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Article

Digestibility and Physicochemical Properties of Brown Rice and Black Glutinous Rice under a Combination of Heat-Moisture Treatments and Citric Acid

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Abstract. Brown rice and black glutinous rice are rich in nutrients and fiber the body needs. The difference between brown and black glutinous rice lies in the starch content, namely amylose, and amylopectin, which can affect digestibility. Low digestibility rice can lower blood glucose levels, so it is needed for people with diabetes and obesity. This study modified brown rice and black glutinous rice with double modification HMT-crosslinking with citric acid and Crosslinking-HMT with various variations to determine the physicochemical properties and the lowest digestibility of brown rice and black glutinous rice. Multiple modifications can reduce digestibility, but a modification of HMT 25%-Crosslinking 20% showed the lowest digestibility in black glutinous rice. Differences in amylose and amylopectin levels in the sample can cause differences in the decrease in solubility and swelling power. The lowest solubility was found in brown rice with the HMT 25%-Crosslinking 20% variation, and the lowest swelling power in the brown rice sample with the HMT 25%-Crosslinking 20% variation. The formation of new covalent bonds after the crosslinking modification process can be identified by FTIR in the 1735 cm^{-1} regi

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1. Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population. There is rice with no pigment and rice with black, red, and brown pigments [1-3]. The deposition of anthocyanins forms the color difference in rice in the rice husk layer [4-6]. The main nutrient content in rice and the primary determinant of rice quality is starch [7-8]. Starch will be broken down into glucose with the help of enzymes in the human metabolic system, and then glucose will be used for energy needs. Rice as a staple food has a weakness, namely rice starch has a high glycemic index.

Consuming foods containing much starch can increase blood glucose levels and will cause obesity, diabetes mellitus, and cardiovascular disorders. The International Diabetes Federation (IDF) in 2021 noted that Indonesia was in the fifth position in the world, with 19.47 million people with diabetes. This has attracted many researchers to develop carbohydrates that have low digestibility. Various developments of carbohydrates with low digestibility using starch samples of rice flour and tapioca flour, brown rice, and modified black glutinous rice in the entire state have not been reported.

The difference between brown rice and black glutinous rice lies in the concentration of amylose and amylopectin. Brown rice belongs to medium amylose starch, and black glutinous rice belongs to very low amylose starch. Brown rice and black glutinous rice are not consumed as daily staples and are only used for processed foods, even though brown rice and black glutinous rice are rich in nutrients and fiber the body needs [9-11]. Consuming rice with a high fiber content can maintain a feeling of fullness because the fiber will expand in the stomach, slows down the digestive process, and no spike in blood glucose. This is very much needed for people with diabetes and obesity.

Recently, several studies have been carried out on the decreased digestibility of several starch sources [12] have performed a single modification with a crosslinking of rice flour starch using citric acid. The result is that the difficult-to-digest starch value after modification increased by 52% compared to starch without modification. This happens because when citric acid is heated, citric acid will form anhydrides due to dehydration and then react with starch molecules to form starch citrate additions.

The other heating process produces additional dehydration of citric acid and causes a crosslinking between starch and citric acid molecules. Multiple modifications have been carried out by [13-14] by using crosslinking using STTP/STMP and HMT in corn flour; the result is that crosslinking-HMT is effective in producing RS in starch and is gelatinized due to strong residual phosphorus interactions and starch structure, thermally stable after HMT-crosslinking. Besides reducing digestibility, modified Heat Moisture Treatment (HMT) and Crosslinking (CL) treatments can also change starch's physicochemical properties, such as solubility and swelling power. Therefore, this study aimed to determine the decrease in digestibility of brown rice starch and black glutinous rice, which had different amylose content and were intact, modified with various variations of citric acid and various variations of HMT.

2. Experimental Section

2.1. Materials

The materials used in this study include brown rice, black glutinous rice, ethanol, NaOH, acetic acid, I_2, KI, citric acid, HCl, -amylase enzyme, DNS (Dinitrocalicylic Acid), and KBr.

2.2. Amylose Content

The amylose content seen in starch was assessed according to colorimetric procedures [15], and samples were analyzed for amylose content by a colorimetric method based on the reaction between amylose and iodine. A 0.1 g rice sample was moistened with 1 mL of ethanol (95%), gelatinized with 9 mL of NaOH, and heated at 100°C for 30 minutes [16]. Cooled for 1 hour, the solution was transferred to a 100 mL volumetric flask, and distilled water was added to the mark. 5 mL of the solution was taken and put into a 50 mL volumetric flask, added 1 mL of acetic acid and iodine solution (2 mL, 0.2% I_2 in 2% KI). The volume was made up to 50 mL with distilled water and stirred, and the absorbance of the sample was measured at 620 nm [9].

2.3. HMT-Crosslinking Starch Modification

Samples of rice and black glutinous rice were double modified in two ways. First, the rice was modified by the HMT method and then crosslinking. For the HMT method, brown and black glutinous rice (30 g) were adjusted for humidity with variations of 15, 20, and 25%, then transferred to a petri dish and tightly closed. The sample was allowed to stand at room temperature for 24 hours. All samples were heated in a hot air oven at 110°C for 1 hour. Then, the cup containing the sample was opened, and the sample was cooled at room temperature for 24 hours. The HMT-modified samples were placed on an aluminum tray and dried in a hot air oven at 40°C for 24 hours [17-18].

Rice samples that had been modified with HMT were further modified by crosslinking. The citric acid (1, 10, and 30% dry rice) was dissolved in distilled water. Next, the pH of the solution was adjusted to 3.5 with 5 M NaOH. The solution was diluted to a final volume of 150 mL with distilled water. Citric acid solution (30 mL) was mixed with 30 g of a rice sample and allowed to stand at room temperature for 24 hours. The mixture was dried in an air oven at 60°C for 6 hours, then dried in a dry oven for 4 hours at 110°C. The dry mixture was rinsed three times with distilled water to remove unreacted citric acid. The washed rice samples were oven-dried at 40°C for 24 hours. Some samples were ground into powder and passed through 150 μm [12].

2.4. Crosslinking-HMT Starch Modification

The citric acid (1, 10, and 30% dry rice) was dissolved in distilled water. Next, the pH of the solution was adjusted to 3.5 with 5 M NaOH. The solution was diluted to a final volume of 150 mL with distilled water. Citric acid solution (30 mL) was mixed with 30 g of a rice sample and allowed to stand at room temperature for 24 hours. The mixture was dried in an air oven at 60°C for 6 hours, then dried in a dry oven for 4 hours at 110°C. The dry mixture was rinsed three times with distilled water to remove unreacted citric acid. The washed rice samples were oven-dried at 40°C for 24 hours [19-20].

Samples of modified brown rice and black glutinous rice were crosslinking, followed by HMT modification. For the HMT method, brown rice and black glutinous rice (30 g) were adjusted for humidity with variations of 15, 20, and 25%, then transferred to a petri dish and tightly closed. The sample was allowed to stand at room temperature for 24 hours. All samples were heated in a hot air oven at 110°C for 1 hour. Then, the cup containing the sample was opened, and the sample was cooled at room temperature for 24 hours. The HMT-modified samples were placed on aluminum trays, dried in a hot air oven at 40°C for 24 hours, partly ground into powder, and passed through 150 μm [17].

2.5. Solubility and Swelling Power

Brown rice flour and modified black glutinous rice (0.1 g, dry weight) were added to 10 mL of distilled water and heated at 50, 60, and 70°C for 30 minutes in a water bath. Then cooled to room temperature, the suspension was centrifuged at 3500 rpm for 15 minutes. The supernatant was collected and dried at 60°C for 6 hours to determine solubility. The precipitate was weighed, and the swelling strength was determined based on the weight gain of the precipitated rice flour and black glutinous rice flour [19].

2.6. Determination of Digestibility of Brown Rice and Black Glutinous Rice

Brown rice flour and 0.1 g black glutinous rice were put in a test tube, and 10 mL of distilled water was added. The sample mixture was heated for 30 minutes at 90 °C. After heating, 5 mL of HCl pH 4 and 5 mL of α-amylase enzyme solution were added. The blank solution was prepared with the same treatment as the previous procedure but did not use starch. Both test tubes (sample and blank) were incubated at 90 °C for 30 minutes. The sample solution and the blank were pipetted 1 mL and put into a test tube. Added 2 mL of DNS solution (dinitro salicylic acid), heated at 100 °C for 10 minutes, and then cooled. The solution was diluted 10x, and the color formed was measured using a UV-Vis spectrophotometer at 520 nm [21-22].

2.7. Fourier Transform Infrared Spectroscopy (FTIR)

Characterization of the samples by FTIR was done by adding samples of red rice flour and black glutinous rice with KBr in a ratio of 1:20. The mixture was crushed using a pestle and mortar to form a fine solid sample. The sample is placed in a container/holder on the FTIR instrumentation device and then operated at a wavelength of 400 to 4000 cm^{-1} [23].

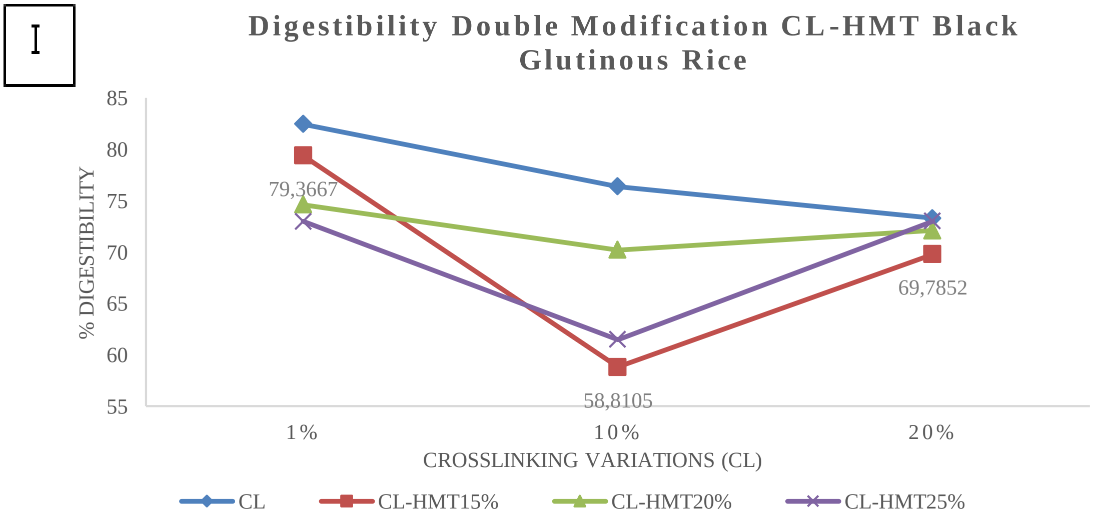
3. Results and Discussion

3.1. Amylose Content

Different amylose content in rice can affect digestibility characteristics. Based on this, 2 samples were selected for this study brown rice and black glutinous rice. Determination of the amylose content in the sample using the iodine calorimetry method and the results obtained that the amylose content in the brown rice sample was 23.49% and the black glutinous rice sample was 14.42% (data not shown). Tests using the iodine calorimetry method can be used because long chains can form complex bonds with iodine. According to [24-25], amylose is classified into 4 types, namely very low (5-12%), low (13-20%), medium (21-25%), and high (25-33%). Based on this, it can be concluded that brown rice is classified as medium amylose, and black glutinous rice is classified as low amylose.

3.2. Digestibility of Brown Rice and Black Glutinous Rice

Results the digestibility of brown rice and black glutinous rice using the α-amylase enzyme can be seen in Figure 1 and Figure 2. Digestibility testing can provide an overview of the ease with starch being hydrolyzed by human digestive enzymes. The digestibility of brown and black glutinous rice before double modification was 98% and 96%, respectively. The content of amylose and amylopectin and the structure of amylopectin can affect the digestibility of starch. In this study, two modification methods were carried out: double modification of HMT-crosslinking and crosslinking of HMT. Double modification of HMT-crosslinking and crosslinking-HMT decreased the digestibility of brown rice and black glutinous rice compared to the samples before modification.



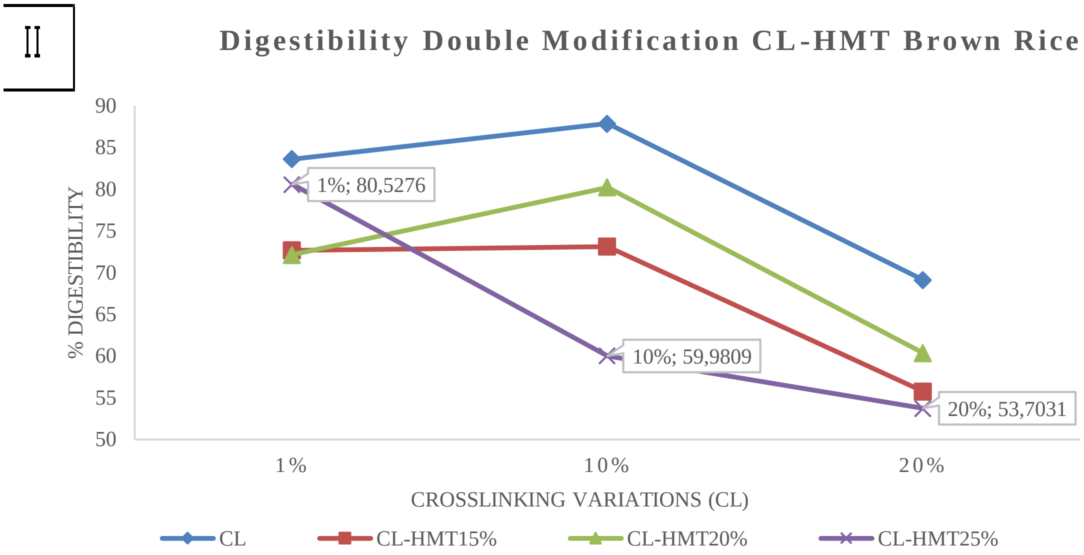


Figure 1. (I) Digestibility of double modified crosslinking (CL)-HMT of black glutinous rice sample, (II) digestibility of double modified crosslinking (CL)-HMT of brown rice sample.

In this study, the combination of double-modified HMT-Crosslinking was found to be more stable in reducing digestibility. The modified sample HMT-crosslinking will occur acid hydrolysis resulting in a low molecular weight chain so that it will resist enzymatic digestion by forming a double helix of amylose-amylose, amylopectin-amylose, and amylopectin-amylopectin so that when further modified with HMT will reduce digestibility but not maximum. While samples modified with HMT can first form interactions during heat-humidity treatment, the starch crystal structure will undergo rearrangement and separate the double helix in the amorphous region without disturbing the granular structure.

Then the samples were modified with HMT, followed by citric acid modification, aiming to reduce digestibility further. When a sample modified by HMT with variations in humidity is reacted with citric acid, an esterification reaction will occur. Heated citric acid will dehydrate to form anhydride, and it will esterify starch to form citric starch. Further heating will crosslinkingdue to intermolecular di-ester bonds [26-27]. This crosslinking will provide low digestibility of digestive enzymes.

Double modification of HMT-Crosslinking with various variations in brown rice samples resulted in a decrease in digestibility with a lower trend when compared to black glutinous rice samples. However, the lowest digestibility was found in black glutinous rice samples with variations of HMT 25%- 20% crosslinking by 33.64%. This is because the brown rice sample included in starch with medium amylose content has a higher amylose content and a longer chain in amylopectin, forming a more stable double helix to reduce susceptibility to the α-amylase enzyme [28-30]. The lowest digestibility in black glutinous rice samples can be attributed to the high amylopectin content. Amylopectin has a shorter chain than amylose, so that it can contribute to decreased digestibility of enzymatic digestion. The decrease in digestibility in the double modification of whole brown rice and whole black glutinous rice indicates that modification with whole rice samples can be carried out [31].

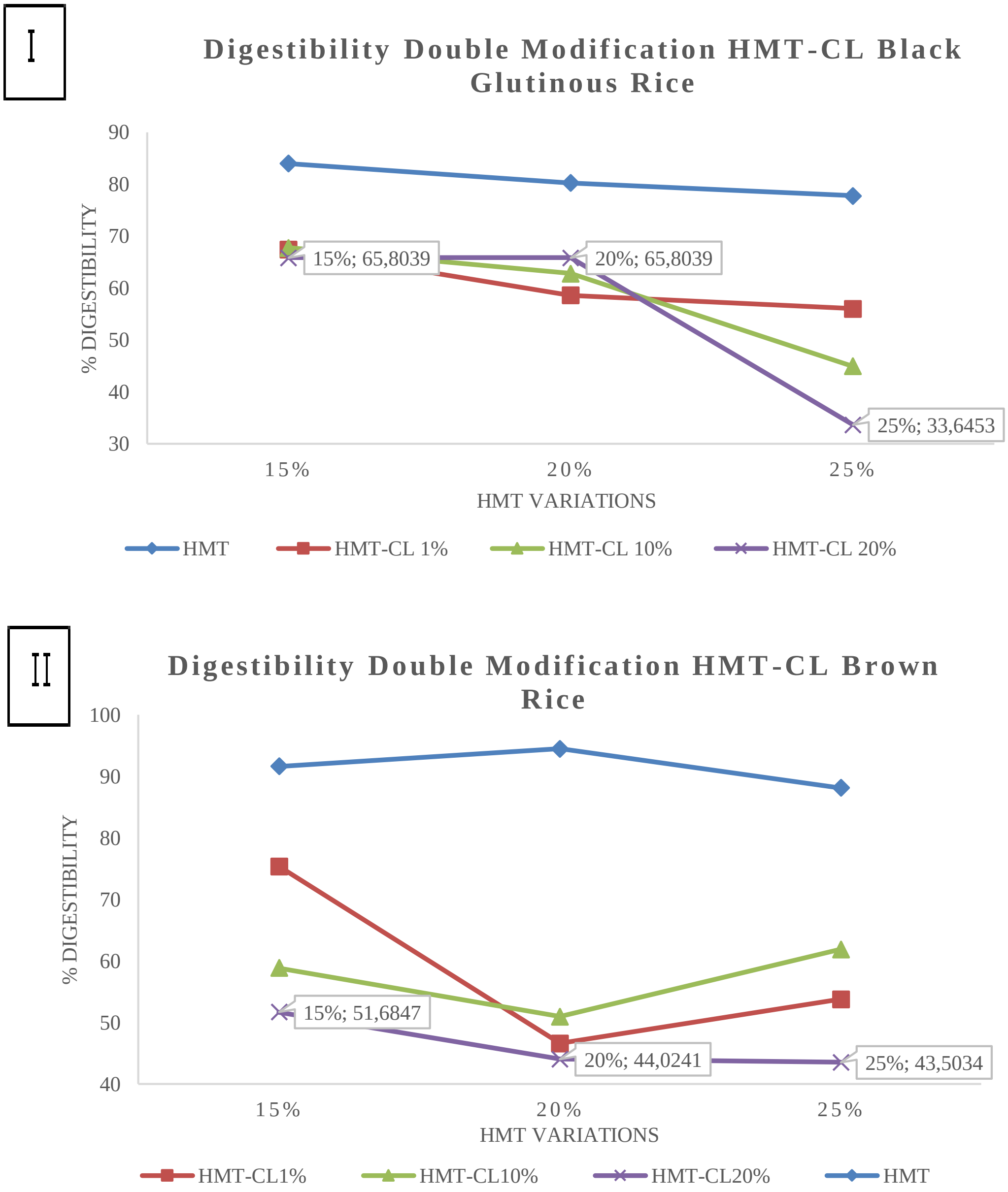


Figure 2. (I) Digestibility of double modified HMT-Crosslinking (CL) of black glutinous rice sample, (II) Digestibility of double modified HMT-Crosslinking (CL) of brown rice sample

3.3. The Solubility of Brown Rice and Black Glutinous Rice

Samples of brown rice and black glutinous rice were double-modified HMT-Crosslinking and Crosslinking-HMT. The two double modifications had lower solubility when compared to the sample before modification, but the double-modified HMT-Crosslinking had much lower solubility, especially in the HMT 25%-20% crosslinking variation. The decrease in solubility occurs because HMT can cause dehydration of the granules and increase chain interactions. After all, rearrangement of the granules results in low solubility. Inter-chain interactions after the HMT process can interact with citric acid to form a cross-link. The new crosslinking between starch granules and citric acid can prevent water absorption so that it can reduce solubility and mark the crosslinking goes well [32-33].

The solubility of modified brown rice HMT 25%-Crosslinking 20% is lower than the solubility of modified black glutinous rice samples HMT 25%-Crosslinking 20%. This is because, during the modification of the HMT-Crosslinking of black glutinous rice samples, inter- and intrahelical hydrogen bonds are broken. The cleavage of the inter-chain and intrahelic hydrogen bonds results in amylopectin in the soluble amorphous region. Meanwhile, the brown rice sample had lower amylopectin levels, thus allowing the double-modified HMT-Crosslinking to have low solubility.

3.4. Swelling Power of Brown Rice and Black Glutinous Rice

The swelling power of the unmodified brown rice starch and black glutinous rice was 11.8368% and 13.7598%, respectively. Multiple modifications were carried out with variations of HMT-Crosslinking and Crosslinking-HMT, each of which was varied by HMT 15; 20;25% and crosslinking 1;10;20%. The swelling power of modified HMT-Crosslinking modified brown rice and black glutinous rice samples decreased to 4.5672% and 6.0064% compared to the unmodified samples. The decrease in swelling power of brown rice and black glutinous rice is almost the same, namely 7%. Although the decrease is the same as 7%, the swelling power of brown rice has the lowest swelling power value, which is 4.5672%.

According to [23], the nature of amylopectin is the main factor of swelling power; amylose acts as a swelling inhibitor. Branched amylopectin chains cause a loose granule structure; the higher the amylopectin content, the more amorphous areas in starch granules that are not dense so that it is easy for water to enter. Meanwhile, amylose, which has a linear structure, is not easily penetrated by water. The decrease in the Swelling power of the sample occurred because the HMT process could increase the interaction between chains due to the rearrangement of granules and crosslinking that form new ester bonds in starch.

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra of samples before modification, single modification, and double modifications are presented in Figure 3. The spectrum broad peaks at 3000-3600 cm^{-1} and 2932 cm^{-1} mark the -OH and -CH stretches; these peak areas are characteristic peaks of starch [34]. There is a change in the spectrum between the sample spectrum before the modification and the sample after the double modification. There is a new peak at 1735 cm^{-1} in the FTIR spectrum related to forming an ester group after a cross-link occurs between the hydroxyl groups of brown rice starch molecules and black glutinous rice with the carboxyl groups of citric acid.

According to [34], on the spectrum of pure citric acid, there are peaks at 1704 cm^{-1} and 1755 cm^{-1} caused by the stretching vibration of C=O in the carboxyl group and interference peaks when the carboxyl groups in the acid are close to each other. In the spectrum of the modified sample, no peaks were seen at 1704 cm^{-1}, and 1755 cm^{-1}, a change in peak to 1735 cm^{-1} could explain that cross-link between citric acid from starch from brown rice and black glutinous rice can occur during heating.

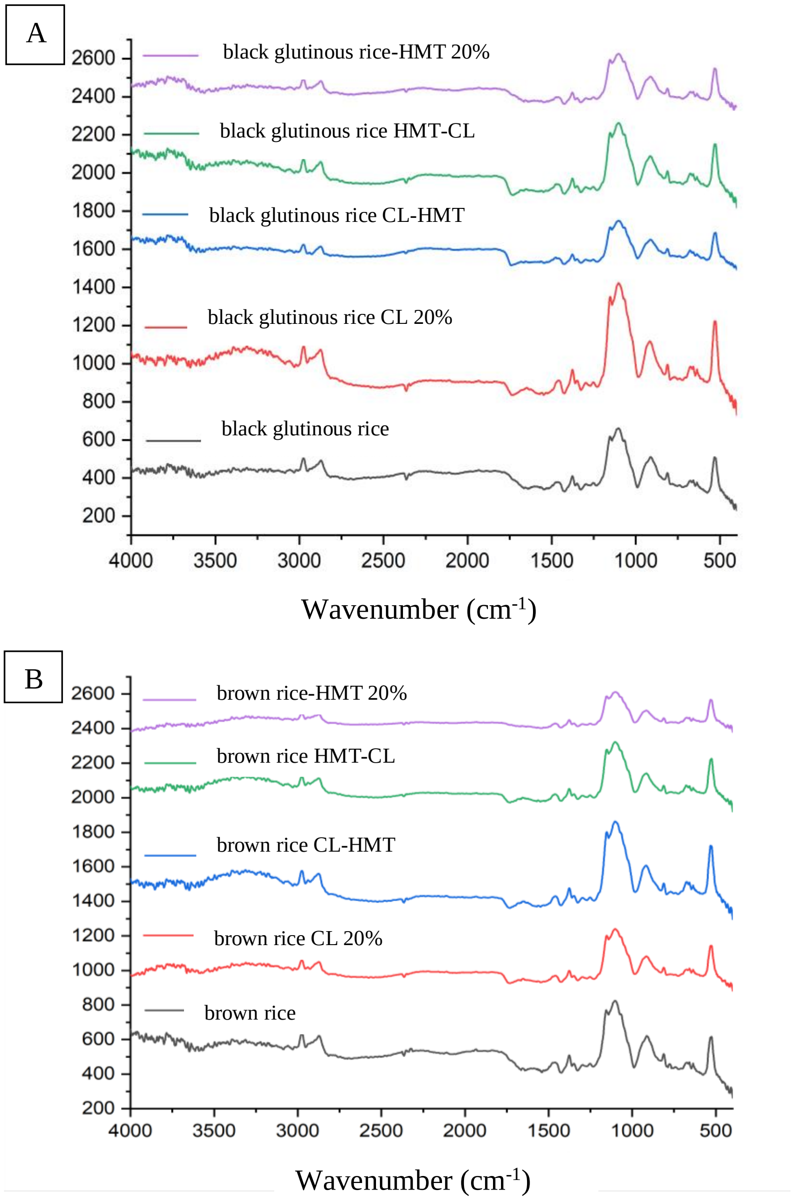


Figure 3. (A) FTIR spectrum of brown rice, HMT-treated brown rice, Crosslink, Crosslink-HMT and HMT-Crosslink. (B) FTIR spectrum of black glutinous rice, black glutinous rice treated with HMT, Crosslink, Crosslink-HMT and HMT-Crosslink.

4. Conclusion

Double modification can be done on brown rice and black glutinous rice. The combination of HMT 25%-Crosslinking20% treatment variations was found to reduce the digestibility of brownand black glutinous rice, with the lowest digestibility of black glutinous rice. The HMT 25%-Crosslinking20% variation treatment can reduce the solubility and swelling power, the lowest decrease in brown rice compared to black glutinous rice. This is due to the difference in amylose content in brown and black glutinous rice. Crosslinking formation after modification with citric acid can be confirmed in the FTIR spectrum at the peak of 1735 cm^{-1}.

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