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Original research

Impact of cooking techniques and refrigeration storage using *in vitro* determination of glycemic index and resistant starch of cooked Mansuli rice

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ABSTRACT

This study investigated the impact of different cooking techniques and refrigeration storage periods on the resistant starch (RS) content and glycemic index (GI) of cooked rice. Mansuli rice was cooked using two methods-pressure cooker and stainless steel pot in induction stove adjusted manually using power setting at a temperature of 800 °C. Cooked rice was stored in refrigerator at 4 °C for 0, 12, 24, and 48 h. Rice cooked in a pressure cooker had a lower resistant starch (RS) but a higher predicted glycemic index (pGI) compared to rice prepared on stainless steel pot. Pressure-cooker cooked rice, RS levels ranged from 1.106% to 5.059% across the four storage durations, whereas stainless steel pot cooked rice, RS content varied between 2.671% to 7.149%. A consistent increase in RS was observed with prolonged refrigeration storage. The predicted glycemic index (pGI) of rice consistently decreased as the refrigeration time extended, dropping from 75.5-62 for rice cooked on pressure-cooker and from 69.75-52.5 for rice cooked in a stainless steel pot. These findings suggest an inverse relationship between RS and GI, highlighting the influence of cooking methods and storage conditions on the starch digestibility of rice.

Keywords: Rice; Cooking techniques; Storage conditions; Glycemic index; Resistant starch.

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1. Introduction

Rice is a staple food that is consumed by about 3.5 billion individual people in the world. Over half of the calories come from rice (Gadal et al., 2019). Cooking temperature and duration reduces the digestibility of the starch present in food items. Starch comprises the chains of amylose made up of α-1,4 connected glucan in a linear chain and amylopectin which contains both α-1,4 and α-1,6 glycosidic linkage, it has a highly branched structure (Cummings & Englyst, 1991). The granular structure, amylose: amylopectin ratio, and crystallinity of starch are its inherent characteristics which promote the digestion of rice starch (Darandakumbura et al., 2013).

Resistant starch (RS) refers to any starch or starch breakdown product which is not digestible in healthy individuals' small intestine (Z. Wang et al., 2023). RS is a fiber fraction based on starch which passes to the large intestine where it does not break down and remains undigested (Lal et al., 2021). Numerous studies have shown that certain starch fractions perform physiological roles similar to those of dietary fiber (Asp & Björck, 1992). Glycemic index (GI), the tendency of carbohydrates presents in foods, which could raise the blood glucose level. Low-GI foods have slower-absorbing and digesting carbohydrate, they assist to stabilize postprandial glucose levels of plasma (Granfeldt et al., 2006). A lower risk of type 2 diabetes mellitus (T2DM) cardiovascular disease (CVD), obesity, pro-inflammatory marker levels, and fasting insulin are linked to low-GI and GL diets not on total carbohydrate (Salmerón et al., 1997; Scazzina et al., 2016), foods and processed goods with low GI and GL can lower the chance of developing illnesses (Lal et al., 2021). FAO/WHO (1998) recommended diabetics and people with impaired glucose tolerance to increase intake of low GI foods (Granfeldt et al., 2006).

Most research focused on individual factors cooking method or storage temperature. This study explored the combined effects cooking methods followed by refrigeration provide more detailed understanding. Existing research focused on short-term storage, few hours after cooked. Investigating how resistant starch and glycemic

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index changed over extended storage periods 48 h could be informative. Many studies relied on *in vivo* digestibility models, complicated by the fact that each person's physiological state is unique, which makes the results difficult to repeat and lack precision, so this study focused on in vitro model. The health benefits of resistant starch are widely known, but there is currently no research had been conducted on how cooking techniques and storage conditions effect on RS and GI of cooked rice that have been retrograded under 4 °C for 0, 12, 24, and 48 h. In this work, the resistant starch and glycemic index of Mansuli rice cooked under pressure cooker and stainless steel pot in an induction stove at 800 °C and stored at 4 °C for 0, 12, 24, and 48 h were compared (Lal et al., 2021).

2. Materials and Methods

2.1. Materials

Pepsin (30 units/mg), pancreatin (4 × USP specifications), fungal alpha amylase (≥ 5 units/mg) were purchased from Sigma-Aldrich and GODPOD (Autospan). A 1-litre rice cooker and stainless steel pot were used to cook rice. Baltra induction cooking stove was used to make consistent cooking temperature. Chemicals, glassware and equipment required were obtained from the laboratory of Central Campus of Technology, Sunsari, Nepal. Munsuli rice were purchased from the local market.

2.2. Preparation method of Mansuli rice

50 g Mansuli rice was soaked in 150 mL water and held for 10 min. Rice was cooked in pressure cooker and stainless steel pot in induction stove set manually at temperature of 800 °C for 20 min and allowed to cool for 5 min.

2.3. α-amylase inhibition assays

The DNS method was employed to quantify the reducing sugar content in the samples, thereby assessing the α-amylase activity following its interaction with inhibitors (J. Wang et al., 2022). A spectrophotometer measured the absorbance of the product at a wavelength of 540 nm, and the sample's inhibitory effect was evaluated based on the absorbance values obtained.

2.3.1. Preparation of α-amylase solution

5 mL of 2.6 mg/mL fungal α-amylase (from *Aspergillus oryzae*) was dissolved in 10 mL of 0.25 M sodium acetate to prepare α-amylase solution for reserve.

2.3.2. Preparation of substrate solution

The cornstarch was dissolved in a buffer containing citric acid, which was then poured into the heated citric acid buffer (not exceeding 100 °C when heated) and kept stirring to prevent gelatinization of the starch. After heating for 2 min, the cornstarch solution was removed and placed at room temperature. Finally, the solution was fixed to 10 mg/mL with citrate buffer for later use.

2.3.3. Preparation of inhibitor solution

The samples were dissolved in ultrapure water and prepared into sample solutions of different concentrations.

2.3.4. Assay protocol

0.2 mL fungal α-amylase solution (2.6 mg/mL) and 0.1 mL sample solution of different concentrations were pre-incubated for 10 min at 37 °C. Then 100 µl of prepared cornstarch solution was added to start the reaction, and the reaction was carried out at 37 °C for 5 min. Then 0.1 mL DNS solution was added and stop the reaction by boiling water bath for 10 min. After cooling in the ice bath to room temperature, the mixture solution was diluted four times and the absorbance value was measured at 540 nm. Buffer solution and acarbose solution were used to replace sample solution as a blank group and positive control group, respectively. All analyses were performed in triplicate and results were presented as percentage (%) of inhibition, calculated using the following relation (Eq. (1)).

$$I\,(\%) = \frac{[1-(C-D)]}{A-B} \times 100 \quad (1)$$

where A, Blank group; B, Blank control group; C, Test group; and D, background control group are the absorbance values measured at 540 nm for each group respectively.

2.4. Determination of resistant starch (RS)

Resistant starch (RS) was determined using techniques suggested by Englyst et al., (1992) with modification. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) using the techniques suggested by Wang et al., (2022) with minor modification. Shortly, 4 g of cooked rice was taken in centrifuge tube and 50 mg guar gum was added and mixed well. 50 mg of pepsin was added along with 10 mL, 0.05 N hydrochloric acid, converts proteins present in the rice into smaller peptones and peptides. Pepsin helps in curdling and breakdown of protein present in rice. Gastric juice contains hydrochloric acid which converts inactive pepsinogen into active pepsin thereby hormones actions are developed (Sembulingam & Sembulingam, 1980). Samples were incubated for 16 h at 37 °C in incubator occasionally manually shaking then 10 mL of 0.25 M sodium acetate and 5 mL fungal α −amylase (2.6 mg/mL) were added and it was placed in water bath maintained at 55 °C for 2 h. After 2 h, 0.5 mL rice sample solution was transferred in the test tube containing 20 mL 66% ethanol and centrifuged for 2 min glucose released was measured called G_{120}. The remainder were vortex mixed and allowed to rest for 30 min at a temperature 100 °C and allowed to cool at 0 °C in an ice bath and 10 mL 7 M KOH solution was added and vortex mixed. The vortex mixed samples were cooled at 0 °C in an ice bath for 30 min with manual shaking. After 30 min, 1 mL of the vortex sample was transferred in a test tube containing 10 mL 0.5 M acetic acid and 0.2 mL fungal α −amylase (26.4 mg/mL) was added in that test tube. The test tube was allowed to rest for 30 min at 70 °C and 10 min at 100 °C simultaneously and allowed to cool to room temperature. On cooled test tube 40 mL of distilled water was added and centrifuged for 5 min. This was the sample solution for the determination of total glucose (TG).

For the determination of both glucose released after 120 min (G_{120}) and total glucose (TG), 0.1 mL sample solution was taken in a test tube and 2 mL GOD-POD reagent was added and kept in water bath maintained at temperature 37 °C for 20 min. In the similar manner, 0.1 mL standard glucose (10 mg/mL) was taken in test

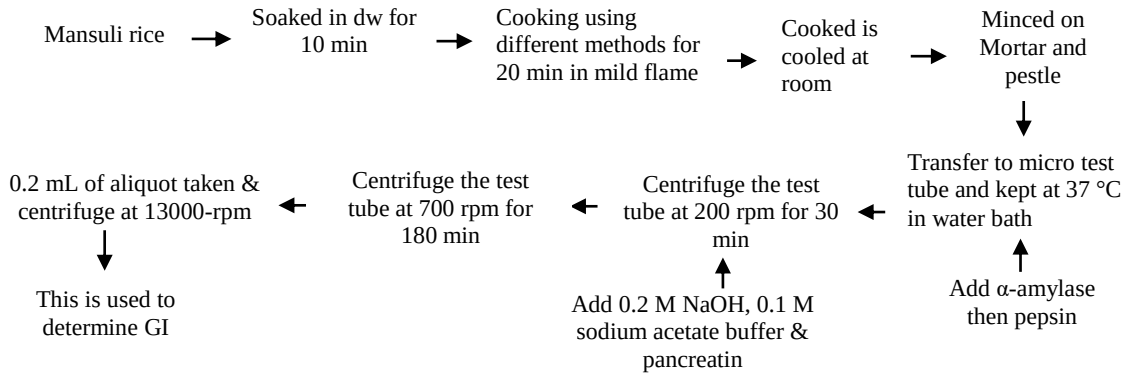

Fig. 1. Steps for determining the glycemic index according to the method of Pautong et al., (2022).

tube and 2 mL GOD-POD reagent was added and kept in water bath maintained at temperature 37 °C for 20 min. Then absorbance was measured at 510 nm using spectrophotometer for G_{120}, TG and standard glucose solution.

RDS, SDS and RS is calculated by using the following equation (Eqs. (2), (3) and (4)).

$$\text{RDS (\%)} = G_{20} \times 0.9 \times 100 \quad (2)$$

$$\text{SDS (\%)} = (G_{120} - G_{20}) \times 0.9 \times 100 \quad (3)$$

$$\text{Resistance starch} = (TG - G_{120}) \times 0.9 \quad (4)$$

TG represents Total Glucose.
G_{20} and G_{120} represents glucose released after at intervals of 20 min and 120 min.

2.5. Determination of Starch hydrolysis index (SHI) and pGI

Starch hydrolysis index was determined by the method suggested by Pautong et al., (2022) with minor modification (Fig. 1). Cooked rice was minced in mortar and pestle to make fine particles and then 25 mg was taken in a test tubes, 0.125 mL α-amylase and 1.25 mL pepsin (1 mg/mL in 0.2 M HCl) were added and kept in water bath maintained at 55 °C for 5 min and centrifuged at 200 rpm for 30 min. Thereby, 1.25 mL of 0.2 M sodium hydroxide solution, 5 mL sodium acetate buffer (pH 6) and pancreatin of 1.25 mL (2 mg/mL) were added then centrifuged for 180 min at 700 rpm. 0.2 mL centrifuged sample solution were taken at 0, 30, 60, 90, 120 and 180 min and transferred in 2 mL micro test tube successively to calculate the glucose hydrolysis. The tube was placed on an ice bath for 10 min and centrifuged at 13,000 rpm for 10 min and after 5 min 0.2 mL sodium acetate buffer and 0.04 mL fungal amylase were added and incubated at 50 °C for 20 min. Glucose contained in the cooked rice was determined using the GOPOD assay at 510 nm through spectrophotometer.

The starch hydrolysis index (SHI) was calculated by dividing the area under the curve (AUC) of the test rice samples by AUC of standard glucose solution (Lal et al., 2024) (Eq. (5)).

$$\text{SHI} = \frac{\text{Area under curve AUC for the test rice}}{\text{Area under curve AUC for standard glucose}} \quad (5)$$

The formula suggested by Goñi et al., (1996) was used to obtain the predicted glycemic index as Eq. (6).

$$\text{pGI} = 39.21 + (0.803 \times \text{SHI}) \quad (6)$$

For area under curve, a glucose hydrolyzed values for each sample were taken of 0, 30, 60, 90, 120 and 180 min for standard solution of glucose. Up to 180 min was taken because this time reading showed 100% glucose was hydrolyzed. So for the rice samples, the reading was also taken up to 180 min. The reading obtained were used for the calculation of AUC using trapezoid rule.

2.5.1. Formula for Trapezoid rule

For using trapezoid rule, AUC at 0 min was not calculated as no glucose hydrolysis would occur, so AUC was calculated from 30 min (Eq. (7)).

$$\text{AUC at 30 min} = \frac{\text{Glucose hydrolysed at 30 min} + \text{Glucose hydrolyzed at 0min}}{2} \quad (7)$$

In the similar manner, AUC at 60, 90, 120 and 180 min were determined. Total AUC of sample is the sum of AUC at different time intervals.

2.6. Statistical Analysis

Triplicate data were performed for analysis of variance (ANOVA) using IBM SPSS Statistics version 27 and Tukey's HSD was used to analyze the significant difference on sample means at 5% level of significance. Microsoft Office 2013 was used for tabulation.

3. Results and Discussion

This study was conducted to evaluate the effects of refrigeration storage and cooking techniques on predicted glycemic index (pGI) and resistant starch (RS) of Ranjeet variety old aged Mansuli rice. Variations in cooking methods and storage duration can significantly

influence RS and pGI levels, which are crucial factors in developing strategies for preparing and storing rice to benefit individuals with different physiological and metabolic conditions, such as diabetes and obesity. Rice was cooked using two methods-pressure cooking and stainless steel pot in induction stove at 800 °C, followed by refrigeration at 4 °C for 0, 12, 24, and 48 h. The glycemic index and resistant starch content were analyzed, and the results are discussed under the following headings.

3.1. Effect of refrigeration storage on resistant starch

Resistant starch developed in freshly cooked and refrigerated rice which were cooked in the pressure cooker and stainless steel pot at 800 °C for 20 minutes and stored for 12, 24 and 48h are shown Table 1.

Table 1. Resistant starch content of freshly cooked and refrigerated rice.

	Resistant Starch			
Samples	**0 h**	**12 h**	**24 h**	**48 h**
Pressure cooker	1.106 ^{a} ± 0.345	2.656 ^{a} ± 0.535	3.820 ^{a} ± 0.360	5.059 ^{a} ± 0.579
Stainless steel pot	2.671 ^{b} ± 0.539	4.135 ^{b} ± 0.680	5.975 ^{b} ± 0.565	7.149 ^{b} ± 0.460

Different superscript letters within the same column indicate significant difference between sample means at 5%.

Pressure cooking and stainless steel pot cooking showed significant effect on the resistant starch i.e., $p < 0.001$ and $p = 0.001$, respectively. The refrigeration storage had a substantial influence on RS of cooked rice. Storage at 1-25 °C can speed up the tendency of retrogradation and the development of RS in rice. Frei et al., (2003) found that 24 h of cooling of cooked rice at 4 °C decreased estimated GI and starch digestibility in vitro. Most studies agrees that cooling carbohydrate-rich foods promotes retrogradation, leading to an increase in resistant starch formation (Hodges et al., 2020).

Resistant starch of freshly cooked rice in pressure cooker was found 1.106%, much higher than the reported value of 0.37 % for medium grain by Chiu & Stewart, (2013). It was observed that freshly cooked rice in an open pan had a resistant starch level of 2.671%. Gu et al., (2019) found that the aging process can drastically alter starch molecular structure. Given that the rice has been aged for a year, this could be the cause of its higher RS content compared to other varieties of medium grain rice that have been previously evaluated. It implies that aging may be used as a sustainable technique to alter the digestibility of rice starch and raise the RS content of cooked white rice (Yi & Li, 2022). RS of freshly cooked rice in pressure cooker increased from 1.106 % to 2.656 %, 3.820 % and 5.059 % after 12 h, 24 h, and 48 h of refrigeration. Similarly, the RS of freshly cooked rice in stainless steel pot increased from 2.671 % to 4.135 %, 5.975 %, and 7.149 % after 12 h, 24 h, and 48 h of refrigeration, respectively. The refrigeration storage caused subsequent retrogradation of starch and raised RS.

3.2. Effect of refrigeration storage on glycemic index

Table 2 Depicts the predicted glycemic index (pGI) of freshly cooked and refrigerated rice which were cooked in the pressure cooker and stainless steel pot at 800 °C for 20 min and stored for 12, 24 and 48 h.

Table 2. The pGI values of freshly cooked and refrigerated rice.

	pGI values			
Samples	**0 h**	**12 h**	**24 h**	**48 h**
Pressure cooker	75.500 ^{a} ± 1.322	70.500 ^{b} ± 0.5	67.000 ^{a} ± 1.833	62.000 ^{c} ± 2.598
Stainless steel pot	69.750 ^{a} ± 0.433	64.300 ^{b} ± 0.608	56.700 ^{c} ± 1.081	52.500 ^{a} ± 0.866

Different superscript letters within the same column indicate significant difference between sample means at 5%.

The pressure cooking and stainless steel pot cooking showed significant effect on the glycemic index levels i.e., $p < 0.001$. The refrigeration storage had a substantial effect on the glycemic response of cooked rice. The pGI of freshly cooked rice in pressure cooker and stainless steel pot was found 75.5 and 69.75, respectively. The refrigeration storage led to subsequent retrogradation of starch with eventual rise in RS of rice thereby lowering the GI with the value decreased from 75.5 to 70.5, 67 and 62 after 12 h, 24 h, and 48 h of refrigeration, respectively for pressure-cooked rice. Similarly, the pGI level of rice cooked in stainless steel pot decreased from 69.75 of freshly cooked sample to 64.3, 56.7 and 52.5 after 12 h, 24 h, and 48 h of refrigeration, respectively. The findings of this study align with the report by Hodges et al., (2020), which discusses how cooking and cooling influence the resistant starch in foods such as potatoes, noodles, rice, and lentils, thereby affecting their digestibility. Ananda et al., (2013) provided evidence for this, reporting a reduction in the glycemic response *in vivo* following a 10 h cooling period at 3°C for cooked white rice. Together with modifications in amylopectin and amylose crystallization, the change in chemical structure could be a factor in starch's indigestibility (Nayak et al., 2014). Hydrogen bonds stabilize the tight structures formed by these retrograded RS molecules (Haralampu, 2000). Food with a lower GI is the result of this altered structure (Granito et al., 2018), that the digestive enzyme (α-amylase) cannot break down starch easily (Englyst et al., 1982). The interaction between starch and dietary polyphenols is crucial in regulating the glycemic response of starchy foods. Polyphenols attach to starch granules via hydrogen bonds and hydrophobic forces, creating a protective barrier that hinders enzymatic breakdown. This interaction limits the access of essential digestive enzymes, such as α-amylase, β-amylase, phosphorylase and protease to the starch, consequently slowing glucose release (Lal et al., 2024).

4. Conclusion

Rice cooked in a pressure cooker had a lower resistant starch (RS) value but a higher glycemic index (GI) compared to rice cooked in stainless steel pot, indicating that the cooking method has a substantial impact on the generation of RS and starch retrogradation. In pressure-cooked rice, RS levels ranged from 1.106% to 5.059% across the four storage durations, whereas in stainless steel pot cooked rice, RS varied between 2.671% and 7.149%. Retrogradation increases resistant starch and lowers glycemic index, benefiting blood sugar control. To ensure safety and maximize these benefits, rice should not be left at room temperature for extended periods and should be consumed within 3-4 days of refrigeration.

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Conflict of interest

The authors declare that there is no conflict of interest.

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