



Study of the response to the glycemic index of rice starch (*Oryza sativa* L) modified by physical and enzymatic treatment

Kajian respon indeks glikemik pati beras (*Oryza sativa* L) yang dimodifikasi perlakuan fisik dan enzimatik

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Article History:

Received: January 29, 2024; Revised: April 07, 2024; Accepted: September 27, 2024; Published: December 05, 2024.

Publisher:



Politeknik Kesehatan Aceh
Kementerian Kesehatan RI

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Abstract

Excessive rice consumption with a high glycemic index has a negative effect on health. This study aimed to determine the effects of physical and enzymatic modifications of rice starch on estimated glycemic index (eIG). This study was conducted between June and November 2023. This experimental study used a completely randomized design (CRD) with four treatments: heat moisture treatment (HMT)-microwave, HMT-autoclave, and enzymatic pullulanase (20 units/g rice starch). The observed parameters included the eIG, amylose content, solubility, swelling power, and thermal characteristics. The eIG values were measured using the *in vitro* method with four replicates for each treatment. The research data are expressed as the average value \pm standard deviation and were analyzed using descriptive methods. The results showed that the HMT-microwave, HMT-autoclave, and enzymatic pullulanase treatments reduced the eIG value of rice starch by 3,06%, 7,19%, and 9,06%, respectively. Thermal analysis using DSC showed that modified starch experienced an increase in the onset temperature (T_o), peak temperature (T_p), and end-set temperature (T_e) compared to normal rice flour. In conclusion, the physical modification of HMT and enzymatic activity can reduce the eIG value of rice starch. This study provides an alternative for the development of functional rice starch-based products with a low glycemic index.

Keywords: eIG, HMT, modification, rice starch, pullulanase

Abstrak

Konsumsi beras dengan indeks glikemik tinggi secara berlebihan berpotensi menyebabkan efek negatif bagi Kesehatan. Penelitian ini bertujuan untuk mengetahui efek modifikasi pati beras secara fisik dan enzimatik terhadap respon estimasi indeks glikemik (eIG). Penelitian dilakukan mulai bulan Juni hingga November 2023. Desain penelitian ini adalah penelitian eksperimental menggunakan rancangan acak lengkap (RAL) dengan empat 3 perlakuan yaitu heat moisture treatment (HMT)-microwave, HMT-autoklaf, dan enzimatik pullulanase (20 unit/g pati beras). Parameter pengamatan meliputi eIG, kadar amilosa, kelarutan dan swelling power, dan karakteristik termal-. Pengukuran nilai eIG dilakukan menggunakan metode *in vitro* dengan 4 kali ulangan untuk tiap perlakuan. Data hasil penelitian dinyatakan dalam nilai rata-rata \pm standar deviasi yang kemudian dianalisa dengan metode deskriptif. - Hasil penelitian menunjukkan bahwa perlakuan HMT-microwave, HMT-autoklaf, dan enzimatik pullulanase mampu menurunkan nilai eIG pati beras masing-masing sebesar 3,06%; 7,19%; dan 9,06%. Analisa termal menggunakan DSC menunjukkan adanya pati termodifikasi mengalami kenaikan suhu onset (T_o), suhu puncak (T_p), dan suhu end set (T_e) dibandingkan tepung beras normalnya. Kesimpulan, bahwa modifikasi fisik HMT dan enzimatik dapat menurunkan nilai eIG dari pati beras. Penelitian ini diharapkan mampu memberikan alternatif pengembangan produk berbasis pati beras fungsional berindeks glikemik rendah.

Kata Kunci: eIG, modifikasi, pati beras, HMT, pullulanase,

Introduction

Rice (*Oryza sativa* L) is the main staple food source of calories for the majority of the Indonesian population. These calories are produced from starch compounds, which make up 90% of rice grains per dry weight. Rice starch is composed of amylose components (18,94 – 28,64%) and amylopectin content (52,41 – 63,52%) (Sari et al., 2020). Rice can be cooked directly into ready-to-eat rice or processed further into flour. Processing rice into flour proceeds via two processes: milling and filtering. Rice flour has been widely used as a raw material for processed food, including in making vermicelli noodles, layered cakes, rice cakes, porridge, and so on (Wang et al., 2019).

A healthy lifestyle has caused changes in people's food consumption patterns, including rice consumption. One of the considerations in determining food consumption patterns is the glycemic index (GI), which is an indicator of the relative glycemic response of humans to carbohydrates in food. Previous research has shown that the GI values of rice products range from 64 to 93. The GI value of rice is determined by many factors, including variety, rice, processing techniques, fiber content, resistant starch, lipids and proteins (Kaur et al., 2016). Other factors that determine the GI value are the ratio between amylose and amylopectin which make up starch, as well as the presence of endogenous phenolic compounds which can also cause changes in the digestibility of rice products (Lal et al., 2021; Ngo et al., 2023).

Consuming high GI foods will increase blood sugar levels, whereas consuming low GI foods is known to lower blood sugar levels (Khoiriyah et al., 2017; Mayawati et al., 2017). High blood sugar levels can trigger type II diabetes mellitus (DM). Based on data from the International Diabetes Federation (IDF) Atlas 2019, Indonesia is ranked 6th as the country with the highest number of type II DM sufferers in the world after China, India, the United States, Brazil and Mexico (International Diabetes Federation, 2019). Due to reduced glucose metabolism during the postprandial phase, diabetics are advised to consume foods with a low glycemic index (IG <55) (Lal et al., 2021).

Several methods have been developed to reduce GI from starch, including genetic, chemical, physical, and enzymatic modifications (Kunyanee & Luangsakul, 2022). However, physical and enzymatic methods are the safest methods to be applied in food consumption (Park & Kim, 2021).

Modifications were made to reduce starch digestibility. A decrease in starch digestibility will reduce the GI value of the carbohydrate source food (Dodi et al., 2023).

Heat Moisture Treatment (HMT) is a widely developed physical modification method for starch production. HMT is carried out by heating starch granules with a water content of less than 35% at a temperature higher than the glass transition temperature but below the gelatinization temperature (ranges between 84-140°C) (Adawiyah et al., 2017). HMT is known to improve starch characteristics, including morphology, crystallinity, gelatinization, thermal stability, retrogradation, and digestibility. HMT modification of buckwheat starch can increase the levels of amylose, slow-digesting starch, and resistant starch, as well as reduce viscosity, swelling power, and solubility. (Liu et al., 2015).

Enzymatic modification of starch can be performed using pullulanase. The pullulanase enzyme breaks the α , 1-6 glycosidic branch chains in amylopectin and pullulan. The main products of the pullulanase enzymatic reaction are maltose and maltotriose. Modification of starch with pullulanase and pre-heating treatment is known to change the starch structure and increase amylose content which causes a decrease in gel strength and estimated glycemic index values (eIG) (Geng et al., 2023).

Although there have been many studies related to starch modification using HMT and enzymatic treatment, there has not been much research regarding the relationship between HMT and enzymatic modification of rice starch and eIG value. This study aimed to investigate the effect of microwave radiation and autoclaving (HMT) and enzymatic (α -amylase and pullulanase in rice starch) treatments on eIG values, amylose content, thermal characteristics, solubility, and swelling power.

Methods

Design

The sample used in this study was rice starch (*Oryza sativa* L) obtained from Nugraha Farma Bandung. Pullulanase (1000 units/ml) was obtained from Megazyme Singapore. This research was carried out at the Agricultural Product Innovation and Entrepreneurship Laboratory, Food and Agricultural Product Quality Control Laboratory, and Functional Food and Nutraceutical Laboratory, Faculty of

Agricultural Technology, Jember University from June to November 2023.

This was an experimental study using a completely randomized design (CRD) with starch modification techniques (HMT-microwave, HMT-autoclave, and enzymatic pullulanase). The observed parameters included amylose content, solubility, swelling power, thermal characteristics, and estimated glycemic index (eIG). Data analysis was performed descriptively, and the data were expressed as the average value \pm standard deviation (STDEV).

Production of Physically Modified Rice Starch using Heat Moisture Treatment (HMT)

The treatment of rice starch HMT refers to research Zheng et al. (2020) with modifications (Figure 1).

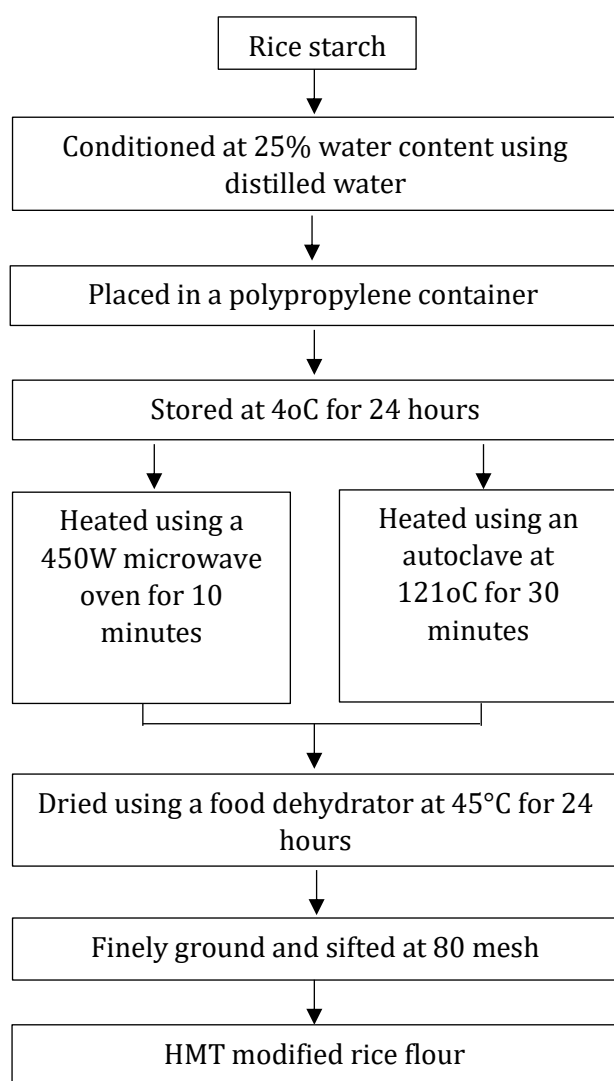


Figure 1. Flow diagram of rice starch modification using the HMT method

Enzymatically Modified Rice Starch Production Process Using Pullulanase

Modification with pullulanase enzyme: Ten grams of rice starch was dissolved in 100 ml pH 5,8 buffer then added pullulanase (20 units/g starch) was added. Incubate at 55 °C for 12 hours (Li et al., 2019). The suspension was dried using a food dehydrator at 45 °C for 24 h. The sample was then ground and sieved through an 80 mesh sieve. The modified rice starch was stored in a closed container at room temperature until further analysis.

Analysis of Amylose Levels

A total of 100 mg of sample was put into a test tube, and then 1 ml of 95% ethanol and 9 ml of 1 M NaOH were added, and the mixture was homogenized using a vortex. The solution was then heated for 10 min and diluted to 100 ml. Take 5 ml of the diluted solution and add 1 ml of 1 N acetic acid solution and 2 ml of 0,01 N iodine solution, as well as distilled water for dilution until the volume becomes 100 ml (AOAC, 2005).

The solution was heated at 30°C for 20 min. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 620 nm. The absorbance was plotted against the amylose standard curve, and the amylose concentration was calculated based on the relationship between sample absorbance and the standard curve. Amylose content (%) is calculated using the equation below (AOAC, 2005):

$$\text{Amylose content (\%)} = \frac{A \times Fp \times Vx}{\text{sample weight (mg)}} \times 100\%$$

Information:

- A = amylose concentration from the standard curve (mg/ml)
- Fp = dilution factor
- V = initial volume (ml)

Solubility and Swelling Power Analysis

0,2 grams of rice starch, dissolved in 10 ml of distilled water and placed in a centrifuge tube. The sample was vortexed for 10 s and then placed in a water bath at 80°C for 30 min. The sample was then cooled to room temperature and centrifuged at 2000 rpm for 15 min. Next, the supernatant was poured into an aluminum box and the sediment was weighed using an analytical balance. The supernatant was dried in

an oven at 105°C for 24 h and then weighed again. The solubility and swelling power values of rice starch samples are calculated using the following equation: (Zheng et al., 2019)

$$\text{Solubility (\%)} = \frac{W_{ss}}{W_s} \times 100\%$$

$$\text{Swelling power (\%)} = \frac{W_{sp}}{W_s(100\% - \text{Solubility})} \times 100\%$$

Information :

W_{ss} = weight of dissolved starch after oven (g),

W_{sp} = weight of starch sediment (g),

W_s = starch sample weight (g)

Thermal Characteristics of Differential Scanning Colorimetry (DSC)

The thermal properties of the rice starch and modified rice starch were analyzed using DSC (differential scanning calorimetry (DSC) (Rigaku DSC 8230). A total of 2,0 mg of the sample was weighed in a pan. The sample was dissolved in distilled water at a starch: water ratio of 1:3 (2 mg sample: 6 µL distilled water). The pan was then tightly closed (sealed) and weighed again. The pan is heated in stages to a temperature of 100°C to observe the peak of gelatinization enthalpy (Rodríguez-Torres et al., 2017).

The sample weight, temperature range, and temperature rise per minute were set in the available software. Temperature and enthalpy profiles (expressed as J/g) were determined using the DSC instrument software. The onset temperature (T_o), peak temperature (T_p), and endset temperature (T_e) are associated with starch gelatinization and retrogradation.

In Vitro Estimation of the Glycemic Index

A 50 mg starch sample was placed into a 30 ml Erlenmeyer flask, and then 10 ml of HCl-KCl pH 1,5 buffer was added. Next, the samples were homogenized by vortexing for 2 min. Then, 0,2 ml of a solution containing 1 mg pepsin in 10 ml of HCl-KCl buffer pH 1,5, was added to each sample and incubated for 60 min at 40 °C. The sample volume was increased to 25 ml by adding 15 ml of Tris-Maleate buffer pH 6,9 (Goñi et al., 1997).

The starch hydrolysis process was carried out by adding 5 ml of Tris-maleate buffer

containing 2,6 IU α-amylase to each sample. The flask was then placed in a shaker-water bath at 37°C and medium speed. A total of 1 ml sample was taken from each flask every 30 min from 0 to 3 h. Next, inactivation of the α-amylase enzyme was performed by heating at 100 °C for 5 min. After cooling, the sample was added to 3 ml of 0,4 M sodium acetate buffer pH 4.75 and 60 µL of amyloglucosidase enzyme and incubated in a shaker-water bath at 60 °C for 45 min. Next, the glucose concentration was measured using a Dumolab GOD-PAP glucose reagent, and the color reaction was measured using a Genesys 10S UV/VIS spectrophotometer at a wavelength of 524 nm. Glucose content was obtained by plotting a glucose standard curve, whereas starch content was calculated by multiplying the glucose content by a conversion factor of 0,9. The rate of starch digestion is indicated by the percentage of hydrolyzed starch content (Goñi et al., 1997).

The starch digestion rate was expressed as the percentage of total starch hydrolyzed at different times (30, 60, 90, 120, and 180 min). A curve was created between the total hydrolyzed starch (%) and time. The area under the curve (AUC) for each sample was calculated. (AUC, 0-180 minutes). The Hydrolysis Index (IH) for each sample was calculated as the ratio between the AUC of each sample and the AUC of the reference (white bread) and was expressed as a percentage. The estimated glycemic index (eIG) is calculated using the equation below: (Goñi et al., 1997)

$$eIG = + 39,71 0,549 * IH$$

Information:

IG = glycemic index (%)

IH = hydrolysis index (%)

Result and Discussion

Amylose levels

The amylose contents of the rice starch and modified rice starch are shown in Table 1. The results in Table 1 show that HMT treatment increased the amylose content of the rice starch. The amylose content of HMT microwave-modified rice starch increased from 18,30% to 21,22%, and the HMT autoclave treatment increased from 18,30% to 18,44. This can be

caused by the influence of temperature and high water content; amylose breaks down and forms shorter amylose, and some long amylopectin forms amylose, transforming its structure during the process (Han et al., 2021).

Table 1. Values of amylose content of rice starch and modified rice starch samples

Starch type	Amylose content (%)
Rice starch	18,30 ±0,19
HMT-microwave	21,22±0,07
HMT-autoclave	18,44±0,19
Pullulanase	19,73±0,05

Data are mean values ± standard deviation

Enzymatic modification of rice starch using pullulanase increased amylose content from 18,30% to 19,73% (Table 1). Pullulanase will specifically cut the α -(1,6)-glycosidic bonds in the amylopectin molecule thereby increasing the amount of amylose content (Dupuis et al., 2014). Other research states that 2% pullulanase enzyme treatment for 1 hour can increase starch amylose levels from 18,56% to 25% (Babu & Parimalavalli, 2018).

Solubility and Swelling Power

The results in Table 2 show an increase in the solubility value of modified rice starch both in physical modification with HMT-microwave and HMT-autoclave, as well as enzymatic modification of pullulanase when compared with normal rice starch. The swelling power value of starch modified by HMT-microwave and HMT-autoclave decreased, but when treated with pullulanase, it increased.

Table 2. Solubility and swelling power values of rice starch and modified rice starch at 80oC

Starch type	Solubility (%)	Swelling power (%)
Rice starch	1,03±0,03	7,36±0,31
HMT-microwave	15,47±0,40	7,06±0,33
HMT-autoclave	21,32±0,27	6,94±0,07
Pullulanase	16,55±0,13	7,51±1,39

Data are mean values ± standard deviation

The HMT-microwave and HMT-autoclave modifications increased the solubility and reduced the swelling power of rice starch. The same thing was shown in the

modification of sweet potato starch HMT for noodle making applications (Pranoto & Kumar Rakshit, 2014). The increase in solubility and decrease in swelling power in HMT modified starch can be caused by the expansion of starch granules, increased molecular bond interactions (amylose-amylopectin), and loss of double helix bonds in starch chains when heated with water (Fonseca et al., 2021).

HMT modification of green banana starch has been proven to reduce swelling power due to the rearrangement of starch molecules, which reduces its hydration power. This is related to a decrease in crystallinity, the formation of amylose-iodine complexes, and the gelatinization temperature of the starch suspension so that it breaks hydrogen bonds and releases water molecules bound from hydroxyl groups (dos Santos Costa et al., 2019).

The results in Table 2 show that treatment with pullulanase enzyme was able to increase the solubility and swelling power of starch. The increase in solubility is due to the breaking of α -1,6 bonds, such as branching parts, such as amylopectin, break and an increase in the number of short chains, such as amylose, which is more soluble than amylopectin. Apart from that, enzymatic modification of starch also occurs when hydrogen bonds are broken and molecules are formed which have many hydroxyl groups so that starch can bind water more easily (Hutabarat & Stevensen, 2023). Corn starch modified with pullulanase showed increased swelling power. This is possible because there is a change in the amorphous part of the starch, making it difficult for water to enter and the starch becomes swollen (P. Liu et al., 2020).

Thermal characteristics

To understand the thermal transition of physically and enzymatically modified rice starch, samples were tested for their thermal properties related to gelatinization, using a DSC instrument. The DSC curve obtained from the research results is shown in Figure 2. The gelatinization temperatures (onset, peak, and end set: T_o , T_p , and T_e), as well as the enthalpy (ΔH) of rice starch and modified rice starch are shown in Table 3.

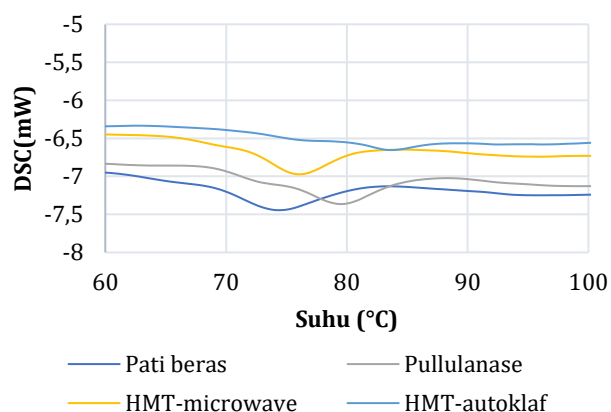


Figure 2. Differential Scanning Calorimetry (DSC) curves of rice starch and modified rice starch

Table 3 shows the modification of starch both physically (HMT-microwave and HMT-autoclave) and enzymatically (pullulanase) by increasing the gelatinization temperature (T_o , T_p , and T_c). The higher peak gelatinization temperature in the HMT treatment indicates that the starch crystals melted more stably than in the other samples.

Table 3. Thermal characteristics of rice starch and modified rice starch

Starch type	Gelatination			
	T_o (°C)	T_p (°C)	T_e (°C)	ΔH_g (J/g)
Rice starch	70,1	74,4	78,7	3,19
HMT-microwave	72,8	76,1	79,8	2,93
HMT-autoclave	81,0	83,7	86,5	0,73
Pullulanase	75,9	79,6	83,6	2,89

Data are mean values \pm standard deviation

HMT-modified rice flour had higher T_o , T_p , and T_e values than unmodified rice flour. HMT produces amylose, amylose-amylopectin, and amylose-lipid interactions, which cause a decrease in the mobility of starch chains in amorphous regions (Xing et al., 2018). This results in swelling and damage to the crystal areas in HMT-starch and requires higher temperatures to increase T_o , T_p and T_c (Kim & Baik, 2022).

The gelatinization enthalpy (ΔH) of the HMT flour was lower than that of the untreated flour (Table 3). This may be caused by the disruption of the double helix present in the crystal region (Chen et al., 2017). A decrease in ΔH by forage treatment was also reported in

potatoes, cassava, corn and peas (Kim & Baik, 2022).

The T_o , T_p , and T_e values of pullulanase enzymatically modified rice starch increased when compared with normal rice starch (Table 3). The same thing was shown in research on potato starch modification using the pullulanase enzyme (Ge et al., 2021). Linear chains after debranching are more likely to produce double helices, which facilitates the formation of crystallites, thereby causing an increase in T_p and T_c (Zhang et al., 2019).

Estimation of glycemic index (eIG)

The hydrolysis curves of rice starch and modified rice starch are presented in Figure 3, along with the hydrolysis index values (IH), and estimated glycemic index (eIG) are presented in Table 4.

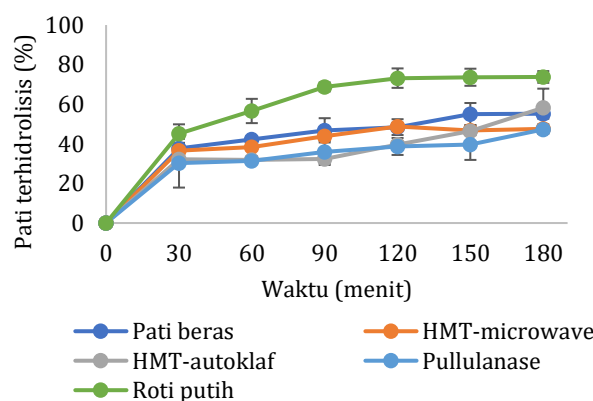


Figure 3. Percentage of hydrolyzed starch from rice starch in various treatments

Table 4. Hydrolysis index (IH) value and estimated glycemic index (eIG)

Starch type	IH	eIG
Rice starch	72,80 \pm 4,80	79,68 \pm 2,64
HMT-microwave	67,24 \pm 2,14	76,62 \pm 1,17
HMT-autoclave	59,70 \pm 1,97	72,49 \pm 1,08
Pullulanase	56,32 \pm 3,82	70,63 \pm 2,10

Data are mean values \pm standard deviation

The results showed that the IH and eIG values of rice starch > HMT-microwave > HMT-autoclave > pullulanase. In previous research, HMT treatment reduced the glycemic index of rice starch and increased the onset temperature when compared with normal rice starch (Kunyanee & Luangsakul, 2022b). The decrease in the hydrolysis rate and glycemic index value in the HMT treatment could be

caused by an increase in the content of slowly digestible starch (SDS) and resistant starch (RS), while reducing the content of starch that can be digested quickly (rapid digestible starch/RDS) (Piecyk & Domian, 2021; Sandhu et al., 2020).

During enzymatic treatment, pullulanase quickly hydrolyzes α -1,6-glycosidic bonds, producing amylose-like linear chains that facilitate starch retrogradation and the formation of type 3 resistant starch (RS), which causes a decrease in starch digestibility. This reduced the starch digestibility. This will have the effect of reducing the IG levels of starch (Kumar et al., 2022). Modification treatment of rice starch with preheating and pullulanase can modify the surface layer of starch granules, which increases the amylose content and breaks some of the desired structures, which results in a decrease in gel strength and the estimated glycemic index (Geng et al., 2023).

Conclusion

Physically modified rice starch (HMT-microwave, HMT-autoclave) and enzymatic pullulanase increased the solubility of normal rice starch. HMT treatment reduced the swelling power of rice starch, but pullulanase enzymatic treatment increased the swelling power. The amylose content increased, and the DSC enthalpy value decreased in all HMT and pullulanase enzymatic treatments. Physical modification using HMT and enzymatic modification using pullulanase reduced the IH and eIG values of starch compared with normal rice starch.

Based on the results of this research, the author suggests carrying out an in vivo test to measure the IG of modified starch. In addition, it is necessary to test consumer acceptance of modified rice starch through a hedonic sensory test.

Acknowledgements

The author would like to thank the Ministry of Education, Culture, Research, and Technology and the University of Jember for the research funding provided for the implementation of this research through KeRis-Dimas grant funding from Jember University's internal funding source number: 3349/UN25.3.1/LT/2023.

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