

The Effect of Gelatinization Rice Storage on Body Fat Percentage and Short Chain Fatty Acids (Acetate) on Obesity

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Abstract

Background: Obesity is a problem in developed and developing countries that has a detrimental effect on health. Gelatinization rice that's stored hot or cold can increase resistant starch and short-chain fatty acids (SCFA) especially acetate (C2). Resistant starch contributes to decreasing glycemic index (IG) of rice and blood glucose levels, while acetate contributes to GLP-1 and PYY secretion which is related to appetite and decreased body fat. **Method:** The experimental study design was a pre-post controlled group design with 39 obese respondents divided into three randomized treatment groups (Control, P1, and P2). The research instrument was a measurement of body fat percentage and SCFA (*acetate*) levels blood plasma and statistically analyze with Saphiro Wilk and Paired t-test or Wilcoxon. **Results:** The analysis results of gelatinized rice storage were significant for body fat percentage ($p = 0.012 < 0.005$) and acetate ($p = 0.000 < 0.05$) in cold storage treatment of 4 °C for 24 hours (P2). **Conclusion:** Gelatinized rice which is kept cold at 4°C for 24 hours can reduce body fat percentage and increase acetate.

Keywords: Obesity, Gelatinization rice, Body fat percentage, Short-chain fatty acids, Acetate.

Introduction

Obesity is a physiological condition due to the excessive accumulation of body fat until it disrupts health¹. The regulation of rice diet can be a solution for obesity but rice consumption is still a problem because of its high glycemic index (GI)². GI rice can be derived through hot or cold storage to change the molecular structure of amylose-amylopectin to resistant starch (retro-gradation) until it's not easily digested³. Heat storage makes thereby reducing carbohydrate content⁴, While Cold storage causes retro-gradation until resistant starch^{5,6}.

Resistant starch is a starch fraction that's difficult to be hydrolyzed by amylase and passes into large intestine into a subtractive (*colonic food*) until it's fermented by micro-biota into SCFA especially acetate (C2)⁷.

Physiological properties of resistant starch are resistant to the amylase and slow release of glucose resulting in low energy intake and increase body fat breakdown^{8,9}. The contribution of acetate affects energy production through the secretion of hormones in intestine such as peptide-1 (GLP-1), glucagon, and peptide YY (PYY)¹⁰. Acetate can increase the secretion of PYY and GLP-1 which contributes to reducing energy intake and reducing body fat¹¹.

Method

Research design in this experiment is an experimental pre-posttest control group design and was carried out in Yogyakarta in October 2018. Sampling technique uses simple random sampling from affordable populations that met the criteria: healthy people, ages 18-58 years, BMI ≥ 25 kg / m², accustomed to eating rice, and have normal blood glucose levels. The minimum sample size was calculated 12 people were obtained in each group and added 10% to avoid drop out. Total samples size is 39 people and divided into three treatment groups randomly (K, P1, and P2). Control group (K) that's given gelatinized rice without storage, P1 that's given gelatinized rice stored hot at 70 °C (*rice cooker*) for 24

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hours, P2 that's given gelatinized rice which kept cold at 4°C (*refrigerator*) for 24 hours.

Gelatinized rice is IR64 milled rice cooked using a rice cooker (Yongma) at a temperature of 100 °C for 45 minutes with a water ratio of 1:2 measured using a food thermometer. Heat storage is a method of heat storage using a Yongma brand rice cooker at a stable temperature of 70-75 °C, measured using a food thermometer and a green on/off button indicator according to the specified time duration. Cold storage is a method of cold storage using a Fagor brand refrigerator at a stable temperature of 4 °C, measured using a hygrometer and an indicator of the engine temperature regulation for a specified duration of time.

The research procedure begins with subject screening and health examination. Then determining subjects if they meet the inclusion, exclusion criteria and are willing to fill in informed consent. Subjects were asked to take three days of diet program to consume 200

gr of gelatinized rice + 50 gr of fish + 240 ml of mineral water (morning, afternoon, and evening) in a single room and monitored. Data collection on day 1 at 07.00 in the morning after fasting for 8-10 hours was measured by body composition before breakfast. Day 2 and day 3 they were not measured, at day 4 their body composition was measured as on day 1 and 10 ml of blood was taken on 60 minutes after breakfast. Recall food intake for three days.

Primary data containing pre-post body fat percentage was carried out using BIA (*Bioelectric Impedance Analysis*) Amron HBF-375 Carada Scan brand, high blood plasma acetate levels carried out using VFA-GCMS method (*Volatile Fatty Acid - Gas Chromatography, Mass Spectrometry*), plasma blood sampling by Yogyakarta Parahita Diagnostic Center Laboratory and food recall is conducted three days with Nutrisurvey. Statistically analyzed using SPSS version 22 with Saphiro Wilk test and Paired t-test or Wilcoxon.

Results and Discussion

Table 1. Characteristics of Subjects

Variable	Value		
	K	P1	P2
n	13	12(1)	12(1)
Sex (Male/Female)	2(15.38%)/11(84.61%)	6(50%)/6(50%)	8(66.66%)/4(33.33%)
Age (year)	(27.92 ± 6.61)	(32.83 ± 7.17)	(31.67 ± 7.46)
IMT (kg/m ²)	(28.97 ± 2.92)	(28.74 ± 1.87)	(29.66 ± 4.81)
Energy intake (%)	90.60	97.21	95.95
Carbohydrate intake(%)	83.41	91.92	89.08
Fat intake (%)	93.60	94.08	92.26
Dietary fiber intake (%)	82.01	96.00	93.18

Table 1 shows the characteristics of the subjects based on their age, sex, BMI, and average intake of nutrients (energy, fat, carbohydrates, and fiber) during the diet program. Research subjects are employees who work in the hospital environment at RSPAU Dr. S. Hardjolukito until it has the same relative activity. The

number of subjects in each group was 13 people, but for the treatment groups P1 and P2 a drop out occurs one person from each group. The average nutrient analysis is relatively the same and meets the nutritional adequacy standard (RDA) for adults.

Table 2. Body Fat Percentage Pre and Post in Treatment Group

Group	Body fat percentage		Value	
	Pre Mean \pm SD	Post Mean \pm SD	%	p
K	33.03 \pm 3.50	34.45 \pm 3.70	0.95	0.166 > 0.05
P1	30.78 \pm 5.32	31.09 \pm 4.98	1.02	0.304 > 0.05
P2	32.66 \pm 5.16	31.00 \pm 5.24	-2.02	0.012 < 0.05

Table 2 shows the mean body fat percentage pre and post-treatment between control groups, P1, and P2 are decreased. The results of statistical analysis showed the mean body fat percentage pre (32.66 \pm 5.16) and post (31.00 \pm 5.24) P2 treatment group showed significant differences compared to the control group, this shows that administration of cold storage gelatinization of 4 °C for 24 hours ($p = 0.012 < 0.05$) had a significant effect on body fat percentage, while mean body fat percentage pre and post-treatment group P1 ($p = 0.304 > 0.05$) was not significantly different from the control group ($p = 0.166 > 0.05$). The average body fat percentage difference is illustrated in the following graph:

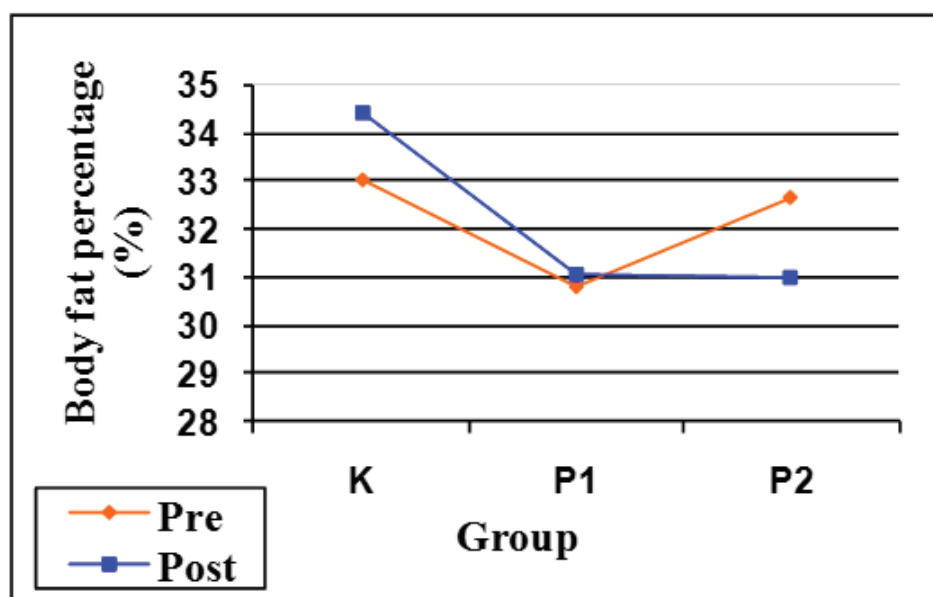


Figure 1. The differences between the effect of gelatinized rice storage on Pre and Post body fat percentage among treatment groups.

Gelatinization rice which is kept cold at 4 °C for 24 hours has been proven to significantly reduce body fat percentage by 2.02%, it's suspected that during cold storage a long decreased temperature can cause retro-gradation until it produces low digestibility and GI values and increases starch resistance¹². The mechanism of retro-gradation is re-binding of amylose-amylopectin molecules that come out of starch granules

that have broken due to a decreased temperature, forming microcrystalline webs and precipitates which are irreversible^{13,14}. Cold storage causes retro-gradation of amylose and amylopectin molecules into strong microcrystalline granules to become starch resistance, thus changing their physicochemical characteristics and digestibility¹⁵.

Body fat percentage reduction is thought to be a direct mechanism that's the physiological effect of resistant starch itself which affects the absorption of blood glucose and body fat¹⁶, while indirectly resistant starch will increase SCFA in the intestine which acts to stimulate hormones PYY and GLP-1 to slow down food absorption and increase energy breakdown¹². High content of resistant starch causes digestive process in the stomach and the rate of emptying the stomach to run slowly until the digestible food in stomach reaches the intestine slower, consequently the absorption of blood glucose in intestine occurs slowly until fluctuations in blood glucose are relatively small and increase energy expenditure^{3,17}. Resistant starch has a low GI and is difficult to digest by amylase, causing slow absorption which results in low blood glucose and increases body fat breakdown¹⁷.

Gelatinization rice is stored hot at 70 °C for 24 hours doesn't significantly reduce body fat percentage, although the average blood glucose level decreased, this is suspected of the increasing temperature for a long time cause oxidation occurs, carbohydrates are broken down into CO₂ and H₂O so carbohydrate content decreases, but doesn't reduce IG and its digestive value⁴. The effect of high temperature can increase the formation rate of hydrogen bonds of starch molecules with water to form a gel structure, but high temperatures also damage the bonds between starch molecules thereby increasing their digestible value^{13, 18}.

Table 3. Acetate Levels between Groups

Group	Mean ± SD	p
K	0.00 ± 0.00	0.000 < 0.05
P1	0.00 ± 0.00	
P2	0.34 ± 0.36	

Table 3 shows mean acetate levels in the P2 treatment group are increased, whereas in the Control and P1 treatment groups there was no increase (not detected). Statistical analysis showed significant differences in treatment group P2 (0.34 ± 0.36), but not significantly different in treatment group P1 (0.00 ± 0.00) compared to the control (0.00 ± 0.00), this shows that administration of cold storage gelatinized rice at 4 °C for 24 hours (p < 0.05) has a significant effect on acetate, The average

acetate level is illustrated in the following graph:

Figure 2. The effect of storing gelatinized rice on acetate among treatment groups.

Gelatinized rice which was kept cold at 4 °C for 24 hours was proven to significantly increase acetate 0.34 mMol, while the P1 treatment group was not detected (0). Acetate measurement results are still in the low category, it's suspected that the component of resistant starch in cold storage has not been formed optimally given the intervention program is only three days until the proportion of acetate formation is relatively small¹⁹. The mechanism of acetate formation is determined from resident starch fermented in the colon, while the role of microflora in the human digestive system requires a long time of adaptation to resistant starch until it can produce acetate and encourage the release of hormones GLP-1 and PYY²⁰. Absorption of acetate in the colon may vary until the location of fermentation and production of acetate greatly determines the circulating concentration of it in blood plasm²¹.

Gelatinized rice which stored hot at 70 °C for 24 hours doesn't increase acetate significantly; it's allegedly that hot storage cause carbohydrate oxidation until it doesn't produce starch resistant as raw ingredients for acetate establishment, other allegation said that it's because the amount of acetate circulate in plasma is too small until it can't be detected by tool's system. Hot storage mechanism for a long period will cause brownish coloration or Maillard reaction; a reaction that happened among carbohydrates with an amino acid as a result of heating²². Maillard reaction causes probiotic bacteria (BAL) difficult to grow when arrived at the colon, because there is a few sugar content that could be used by prebiotic bacteria for its growth, until acetate, as a product from the fermentation of resistant starch is not produced maximally^{22,23}.

Molecular mechanism of the parting of acetate allegedly through two pathways: first the G protein-coupled receptors (GPCRs) bind with micro-biota products that contribute to the regulation of food intake and fat formation¹¹; second, acetate binds to the enteroendocrine cell receptor, then it will secrete PYY, Glucagon, and GLP-1²³. GLP-1 and PYY secretion occurs in L cells located along the small intestine and large intestine, the numbers are increasing from

jejunum, ileum to the colon, and rectum¹¹. Glucagon works to maintain blood glucose levels in a state of fasting, while GLP-1 increases the ratio of insulin to glucagon which results in inhibition of hepatic glucose production until blood glucose levels decrease²⁴. PYY related to appetite regulation influences energy intake and expenditure through the hypothalamus and brain stem, low in concentration before eating, and increasing after eating²⁵. PYY will slow down food absorption and through the hypothalamus suppress hunger^{11,25}. Overall acetate affects PYY and GLP-1 to improve glucose homeostasis, increase energy expenditure, fat oxidation, and reduce lipolysis, thereby reducing body fat and body weight²⁶.

Conclusion

Cold storage in gelatinized rice has a significant effect on body fat percentage and acetate levels in obesity. Giving gelatinization rice that had been kept cold at 4 °C for 24 hours in the storage can reduce body fat percentage by 2.02% and increase blood plasma acetate 0.34 mMol.

Conflict of Interest; Nil

Source of Funding; Own

Ethical Clearance; Ethical clearance obtained from the Health Research Ethics Commissioner of RSUD dr. Muwardi Faculty of Medicine, Sebelas Maret University Surakarta, Indonesia, Number: 538 / IV / HREC / 2018.

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