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The Effects of Water Addition and Steaming Duration on Starch Composition of Wheat Pollard

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Abstract

The objective of the study was to determine the effects of water addition and steaming duration on starch composition of wheat pollard including starch, amylose, amylopectin and starch resistant. Water was added towheat pollard (0, 30 and 60%) and autoclaved at 121 °C (15 and 30 min) afterward. The pollard then was oven-dry at 70°C. The study used a complete randomized design of 3x2 factorial with 3 replications. The addition of water and steaming duration showed a very significant (p<0.001) impact on the amylose and starch resistant parameters but not the starch and amylopectin. The addition of water increased the starch content from 38.71±0.10%. The starch content of a 15-min autoclave was higher (44.54±11.34%) than that of a 30-min (43.43±11.16%). The amylose levels decreased with addition of water and duration of autoclave. The amylose decrease from 5.23±0.10 to 4.03±0.32%. Addition of water increased the amylopectin level from 33.50±0.10 to 52.90±0.70% while the amylopectin level was heated at 15 minutes (39.79±11.76%) and decreased to 39.20±11.44% after treatment with steam for 30 minutes. Addition of water increased the starch resistant level from 8.75±1.06% to 15.40±1.51% while heating for15 minutes had the highest starch resistant of 16.65±0.04% compare to the longer steaming duration. This result concluded that the addition of water and the duration of steam greatly affects the content of starch, amylose, amylopectin and starch resistant of the wheat pollard. The best results of this study was the addition of 30% moisture content with a steam duration of 15 minutes which results in the highest starch resistant of 16.65± 0.04%.

Keywords: *amylose; moisture content; starch; starch resistant; steam; wheat pollard*

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INTRODUCTION

Water is a good conductor and is able to influence the structure of starch of wheat pollard. The moisture content of wheat pollard greatly affects its gelatinization. Howling (1980) states that gelatinization process occurs when water penetrates starch granules due to the disruption of hydrogen bond during moist-heat process. The bond between water and either amylose or amylopectin causes starch granules swelling. The swelling of granules is due to a changing granule size which then collapsed. The collapis caused by the leaching out of amylose and amylopectin

(Wurzburg, 1989). Kruger and Matsuo (1996) stated that autoclaving breaks the chemical bond and causes variance degradation effects that increasing the digestibility of starch. Bjorck *et al*. (1985) and Hancock *et al*. (1991) stated autoclave treatment increases gelatinization and decreases anti-nutrition.

Heating up process using autoclave affects retrogression process of starch. The retrogression process changes the starch structure into the crystalline structure so that the starch is uneasy to dissolve in water (Sajilata *et al*., 2006).

Wheat pollard is a feed material rich of amylase and amylopectin. This pollard is a by-product of a wheat milling process with amylose and amylopectin content are 25 and 75%, respectively. These type of polysaccharides cause gelatinization (Yono *et al*., 2000). Treatment on wheat pollard is expected to change the structure, physical shape and chemical components of wheat pollard thus affecting the quality of wheat pollard.

This study aimed to examine the effect of water addition and steaming duration on the starch composition of wheat pollard including the levels of starch, amylose, amylopectin and starch resistant. The wheat pollard shows a potential as prebiotic source seenit contains resistant starch. The results of this study were expected to be applied to high starch content food/feed which is difficult to be digested in the digestive system.

METHOD

Material

The materials used in this research were wheat pollard, pure amylose, 95% ethanol, 1 N NaOH, 1 N acetic acid, iodine solution, aquadest, whatman 42 filter paper, 25% HCl, 45% NaOH, 0.1 M phosphate buffer, α-amylase enzymes, 1 N HCl, 1% enzyme pepsin, beta amylase enzyme. The equipment used was an autoclave, analytical scales, aluminum foil, test tube, gourd flask, spectrophotometer, 250 ml cup glass and Erlenmeyer.

The Experiment

Water was added to the wheat pollard to obtain 0, 30 and 60% content and placed afterward into the autoclave which set to 121°C for 15 and 30 min as variables. The addition of water calculated by the following formula:

$$WC\% = \frac{((MC\ x\ W)+A)x100\%}{W+A} \quad (1)$$

where:
WC was water content sought
MC was moisture content of materials
W was weight of material
A was water addition

Wheat pollard is then fed into the autoclave until the temperature reaches 121°C. After reaching temperature 121°C wait for 15 and 30 minutes, then autoclave is turned off. After the autoclave became cool, the wheat pollard is removed and dried in an oven at 70°C.The dried pollard grounded before analyzed for the starch, amylose, amylopectin and the resistant starch content. The measurement of the value of starch, amylose, amylopectin and resistant is done as follows:

Analysis of Starch

A total of 5 g of sample is put into 250 ml beaker glass, and filled 50 m of Aquadest then stir for 1 hour. The suspension is filtered with Whatman 42 filter paper and washed with Aquadest up to 250 ml filtrate volume. The filter result is then transferred into Erlenmeyer by washing 200 ml of Aquadest and 20 ml of 25% HCl added, then cover with an inverted coolant and overheat boiling water for 2.5 hours. After cold neutralize with 45% NaOH solution and dilute to 500 ml volume, then strain with Whatman 42 filter paper. Determine the sugar content expressed as glucose from the obtained filtrate. The weight of glucose multiplied by 0.9 is the weight of starch.

Analysis of Amylose Levels

a) Standard Curve Creation

A total of 40 mg of pure amylose is weighed and fed into the test tube, then 1 ml of 95% ethanol and 9 ml of 1NNaOH. Heat in boiling water for 10 minutes until all the ingredients form a gel, then cooled. Move whole mixture into a 100 ml flask and then take 1, 2, 3, 4, and 5 ml of the solution and put it into a 100 ml flask. Add 1N of Acetic Acid each 0.2, 0.4, 0.6 and 1 ml then add each 2 ml Iodine solution leave for 20 minutes. The intensity of the formed blue color is measured by a spectrophotometer at a wavelength of 625 nm and make a standard curve, amylose concentration versus absorbance.

b) Measurement of Amylose

As many as 100 mg of sample is inserted into the reaction tube then added 1 ml 95% ethanol and 9 ml of 1N NaOH. Heat in boiling water for 10 minutes until gel is formed. Move the entire gel into 100 ml flask. Pipette 5 ml of the solution then put in 100 ml poultice flask and add 1 ml of 1N acetic acid and 2 ml of Iodine solution then shake and let stand for 20 minutes. Measure the intensity of the color formed by spectrophotometer at 625 nm wavelength and calculate the amylose content.

Measurement of Amylopectin

Amylopectin is the result of a reduction of amylum reduced amylose.

Measurement of Starch Resistant

Starch resistant was measured using multienzyme method. A total of 0.5 g of sample was included in Erlenmeyer glass, then 25 ml of a 0.1M Phosphate buffer solution with pH 7 and stirred to form a suspension. Then 0.1 ml of enzyme α-amylase added into Erlenmeyer containing the sample. Erlenmeyer glass is then covered with aluminum foil and incubated in a water bath with a temperature of 100°C for 15

minutes while 2rring. The sample was removed and cooled and 20 ml of distilled water and 5 ml HCl 1 N was then added 1 ml of 1% pepsin enzyme into Erlenmeyer 8glass containing sample, sealed and incubated in a water bath at 40°C for 1 hour. Erlenmeyer glass was then removed 20 ml distilled water, 5 ml NaOH 1N, 0.1 8 β-amylase enzyme was then closed and incubated in a water bath rocked at 40°C for 1 hour. Strain using filter paper, then the residue is dissolved and analyzed levels of starch.

A complete randomized design of 3x2 factorial designs with 3 replications was applied in this study. The first factor is the water addition (0, 30 and 60%) and the second factor is steaming duration (15 and 30 min) 11the result showed the real effect then continues with D*uncan's multiple range test (*DMRT) (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

The addition of water to the steaming process has a significant effect on amylose, starch, amylopectin and starch resistant. The mean interaction among the variables on the parameters of starch, amylose, amylopectin andresistantstarchare presented in Table 1, 2, 3 and 4.

Starch

Starch consists of amylose and amylopectin in which ar26opectin is in bigger proportion than amylose. Differences in the characteristics of starch granules greatly affect the 9ysical, chemical and functional properties of starch. In the structure of starch granules, amylose and amylopectin are arranged in a ring. Amylose and amylopectin in starch granules are associated with hydrogen bonds (Sajilata *et al*., 2006). Table 1 illustrates the addition of water and the duration of steam affects the content of wheat pollard starch.

Table1. Starch content of wheat pollard in various water addition and steaming duration

Water Addition (%)	Starch content (%) Time of Steam (Minutes)		
	15	30	Average
0	38.71±0.31	38.71±0.43	38.71±0.10^{c}
30	37.30±0.19	35.40±0.31	36.35±1.34^{b}
60	57.61±0.06	56.17±0.12	56.89±1.02^{a}
Averag4	44.54±11.34^{a}	43.43±11.16^{b}	

Different superscript in the same rows and column is a significant difference (P<0.001).

Addition of water 60% and 15 minutes the steaming time resulted in the highest starch content of 57.61+ 0.06% (Table 1). Water was a heat conductor so it was influenced the structure of starch in wheat 3llard. Addition of water up to 60% triggers gelatinization process. The gelatinization process occurs when the starch granules are heated in water, then the heat energy will crack the hydrogen bond and water will penetrate into 19starch granules. The penetration of water further forms hydrogen bonds with amylose and amylopectin. The penetration of water into 23the granular causes swelling of the starch granules. The size of the granular will increase to some extent before the starch granules finally break. This break of the granules causes the amylose and amylopectin part to diffuse out. This gelatinization begins with an irreversible swell of starch granules in hot water and it is terminated at the time of losing its crystalinity properties (Wurzburg, 1989).

In the autoclave, treatment retrogradation occurs due to the heating process followed by the cooling process (Sajilata *et al*., 2006). Marsono (1998) states that retrogradation can change the starch structure and contribute to the formation of the new crystalline structure which is not easy to be dissolved. The gelatinous starch that was mixed the granule starch occurred a formation of the net-structure that created three-dimensional formations ofgel starch. The complex structures were expected exchange in the digestive organ, so it can be used as a prebiotic.

Amylose Content

Amylose is part of a straight chain that can rotate and forn5 double spiraling region. Hydrogen attached to outer surface of the single-stranded amylose on the O_2 and O_6 atoms. The amylose straight chain which 9rms the crystalline double vine is resistant to amylase. In the structure of starch granules, amylose and amylopectin are arranged in a ring. The number of rings in a starch granule is approximately 16 pieces, consisting of an amorphous layer ring and a semi-crystal layer ring (Hustiany, 2006). The amylose of wheat pollard due to the addition of water and different steam duration can be seen in Table 2.

Table2. The amylose of wheat pollard due to the addition of water and different steam duration

Water Addition (%)	Amylose content (%) Time of Steam (Minutes)		
	15	30	Average
0	5.23±0.11^{a}	5.23±0.21^{a}	5.23±0.10^{a}
30	4.76±0.01^{ab}	3.65±0.04^{b}	4.21±0.78^{b}
60	4.25±0.14^{ab}	3.80±0.11^{b}	4.03±0.32^{b}
Averag4	4.75±0.49^{a}	4.23±0.87^{b}	

Different superscript in the same rows and column is significant difference (P<0.001).

Treatment of water addition and steaming duration gave a very significant impact 7decreasing amylose wheat pollard content (Table 2). Amylose is a straight chain consisting of glucose molecules with α-(1,4) -D-glucoseas a linking. The length of the polymer is influenced by the source of starch which affects the amylose fiber molecule (Moorthy, 2004). The amylose changeswere inline with an increase in starch resistant content (Table 4). Amylose content decreased from 5.23±0.10% to 4.21±0.78% and 4.03±0.32% in addition of 30% and 60% water, respectively. This was followed by a successive increase of starch resistant from 8.75±1.06% to 15.10±2.19% and 15.40±1.51%, accordingly. Taggart (2004) states that amylose has the ability to form crystals because of its simple polymer

chain structure. This simple structure allows to form strong molecular interactions. This interaction occurs in the hydroxyl group of amylose molecules. Amylose is a dynamic fraction which allowed the starch to be moveabledepends on the type of starch. Thus, increasing level of starch resistant is strongly influenced by the amylose content of the ingredients. This supports the research of Huang and Rooney (2001) and Eerlingen *et al*. (1993) who reported that the highest resistant starch was formed in amylose retrogradation and amylose crystallization process.

Amylopectin Concentration

Addition of water and steaming duration had a significant effect on the percentage of wheat pollard amylopectin (Table 3).

Table 3. The impact of water addition and steam duration on amylopectin of wheat pollard

Water Addition (%)	Amylopectin content (%) Time of Steam (Minutes)		
	15	30	Average
0	33.48±0.20	33.48±0.22	33.50±0.10^{c}
30	32.54±0.19	31.75±0.27	32.10±0.56^{b}
60	53.36±0.08	52.37±0.23	52.90±0.70^{a}
Average	39.79±11.76^{a}	39.20±11.44^{b}	

Different superscript in the same rows and column is significant difference (P<0.001).

The amylopectin content was obtained as differences of the starch and amylose content. Amylopectin levels increase with increasing water from 33.50±0.10 to 52.90±0.70% after addition 60% water. When heated in water, the starch forms a transparent layer, solution with high viscosity and shaped layers such as string strands called amylopectin. Belitz and Grosch (1999) suggested that amylopectin does not tend to be retrograded and does not form gels, except at high concentrations. Combination of water addition (60%) with 15 min of steam durations resulted the highest percentage of amylopectin which was 53.36±0.08%. Extending steam treatment into 30 minutes decreased the amylopectin to 52.37±0.23%.

Resistant Starch

Wulan *et al*. (2007) stated that resistant starch (RS) is an undigested carbohydrate during digestion process that supported the body health. Resistant starch does not contain mineral, does not cause flatulence and provides better texture and appearance. One potential commodity developed into resistant starch is pollard grain. The effect of water addition and steaming duration on the resistant starch of pollard wheat is presented in Table 4.

Table 4 shows the increase of starch resistant level after treatment with water and steam. Increasing water will increase starch resistant. Treatment with 60% water increased the resistant starch from 8.75±1.06% to 15.40±1.5%. Sajilata *et al*. (2006) reported that the content of starch resistant affected by various processing processes such as roasting, boiling with high temperature, cooling and reheating resulting in repeated retrograde process. The changes influence the physical properties such as swelling, viscosity, gel formation and water-binding ability. Material which resistant from digestion can be used as a prebiotic. The RS can significantly modulate intestinal flora, resulting in short chain fatty acid (SCFA), especially propionate and butyrate which have the potential to decrease the risk of developing colon cancer (Brouns *et al*., 2002). The RS could improve the health status of gastro-intestinal tract (GIT) because it has the properties of fecal bulking, potentially diluting toxins and increasing the production of SCFA. The RS can also be used as a prebiotic to stimulate the growth of some beneficial microbes such as *Bifidobacterium* because RS can be used as a substrate for probiotic organisms as well as maintaining colon health and increase the rate of crypt cell production (Bird *et al*., 2000).

Table 4.The impact of water addition and steam duration on resistant starch of wheat pollard

Water Addition (%)	Resistant Starch content (%) Time of Steam (Minutes)		
	15	30	Average
0	9.50±0.05^{e}	8.00±0.05^{f}	8.75±1.06^{c}
30	16.65±0.04^{a}	13.55±0.05^{d}	15.10±2.19^{b}
60	16.47±0.04^{b}	14.33±0.04^{c}	15.40±1.51^{a}
Average	14.21±42.62^{a}	11.96±35.88^{b}	

Different superscript in the same rows and column is real difference (P<0.001).

CONCLUSION

Compositions of starch, amylose, amylopectin and starch resistant in wheat pollard was affected by water addition and steaming process. This study showed treatment with 30% water addition and steam process 15 minutes give the high level of starch resistant 16.65±0.04%.

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