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The Effect of Annealing Modification on Increasing Glucomannan Content of Porang (*Amorphophallus Muelleri Blume*) Flour

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***Abstract*— This study aims to determine the effect of annealing on the glucomannan, protein, and water content of *porang* (*Amorphophallus Muelleri Blume*) flour. The material was a *porang* tuber from a farmer in Lubuk Pakam Regency in the second plant period. The method was completely randomized with temperature treatments of 30 °C, 40 °C, and 50 °C, and time; 3 hours, 4 hours, and 5 hours—parameters observation consisting of water content, protein, glucomannan, and yield. Annealing time significantly affects water content, glucomannan, and yield. It is related to the longer the time; the more water-soluble compounds were lost. It increases the amount of glucomannan and decreases the yield. The temperature significantly affects water content, protein level, glucomannan content, and yield. At the temperature reaches 40 degrees Celsius, the number of glucomannan increases, which is associated with the beginning of the gelatinization process. At the onset of gelatinization, the starch granule structure weakens, allowing it to be readily crushed and liberated from glucomannan. This study indicated that glucomannan content decreased significantly as the temperature increased to 50 °C. It is hypothesized that the gelatinization temperature of *porang* starch is low; therefore, gelatinization is complete at around 50 °C. However, it needs further research. The protein level decreases by increasing temperature due to protein denaturation. The annealing process at 40 °C for 5 hours gives the high glucomannan content. Glucomannan has a strong relationship with yield. The annealing process promised to be used in glucomannan production from *porang* tuber.**

***Keywords*— Porang; annealing; glucomannan; yield; gelatinization; starch.**

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I. INTRODUCTION

Porang (*Amorphophallus muelleri Blume*) is a locality-specific commodity from Indonesia. West Java, Central Java, East Java, Lampung, West Nusa Tenggara, and South Sulawesi are centers for *porang* plants [1]. Porang is a source of glucomannan [2]. Extracting glucomannan from tubers, leaves, steam, and other sources is possible [3]. Porang must first be made into chips or flour to extract glucomannan. Chips are slices of *porang* tubers that resemble chips or cassava and are then dried. In making chips, the fresh tubers are sorted first by separating the ones that are not damaged or defective, then peeling, washing, and soaking in the water while waiting for the following process to prevent browning. The tubers were then sliced, soaked, and dried [4]. It is traditionally dried using sunlight. On a modern scale, drying uses more sophisticated tools and machines in a controlled environment. The quality of *porang* flour is determined by its glucomannan content.

Glucomannan is a neutral polysaccharide consisting of mannose and glucose in a ratio of 1.6:1 [3], [5]. Glucomannan cells are significant in contrast to starch and others, which are small in size. The size of glucomannan cells, ranging from 0.25 to 0.75 mm, is equivalent to 5–10 times the size of normal cells, are oval, have a rigid structure, and are difficult to destroy [6]. Biocompatibility, non-toxicity, and safety are all characteristics of glucomannan. Because of these things, glucomannan is used in the food, beauty, and medical industries [7]. The ability of glucomannan to gel quickly in hot temperatures makes it popular in the industry [5].

The transformation of *porang* into *porang* chips or *porang* flour can preserve the glucomannan content for subsequent purification or extraction until pure glucomannan is achieved. In principle, processing *porang* into chips or *porang* flour removes impurities surrounding the glucomannan by washing and slicing. Before flour crushing and sieving, commercial glucomannan granules were removed by slicing, drying, and grinding to eliminate contaminants sticking to the granules

[8]. In its fresh form, glucomannan is surrounded by impurities. The drying process helps impurities adhere to the surface of the granules so that they are easily separated in the purification process [6]. Glucomannan contains impurities such as starch, cellulose, and nitrogen.

Physical change is a method that is often used to change the way that starch works. In particular, heat changes the starch granules either partially or totally. Physical modification is considered quite good and promising compared to chemical modification because it requires low cost and safety and does not use chemical reagents [9] [10]. Physical changes can be made in several ways, including pre-gelatinization, heat-moisture treatment (HMT), annealing, hydrothermal processes, and changes that do not involve heat. Heat treatment and annealing are the most popular [9] because they are thought to be the best at changing the properties of starch, such as its relative crystallinity, ability to absorb water, and ability to form a paste without damaging the molecules [11].

Annealing is a hydrothermal process by conditioning starch over 70% (v/v) or intermediate (40%, v/v) water content at temperatures above the glass transition temperature and below the starch gelatinization temperature [9], [12] over a period varying from minutes to days. The annealing principle was widely used to improve starch properties. The annealing process, in principle, changes the morphological properties, thermal viscosity, and pasting properties of various starches. It makes native starch better than before. Annealing can change the physical, functional, and rheological properties [13], crystallinity structure and degree of crystal formation in starch [14], thermal properties [13], [15], physicochemical properties [16], as well as starch digestibility [13], [16]. Research examining the effect of annealing on glucomannan content has never been studied before. Even though the annealing procedure altered the structure of the starch, it will be easier to separate it from the structure of the glucomannan, which could increase the glucomannan content.

II. Materials and Methods

A. Material

The primary material used is Porang tubers from the Porang plant that have been planted for over two years and obtained from farmers in Lubuk Pakam District, Deli Serdang Regency, North Sumatra Province. Other ingredients used are aqua, water, and salt. In comparison, the equipment used is a tuber slicer, a dryer, a sieve, a drying oven, a digital scale, a blender, a knife, a baking sheet, a drying oven, and glassware for analysis.

B. Experimental Design

This study used a factorial, Completely Randomised Design method consisting of two factors; temperature(°C) and time (h). Nine treatment combinations were obtained based on the number of factors and the treatment level tested, and each was replicated three times. The temperatures in this study were 30 °C, 40 °C, and 50 °C, and the times used were 3 hours, 4 hours, and 5 hours.

C. Preparation

A total of 1 kg of *porang* tubers for each treatment were peeled, washed, and drained. Clean Porang tubers are sliced to a thickness of 2 mm (chips). Washed using aqua and drained. The clean chips are soaked with a 5% salt solution to remove the calcium oxalate content. The chips were rewashed to remove the remaining salt using distilled water. Implementation of annealing refers to [18] with modification, with some modifications. We weighed the chips dryly into polyethylene bags and added water in two ratios of 1:2 wt/vol. They were then placed in an oven (UN55 PLUS, Memmert) and heated at the appropriate temperature and time. The temperature used is a temperature below the initial gelatinization temperature. In an oven, the chips were dried in the air at 35°C for 48 hours. Then ground into powder and passed through a 75-m sieve. Flour was stored in a closed container at room temperature in the refrigerator until used for analysis.

D. Water Content

The water content was determined by the oven-drying method [19]. The aluminum crucible was dried in an oven at 130 ± 3 °C. The cup is then cooled in a desiccator and weighed (W2). About 2 g of the sample was weighed (W) and put into a cup. The cup containing the sample was dried in an oven at 130 ± 3 °C for 1 hour (1 hour drying period starts when the oven temperature is 130 C). The cup with the dried sample was put in a desiccator to cool it down. It was weighed as soon as it reached room temperature (W1). Water content using the following formula:

$$\text{Water content (\%bb)} = \frac{\text{W}-(\text{W1}-\text{W2})}{\text{W}} \times 100\ \% \quad (1)$$

Which:
W = sample weight (g)
W_1 = cup weight+ dried sample (g)
W_2 = weight of empty cup (g)

E. Protein Analysis

Protein content was measured by the Kjeldahl method [19]. A 0.1 g sample was placed in a 30 ml Kjeldahl flask and mixed with 2.5 ml of concentrated H2SO4, 1 g potassium sulfate, and a boiling stone. The sample is boiled for 1–1.5 hours or until the liquid is clear. The flask and contents were cooled before being transferred to a distillation apparatus and treated with 15 ml of 50% NaOH solution before being rinsed with distilled water. An Erlenmeyer flask containing 0.02 N HCL was placed under the condenser. Previously, a mixture of 0.02% methyl red in alcohol and 0.02% methyl blue in alcohol was added in a ratio of 2:1. The condenser tube's end must be placed in the flask holding the HCl solution before being distilled. The total distillate collected was about 25 ml. A small amount of distilled water was used to rinse the condenser's tip, and the distillate and rinse were combined with an Erlenmeyer before being titrated with 0.02 N NaOH until the color changed from green to purple. The blank determination was carried out with all the same procedures without samples.

$$\text{Nitrogen (\%)} = \frac{(Y-Z)x\ N\ x\ 0.014}{\text{W}} x\ 100\ \% \quad (2)$$

Which:
W = sample weight (g)
Y = ml NaOH titer for blank
Z = ml NaOH titer for sample
N = normality of NaOH

F. Glucomannan Analysis

Glucomannan levels were carried out using the gravimetric method in [19]. The sample was weighed at 0.5–1 g into a 50 mL test tube. Dissolved with 50 mL of Aquades, rotated, and shaken using the ultrasonic method at 60 °C for 1 hour. The solution was put into the incubator overnight at 60 °C. The solution was centrifuged to obtain a clear liquid, then accommodated in a 200 mL Erlenmeyer and 50 mL ethanol pa. Shake gently until a precipitate appears—filter with filter paper whose weight is known. Then wash the Erlenmeyer and filter paper using ethanol and dry them in an oven at 100 °C for about 30 minutes. The filter paper and precipitate were weighed. The difference in the blank paper's weight minus the paper's and sediment's weight calculates glucomannan levels.

G. Yield

Measuring flour yield based on flour weight after annealing [20]. The percentage yield is calculated by dividing the difference between the weight of the flour and the weight of the chips by the weight of the chips.

H. Statistical Analysis

The data obtained in the study were processed by ANOVA and *Least Significant Data* (LSD) further test, regression analysis, and Principal Component Analysis (Principle Component Analysis). The software used is MS Excel, SPSS V.20, and SMARTSTATXL".

III. Result and Discussion

The annealing technique is carried out by conditioning the starch at a high water content and heating it below its gelatinization point. The temperature is above the glass transition and below the gelatinization, time-varying from minute to day [11]. In this study, starch was conditioned at temperatures; 30 °C, 40 °C, and 50 °C for 3, 4, and 5 hours.

Fig. 1 Porang from different temperatures and times of annealing treatment in chip and flour form

Fig. 1 visually shows the noticeable change in the color of the chips and flour. The longer the time, the lighter the color. While visually, the higher temperature does not have much effect on the color. The mechanism of annealing modification involves increased starch chain interactions, which begin with crystal structure disruption, followed by double helix structure dissociation and crystal reassociation disruption [11]. Starch granules' dehydration and molecular rearrangement may be responsible for the microstructural changes [21]. Some researchers reported no significant morphological changes in starch, especially in the integrity and starch shape [22], granule size [13], and granules presented [15]. In this study, the effect of annealing on starch morphology was not observed.

A. Water Content

Water content is a parameter that has a significant role in the stability of a product's quality. Statistical analysis showed a very significant effect of temperature and soaking time on the moisture content of porang flour ($p<0.01$). The higher the temperature used, the higher the water content. Likewise, the longer the time, the higher the water content. The statistical analysis results also did not show a significant effect of interaction between temperature and soaking time on the moisture content of *porang* flour.

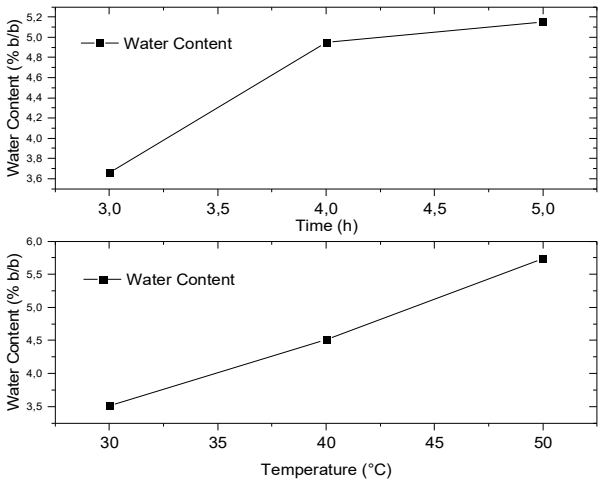

Fig. 2 The effect of temperature and time on the water content of annealing *porang* flour.

Starch is the main component in *porang* flour. During heating, the starch granules will absorb water and swell.

Water absorption has a positive correlation with the swelling capacity of the granules. This situation indicates the beginning of gelatinization. Gelatinization is characterized by changes in intermolecular associations at O-6 from amylose and OH-2 from amylopectin molecules. Hemiacetal hydrogen (>O) and the hydroxyl group of starch are responsible for changing the tetrahedral arrangement of hydrogen bonded to water molecules [23]. In annealing, the flour is heated below the gelatinization temperature. However, the start of gelatinization already occurred when the temperature was increased. The degree of change in the structure and properties of starch depends on the water content, temperature, and duration of treatment [24]. Therefore, the higher the temperature and the duration of soaking, the more accumulation of chemically bound water must be removed, affecting flour's water content. Increases The decrease in water content was due to the higher weight of the dried *porang* chip, the more accumulated water content that had to be evaporated from the chip [25].

B. Protein Content

Before the protein content, glucomannan, and yield were analyzed, an initial analysis was carried out on *porang* chips (without treatment). The analysis found that *porang* chips contained 16.76% protein and 14.27% glucomannan. Statistical analysis showed a very significant effect of the temperature on the protein content of *porang* flour ($p< 0.01$). Meanwhile, the time did not significantly affect protein content ($p> 0.01$). Fig. 3 shows that the protein content of *porang* flour decreased with a longer time.

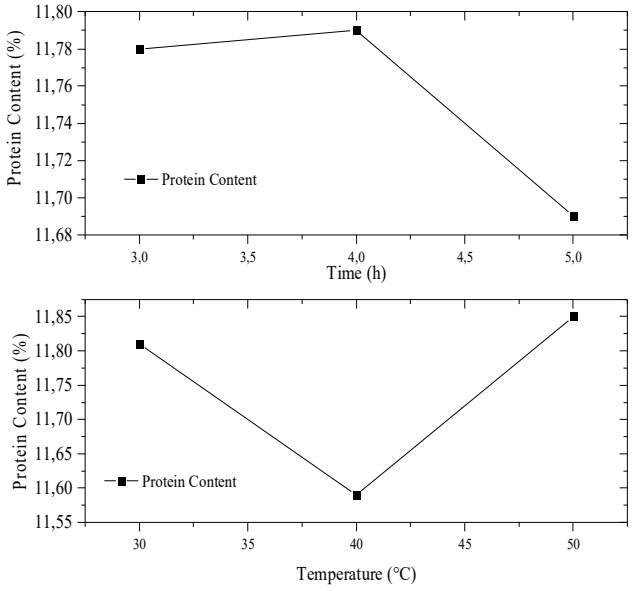

Fig. 3 The effect of temperature (above) and time (below) on the protein content of annealing porang flour.

At the time of soaking, water-soluble proteins will dissolve. The longer the time, the more dissolved protein was, resulting in low protein content. The protein content showed a decrease at the annealing temperature of 40 ºC. The occurrence of protein denaturation is probably due to heat. When heated, the protein can be denatured. The heating process allows the interaction of carbohydrates, protein, and fat molecules. The activity of the polyphenol oxidase enzyme can also cause protein content to decrease. It causes a decrease in the value of fat and protein [26]. Temperature affects protein denaturation in enzymatic hydrolysis. The hydrolytic degradation of β-mannanase may be inactivated with increasing time [27].

Statistical analysis also showed a very significant effect of the interaction between temperature and time. In the interaction of 50 °C temperature and times of 3, 4, and 5 hours, protein content showed a decreasing pattern with treatment time. The highest protein content was obtained from the treatment interaction at 40 °C for 4 hours and at 50 °C for 3 hours (11.99± 0.11%). At 50 °C, the highest protein content was 11.85±0.14%. Although the protein content of the various treatment interactions did not show a particular pattern, the difference was that, in general, the protein content of each treatment was lower than without treatment (*porang* chip protein content).

The results of this study indicated that the annealing treatment of *porang* flour reduced the flour's protein content. Compared with *porang* chips, the annealing modification reduced the protein content of *porang* flour by 15.89%. Proteins are macromolecules composed of several atoms, such as oxygen, nitrogen, carbon, and amino acids. In the glucomannan structure, nitrogen is one of the impurities in several acid amines. Normal constituents of the glucomannan structure include starch, cellulose, and nitrogen around idioblasts containing glucomannan grains [6], [28]. It is thought that there will be less material in normal cells and more glucomannan that can be taken from *porang* chips or flour.

C. Glucomannan Content

Glucomannan is one of the essential raw materials for the food, pharmaceutical, cosmetic, and chemical industries. *Porang Amorphophallus muelleri Blume* is a type of tuber plant that is rich in glucomannan. It needs an advanced process to get glucomannan from *porang* flour. This study examined the effect of annealing on the glucomannan content of dried *porang*.

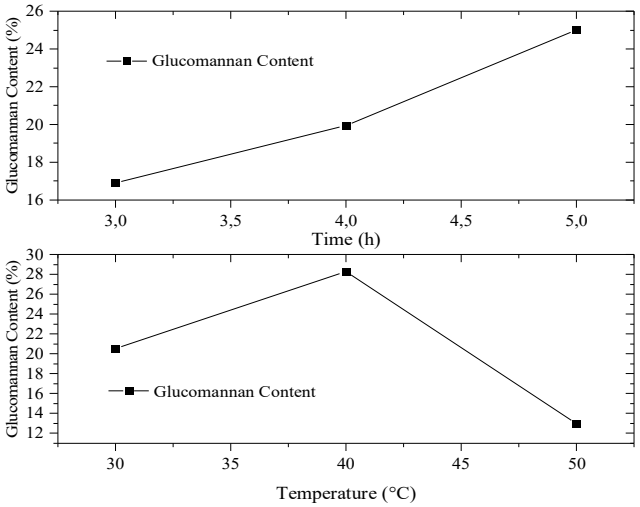

Fig. 4 The effect of temperature (above) and time (below) on glucomannan content of annealing porang flour.

The statistical analysis showed a significant independent effect of time on the glucomannan content of annealing *porang* flour ($p<0.01$). The content of glucomannan increases

with time (Fig 4 above). At 5 hours, the glucomannan content reached 25.02±9.43%. It is due to a large number of impurities, such as protein and starch, that dissolves into the water. The longer the time, the longer the interaction with water, and the number of vitamins and compounds easily soluble in water will increase. With reduced impurities, glucomannan is easier to extract. According to [29], the lowest glucomannan content was in the treatment without soaking. It is because the soaking treatment can reduce the impurities like calcium oxalate. During the soaking process, there was a reduction in the amount of calcium oxalate. The longer the object is submerged, the greater the water pressure exerted on the cell walls. It causes the calcium oxalate crystals to be expelled from the cells and discarded along with the soaking water [30]. Porang, which was directly dried in the sun, still contains high calcium oxalate.

Statistical analysis of the independent effect of temperature on glucomannan content showed a very significant effect ($p<0.01$). The glucomannan content increased with increasing annealing temperature from 30 °C to 40 °C (fig 4, below). When the temperature was increased to 50 °C, the glucomannan content decreased drastically. The highest glucomannan content was 28.31 ± 4.38% at 40 °C. It was thought to be related to the gelatinization of starch at 30 to 40 degrees Celsius; starch gelatinizes due to extra water and an increase in temperature. During the *gelatinization* of water molecules with a hexagonal shape, including intramolecular and intermolecular interactions, hydrogen bonds transform the majority of water molecules into a tetrahedral hydrogen network linked to specific water molecules [23]. Hemiacetal hydrogen (>O) and the hydroxyl group of starch are responsible for altering the tetrahedral arrangement of hydrogen attached to water molecules, which affects intermolecular interactions at O-6 of amylose and OH-2 of amylopectin during *gelatinization*. In the initial phase of *gelatinization*, water will be imbibed, which causes swelling of the starch granule size. Puelles-Roman et al. [13] reported annealing modified starch granule size enlargement.

The annealing procedure increased the annealed starch preparations' swelling strength by three times and their resistance to amylolysis by about twofold [12]. Annealing leads to reconstruction in the structure of starch granules because of swelling in the amorphous region [15]. The annealed starch also showed multiple pores and an increase in pore size, weakening the structure of the starch granule network. This condition facilitates the destruction and separation of glucomannan from impurity molecules. The longer the time starch is in contact with water, the more water is imbibed and the weaker the structure of the starch network. This event is associated with a weak and easily destroyed starch structure, releasing glucomannan. Meanwhile, at 50 °C, the glucomannan content of *porang* flour decreased. This is probably due to the imperfect coagulation process. The coagulation process is influenced by heating, stirring, and adding electrolytes [31]. In addition, it is suspected that *porang* starch has a low gelatinization temperature. It needs to be proven again by measuring the RVA profile of *porang* starch.

The interaction between temperature and time significantly affected glucomannan levels ($p<0.01$). Interaction analysis revealed that the glucomannan content was lower at 50 °C for 3, 4, and 5 hours than in the other treatments. The glucomannan content tends to be high at the interaction temperature of 40 °C for 3, 4, and 5 hours. The highest level of glucomannan was at the treatment interaction at 30°C for 5 hours. Compared to the annealed modified *porang* chip, which had the same amount of glucomannan, the new chip had up to 121% more glucomannan.

D. Yield

Yield is the percentage ratio between the final weight and the initial weight. The yield was calculated as the ratio between the weight of *porang* flour and the weight of *porang* chips. The drying process, the wind, and some are attached to the drying area is the factor that influences yield. In addition, some parts cannot be mashed in refining or flouring chips using a blender, and some of the flour is scattered in the blender. In the sifting process, some flour flies and sticks to the flour-holding material, which causes a decrease in the yield percentage. Water content affects the yield of flour produced; the lowest water content is in the technique without soaking [29]. From processing fresh *porang* tubers to *porang* chips, the yield ranges from 13.16% to 16.99%, meaning that 1 kg of *porang* tubers will get 131.6 g–169.9 g of *porang* chips. The *porang* chips were then treated with annealing, and the yield of annealing *porang* flour was calculated.

The statistical analysis showed no significant effect between time and yield ($p>0.01$). The yield decreased with the increasing length of time. It is related to the more lost water-soluble compounds—causing lower yield. Statistical analysis showed that temperature independently significantly affected the yield ($p<0.01$). The yield tends to decrease with the increasing temperature from 30 °C to 40 °C. Moreover, the yield suddenly rose sharply when the temperature increased to 50 °C. The yield of *porang* at 50 °C was 77.84 2.92%. This condition is thought to be related to the increased protein content at 50 °C (fig 5.).

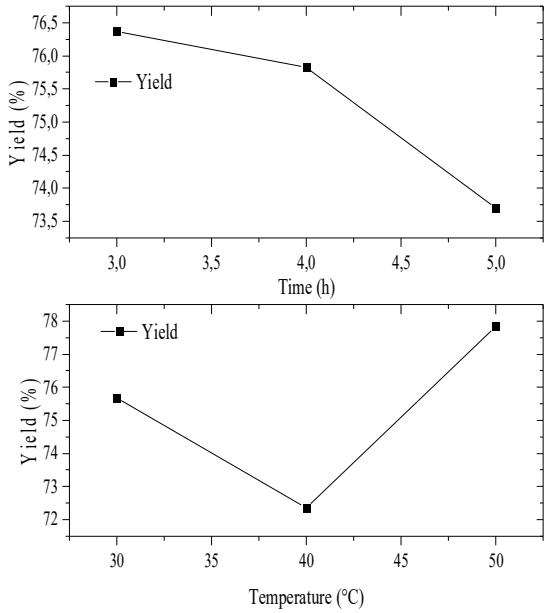

Fig. 5 The effect of temperature (above) and soaking time (below) on the yield of annealing porang flour.

It is suspected that other factors outside the observed variables affect the increase in protein content at a

temperature of 50 °C. The effective interaction between temperature and time did not show a significant effect ($p > 0.01$). The interaction of temperature, 50 °C, with the length of time showed high yields for all treatment combinations. The highest yield in the combination treatment was 79.07±0.21% after 3 hours at 30 °C.

According to [29], if the water content in a material is low, the flour yield will increase, but if the water content in a material increases, the resulting yield will be less. Fig. 5 shows that at a temperature of 30–50 °C, the longer the soaking time, the lower the yield. On the other hand, at a temperature of 50°C, the longer the soaking, the higher the yield, and the possibility of the *porang* flour no longer absorbing water. It proves that the water content affects the resulting flour yield. Soaking will cause the chips to absorb more water. Supposedly it decreases because the cells have been saturated to accommodate water and reach a balance. In that case, the swollen starch granules have large cavities that facilitate water evaporation during drying. The low yield can also be caused when the processing refining using a blender leaves some parts that can no longer be mashed, causing a reduced percentage yield [24].

E. Pearson Correlation

A Pearson correlation test was done to see if there was a link between the amount of water, protein, glucomannan, and yield. The analysis showed a significant negative correlation between yield and glucomannan (Table 1). The result shows that glucomannan influenced the increase in yield with a correlation value of 0.79. The closer to 1, the stronger the correlation. The amount of glucomannan obtained per unit increases proportionally as the yield decreases. More glucomannan extracted from flour does not influence the water and protein content. It is clear because nitrogen is a small component of protein. *Nitrogen* is an impurity that will affect the levels of glucomannan. Therefore, the presence of protein was not significantly related to glucomannan levels.

The annealing modification process, in principle, improves characteristics that can be carried out in several physical ways, including the temperature above the gelatinization and the temperature below the gelatinization. The modification process by heating under gelatinization can improve the characteristics. *Annealing*-modified starch has a lower rate of development than native. This situation was due to the increased crystallinity of the *annealed modified starch* [14][31]. This change in how starch works makes it easier to crush and get it out of the glucomannan structure.

TABLE I
PEARSON CORRELATION MATRIX

	Water Content (% b/b)	**Protein (%)**	**Glucomannan (%)**	**Yield (%)**
Water Content	1.00	-0.12	0.26	0.28
Protein	-0.12	1.00	-0.13	0.25
Glucomannan	0.26	-0.13	1.00	-0.79*
Yield	0.28	0.25	-0.79*	1.00

Notes: *) Significant at the 0.05 level of significance, the figure in the body of the table is the value of the correlation coefficient.

IV. CONCLUSION

Annealing time significantly affects water content, glucomannan, and yield. It is related to the longer the time and the more water-soluble compounds were lost. It increases the amount of glucomannan and decreases the yield. The temperature significantly affects water content, protein level, glucomannan content, and yield. When the temperature goes up to 40 °C, the amount of glucomannan goes up, which is related to the start of the gelatinization process. The starch granule structure weakens at the beginning of gelatinization, so it is easily crushed and released from glucomannan. This study found a sharp decrease in glucomannan content when the temperature increased to 50 °C. It is suspected that *porang* starch has a low gelatinization temperature, so, at 50 °C, starch has total gelatinization. However, this must be proven again by knowing the gelatinization profile of porang starch—the protein level decreases by increasing temperature due to protein denaturation. This study found an increase in the glucomannan content through the annealing process at 40 °C for 5 hours.

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