COST EFFECTIVE PROCESS DEVELOPMENT OF LOW GLYCEMIC INDEX FOR RICE PRODUCT

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Abstract
The high glycemic index (GI) value of white rice generates a more robust postprandial glucose response as compared to other grains. The scope of this work is to study the effect of retrogradation treatment on the digestibility of rice starch because of the impact of rice on health, mainly due to higher GI. The present study deals with the effect of retrogradation treatment on the structural characteristics and in-vitro digestibility of rice starch. The parboiled rice was retrograded for seven different time intervals, which were designated as RET1, RET2, RET3, RET4, RET5, RET6, and RET7, respectively. The study indicated that RET7 rice was with the lowest GI value of 30.05±0.17 when digested for 180 min. &further treated rice was puffed to expanded rice. The proximate content, GI, morphological structure melting enthalpies, and sensory analysis of parboiled (gelatinized) and RET7 rice puff was estimated. The results indicated that the GI of RET7 rice puff was reduced by 56.28% significantly as compared to GI of parboiled rice puff due to modification in crystalline morphology, thereby reducing enzymatic digestion. Melting enthalpies of parboiled -5.09 J/g and RET7-0.43 J/g rice starch, which suggests, digestion of retrograded rice requires more energy as compared to parboiled rice due to the formation of ordered structure. The sensory analysis also indicated an enhanced character of retrograded rice compared to parboiled rice. Therefore a reduction in GI by retrogradation for a specific period can be an essential tool for devising low GI food products.

Keywords: Retrogradation, Parboiled rice, Glycemic Index, Digestibility, Crystalline.

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INTRODUCTION

The increasing prevalence of diabetes and chronic disease in modern times has suggested more considerable research attention. Diabetes mellitus is a group of the disease characterized by a high sugar level in the bloodstream. The dramatic increase in diabetes has occurred in all countries, and rural as well as urban areas.(Ogurtsova, Rocha, Huang, Linnenkamp, & Guariguata, 2017).Consumption of white rice and its products by people with diabetes can result in vascular damage affecting the heart, eyes, kidneys, nerves, and prevalence of specific complications(Cho et al., 2018).Rice is a primary dietary staple food and significant single food source of carbohydrate of 3.5 billion people and contributes to the one-fifth calories consumed worldwide. Rice refers to two species (Oryza sativa and Oryza glaberrima) of grass, indigenous to tropical and subtropical southeastern Asia and Africa (Taylor, 2008).

Studies carried on the Glycemic index of rice showed that the mean Glycemic index of white rice was 64, brown rice was 55, and barley was 25(Hu, Pan, Malik, & Sun, 2012). The Glycemic Index (GI) indicates the rate at which carbohydrates in foods are released. It allows ranking of foods from those which give rise to the highest blood glucose and insulin responses (high glycemic food) to those associated with the lowest blood glucose and insulin responses (low GI foods), which are more slowly digested, absorbed and metabolized as compared to high GI food GI of rice depends on variety, and effects on GI appear to be mainly mediated by its amylose content. Due to the high Glycemic index value consumption of white rice generates more robust postprandial glucose response as compared to other grains. Hence, it is necessary to devise a product of rice that does not generate a more robust postprandial glucose response after consumption by people who have diabetes

(Agustin, Franceschi, Jenkins, Kendall, & La Vecchia, 2002).

Starch is a pure polymer of glucose that is slowly digested, or the resistant starches which pass undigested into the colon are associated with health benefits through better glucose control and promoting the growth of beneficial microflora of colon. Based on glucose response after consumption of food, they categorized as high GI (GI > 70), low GI (GI < 55), and intermediate GI foods (Zhang & Hamaker, 2009). Several studies have shown that a low GI diet not only improves certain metabolic disorders of insulin resistance but also reduces insulin resistance.(Bharath Kumar & Prabhasankar, 2014)The current study aims in the development of a new type of physical modification of starch and formulation of snack from modified rice.

Currently, Retrogradation is a spontaneous process that sometimes leads to modification in structural, mechanical, or organoleptic properties of certain starch-based products. (Karim, Norziah, & Seow, 2000). The retrogradation has proven to result in hardening and reduction in the stickiness of parboiled rice and breakfast cereals, also decreases the digestibility of soluble starch and increases the consistency of reconstituted product.(Boers, Seijen ten Hoorn, & Mela, 2015). Low GI of rice products of the study was expected to the development of a cost-effective process.

MATERIAL AND METHODS

Material

Indrayani variety paddy was purchased from Pune, India, Anthrone- Millipore Sigma, H_2SO_4, D-Glucose-, Sodium tartrate-, Copper sulfate, Sodium hydroxide-, Bovine Serum Albumin, HCL, Sodium Acetate, Magnesium Chloride, Calcium Chloride, Amyloglucosidase, Pepsin chemicals and reagents procured from Sigma Aldrich, Himedia and Merck.

Methods

Sample Preparation

2 kg of paddy was added to 2-3 liters tap water previously heated to ambient temperature; the vessel was covered and left for 3 hrs. The water was drained off, and the paddy was cooked by steaming in an autoclave to achieve complete gelatinization. 1 kg of steam parboiled rice (GEL) was tray dried and kept at RT and grind it to make flour.

Retrogradation Treatment

The remaining 1 kg parboiled rice was divided into seven parts (RET1, RET2, RET3, RET4, RET5 RET6, and RET7) and refrigerated with water. The retrogradation was carried out, and each sample was removed after a specific period. All samples after retrogradation were dried in the tray drier. The dried paddy was milled to separate the husk from the grain. Milled rice was puffed in a preheated sand until the crackling sound just ceased.(R. Chinnaswamy, 1983). The analysis of expanded rice and rice flour was carried out by the methods mentioned below.

Determination of Color

The color was analyzed by estimating ΔE values for gelatinized and retrograded rice puff through L*a*b* values calculated by using color analysis app version 7.0.0. The ΔE value was calculated by the formula given below(Nagareddy & Kingdom, 2014).

$$\Delta E = \sqrt{(\delta L *)2 + (\delta a \ *)2 + (\delta b \ *)2}$$

Determination of Glycemic Index

Milled samples were ground and sieved to obtain particles in the range of 400–600 microns.500 mg sample was cooked by the addition of 5 ml of water and subsequent boiling over 100 °C water bath for another 30 minutes. After cooking, it kept at RT, and rice clumps were first broken down by subsequent stirring at 350 pm. The following were added consecutively, with their corresponding incubation time: 5 ml pepsin (1 mg/ml in 0.01 M HCl) for 30 minutes, 5 ml 0.02 M NaOH for 1 minute, and 20 ml 0.2 M NaOAc buffer (pH 6.0, 0.49 mM MgCl2, 4 mM CaCl2) for 10 minutes. A 200 µL aliquot (duplicate) was transferred to a microfuge tube (labeled as time 0). Afterwards, 5 ml pancreatin/ amyloglucosidase (AMG) solution (2 mg/ml pancreatin and 28 U/ml AMG in 0.2 M NaOAc buffer pH 6.0 with 0.49 mM MgCl2 and 4 mM

CaCl2) was added to the test tube. At the following sampling points, 200 µL aliquots (duplicate) were obtained: 5, 10, 20, 30, 45, 60, 90, 120, and 180 minutes after the addition of the pancreatin/AMG solution. Aliquots were rapidly immersed in the ice bath to stop enzyme digestion, centrifuged at 13,000 rpm (4 °C) for 10 minutes to collect the supernatant, and stored at −20 °C before measuring the glucose. Glucose was measured based on Aurrex's D-glucose assay kit (K-GLUC). In 2 ml microfuge tubes, 50 µl solutions (with proper dilution) was transferred and 5 µl AMG solution (300 U/ml AMG in 0.2 M NaOAc buffer pH 6.0 with 0.49 mM MgCl2 and 4 mM CaCl2) was added. The mixture was incubated for 20 minutes in 50 °C. GOPOD reagent (1.5 ml) was added and the solution was incubated at 50 °C for 20 minutes. Absorbance was read at 510 nm using water as blank. The glucose concentration was calculated by the formula given below. The trapezoid method was used for the determination of glycemic index which was calculated by the below equation (Egba, Omeoga, Okafor, Adimuko, & Akokwu, 2017)

Glucose in mg (%) =Absorbance of sample / Absorbance of standard × 100

GI of Test Food = AUC of test food / AUC of reference food ×100

$AUC= (G_i - G_o) + (G_{i-1} - G_o) / 2 \times \Delta t$
Where:
AUC = Area under the curve; G_i is glucose concentration at a particular time, G_0 is starting glucose concentration, and Δt = time interval between two values (Langová et al., 2008)

Determination of Structural Arrangement in Gelatinized and Retrograded Starch
Flour was prepared from full grain by grinding in a laboratory-scale mill until a particle size lower than 500 mm was obtained (1 min). (Mariotti, Alamprese, Pagani, & Lucisano, 2006). Puffed grains were observed after freeze-drying, Samples were mounted on aluminum stubs using double-sided adhesive tape, and they were coated with a thin layer of under vacuum. The samples on the stub were operated at 10 kV Their ultra-structure was imaged in the scanning electron microscope.

Determination of Functional Temperatures and Bond Enthalpies of Gelatinized and Retrograded Starch:
The gelatinized and retrograded rice grains were ground in the laboratory scale mill until the particles pass through a 100-mesh sieve. To determine the degree of retrogradation and gelatinization of samples, a Differential Scanning Calorimeter (DSC 60, Shimadzu, Japan) was used and calibrated with indium. The total weight of l0 mg samples was placed in DSC pans, and it was run at a temperature rate of 20^{o}C per min.
The degree of retrogradation (%) was calculated using the below equation

$$(\%R)= \frac{\Delta H\ retrogradation}{\Delta H\ gelatinization} \times 100$$

Where $\Delta H\ retrogradation$ is the enthalpy of retrogradation of the sample (J/g) andΔH gelatinization is the enthalpy of gelatinization of the sample (J/g)(Kingcam, Devahastin, & Chiewchan, 2008).

Statistical Analysis:
The data were expressed as means of triplicate determinations. Statistical significance was assessed with one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using SPSS 17.0. Treatment means were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

In vitro starch digestion of gelatinized (GEL) rice at a different time interval and their corresponding GI:
The GI of gelatinized rice samples after digesting for the different periods. It is found that the first hour is most crucial in differentiating digestibility, and by the second hour, starch hydrolysis is completed indicated by an almost constant value of GI in the chart. The gelatinized rice exhibited maximum hydrolysis at 45 min showing 73.83 GI. The

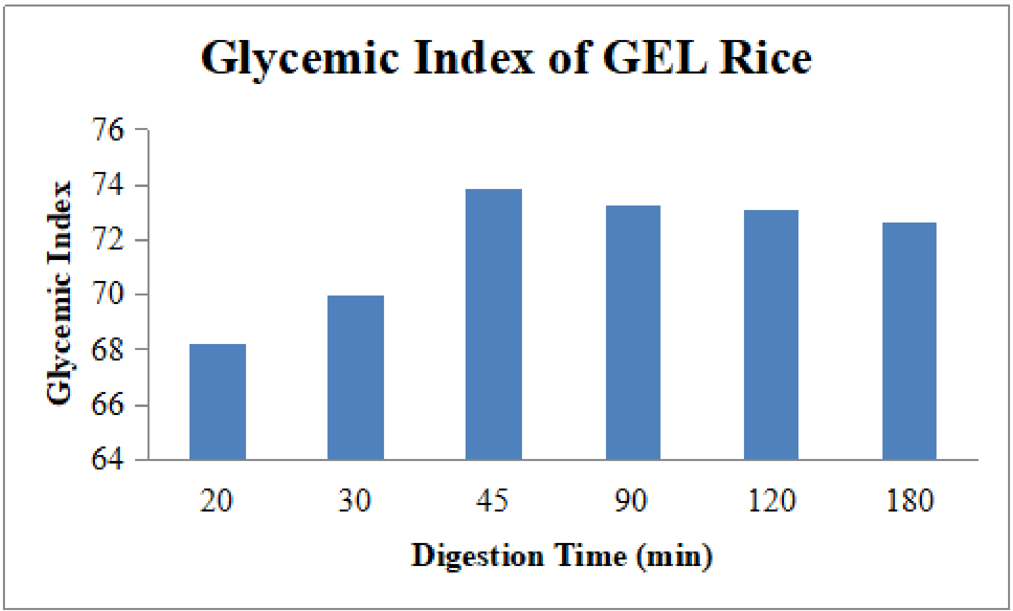


Figure 1. Digestion of gelatinized rice at a different time interval and their corresponding GI

hydrolysis of starch is analogous to clinical GI determination, where blood glucose is measured for a period of 2 hrs. After 2 hrs. of enzymatic treatment starch samples reached maximum digestion, and the differences in the maximum levels became minor which was in agreement with the(Chung, Lim, & Lim, 2006)

Effect of Retrogradation time on Glycemic Index:

The glycemic patterns of retrograded samples are shown in Fig 2. It is observed that the glycemic index of retrograded samples is significantly affected by storage conditions. The retrograded starch samples hydrolyzed faster, reaching its maximum GI value within 20 min which was also observed by (Chung et al., 2006). The results show that RET7 exhibited the highest resistance to digestion at all temperatures, henceforth showing low GI. It is also observed from the Duncan chart that all the retrograded samples were significantly different at $P < 0.05$ from each other, and RET7 was found to be the best sample with the lowest GI. Hence, RET7 was chosen to make rice puff.

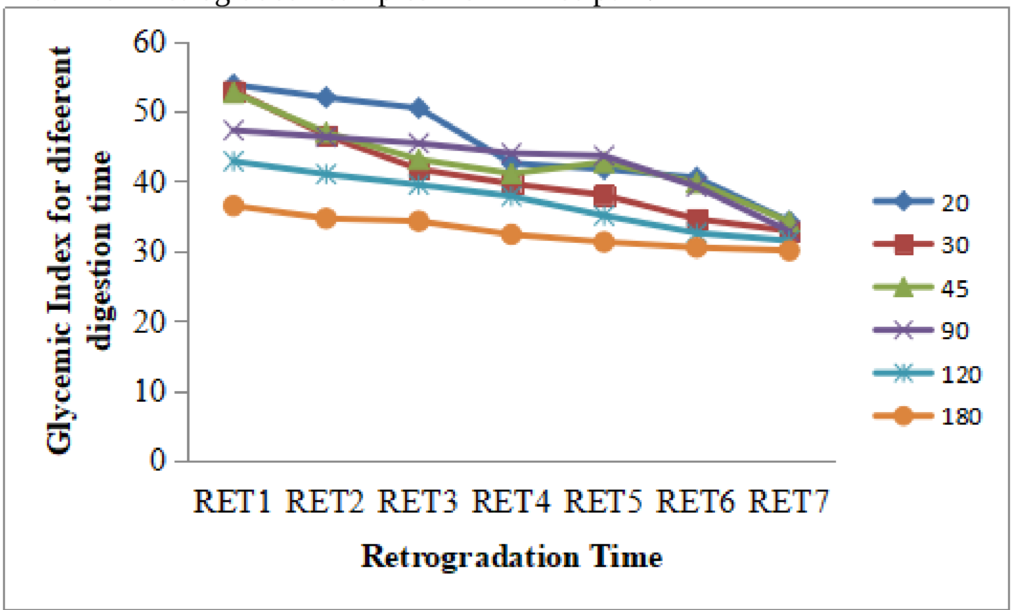


Figure 2. Digestion kinetics of retrograded rice with varying time

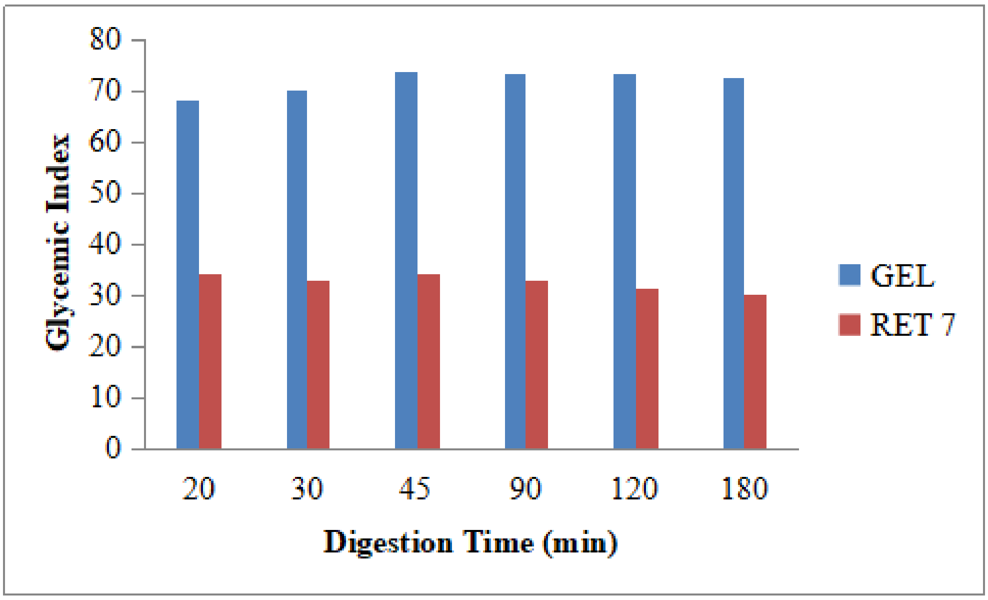


Figure 3. Glycemic Index chart for gelatinized rice and retrograded rice digested at a different time interval

Gelatinized rice vs. Retrograded rice GI:

The GI chart for gelatinized and retrograded rice (RET7) samples is shown in Figure 3. The gelatinized rice showed maximum GI after 45 min of hydrolysis, which on further hydrolysis showed a reduction in GI, reaching approximately a constant value. The retrograded rice sample exhibited a significant difference in GI as compared to the gelatinized rice sample. This is following the concept reported by (Chung et al., 2006) that as gelatinization progresses due to exposure of heat, the intra and intermolecular hydrogen bonding between the starch chains are disrupted, resulting in the formation of amorphous structure. This structure is more susceptible to digestion by digestive enzymes and thus shows more GI. The low GI in retrograded rice samples is due to the recrystallization of amylopectin, which results in the formation of resistant starches, henceforth decreasing the susceptibility of digestive enzymes to starch digestion. The reduction in GI of retrograded rice sample was observed from 73.12 to 31.46, which corresponds to a 56.98% reduction.

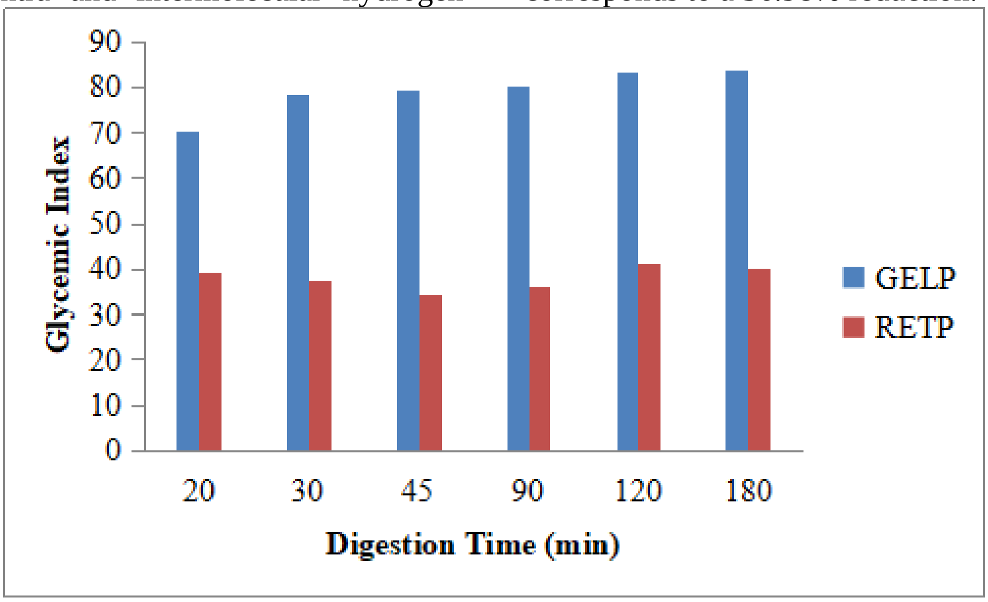


Figure 4. Glycemic Index chart for gelatinized rice puff (GELP) and retrograded rice puff (RETP) digested at a different time interval

Gelatinized rice puff (GELP) vs Retrograded rice puff (RETP):

Figure 4 shows the comparison block between GELP and RETP GI. From the above results, it is observed that the GI of RETP showed a significant difference for digestibility as compared to GELP. The GI of RETP and GELP at 45 min was observed to be 34.58 and 79.09, respectively, which corresponds to 56.28% reduction due to storage**.**

Comparison of GI between retrograded; gelatinized rice flour and retrograded; gelatinized puff:

Figure 5 indicates the estimated GI for GEL, RET7, GELP, and RETP at different digestion times. It is observed that on puffing, the GI of GEL and RET7 samples were significantly increased. The increment in GI after puffing GEL and RET7 samples suggest the effect of processing on the structure of starch granules. (Brand & Nicholson, 2006) in their studies suggested that during puffing, when the samples were heated at more than 100°C the water in the sample suddenly burst into steam, imparting porous and cellular structure to the product. The formation of the porous structure increases the affinity to digestive enzymes and hence increases the GI.

Morphological Structures of Retrograded and Gelatinized Rice Puff

The scanning electron micrographs at a magnification of 200 X, 2.00 KX, 5.00 KX and 10.00 KX of the RETP and GELP are illustrated in Fig 6A, Fig 6B, Fig 6C, Fig 6D, Fig 6E and Fig 6F respectively. The RETP and GELP granular structure exhibited significant variations in shape when viewed by scanning electron microscopy. The puffing treatment radically changed the ultra-structure of rice starch in both the samples (Mariotti et al., 2006). Reported that the internal structure of rice grain was compact due to the presence of the aleurone layer at the periphery of grain, imparting regular structure and shape. The current SEM images of the puffed rice indicate the absence of the aleurone layer, which may be due to processing treatments, thereby resulting in the formation of irregular structures (Fig A and B).The results from Fig C and D at higher magnification showed that the surface of both the samples had shriveled appearance, which may be due to combined effect of gelatinization and desiccation of starch upon drying and puffing Apart from shriveled appearance it is also observed that the surface of retrograded starch (Fig E) was comparatively smoother than the surface of gelatinized starch (Fig F).

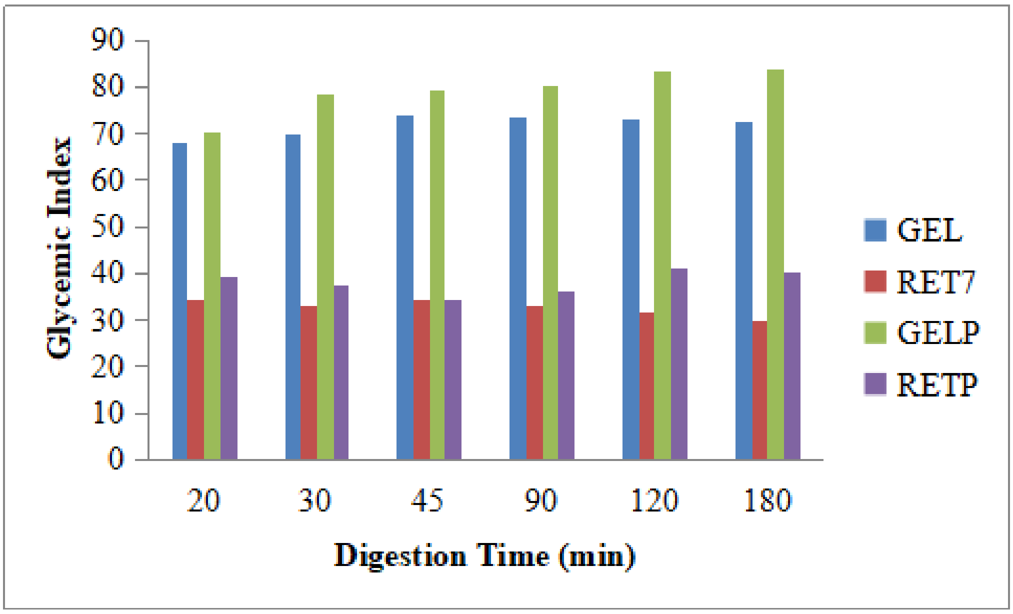


Figure 5. GI of GEL, RET7, GELP, and RETP at different digestion time.

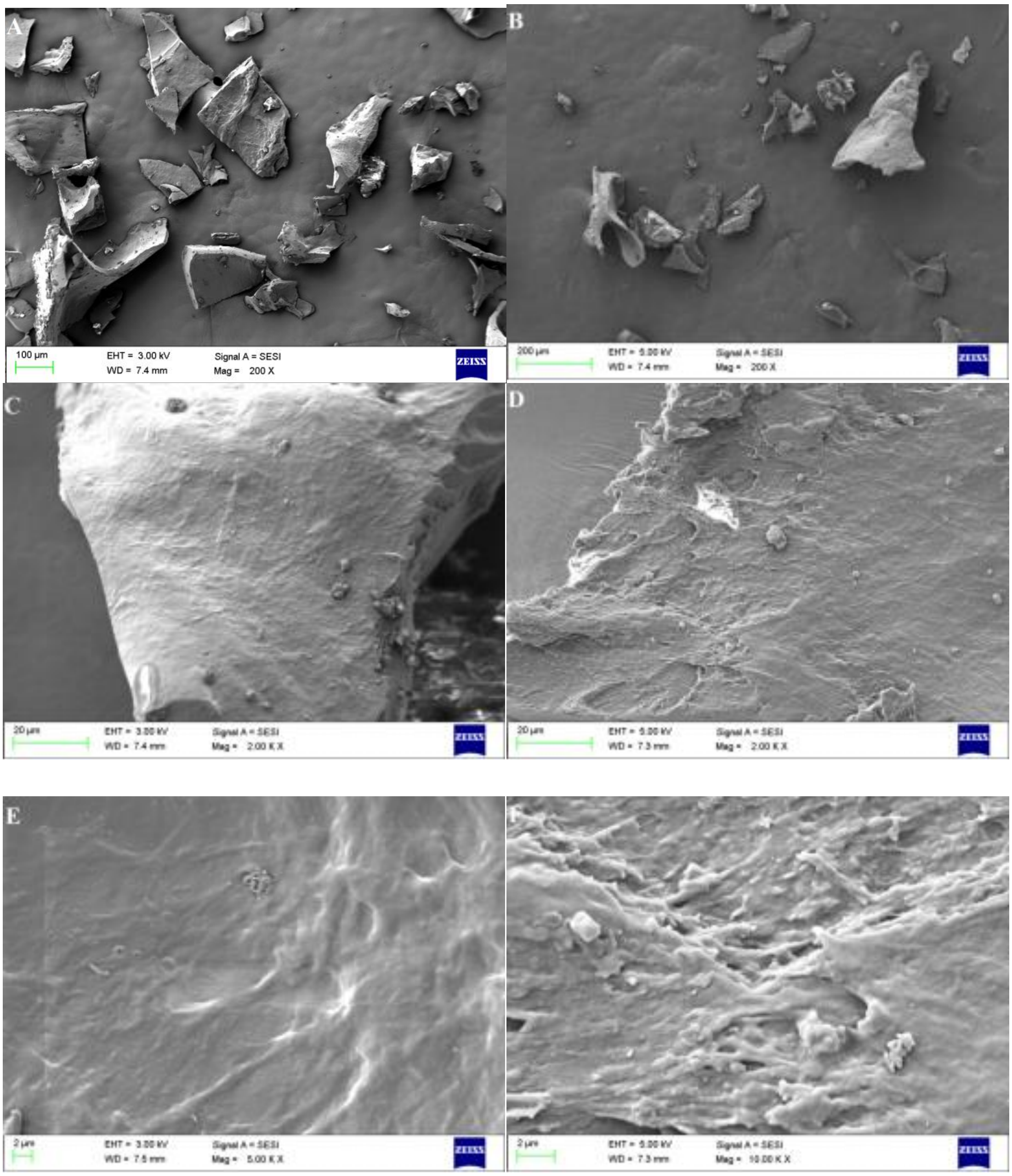


Figure 6.Morphological structure for retrograded (Fig A, C, E) and gelatinized (Fig B, D, F) rice puff

The smooth glassy surface on the retrograded starch is likely to be due leaching of amylose during heating and recrystallization of the amylopectin to form a semi-crystalline glass-like structure on cooling (Dang & Copeland, 2004). The smooth surfaces of retrograded starch are imperfect crystallites that may act as a barrier and restrict water and enzyme penetration, thus decreasing starch digestibility due to the formation of RS yield (Xie, Hu, Jin, Xu, & Chen, 2014),. The smooth surface (Fig E) decreases the digestibility due to a reduction in contact of starch granules with enzymes, whereas rough texture (Fig F) provides more surface area, which in turn enhancesthe activity of enzymes. The results showed that there was a significant difference in the granular integrity of retrograded and gelatinized starch (Fig C and Fig D). This may be due to syneresis, which is associated with water separation from the gel and recrystallization of amylopectin during retrogradation. The extent of syneresis measures the extent of retrogradation and display high crystallinity due to formation of high solid and compact structure as observed in (Fig C).

Functional Temperatures and Bond Enthalpies of Gelatinized (RGEL) and Retrograded (RRET) Starch

Compared with RGEL starch sample, the onset temperature, conclusion temperature, and melting temperature range of RRET starch sample had the shifted toward a higher temperature. This indicates after retrogradation, gelatinized starch molecules reassociate to an ordered structure due to recrystallization of amylopectin and resulting in the formation of a structure that was different from the native structure. The endotherm peak of RRET starch was comparatively higher than the RGEL starch. This is observed because gelatinized starch do not have crystallites due to the conversion of the crystalline region of starch into the amorphous region, whereas the endothermal peak in retrograded starch is due to melting of recrystallized amylopectin. In the present study, RRET starch sample showed highest ΔH value of (-0.43 J/g) with a degree of retrogradation of 8.447%, suggesting that reassociation of starch molecules forms a more robust matrix or network, consisting of perfect crystallites of amylopectin which act as resistant starch formed during retrogradation. Eerlingen, Van Haesendonck, De Paepe, & Delcour (1994) also reported that increased retrogradation extents (high melting temperatures, melting enthalpies, and higher crystallinity levels) caused reduced enzyme susceptibility to pancreatic α-amylase and amyloglucosidase at 37°C. In other words, the higher ΔH value of RRET starch sample indicates that due to retrogradation, the digestible starches are converted into resistant starches, thereby reducing enzymatic activities towards these starches.

Color Analysis:

ΔE value between the gelatinized and retrograded rice puff. It is observed that there is a difference in the color of both the samples, which can be due to various exposure time to heat while puffing for both the samples.

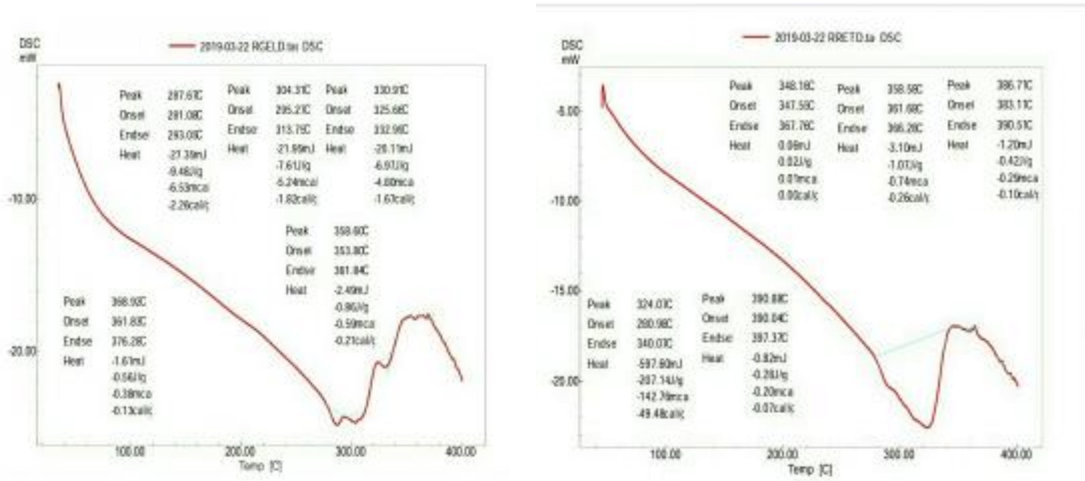


Figure 7. DSC thermogram for gelatinized rice (left) and retrograded rice (right) starch

Samples	*L**			*a**			*b**			*ΔE*
	*L₁**	*L₂**	*L₃**	*a₁**	*a₂**	*a₃**	*b₁**	*b₂**	*b₃**	
GELP	*55.6*	*42.0*	*52*	*-3.6*	*1.5*	*-4.8*	*19.7*	*25.4*	*24.4*	*29.50*
RETP	*40.4*	*53.4*	*58.5*	*8.6*	*-4.8*	*-4.7*	*32.6*	*-24.5*	*23.8*	

Table 1 Precision parameters for determination of bulk color of gelatinized and retrograded rice puff

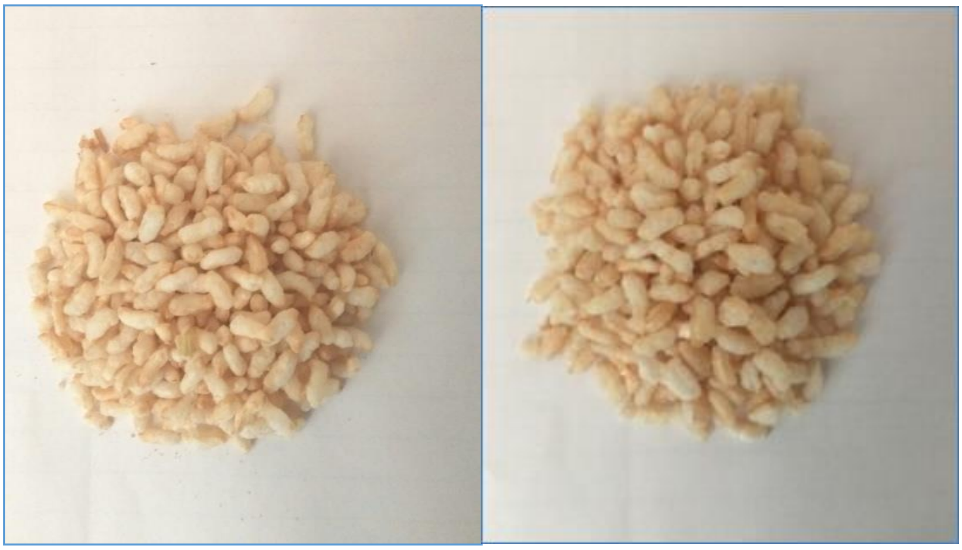
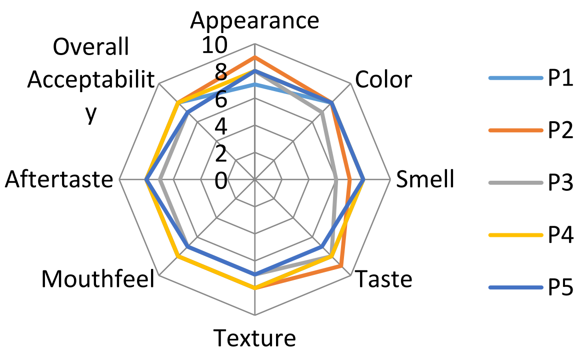
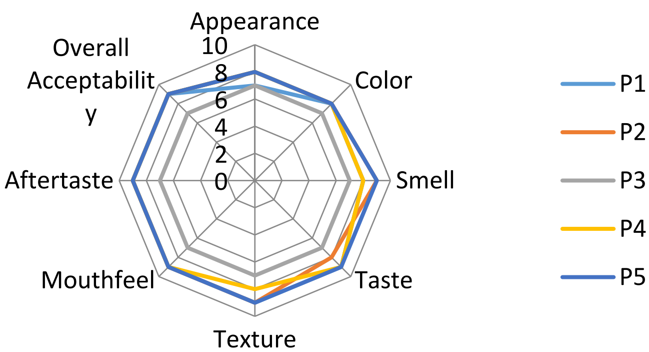


Figure 8. Image of Gelatinized (left) and Retrograded (right) rice puff



A



B

Figure 9. Radar chart for sensory analysis of gelatinized: A- rice puff and B- retrograded rice puff.

Sensory Analysis of Gelatinized and Retrograded rice:

Sensory analysis of gelatinized and retrograded rice puff was done by panel of 5 peoples using hedonic scale of 1 to 9. The outcomes for both samples are represented in radar chart Figure 9A and Figure 9B. From the radar chart it can be reported that there was not any significant difference between both samples. Hence they are accepted.

CONCLUSION

Our study showed that it would be valuable to re-evaluate the indigenous knowledge of rice. The results clearly demonstrated that starch retrogradation and its possible application in the modification of diets for diabetic person, as research showed optimum retrogradation treatment can recrystallize the distorted amylopectin structure during gelatinization. Gelatinized and retrograded rice exhibit variation in digestion kinetics. It's distinguish that on Puffing the GI of GEL and RET sample were notably increase. The morphological structure showed that surface of retrograded starch comparatively smoother than the surface of gelatinized starch. The retrograded rice starch showed higher onset temperature, melting temperature range and melting enthalpy in comparison to gelatinized rice starch. The results show that RET7 exhibited the highest resistance to digestion at all temperature henceforth showing low GI. This study suggested that crystallites formed during retrogradation provide structural rigidity which effects the enzymatic digestion. Hence it can be concluded that retrogradation post gelatinization for specified period can influence on the glycemic index of rice, which can be an important tool for the development of low GI product.

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