Effect of Cooling Methods and Drying Temperatures on the Resistant Starch Content and Acceptability of Dried-Growol

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Research Article

Effect of Cooling Methods and Drying Temperatures on the Resistant Starch Content and Acceptability of Dried-Growol

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Abstract

Background and Objective: Growol is a local staple food made from cassava that is processed by spontaneous fermentation in water for 3-5 days. Previous research has shown that drying Growol could extend its shelf life, and fermentation during processing could increase the amylose content. Increasing the amylose would produce high retrograded starch, which is a resistant starch (RS). The aim of this study was to evaluate the effects of different cooling methods and drying temperatures on the amylose content, the resistant starch content and the acceptability of dried-Growol. **Materials and Methods:** The cassava used in this research was the Martapura variety, which was fermented for 24 h and cooked by use of an autoclave for 15 min. Samples were then either cooled to room temperature with no subsequent storage or stored in a refrigerator at 4-7°C for 24 h and then dried with temperature variations of 40, 50 and 60°C. The dried Growol samples were analyzed for their moisture content, starch, amylose, X-ray diffraction, resistant starch content and their acceptability by hedonic test. **Results:** The research showed that varying the cooling method and drying temperature affected the amylose and RS content, and the acceptability of dried Growol. Cooling at refrigerator resulted dried-Growol with high RS and acceptability. **Conclusion:** Acceptable dried-Growol was obtained by cooling by refrigeration and drying at temperatures of 50-60°C. This Growol had an amylose content of 42.92-44.63%, the degree of crystallinity was 25.81-26.16% and it had an RS content of 16.55-17.04 g/100 dry matter.

Key words: Amylose, fermented-cassava, Growol, resistant-starch, retrogradation,

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Growol is a staple food made from cassava with a fermentation stage as part of the process. Fermentation is achieved by soaking the cassava in water for 3-5 days[1]. During fermentation lactic acid bacteria grows to produce lactic acid. Putri *et al.*[2] stated that the lactic acid bacteria types predominantly found in Growol are *Lactobacillus plantarum* and *Lactobacillus rhamnosus*. Wariyah and Sri Luwihana[3] found that the total *Lactobacillus* in Growol was 4.7×10^3 CFU g^{-1}. Because of its lactic acid content, Growol has been proven to be effective in preventing diarrhea, if consumed daily[4].

A problem with Growol is its short shelf life of only 3-4 days, after which it quickly becomes moldy. Luwihana and Wariyah[5] have preserved Growol by drying. Dried Growol has a storage life of about 4-6 months. However, the potential pro-biotic quality of Growol was greatly reduced during drying. The dried Growol had a white colour, appropriate to the cassava type used, but the texture was very hard. This texture indicated the retrogradation in gelatinized starch of the Growol. According to Sajilata *et al.*[6], starch will gelatinize during heating, and when cooled the gelatinized starch becomes retrograded and is marked by a hard texture. Retrograded starch is a resistant starch (RS), that is the part of starch that cannot be broken down enzymatically into glucose[7] and RS is among a number of starches and degradation products that cannot be digested by the human small intestine. The advantage of resistant starch formation is that RS can suppress hyperlipidemia[8] by lowering blood triglyceride, and Si *et al.*[9] stated that RS can control weight and fat mass in adipose tissue, suppress oxidative stress and it has a hypoglycemic effect that is beneficial in preventing obesity.

There are four kinds of RS: RS1, RS2, RS3 and RS4. RS3 is a fraction of resistant starch consisting of retrogradation amylose which is formed during the cooling of the gelatinized starch[6]. RS3 is formed from amylose, so that a greater amount of RS3 is produced by increasing the amylose[10]. The level of starch re-crystallization of gelatinized amylose is affected by storage temperature and time. Cooling temperatures will accelerate RS3 formation and total crystallinity more effectively than storage time[7]. The objectives of this study were to evaluate the effect of cooling methods and drying temperatures on amylose content, degree of crystallinity, resistant starch content and acceptability of dried-Growol.

MATERIALS AND METHODS

Materials: This study used the Martapura local cassava variety as a raw material for Growol production. The cassava was obtained from farmers at Kalirejo village, Kokap sub district, Kulon Progo Regency, Special Region of Yogyakarta, Indonesia. The cassava was harvested at the age of about 10-12 months and was used no more than two days after harvesting. Chemicals used for analysis of the starch content and amylose were: KOH (Merck, 85%), indicator phenolphthalein C_2OH_4OH (Merck, Darmstadt, 1%), ethanol (Merck, 100%); HCl (Merck, 37%) with pro-analysis qualification.

Equipments: The equipment used in this research was: a set of cassava fermentation equipment, autoclave (Pressure sterilizer model 1925X, Wisconsin Aluminium Foundry C. Inc. 838 South 16th St. Manitowoc, WI 54220), steamer pan (Bima Stainless Steel), UV Vis Spectrophotometer (Shimadzu UV mini 1240) for analysis of amylose and starch, balance scales (OHAUS Pioneer PA214), oven (Memmert DIN 40050 IP 20), vortex (Maxi Mix II TY 37600), X-Ray Diffraction analysis with Rigaku Miniflex 600, a set of sensory testing equipment and glassware for chemical analysis from Pyrex Iwaki (Iwaki glass under LIC).

Methods: The fresh cassava was analyzed for its water content using the static gravimetric method and for its starch content with the Direct Acid Hydrolysis method[11]. Amylose content was analyzed by the colorimetric method[12]. Processing of Growol refers to Wariyah and Sri Luwihana[3] (2015) and Wariyah *et al.*[13] with cooking modifications. The cassava was peeled and cut into 5 cm lengths, then soaked in water at a cassava/water ratio of 1/3 (w/v) with a soaking/fermentation duration of 24 h; the sample was made in duplicate. The fermented cassava was analyzed for moisture content, starch and amylose content. Furthermore, each of the samples was cooked using two autoclave cycles at 121°C for 15 min[14]. Samples were then either cooled to room temperature with no subsequent storage or stored in a refrigerator at 4-7°C for 24 h, after which they were dried with temperature variations of 40, 50 and 60°C. The dried Growol samples were analyzed for moisture content, degree of crystallinity and RS content using the AACC International method (AACC 32-40.01)[7] and the acceptability of the dried Growol was determined by Hedonic Test[15].

This research used Completely Randomized Design with the factors of cooling method and drying temperature. The difference between treatments was determined by F test then any significant difference between samples was determined by Duncan's Multiples Range Test (DMRT) and analyzed by SPSS 13.0.

RESULTS AND DISCUSSION

Chemical properties of cassava and fermented cassava: The Martapura variety of cassava used in this study was harvested at 10-12 months of age. The water content, starch and amylose were 73.78±0.67, 21.79±0.48 and 16.52±0.80%, respectively. The water content, starch and amylose of the cassava were affected by its variety, harvesting age and planting location. According to Susilawati *et al.*[16], the later the harvest, the greater the amylose and starch content, but the lower the water content. The Kasetsart cassava variety, which was harvested between the ages of 7-10 months contained between 14.33-35.93% starch with an amylose content of 12.37-18.91%.

The water content of fermented cassava was 59.25±1.74%, starch 38.24±4.22% and amylose 20.26±3.70%. The water content of the fermented cassava was lower than that of the fresh cassava, because it had been pressed before being steamed to decrease the water content to a required level, so that the resultant Growol texture was hard and had a rice-like appearance. The starch content of the fermented cassava was lower than the fresh cassava, but the amylose content was higher. According to Putri *et al.*[2], during the fermentation there is hydrolysis of the cassava starch, which can increase the amylose but this amylose increase is affected by the *Lactobacillus* (L.) strain that grows during fermentation. In the 48 h cassava starch fermentation, *L. amylophilus* gave the highest amylose content (34.02%), higher than *L. plantarum* (32.18%). In this study fermentation was carried out on fresh cassava rather than fermented cassava, to give a lower amylose content. In addition, the bacteria that grow primarily are *L. plantarum* and *L. rhamnosus*[17], which turn out to produce lower amounts of amylose.

The RS content of the fresh cassava was 9.42±0.53 g/100 g dry matter and on the fermented cassava was 10.92±1.33 g/100 g dry matter, or about 2.47% (wb) on fresh cassava and 4.45% (wb) on fermented cassava. According to Ogbo and Okafor[18] the RS content of Tropical Manihot Species (TMS) of 6 varieties was between 5.70-7.07g/100 g, whereas the RS content of cassava with a fermentation time of 48 h was between 4.13-4.79 g/100 g. This study used the Martapura cassava variety with a fermentation time of 24 h. According to Putri *et al.*[17], fermentation which takes too long will first cause starch to hydrolyze into sugar, then produce lactic acid. Therefore, the amylose and RS contents were higher.

Chemical properties of dried-Growol: Each of the fermented cassava samples had been pressed and minced, then was cooked in the autoclave for two cycles. The resulting Growol was cooled, either at room temperature or by being stored in a refrigerator at a temperature of 5-7°C for 24 h before drying. The result of the dried Growol analysis is shown in Table 1.

The results showed that the moisture content of dried Growol was not significantly different. Dried Growol is a product similar to artificial rice, so drying was directed toward reaching a moisture content of less than 15%. In dry materials such as rice, the maximum water content requirement is 15%[19], therefore the drying yield of Growol is still suitable as a rice-like food.

Table 1 shows that the starch content of dried Growol was not significantly different. This was because the drying method was by water evaporation only, therefore it did not change the starch component chemically. However, the amylose levels and RS in the dried Growol differed significantly. According to Sajilata *et al.*[6] the starch undergoes gelatinization during heating and then is retrograded during cooling/drying, which is characterized by a hard texture. Cooling in a refrigerator at a temperature of about 4°C causes the degree of crystallinity or retrogradation to be greater than by cooling at room temperature (Table 2 and Fig. 1), although analysis of the amylose using iodine showed that the amylose content in the Growol cooled in the refrigerator was less. William *et al.*[12], stated that interaction between amylose and an iodine solution produced a blue colour; the more amylose, the greater the interaction, thus the intensity of the blue color deepened. However, Nwokocha and Ogunmola[20] stated that the interaction of amylose with iodine will decrease in retrograded amylose.

Crystallinity of dried Growol: Figure 1 shows the X-ray diffraction (XRD) pattern of cassava flour which had peak characteristics with angles 2θ at 15°, 17.43° and 22.84°. These characteristics were similar to white bread and most other cereal starches which have peaks of 2θ angles of 15, 17, 18 and 23°. Shah *et al.*[21] also found that oat starch had peak angles 2θ at 15 and 23°. As shown in Fig. 1, it was seen that the amorphous zone was larger than the crystalline zone. After being processed into dried Growol through cooking in the autoclave and cooling, there were changes in the area of the

Table 1: Amylose and resistant starch of dried Growol

Cooling methods	Drying temperature (°C)	Moisture** (%)	Starch** (%)	Amylose* (%)	Resistant* Starch g/100 g dry matter
Room temperature	40	11.45 ± 0.29	82.34 ± 4.93	46.47 ± 0.46^{bc}	13.06 ± 0.27^{a}
	50	12.24 ± 0.90	78.13 ± 4.13	50.24 ± 3.70^{c}	15.62 ± 0.49^{bc}
	60	13.24 ± 0.26	81.81 ± 5.82	49.83 ± 0.51^{c}	14.68 ± 0.91^{b}
Refrigerator	40	12.67 ± 0.36	81.82 ± 4.05	37.80 ± 0.40^{a}	17.83 ± 0.77^{d}
	50	13.00 ± 1.08	81.50 ± 0.90	42.92 ± 3.48^{b}	16.550 ± 0.58^{cd}
	60	12.74 ± 1.24	82.63 ± 1.64	44.63 ± 0.63^{b}	17.04 ± 0.67^{d}

*Means in a column with similar superscript, no significant difference at $\alpha = 0.05$, **ns

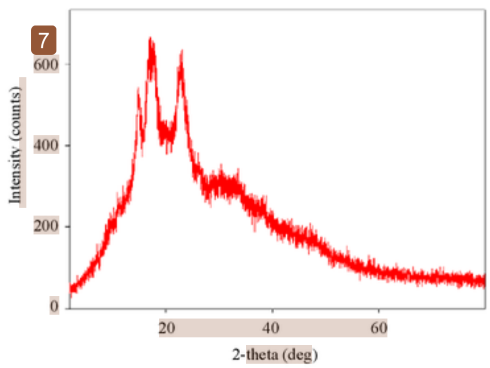

Fig. 1: X-ray diffractogram of cassava flour

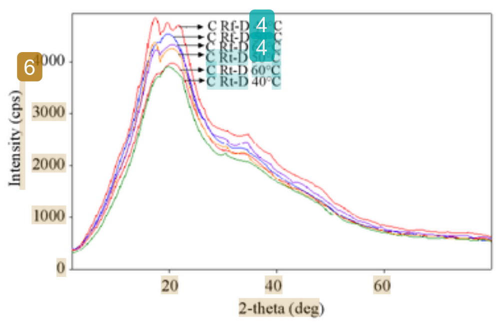

Fig. 2: X-ray diffractograms of dried-Growol subjected to cooling in a refrigerator (C Rf), at room temperature (C Rt) and being dried (D) at temperatures of 40, 50 and 60°C

Table 2: Degree of crystallinity of dried Growol

Cooling method	Drying temperature (°C)	Degree crystallinity* (%)
Room temperature	40	21.62 ± 1.66^{a}
	50	24.58 ± 1.82^{ab}
	60	23.58 ± 1.04^{ab}
Refrigerator	40	27.41 ± 0.88^{b}
	50	26.16 ± 2.53^{b}
	60	25.81 ± 1.55^{b}

*Means in a column with similar superscript are not significantly different at $\alpha = 0.05$

amorphous and crystalline zones (Fig. 2). The crystalline zone became larger, especially for Growol which had been cooled in the refrigerator at temperatures between 4-7°C, with characteristic peaks at angles 2θ at 17.30° and 23.25°. According to Sullivan *et al.*[7], retrogradation or re-crystallization of starch occurs during storage, but the level of retrogradation differs depending on storage conditions. Storage at 4°C showed the highest degree of crystallization in white bread. Something similar happens with Growol. Growol stored in a refrigerator at temperatures of 4-7°C showed a higher degree of crystallization than Growol stored at room temperature (Table 2). The drying temperature did not significantly affect the level of crystallinity.

Starch retrogradation occurs very quickly in amylose portions because the linear structures facilitate cross-linking through hydrogen bonds. Sajilata *et al.*[6] found that the rate and extent to which a starch may be essentially retrograded after gelatinization depends on the amount of amylose content. Two cycles autoclaving of wheat starch may result up to 10% RS. The level obtained appeared to be strongly related to the amylose content and the retrogradation of amylose. The amylose level is identified as the main mechanism for the formation of RS that can be generated in large amounts by repeated autoclaving. The degree of crystallinity of dried Growol processed by cooling in a refrigerator was relatively high compared with dried Growol which had been cooled at room temperature. The increase in Growol crystallinity produced by cooling in a refrigerator was caused by the higher retrogradation level and was indicated by the higher levels of RS and lower levels of amylose. Nurhayati *et al.*[22] and Ashwar *et al.*[14] conducted experiments with banana flour starch and rice reported that cooling in a refrigerator resulted in higher retrograded starch than cooling at room temperature. This was due to the greater re-association between the amylose straight chains. This was in line with the increase in RS of dried Growol which was obtained when cooled in a refrigerator.

Acceptability of dried Growol: Dried Growol was tested for its acceptability, on a scale of one to seven, based on its aroma, colour, texture and overall consumer preference.

Table 3: Acceptability of dried Growol

Cooling method	Drying temperature (°C)	Aroma**	Colour**	Texture*	Overall* preference
Room temperature	40	3.06	3.28	4.17^{b}	3.28^{ab}
	50	3.06	3.06	4.61^{b}	3.78^{b}
	60	3.17	2.94	3.39^{a}	3.06^{ab}
Refrigerator	40	3.11	3.22	3.11^{a}	3.11^{ab}
	50	3.00	2.61	2.83^{a}	2.61^{a}
	60	2.94	2.56	3.00^{a}	2.83^{a}

*Means in a column with similar superscript are not significantly different at α =0.05, **ns

Number 1 means very well liked, 4 neutral and 7 very much disliked. The resulting test of preference for dried Growol (Table 3) showed a significant difference in texture and overall consumer preference, while aroma and color were not significantly different.

Growol aroma is typically acidic, this is caused by organic acids, especially lactic acid, which forms during fermentation. In this study, for all treatments the fermentation was carried out for 24 h, so the acid formed during fermentation was estimated to be no different. According to Wariyah *et al.*[13], the titratable acidity of fermented cassava with a 24 h fermentation time was 0.38±0.02% (wb). Whereas dried Growol colour was governed by the colour of the cassava starch used as the Growol raw material. The cassava used for this research was the same Martapura variety which has a white flesh colour, so the Growol color was also white; consumer preference of dried Growol was not significantly different.

The preference level of the dried Growol texture was significantly different with variations in cooling and drying temperatures. The texture of Growol cooled in a refrigerator was preferred compared to that cooled at room temperature. Besides the treatment factor, the texture was also influenced by the composition of the product[23]. Table 1 shows the difference in amylose content and dried Growol RS, so that the higher the amylose content in the dried Growol, the harder the texture becomes and it is less well preferred. Visually, dried Growol which had been processed by cooling in a refrigerator was shaped like a small grain in separate granules. This was because before drying, crystallization of Growol had occurred due to starch retrogradation being higher during cooling, so the resulting dried Growol in the form of small granules similar to rice was preferred over dried Growol which had been cooled at room temperature. Characteristically, Growol cooled at room temperature was sticky, large and irregular in size and shape. As a result, when it was dried it appeared to be a hard dry lump and was less well preferred. Based on the preference of aroma, color and texture and the content of the RS, the preferred dried Growol was that which had been treated by cooling in a refrigerator at a temperature of 4-7°C and dried at temperatures between 50-60°C.

CONCLUSION

Dried Growol processed by cooking in an autoclave and cooling in a refrigerator for 24 h, then drying at temperatures between 50-60°C, produced dried Growol with high crystallization, high resistant starch (RS) content and high acceptability. The Growol had an amylose content of 42.92-44.63%, the degree of crystallinity was 25.81-26.16% and the RS was 16.55-17.04 g/100 dry matter.

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