophotometric method of Chapagain et al.[6] Three grams of flour was defatted using *n*-hexane by electric shaking overnight and centrifuging at 3500 rpm for 18 min. As much as 30 mL methanol was added to the defatted cake on reaction tubes and left on the shaker overnight, followed by centrifugation at 3500 rpm (or g) for 18 min. The second and third extractions by methanol were also carried out with *n*-hexane. At the end, all supernatants of methanol extracts were mixed and the methanol was evaporated under reduced pressure using a rotary evaporator to remove solvent. Finally, a yellowish crystal powder of crude saponin was obtained. The diosgenin level was determined by measuring absorbance at 430 nm, based on the color reaction with anisaldehyde, sulfuric acid, and ethyl acetate.

### Morphological properties

For examination by scanning electron microscopy, control and three cycle autoclaved–cooled water yam flours were placed on a double stick scotch tape and mounted on aluminium specimen holders. The samples were gold coated and scanned using a Hitachi TM3000 scanning electron microscope.

### Starch digestibility (in vitro)

Enzymatic hydrolysis was performed as described by Anderson et al.[22] Flour (1 g) was gelatinized with 100 mL water, and 2 mL of the solution was incubated with α-amylase in 5.0 mL phosphate buffer, pH 7.0 in a shaking water bath at 37°C for 30 min and in 2 mL of dinitrosallicylic acid (DNS) at 100°C for 12 min prior to absorbance determined by spectrophotometer at 520 nm. Hydrolysis degree was defined as the amount of soluble saccharides (mg) per 100 mg of sample (%).



## Statistical Analysis

A 3 × 4 factorial experimental in completely randomized design was used in this study. The independent variables were three varieties of water yams and four autoclaving–cooling cycles (0, 1, 2, and 3 times). All analyses were carried out in three replications. The data was evaluated for significant differences by Duncan's Multiple Range Test (DMRT) in their means with analysis of variance (ANOVA; $p = 0.05$).

# RESULTS AND DISCUSSION

## Chemical Characteristics of Water Yam Flours

The moisture content of the water yam flours ranged from 5.58 to 6.24% (Table 1). Moisture content between 4.40 and 5.86% have been reported previously for purple and yellow water yams, respectively.[25] Ash content of the three types of *D. alata* ranged from 2.31 to 4.16% and fat content ranged from 0.28 to 4.16%. Similar values for ash content (0.70–2.10%) and fat content (0.10–0.60%) have been reported by Baah et al.[2] Protein content of the *D. alata* types ranged between 3.83 and 6.49%. Similarly, protein content of 4.30–11.95% and 5.07–9.05% was obtained for *D. alata* in earlier studies.[2,3] This result was lower than that of our previous study, that yellow and purple water yams had 8.33 and 6.00% of protein, respectively.[25]

Starch was a major component of the water yam flour (Table 1), varying from 67.80 to 70.76% on a dry weight basis (db). This result was similar to the result of Wireko-Manu et al.[3] and Baah[2]

TABLE 1
Proximate composition of purple, yellow, and white water yam flour

| *Components* | *Purple water yam* | *Yellow water yam* | *White water yam* |
|---|---|---|---|
| Moisture (%db) | 6.24 ± 0.21 | 5.85 ± 0.01 | 6.63 ± 0.21 |
| Ash (%db) | 2.31 ± 0.04 | 3.93 ± 0.15 | 4.16 ± 0.02 |
| Protein (%db) | 6.49 ± 0.09 | 4.40 ± 0.17 | 3.83 ± 0.12 |
| Fat (%db) | 0.60 ± 0.01 | 0.41 ± 0.01 | 0.28 ± 0.01 |
| Carbohydrate(by different) | 84.36 | 85.41 | 85.10 |
| Starch (%db) | 68.16 ± 0.81 | 67.82 ± 0.15 | 70.76 ± 0.81 |
| Dietary fiber (%db) | 13.53 ± 0.34 | 13.93 ± 0.13 | 14.38 ± 0.28 |

who reported that starch content of *D. alata* flour was 60.42–77.56% and 68.40%, respectively. Dietary fiber of three types of water yam flours was 13.53–14.38%. This result was higher than that reported by Baah,[2] that *D. alata* had 6.90% of dietary fiber. High starch, especially amylose, in the water yam made it possible to process as a high RS food or a functional food.

Chemical characteristics of the yellow water yam were similar to the white water yam especially in carbohydrate content. Purple water yam flour had the highest protein and fat content. Meanwhile, white water yam flour had low protein and fat content and a high level of starch. The variation may be due to genetic composition of the varieties and environmental conditions.

The types of water yam and autoclaving–cooling cycle significantly affected protein, fat, amylose, and sugar content of modified flour. It was found that *D.alata* varieties had amylose content ranging from 17.59 to 22.66% and sugar content ranging from 1.92 to 6.41% (Fig 1). Similar values for amylose content (21.69–31.56%) and sugar content (2.43–6.91%) have been reported by Wireko-Manu et al.[3] It was found that an autoclaving–cooling treatment significantly affected the protein and fat content of water yam. The treatment slightly decreased protein and fat content because they disrupted during heating and leached out during autoclaving–cooling process. This phenomena is similar for all water yam varieties; however, the previous study showed that pressure cooking (autoclaving) and cooling treatment did not affect protein and fat content of arrowroot starch. This process slightly affected physicochemical properties, such as decreased protein and fat, and increased amylose, but the changes were insignificant.[26]

The autoclaved–cooled flour had higher amylose content than the control flour either purple, yellow, or white water yams, significantly (Fig. 1). This result is in accordance to the result of Sugiyono et al.[26] in which three cycles of autoclaving–cooling of arrowroot

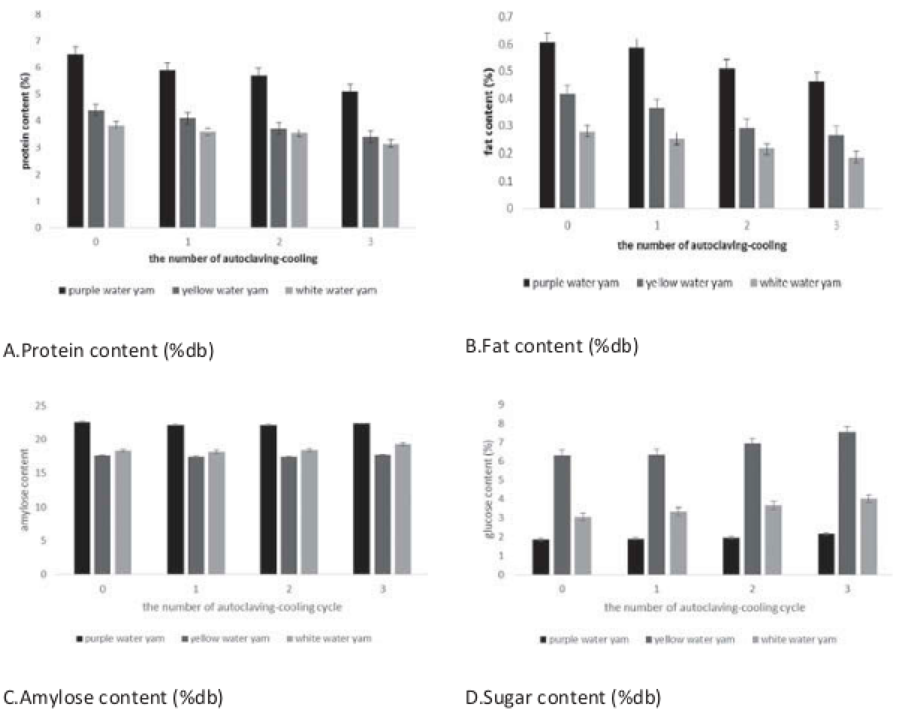


FIGURE 1 The protein, fat, amylose, and sugar content of autoclaved-cooled purple, yellow, and white water yam (% dry basis).

starch resulted in a new amylose molecule formation from de-polymerization process of amylose and amylopectin. Increasing in short-chain amylose content would increase the amount of reducing end molecules, which is measured as reducing sugar and implied in higher sugar content.

## Bioactive Compounds

Table 2 showed bioactive compounds of autoclaved–cooled purple, yellow, and white water yam flour, compared to the control flour. WSP content of autoclaved–cooled water yam flour ranged from 0.10 to 0.28%, while the control flour ranged from 0.12 to 0.49%. This result is similar to WSP yield in previous research, which are yellow and purple water yam flours had WSP 0.10 and 0.12%, respectively.[25] The varieties of water yams affected WSP content but autoclaving–cooling cycle insignificantly affected WSP. The modified yam flour had less WSP content, presumably due to leaching out of WSP during the autoclaving–cooling treatment.

The control water yam flour had a dioscorin content ranging from 0.32 to 1.22%, meanwhile dioscorin of autoclaved–cooled flour was ranging from 0.12 to 0.23%. The treatment slightly decreased the dioscorin content because dioscorin was a water soluble protein that possibly leached out during the autoclaving–cooling process. Dioscorin content of water yam flour is ranged from 0.13 to 0.25% depending on the types of water yam, with 0.25% dioscorin for purple water yam and 0.13% for yellow water yam.[23] SDS-PAGE analysis in this study showed an apparent band in 26 kDa which meant that water yam flour protein contained dioscorin. Harijono et al.[25] reported that dioscorin appeared as a single protein band with molecular weight of about 25 kDa.

The types of water yams and autoclaving–cooling cycle significantly affected diosgenin content of modified flour. The diosgenin content ranged from 0.30 to 3.19 mg/100 g in autoclaved–cooled flour, while the control flour ranged from 0.99 to 4.80 mg/100 g. This result is higher than the diosgenin content in a previous study which was 0.029 mg/100 g in purple water yam and 0.0049 mg/100 g in yellow water yam.[25] Perhaps, during autoclaving–cooling, diosgenin was hydrolized from saponin with hydrophilic properties. Therefore, the diosgenin level decreased. According to Savikin-Fodulovic et al.,[27] diosgenin and related steroidal saponins were commercially obtained from the tubers of various *Dioscorea* species. *Dioscorea* root contains a higher level of sapogenin (3–7% combination of diosgenin and yamogenin) especially in its mature stage. Autoclaving–cooling and water yam types affected the concentration of bioactive compounds. The autoclaved–cooled water yam flour had less WSP, dioscorin, and diosgenin contents. It was assumed that bioactive compounds leached out during autoclaving–cooling.

TABLE 2
Bioactive compound of autoclaved-cooled purple, yellow, and white water yam flour (WYF)

| *Treatment* | *Water soluble polysaccharide (%)* | *Dioscorin** (%)* | *Diosgenin (mg/100 g)* |
|---|---|---|---|
| Control purple WYF | 0.19 ± 0.01 | 0.77 | 4.78 ± 0.02 |
| Autoclaved-cooled purple WYF* | 0.13 ± 0.03 | 0.12 | 3.09 ± 0.08 |
| Control yellow WYF | 0.12 ± 0.01 | 0.32 | 1.48 ± 0.05 |
| Autoclaved-cooled yellow WYF* | 0.10 ± 0.01 | 0.23 | 1.09 ± 0.09 |
| Control white WYF | 0.49 ± 0.11 | 1.22 | 0.98 ± 0.01 |
| Autoclaved-cooled white WYF* | 0.28 ± 0.02 | 0.12 | 0.27 ± 0.05 |

*After three cycles autoclaving-cooling treatment.
**No replication.

## Starch Granule Morphology

The Scanning Electron Microscope (SEM) images of the starch granules of modified water yam flour were obtained and compared to that of control flour (Fig. 2). The control water yam flour, without autoclaving–cooling treatment, consisted of granules with average size being about 22.1–45.8 µm. This result was in accordance to the result of Harijono et al.,[25] that the average granule size of purple water yam was 19–46 µm and yellow water yam was 27–37 µm. Baah[2] reported that *D. alata* had fairly large granule (5–50) µm and shape variation, which were oval or egg-shaped, elongated rounded squares, or mussel-shell-shaped, sometimes with one side flattened. The autoclaved–cooled flour had bigger granules, the size was 50.1–75.0 µm in purple water yam flour, 2.53–88.93 µm in yellow water yam flour, and 52.3–98.0 µm in white water yam flour. Perhaps, during autoclaving–cooling some starch granules absorbed water and swelled.

Moorthy et al.[27] reported that large variability in shape exists among yam starches, round, triangular, oval, and elliptical. Granule size was reported to range from 20 to 140 µm and from 10 to 70 µm for *D. alata* and *D. rotundata*, respectively.[28] Sizes of starch granules from *D. alata* and *D. rotundata* varied from 13 to 52 µm and from 19 to 50 µm, respectively.[29] The morphology of chemically modified granules depends on botanical source of starches.[30]

Figure 2 clearly illustrated that the autoclaved–cooled of water yam flour altered the starch granule morphology. The control flour exhibited a granular appearance (Fig. 2a, 2c, and 2e), the granular structure disappeared and an irregular shape was formed in the autoclaved–cooled flour (Fig. 2b, 2d, and 2f). This result was similar to the previous research. Sievert et al.[31] stated that starch granule in amylomaize after one autoclaving–cooling cycle showed a completely different image. The granular structure disappeared and the starch granule had bigger, irregularly shaped particles with a continuous spongy-like porous network. SEM examination showed that water yam flour had heterogeneous starch granules.

## RS Content

The types of water yams and autoclaving–cooling cycle significantly affected RS content of modified flour. As shown in Fig. 3, RS level of control water yam flour ranged from 4.06 to 5.22%. These values were less than those found in the autoclaved–cooled flour (7.14–9.04% of RS), but no significant difference. The white water yam flour had the greatest RS content (5.22%) because of its higher starch content (70.76%) and dietary fiber content (14.38%), more than the other water yam types (Table 1). The previous research[30] showed that insoluble dietary fiber could increase the formation of RS by using a high-pressure autoclave. According to Liu et al.,[32] under the treatment of conventional heat moisture, the starch-soluble dietary fiber conjugated and almost completely resisted to the hydrolysis of the amylolytic enzymes and has potential to be used as a new dietary supplement in the food industry. The least RS content was found in purple water yam flour (7.14%) because of its higher protein and fat content, which was 6.49 and 0.61%, respectively (Table 1). Starch–protein interaction has been believed to reduce RS contents as observed in the cases of potato starch and the addition of albumin when autoclaved and subsequently cooled at –20°C.[30] RS content from amylose-lipid complex samples were lower than RS content from the autoclaved and cooled control. In the formation of complexes, lipids or proteins compete with amylose chains that are responsible for the formation of RS. Amylose-lipid complexes are enzyme-degradable and an increase in amylose complexes reduced yields of RS. Amylose–lipid complexes can also be formed during food processing (autoclaving and cooling) and affected retrogradation process to a lower extent.[33]

Different amounts of RS content in three cultivars of water yams could arise from heat treatment during the autoclaving–cooling process. Increasing the number of autoclaving–cooling cycles increased the yield of RS. RS content was significantly increased to 7.55, 7.14, and 9.04% in purple,

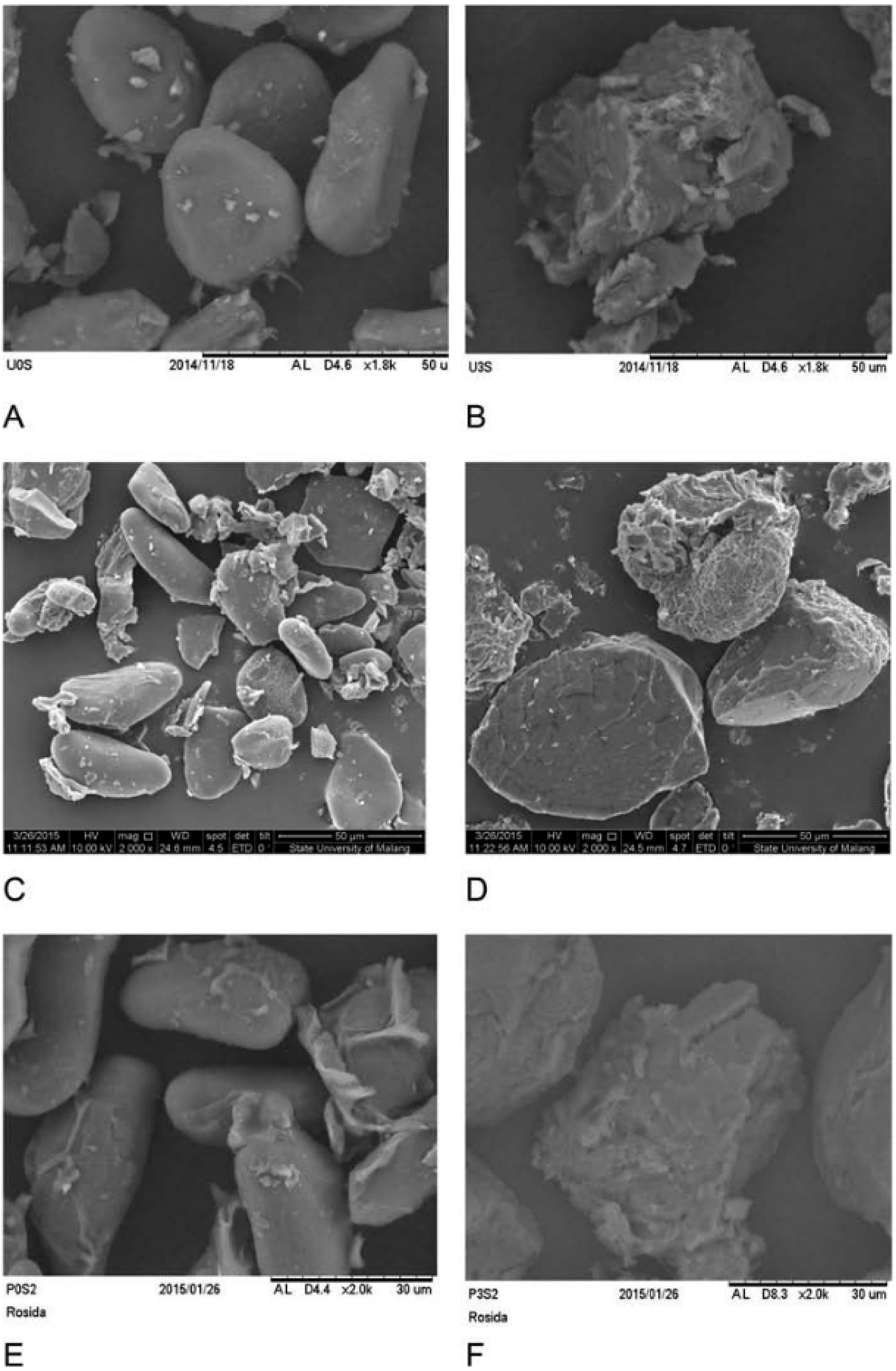


FIGURE 2 Starch granule of A: control and B: autoclaved-cooled purple water yam flour; C: control and D: autoclaved-cooled yellow water yam flour; E: control and F: autoclaved-cooled white water yam flour.

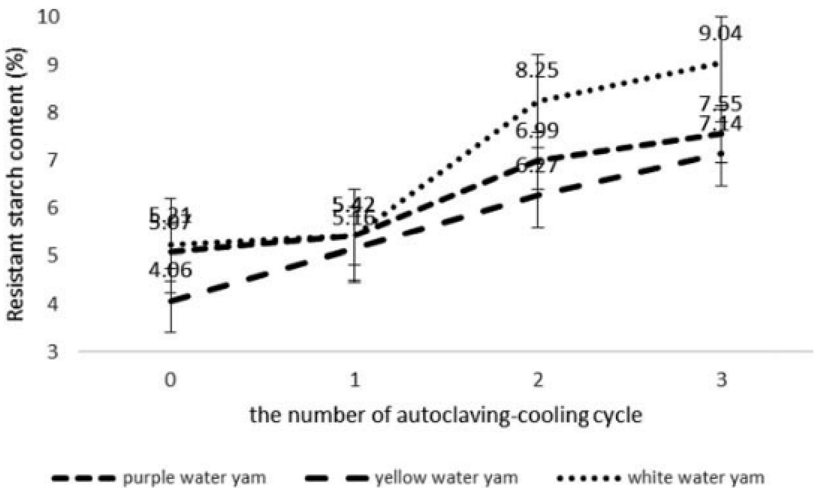


FIGURE 3 RS content of control and modified purple, yellow, and white water yam flour (treated by autoclaving-cooling with 1, 2, and 3 cycles).

yellow, and white water yam flour, respectively. According to Shankon et al.,[34] when autoclaved at 110°C the starch was completely gelatinized. Amylose was leached from the granules into the solution as a random coil polymer, whereas the crystalline regions of clusters of branched amylopectin chains had disappeared, which might be hard to digest by enzymes. Cycling to autoclaving temperature is favorable for the formation of extremely stable RS.[9,10] The reported levels of RS produced by autoclaving–cooling treatment were about 10% in cassava starch[14] and 19% in banana starch.[35]

The autoclaving process could hydrolyze the outer chain of amylose and amylopectin in crystalline area.[36] Increasing the number of autoclaving–cooling cycles increased the hydrolysis of amylose and amylopectin and formation short-chain amylose fractions. Increasing the amount of short-chain amylose fractions possibly made the retrogradation and recrystallization process happen during the cooling process. Amylose fraction bound each other through hydrogen bonds and formed double helices structure. Double helices structure bound each other and formed crystallite, and hence, produced recrystallization of amylose fractions.[9] Chung et al.[37] stated that the amylose–amylose interactions, which are much stronger than those of amylose–amylopectin, may have continued to exist after gelatinization. Thereby this interaction is partly restricting accessibility of starch chains to the hydrolyzing enzymes.

### Starch Digestibility (*in Vitro*)

Figure 4 presents the starch digestibility (in vitro) of purple, yellow, and white water yam flours as a result of the autoclaving–cooling cycle treatment. The starch digestibility varied among treatments due to its RS content. The flour with the highest RS content had the lowest starch digestibility, as expected. White water yam flour had the highest RS content (9.04% db) and the lowest starch digestibility (49.94%).

As shown in Fig. 4, autoclaving–cooling cycles decreased the starch digestibility. The control flour (without autoclaving–cooling treatment) had high starch digestibility (63.91–76.02%). In this study, RS was distinctly increased after autoclaving–cooling treatments, indicating the lower digestibility of the autoclaved–cooled flours. Increasing the number of cycles up to three lowered the starch digestibility to 48.47–49.94%. But the changes in the digestibility values at different water yam types and autoclaving–cooling cycles were not significant ($p > 0.05$).

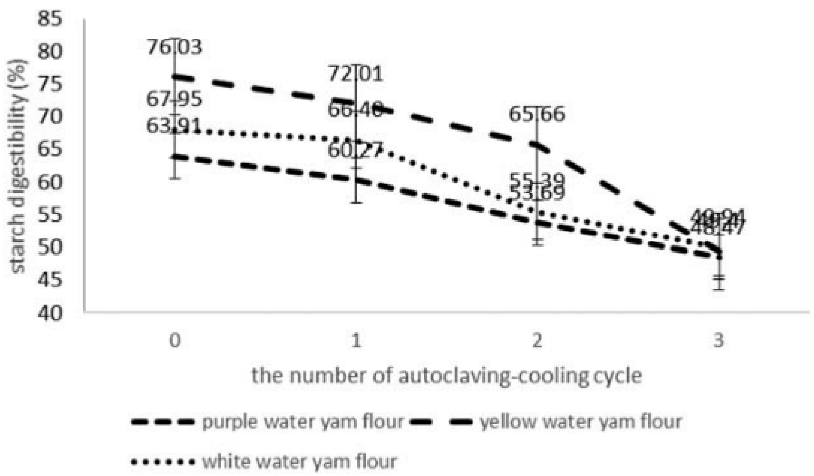


FIGURE 4 Starch digestibility (in vitro) of control and modified purple, yellow, and white water yam flour (treated by autoclaving-cooling with 1, 2, and 3 cycles).

In a similar study, Sugiyono et al.[26] stated that three cycles of autoclaving–cooling of arrowroot starch would reduce starch digestibility from 70.69 to 48.49%. The autoclaving–cooling process decreased starch digestibility of modified arrowroot starch due to the increasing of the formation of amylose as the result of de–polymerization process during autoclaving–cooling process. Hence, the formation of retrograded starch is more possible and lowers starch digestibility. The cooling of starchy foods which have been heated in excess water leads to the formation of crystals which hinder the digestion process. Sometimes the cooking in water in the preparation of starchy foods for consumption renders the digestion of food[9] The autoclaved–cooled white water yam flour had the lowest starch digestibility (49.94%) of all the flours due to its biggest granule size (52.3–98.0 µm; Fig. 2f). Particle size affects digestibility of the yam starches, being that smaller starch granules are more digestible than the larger ones.[38]

## CONCLUSION

The result of the present study indicated that water yam types and autoclaving–cooling cycles affected the physicochemical properties of the flour. The autoclaving–cooling treatment increased the RS content and lowered digestibility of starch (*in vitro*) and altered the outer structure of starch granule due to gelatinization and retrogradation process. These treatments significantly affected the protein, fat, amylose, and sugar content of water yams. Autoclaved–cooled purple, yellow, and white water yam flour had high RS content (7.14, 7.55, and 9.03% db, respectively) hence made it possible to process as high RS food or a functional food.

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