

# The Effect of Processing and Cooling Methods on *Coleus tuberosus* in vitro Starch Digestibility

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## ABSTRACT

*Coleus tuberosus*, also known as black potato, is one of the Indonesian local tubers consumed as a carbohydrate substituent. Therefore, this study aimed to examine the effect of processing and cooling methods on the in vitro digestibility of black potato starch. Furthermore, two factors Randomized Block Design with a 2x3 experimental design was used, which consisted of processing methods (boiling, roasting, and microwave) and cooling at room temperature and 4 °C for 24 hours with 3 repetitions. Black potato flour was compared with the raw form, by assessing some parameters, namely Resistant Starch (RS), Slowly Digestible Starch (SDS), Rapidly Digestible Starch (RDS), and Glycemic Index (GI). Also, the analysis of total starch, moisture, and color was performed, hence raw black potatoes generally have 10% resistant starch (%wb). Different treatments of cooking and cooling had a significant effect ( $\alpha = 0.05$ ) on moisture content, total starch, RS, RDS, SDS, GI, brightness (L), and yellowness (b). Black potatoes subjected to the processing method followed by cooling had lower RDS and increased RS content. Furthermore, refrigeration at 4°C for 24 hours reduced the digestibility of black potato starch more than cooling at room temperature. Contrarily, microwaved black potato cooled at room temperature showed a higher digestion rate compared to the raw counterpart. Conclusively, processing followed by cooling reduces the GI and increases the RS content of *Coleus tuberosus*.

**Keywords:** *Coleus tuberosus*; cooling; in vitro digestibility; processing; resistant starch

## INTRODUCTION

The prevalence of diabetes patients in Indonesia is 2.1% of the total population or specifically, up to 6 million people are suffering from the disease (Indonesian Ministry of Health, 2013). Furthermore, their number is estimated to potentially reach 21.3 million in 2030 (Soewanda and Pramono, 2011). This tends to occur in the absence of preventive efforts. The means of preventing diabetes include consumption of carbohydrates with low glycemic index which often digest without increasing blood sugar drastically (Zhang *et al.*, 2009). Besides, starch digestibility is defined as its ability to be digested in human intestines and the

physiological effects created on the blood sugar after consumption. Resistant starch has been proven to be beneficial for people with type 2 diabetes (Kwak *et al.*, 2012), as it is not digested in the small intestine but enters the large intestine and becomes fermented into short-chain fatty acids (Jiang *et al.*, 2020). The urge to raise carbohydrate source consumption with higher RS is needed in society.

*Coleus tuberosus* locally known as black potato or 'kleci' potatoes is one of the widely available tubers in Indonesia, and the raw form contains 10% resistant starch (Nugraheni *et al.*, 2014). This plant has been reported to have a good effect on glucose levels, blood lipids, and short-chain fatty acids in mice (Nugraheni

et al., 2015). Hsum et al. (2010) also reported it to contain maslinic acid possessing anti-cancer activity. In addition, the tuber has compounds that possess antioxidant and anti-cancer properties (Nugraheni et al., 2011). Its capability to decrease blood glucose and lipid levels is much better when boiled or made into flakes (Nugraheni et al., 2015).

Generally, *C. tuberosus* is potentially prepared into various dishes and usually processed by frying, boiling, roasting, or mixing with vegetables. The varied cooking processes affect its digestibility, such that almost all techniques used promote starch hydrolysis (Singh, et al., 2010). Also, these processes tend to enhance the resistant starch content. According to Inan Eroglu and Buyuktuncer (2017), starch is either boiled, steamed, fried, roasted, microwaved, autoclaved, or baked during cooking, which in combination with a period of cooling leads to higher RS levels' development due to retrogradation. Furthermore, starch retrogradation is usually accompanied by physical changes such as swelling, gelling, pasting, alteration of functional and mechanical properties, including disorganization of starch granule structures and crystalline formation (Matignon and Tecante, 2017).

*Coleus Tuberosus* is a local commodity that has not been commercialized, while its preparation for consumption only uses simple methods. However, the related foods processed using different methods tend to have different resistant starch levels, because of subsequent changes in structural and chemical composition. The black potato tuber is expected to be used as a functional food or rice substitute, good for people with diabetes and obesity. Therefore, this study was conducted to determine the effect of processing followed by cooling on in vitro digestibility of *C. tuberosus*.

## MATERIALS AND METHODS

### Raw Materials

The plant samples used were obtained from Jaten Village, Jogorogo District, Ngawi Regency, Indonesia, at maximum days of harvesting in the dry season at 2-3 cm diameter.

### Chemicals and Enzymes

The materials used included 25% HCl solution, 45% NaOH solution, petroleum ether solution (PE), 0.2 M HCl-KCl buffer solution of pH 1.5, 2 M KOH solution, pepsin (Sigma-Aldrich), pancreatin  $\alpha$ -amylase enzyme (Sigma-Aldrich), 300 U/mL amyloglucosidases (Sigma-Aldrich), 100 mM sodium maleate buffer solution of pH

6, and 0.2 M tris maleate buffer solution of pH 6.9. Also, 1.2 M sodium acetate buffer solution of pH 3.8, 100 mM sodium acetate buffer solution of pH 4.5, 0.4 M sodium acetate buffer solution of pH 4.75, aquadest, 99%, 50% and 10% (v/v) ethanol, as well as Nelson, Arsenomolibdat and DNS reagents were used, with all chemicals being of analytical grade.

### Experimental Design

Two factors Randomized Block Design (RBD) with 2x3 experimental design was used, which were the preparation methods (boiling, microwave, and roasting) and cooling (room temperature and refrigeration at 4 °C for 24 hours), where each treatment was performed with 3 replications.

### Sample Treatment

#### Raw *C. tuberosus*

*Coleus Tuberosus* was treated, cooled to room temperature, and refrigerated at 4 °C for 24 hours. Then, the tuber was washed and stripped to clean the dirt that sticks to the skin followed by mashing into a raw puree.

#### *C. tuberosus* flour

The sample was washed, stripped, and sliced, then dried at 60 °C for 7 hours with the cabinet drier, followed by grinding using a blender and sieving with 80 mesh sieve. The flour was treated, cooled to room temperature, and refrigerated at 4 °C for 24 hours.

#### Boiled *C. tuberosus*

*Coleus Tuberosus* was washed, then boiled for 50 minutes in 100 mL of water at 100 °C in a household pan and dried afterward. It was further treated, cooled to room temperature, and refrigerated at 4 °C for 24 hours, followed by peeling and mashing into a puree.

#### Roasted *C. tuberosus*

The potato tuber was washed and roasted for 30 minutes at 200 °C in Cosmos CO-9919R household roasting condition, then cooled to room temperature and refrigerated at 4 °C for 24 hours, after which it was peeled and mashed into a puree.

#### Microwaved *C. tuberosus*

*Coleus Tuberosus* was washed and microwaved with 30 V power level for 3 minutes in Samsung M 745

household microwave settings. This was treated, cooled to room temperature, and refrigerated at 4 °C for 24 hours, then peeled and mashed.

### Moisture Content, Total Starch, and Color Analysis

Moisture content was determined by using thermogravimetry analysis (AOAC, 2005) and total starch was assessed by acid hydrolysis using the nelson somogy method (AOAC, 1990), while color was analyzed with a color reader (Susanto and Yuwono, 1998).

### Starch Digestibility (In Vitro) Analysis (Goñi et al., 1997)

To carry out the analysis, 50 mg puree samples were put into some test tubes. Next, 10 mL HCl-KCl buffer and 2 mL pepsin solution (1 mg pepsin in 10 mL HCl-KCl buffer of pH 1.5) were added and vortexed. The mixture was incubated at 40 °C for 60 minutes and up to 25 mL tris-maleate buffer of pH 6.9 was added. The 5 mL tris-maleate buffer containing 2.6 IU  $\alpha$ -amylase was added and vortexed, then the mixture was incubated at 37 °C. Every 30, 60, 90, 120, 150, and 180 mins, 0.2 ml of the mixture was collected then heated for 5 mins to inactivate the enzyme present. Into this, 60  $\mu$ l amyloglucosydases (300 U/mL) and 2 mL of 0.4 M sodium acetate buffer with pH 4.75 was added, then mixed thoroughly. The solution was incubated at 60 °C for 45 mins, after which 2 ml dinitrosalicylic acid was added and vortexed, followed by heating for 15 mins and glucose content was later measured with spectrophotometer  $\lambda$  540 nm.

### Rapidly Digestible Starch (RDS) Analysis (Englyst et al., 1992)

Glucose data from the 30, 60, and 90 minutes digestibility in vitro analysis were collected and multiplied with 0.9 (the starch conversion factor).

### Slowly Digestible Starch (SDS) Analysis (Englyst et al., 1992)

Glucose data from 120, 150, and 180 minutes digestibility in vitro analysis were collected and multiplied with 0.9 (the starch conversion factor).

### Resistant Starch (RS) Analysis (Englyst et al., 1992)

Resistant starch was determined as:

$$RS = TS - (SDS + RDS) \quad (1)$$

### Glycemic Index (GI) Analysis (Goñi et al., 1997)

Each % starch hydrolysis was measured as:

$$\% \text{ starch hydrolysis} = \frac{\text{glucose concentration every 30 minutes}}{\text{total starch}} \times 100\% \quad (2)$$

Hydrolysis index (HI) and glycemic index (GI) was calculated as:

$$HI = \frac{\text{area under curves (AUC) sample}}{\text{area under curves (AUC) white bread}} \times 100\% \quad (3)$$

$$GI = 39.71 + (0.549 \times HI) \quad (4)$$

### Data Analysis

Data analysis was performed with ANOVA method using Minitab 17 followed by Duncan's multiple range test ( $\alpha = 0.05$ ) for the treatment factor type of processing and cooling.

## RESULTS AND DISCUSSION

### Moisture and Total Starch

The moisture content of *C. tuberosus* after being treated with six types of cooking and cooling processes ( $\alpha = 0.05$ ) can be seen in Table 1, which was influenced by the methods used. The lowest water content was discovered in the roasted sample at 200 °C followed by the one refrigerated (52.95%). But, the highest was in samples boiled and cooled at room temperature (72.30%). Besides, the low humidity of the air in the roaster created a vapor pressure gradient causing water to move from the inside of the material to the surface and which eventually evaporated (Revlisia, 2012). The boiling process caused water to enter the sample because of the vapor pressure gradient (Fitriani, 2008) and cell damage (Amon et al., 2011).

According to Table 1, different processing methods affect total starch ( $\alpha = 0.05$ ). Based on temperature and water activity, thermal processes enhance starch hydrolysis rate by gelatinization (Alsaffar, 2011). Contrarily, Eleazu et al. (2017) and Capriles et al. (2008) said differences in cooking methods did not significantly affect total starch in food ingredients. The highest total starch was obtained by boiling *C. tuberosus* followed by cooling to room temperature (86.59%), and this is possibly due to the complete gelatinization process in boiling with adequate temperature and water availability. When starch granules in the water are exposed to heat, intermolecular and intramolecular hydrogen bonds between their chains are disrupted, allowing granular enlargement and disintegration. This makes starch to be easily hydrolyzed by enzymes, however, its content is lessened in roasters and microwaves.

Table 1. The moisture content and total starch of *C. tuberosus* after processing and cooling

Processing and cooling methods of <i>Coleus tuberosus</i>	moisture (%wb)	total starch (%db)
Raw, cooled at room temperature	71.29 ± 2.31	84.64 ± 5.17
Raw, refrigerated at 4 °C for 24 hours	9.82 ± 0.70	58.74 ± 1.73
Flour, cooled at room temperature	69.16 ± 5.43	74.39 ± 4.70
Flour, refrigerated at 4 °C for 24 hours	10.41 ± 0.30	61.81 ± 1.01
Boiled, cooled at room temperature	72.30 ± 4.90 <sup>b</sup>	86.59 ± 6.44 <sup>c</sup>
Boiled, refrigeration at 4 °C for 24 hours	64.96 ± 0.92 <sup>ab</sup>	71.57 ± 2.14 <sup>ab</sup>
Roasted, cooled at room temperature	60.38 ± 0.88 <sup>ab</sup>	71.41 ± 1.87 <sup>ab</sup>
Roasted, refrigeration at 4 °C for 24 hours	71.50 ± 2.76 <sup>b</sup>	75.33 ± 1.90 <sup>bc</sup>
Microwaved, cooled at room temperature	52.95 ± 8.78 <sup>a</sup>	61.32 ± 3.47 <sup>a</sup>
Microwaved, refrigerated at 4 °C for 24 hours	55.32 ± 9.87 <sup>ab</sup>	63.14 ± 0.77 <sup>ab</sup>

The abc = values with the small annotation have no significant difference ( $\alpha = 0.05$ )

### In vitro Digestibility Analysis

*Coleus Tuberosus* digestibility analysis was conducted after cooking and cooling to determine rapidly digestible starch, slowly digestible starch, and resistant starch according to Goñi *et al.* (1997), using in vitro method (Table 2). The result showed the treatments affected black potato resistant starch levels ( $\alpha = 0.05$ ). Based on Table 2, the cooking process followed by cooling extended the resistant starch levels in *C. tuberosus*. Previous study reported type III resistant starch formation in food during the treatment process

(García-Alonso *et al.*, 1999; Rendon-Villalobos *et al.*, 2002; Tovar *et al.*, 2002; Gonzáles-Soto *et al.*, 2006). Resistant starch content in the sample after cooking and cooling was higher than in the raw potato (38.18%), except in microwave processing which was cooled at room temperature (29.24%). When cooling is carried out after processing, retrogradation occurs to ensure starch structures become more resistant to enzymatic digestion (Chung *et al.*, 2006). The amount of resistant starch in processed foods compared to the raw material depends on the processing method employed (Alsaffar,

Table 2. In vitro digestibility analysis of *C. tuberosus* after cooking and cooling

Processing and cooling methods of <i>Coleus tuberosus</i>	Resistant starch (RS) (%db)	Rapidly digestible starch (RDS) (%db)	Slowly digestible starch (SDS) (%db)	Glycemic index
Raw, cooled at room temperature	38.18 ± 1.90	23.48 ± 0.82	22.98 ± 1.22	64.54 ± 1.07
Raw, refrigerated at 4 °C for 24 hours	41.19 ± 0.31	16.79 ± 0.09	16.41 ± 0.23	59.85 ± 0.15
Flour, cooled at room temperature	43.84 ± 0.78	7.40 ± 0.39	7.50 ± 0.43	51.12 ± 0.52
Flour, refrigerated at 4 °C for 24 hours	50.37 ± 0.94	5.65 ± 0.43	5.79 ± 0.51	48.11 ± 0.68
Boiled, cooled at room temperature	49.91 ± 1.20 <sup>d</sup>	18.46 ± 0.45 <sup>c</sup>	18.21 ± 1.64 <sup>bc</sup>	58.93 ± 0.48 <sup>ab</sup>
Boiled, refrigerated at 4 °C for 24 hours	49.45 ± 0.56 <sup>d</sup>	12.79 ± 0.77 <sup>a</sup>	13.09 ± 0.64 <sup>a</sup>	57.11 ± 0.56 <sup>a</sup>
Roasted, cooled at room temperature	40.14 ± 0.71 <sup>c</sup>	15.50 ± 0.42 <sup>b</sup>	15.93 ± 0.51 <sup>ab</sup>	59.60 ± 0.44 <sup>b</sup>
Roasted, refrigerated at 4 °C for 24 hours	35.01 ± 0.19 <sup>b</sup>	12.89 ± 0.15 <sup>a</sup>	13.42 ± 0.06 <sup>a</sup>	59.17 ± 0.17 <sup>ab</sup>
Microwave, cooled at room temperature	29.24 ± 1.76 <sup>a</sup>	20.98 ± 0.86 <sup>d</sup>	21.18 ± 0.91 <sup>c</sup>	66.48 ± 1.24 <sup>c</sup>
Microwave, refrigerated at 4 °C for 24 hours	36.52 ± 0.14 <sup>b</sup>	13.19 ± 0.06 <sup>r</sup>	13.43 ± 0.08 <sup>a</sup>	58.76 ± 0.11 <sup>ab</sup>

The abc = values with the same small annotation have no significant difference ( $\alpha = 0.05$ )

2011; Englyst *et al.*, 2007). Therefore, the potato sample's resistant starch was reduced after being treated. In general, refrigeration led to higher resistant starch compared to cooling at room temperature. Starch retrogradation increases with longer storage time and constant temperatures as both parameters are its main factors (Fu *et al.*, 2013; Xie *et al.*, 2014).

The treatment of heating followed by cooling has a significant effect on the RDS levels in *C. tuberosus* ( $\alpha = 0.05$ ). Foods containing lots of RDS quickly raise blood glucose and insulin levels (Miao *et al.*, 2013). Notably, the highest RDS was detected in the microwave treatment, cooled at room temperature (20.98%) where the sample had the lowest resistant starch. According to Patindol *et al.* (2010), RS and SDS are not collinear, foods containing high levels of RS do not necessarily have high SDS levels, and vice versa. In general, the amount of RDS in *C. tuberosus* (12.79-20.98%) reduced after heating and cooling treatment compared to the initial sample (23.48%). Refrigeration led to a lower RDS value in raw *C. tuberosus* compared to room temperature, while the RDS value after treatment is still higher than that of the flour subjected to both cooling methods.

The SDS is a starch that can be digested in the small intestine at a slower rate than RDS. According to Table 2, the different cooking processes followed by cooling affected the SDS levels in *C. tuberosus* ( $\alpha = 0.05$ ). The

microwave treatment with cooling at room temperature had the highest SDS compared to other treatments (21.18%), while refrigeration caused the SDS level to be lower than when cooled at room temperature. In addition, some studies stated consuming foods with high levels of SDS stabilizes blood sugar levels in the body (Vinoy *et al.*, 2013; Peronnet *et al.*, 2015).

Table 2 shows the treatments also affected the glycemic index (GI) of *C. tuberosus* ( $\alpha = 0.05$ ). Jenkins (1981) introduced the GI first, by grouping foods based on the physiological effects on blood glucose levels after consumption. Every food entering the body is digested at different speeds, hence leading to various blood glucose levels. The GI value in this study was around 57 to 66, where a different cooking process produced a different glycemic index. The lowest GI value of 57.11 was obtained from black boiled potatoes with 24-hour cooling at 4 °C. But, the highest (66.48) was in microwaved black potatoes at cooling room temperature. Moreover, cooling time affects RS levels in food as the retrogradation of starch increases when the storage time is longer with constant temperatures (Fu *et al.*, 2013; Xie *et al.*, 2014). The higher the RS level, the lower the GI in the foods consumed because resistant starch is unable to be digested, hence glucose levels in the blood are not increased.

The values of RDS, SDS, RS, and GI of potato flour are based on physiological effects of glucose digestibility

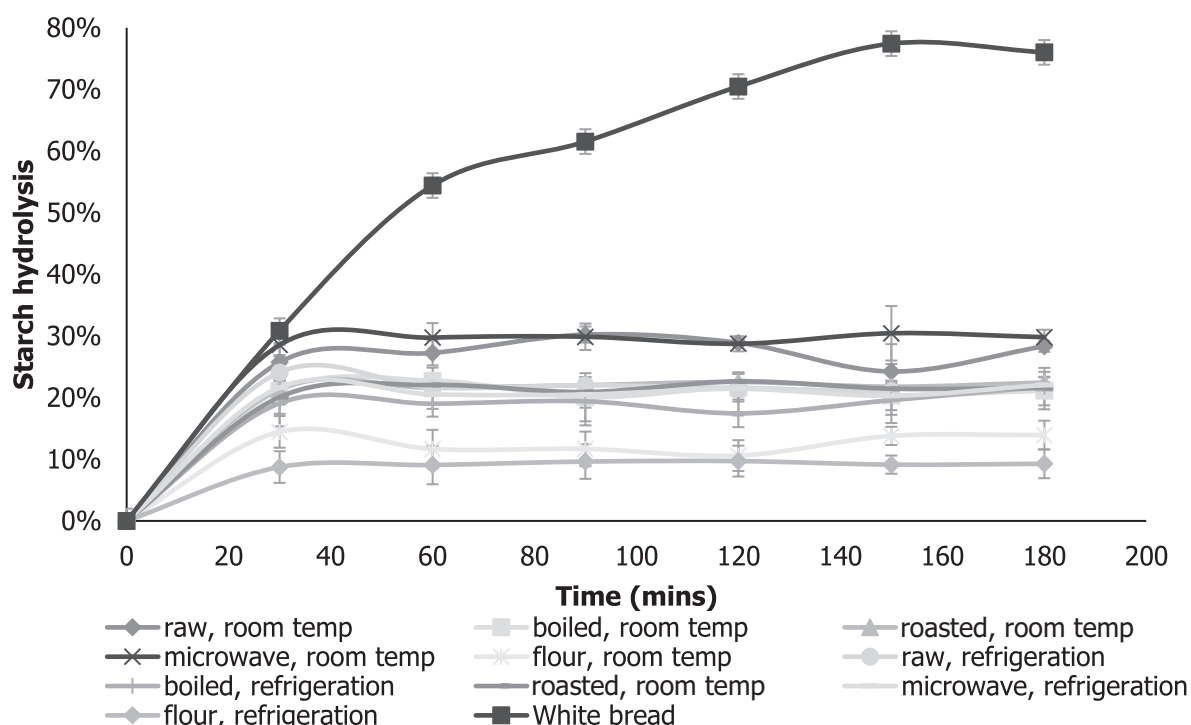


Figure 1. Percentage (%) hydrolysis of *C. tuberosus* after processing and cooling compared to white bread



Table 3. Lightness and yellowness of *C. tuberosus* after processing and cooling

Processing and cooling methods of <i>Coleus tuberosus</i>	Lightness (L)	Yellowness (b*)
Raw, cooled at room temperature	72.7 ± 0.66	29.63 ± 0.74
Raw, refrigerated at 4 °C for 24 hours	78.5 ± 0.97	18.87 ± 0.26
Flour, cooled at room temperature	71.1 ± 1.34	30.13 ± 0.93
Flour, refrigerated at 4 °C for 24 hours	79.7 ± 0.14	18.37 ± 0.12
Boiled, cooled at room temperature	62.2 ± 1.69 <sup>ab</sup>	14.30 ± 0.86 <sup>ab</sup>
Boiled, refrigeration at 4 °C for 24 hours	63.0 ± 2.79 <sup>ab</sup>	11.40 ± 0.78 <sup>a</sup>
Roasted, cooled at room temperature	69.4 ± 1.31 <sup>bc</sup>	19.57 ± 2.18 <sup>ab</sup>
Roasted, refrigeration at 4 °C for 24 hours	62.7 ± 0.97 <sup>ab</sup>	20.67 ± 3.56 <sup>b</sup>
Microwave, cooled at room temperature	60.4 ± 2.92 <sup>a</sup>	19.43 ± 3.29 <sup>ab</sup>
Microwave, refrigeration at 4 °C for 24 hours	71.6 ± 3.06 <sup>c</sup>	19.43 ± 2.55 <sup>ab</sup>

The abc = values with the same small annotation have no significant difference ( $\alpha = 0.05$ )

following the in vitro method (Goñi, 1997). In general, foods containing high GI raise blood glucose levels quickly, and those with low IG levels raise the glucose slowly (Atkinson *et al.*, 2008; Rimbawa and Siagian, 2004; Haree-Brun *et al.*, 2008). The hydrolysis level of each treatment was plotted as a sample area under the curve (AUC) compared to the standard AUC food reference for white bread. Percentage starch hydrolysis per 30 minutes sample can be seen in Figure 1 which shows that the rate was lower in the *C. tuberosus* subjected to processing and cooling compared to white bread. The increase in hydrolysis occurred in the initial 30 minutes, but it tended to be more stable afterward.

### Color Analysis

According to Table 3, the six treatment factors of cooking processes followed by cooling affected the brightness and yellowness value ( $\alpha = 0.05$ ), but had no effect on the redness level (data not shown). The lowest brightness value (60.4) was in the *C. tuberosus* roasted for 24 hours with cooling at 4 °C. This was possibly caused by the 200 °C temperature used for the roaster. However, the highest brightness value (69.4) was in the microwaved sample with cooling at room temperature. The lowest yellowness value (11.4) was obtained from roasted *C. tuberosus* with cooling at room temperature, while the highest (20.7) was in the boiled sample with 24-hour cooling at 4 °C. Generally, the processing decreases brightness and yellowness that are caused by the browning reaction due to polyphenol dehydrogenation (Le Bourvelec *et al.*, 2004).

### CONCLUSION

In summary, this study showed food processing and cooling affect the digestibility of a food ingredient. The heating process with cooling increases RS and SDS, but reduces GI and RDS levels in *C. tuberosus*. Furthermore, the lowest digestibility was achieved through boiling followed by refrigeration and the highest was through microwaving followed by cooling at room temperature. Generally, the cooling process with refrigeration has a greater effect in lowering starch digestibility than cooling at room temperature. Further analysis is recommended to be carried out on changes in starch structure due to *C. tuberosus* processing.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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