LOW GLYCEMIC INDEX SIMULATED FLOUR FORMULATION FROM RETROGRADED FOX NUT (*Euryale ferox*) STARCH

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Abstract
Fox Nut (Euryale ferox) or Gorgon Nut, popularly referred to as Makhana, has been wide employed in traditional foods to cure kind of health diseases associated to blood glucose. Based on the studies saying lower glycemic index (GI) of it, we sought to determine if Fox Nut really has a low GI and whether it can be further lowered by giving treatments and by use of gluten(protein) and Guar gum. The retrogradation of starch isn't a straight forward process, it manifests itself in completely different processes such as inflated firmness and recrystallization of gelatinized starch gels with time. The elements of starch, amylopectin and amylose, have completely different roles in retrogradation. The fox nut starch was gelatinzied by giving them heat treatment at 60°C,70°C & 80°C for time varying from 5 to 20 minutes and were successively retrogradaded at 4°C from a time spanning from five minutes to seventy two hours. These were then dried 70°C-80°C and powdered for further use. The following treated fox nuts where then subjected to digestion and their GI were determined by a spectrophotometric assay. The effective temperature for gelatinization was 70°C and time of retrogradation was one night at 4°c, gluten 10% and Guar gum 2% was able to reduce the GI to lowest was determined.The control used was the whole wheat flour to which a 60±5% reduction in GI was observed.

Key words: Fox Nut, Euryale ferox, Makhana, Low Glycemic Index, Gelatinization, Retrogradation, Simulated flour

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INTRODUCTION

Makhana (*Euryale ferox*) is considered as aquatic crop and considered as special food due to its low fat content, high content of carbohydrate, protein, and minerals, it is one among the foremost common dry fruits utilized by the people. The calorific value of raw seeds is considered as 3362 kcal/gm and puffed seeds (3.28 kcal/g) lie on the point of staple foods like wheat, rice, other cereals (Alka Jain1 , H Birkumar Singh and P B Kanjilal,1972). Besides nutritional value, Makhana is also recommended in the Indian and Chinese system of medicine in the treatment of respiratory, circulatory, digestive, excretory, and reproductive systems disorder (ALLISON M. HODGE, MENVSC1 DALLAS R. ENGLISH, PHD1 KERIN O'DEA, PHD2 GRAHAM G. GILES,2002).

Retrogradation of starch can be considered as a modification process to change the properties of stitches. The changes that the starch gels undergo are manifested by apparently different processes. Their rheological properties change, as evidenced by increased firmness or rigidity. Loss of water-holding capacity and restoration of crystallinity also become evident and increase in aging. These processes are collectively called retrogradation and are thought to exert a significant influence on texture leading to a decreased acceptability of many starch-containing foods. The retrogradation of starch is complex chemistry: which may cause increased firmness and recrystallization effects. Amylopectin and amylose are significant components of starch modification (ANUBHA KUMARI, IS SINGH*1 , LOKENDRA KUMAR1 , AMIT KUMAR, RAMESH KUMAR AND VK GUPTA,1952).

The Glycemic index is a system that classifies carbohydrate-based foods according to their ability to elicit blood glucose response (B. N. M. Yusof, R. A. Talib, N. A. Kamaruddin, N. A. Karim, K. Chinna and H. Gilbertson,2009). It is measured as a relative increase in the area of glycemic response curve brought about by the available carbohydrate of food as compared to an equivalent portion of glucose. Foods with GI less than or equal to 55 qualify as low GI foods while foods with GI in the range of 56–69 and GI greater than or equal to 70 are considered as medium and high GI foods, respectively. High intake of rapidly digestible high GI foods can lead to excessive insulin stimulation and pancreatic beta-cell dysfunction, increasing the risk of glucose intolerance and type 2diabetes. Low GI foods, on the other hand, by their slower rate of carbohydrate digestion, enable a gradual and sustained release of glucose in the bloodstream. Hypoglycemic or low GI foods are known to have an array of favorable health implications, including prevention and management of diabetes, alleviation of hyperlipidemic conditions (B.S. Khatkar∗, Sheweta Barak, Deepak Mudgil,1977), hypercholesterolemia and cardiovascular diseases (Bilal Ahmad Ashwar, Adil Gani, Asima Shah, Idrees Ahmed Wani and Farooq Ahmad Masoodi,2001). Considering the increasing incidence of type 2diabetes reported around the world, the modification or development of foods with a lower rate and extent of carbohydrate digestibility has assumed great importance in recent times(CJAM Keetels, T.van Vliet, P.Walstra,1996).A database of GI of staple foods needs to be generated, and also new functional ingredients may be explored to lower GI of such foods.

The mechanisms by which the gluten network slows digestion rates of entrapped starch are not fully understood. The most common explanation is that the gluten network entrapping starch granules acts as a barrier to inhibit the accessibility of enzymes. It may also limit water absorption by starch granules, limiting the degree to which the starch can swell and hence gelatinize during pasta cooking in excess water, and limiting the ability of enzymes to access available starch and therefore decreasing the rate of starch digestion (David JA Jenkins, Thomas MS Wolever, A Venketschwer Rao,1987).

The incorporation of guar gum or other similar polysaccharides to carbohydrate-rich test meals or oral glucose loads lowers postprandial hyperglycemia in normal and diabetic humans. The mechanisms are responsible for this effect.

MATERIALS AND METHODS

Material:

Fox Nut (puffed), whole wheat flour purchased from the local market. Food-grade guar gum from iHerb, Gluten (90.0% pure) from Nutrova, Potassium hydroxide from HiMedia, Sodium acetate from HiMedia, Amyl glycosidase enzyme (98.5% pure) from Ubuy India and GOD/POD reagent was procured from Accurex.

Methods:

Gelatinisation of fox nut starch:

About 2g of fox nut sample was taken and were subjected to heat at temperatures of 60°C,70°C and 80°C each for a time interval varying from 5 to 20 mins using a boiling water bath.

Retrogradation of fox nut starch:

The heat-treated nuts were then cooled to 4°C using a refrigerator. The cooling time was varied from 5mins to 72hrs to check the effect of retrogradation on the GI of fox nut starch.

Sample preparation for GI estimation:

After the completion of retrogradation at 4°C, the nuts were dried in a tray drier at 60°C to 70°C for 3 to 4hrs.These were then subjected to grinding using a twin-blade mixer, and thus the sample was formed into a powdered form.

GI Estimation:

60mg of the powdered samples were taken into five (0, 30, 60, 90, market flour) clean and dried test tubes. 300µl of distilled water was added to each test tube and then were placed in a BWB for 30mins at 100°C. After heating, tubes were cooled under tap water, and 2ml of 2M KOH was added to then to start the digestion. For proper mixing, the tubes were agitated using a vortex machine for about 2mins. Tubes were exposed to KOH for 0min,30mins,60mins, and 90mins, respectively. After sufficient exposure, 8ml of 1.2M acetate buffer (ph:3.8) was added to each, and the ph was finalized to around 4.2 to 4.8

using a ph meter. For enzymatic digestion to take place, 60µl of amyl glucosidase enzyme was added, and for the activation of the enzyme, the tubes were placed in a water bath at 60°C for 45mins. After cooling, the tubes were centrifuged to get the supernatant, and GI analysis was carried out using the GOD/POD reagent. The colored solution resulted after the addition of reagent was estimated using a spectrophotometer at 505nm (Sabel Gorii' PhD, Alejandra Garcia-AIonso* BSc, Fulgencio Saura-Calixto* RsProf,1999).

Glycemic Index Estimation for optimization of gluten content:
Once the proper gelatinization and retrogradation conditions for the fox nut starch got optimized, gluten addition was carried out to match the resulting flour with the presently used whole wheat flour. 6% to 12% was added into the selected fox nut powder (As per FSSAI regulation), and its GI estimation was carried out after forming it into a chapatti. The selected fox nut powder was substituted with 6%,8%,10%,12% gluten. The mixture was properly homogenized by using a mortar pestle. The mixtures were sieved before making into a dough, and Indian flatbread (Roti) was made by cooking them for 2-3min at a low flame on each side on a flat metal surface. The GI estimation was done in the same manner as done above, this time exposing the sample to KOH only for 60mins (David JA Jenkins, Thomas MS Wolever, A Venketschwer Rao,1987).

Glycemic index Estimation for optimization of Guar gum content:
After selecting the proper amount of gluten to be added to minimize the GI, for proper flexibility and texture, food-grade guar gum was added, ranging from 1% to 5%, and the chapattis were made. The analysis was carried out using 60mg of the sample from each of the guar gum added chapattis(Kai Lin Ek, Shujun Wang, Jennie Brand-Miller and Les Copelan*,2014).

Differential Scanning Calorimetry:
Fresh doughs were prepared under optimum farinograph conditions for each mixture (farinograph absorption and development time), and an aliquot of each sample (10-14 mg) was exactly weighed in a DSC pan, then hermetically sealed and subjected to a heating program in a Q100 calorimeter. An empty pan was used as a reference. Heating was performed from 20 to 130°C at a rate of 10°C min-1. From thermograms, onset, peak, and final temperatures were obtained (Kaye Foster-Powell, Susanna HA Holt, and Janette C Brand-Miller,2002).

Scanning Electron Microscopy
For qualitative observations of microstructural damage on the starch granules surface, scanning electron microscopy (SEM) was used. Dry granules were sprinkled onto double-sided tape attached to the specimen stubs and coated with a thin layer of gold (300A thickness) using a Pelco 91000 sputter coating system. Samples were then observed using a Supra 55 VP (Carl Zeiss Co., Germany) scanning electron microscope at an acceleration potential of 1 kV. The observations were made using a secondary electron detector "SE" and an "In Lens" detector, characteristic of this microscope. The photographs were taken using an automatic image capture software(Gabriela N. Barrera, Georgina Calderón-Domínguez, Jorge Chanona-Pérezc, Gustavo Gutiérrez-López, Alberto E. León, Pablo D. Ribotta,2013).

Colour Analysis:
The color analysis l, a,b values of samples were determined by camera lens (Make-Zeiss), and color difference Δ E (Delta E) was carried out by "Colour Analysis- Research Lab Tool" Software in comparison with control samples.

RESULTS

Effect of Retrogradation treatment on GI:
The (Figure.1.) and (Figure.2.) represents the GI values for one night, and three nights respectively, not much difference was observed in the readings.

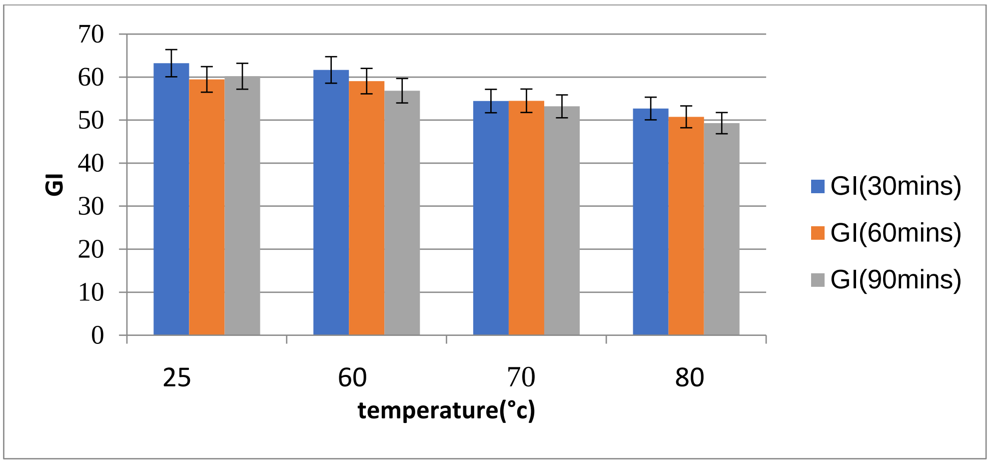


Figure 1. Glycemic index of sample retrograded for 1 night. *(n=3)*

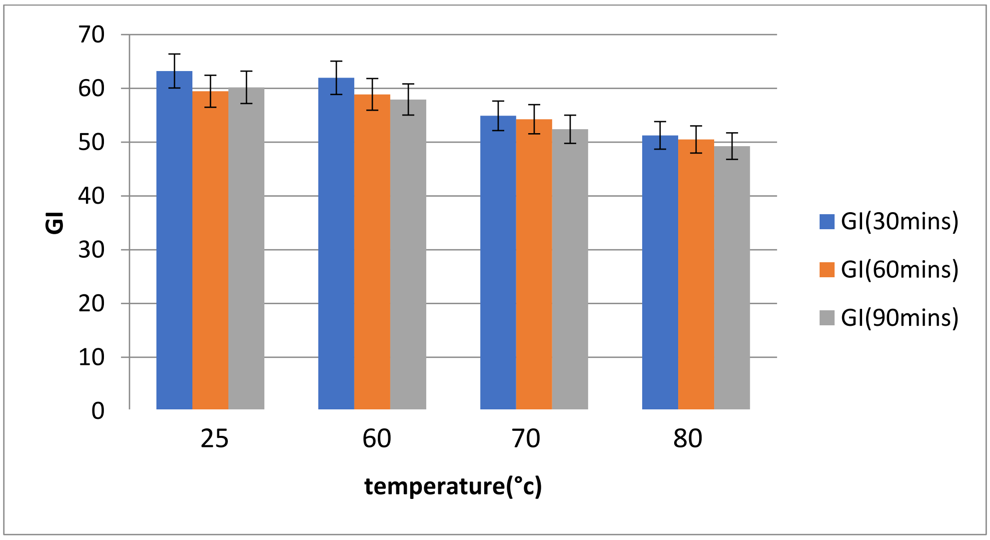


Figure 2. Glycemic index of sample retrograded for three nights. *(n=3)*

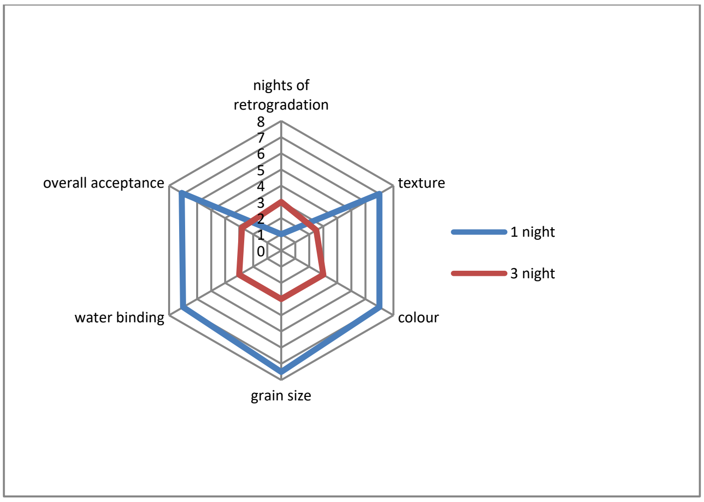


Figure 3. Sensory readings for retrogradation nights selection*(n=50)*

Thus to select the best out of the two, a sensory analysis was carried out (Figure.3), which showed significant differences between the two samples under the study, thus for further analysis, one night retrograded sample was used.

Effect on GI on the addition of gluten to retrograded Fox nut starch

Furthermore, to decrease the GI and increase the dough forming ability of the flour, gluten(protein source) was added to the retrograded starch. Gluten was added in a gradient from 6% to 12%; 6 % was taken as a base value because, as per FSSAI, flour should not contain less than this value. (Figure. 4) shows the GI values obtained after the addition of gluten. A decrease in GI value was observed up to 10% gluten addition(for 8g of sample size). A decrease of up to 9.25-10.0% was observed in the GI values after the addition of gluten to the starch [7]. 6%, 8%, and10%, a sensory analysis was carried out for the three samples by considering various parameters. (Figure.5) shows the sensory values and on observing shows that 10% gluten is significantly different from 6% and 8%, and thus, fox nut starch powder with 10% gluten was used for further analysis.

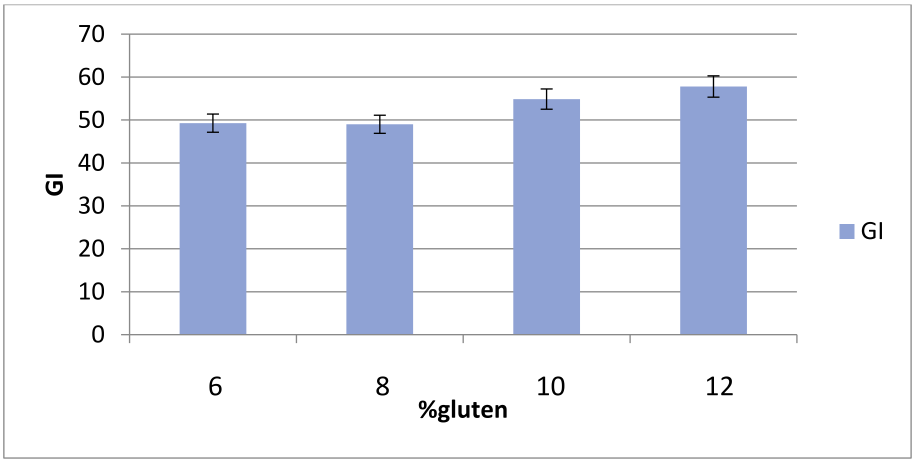


Figure 4. GI after gluten addition to retrograded starch *(n=3)*

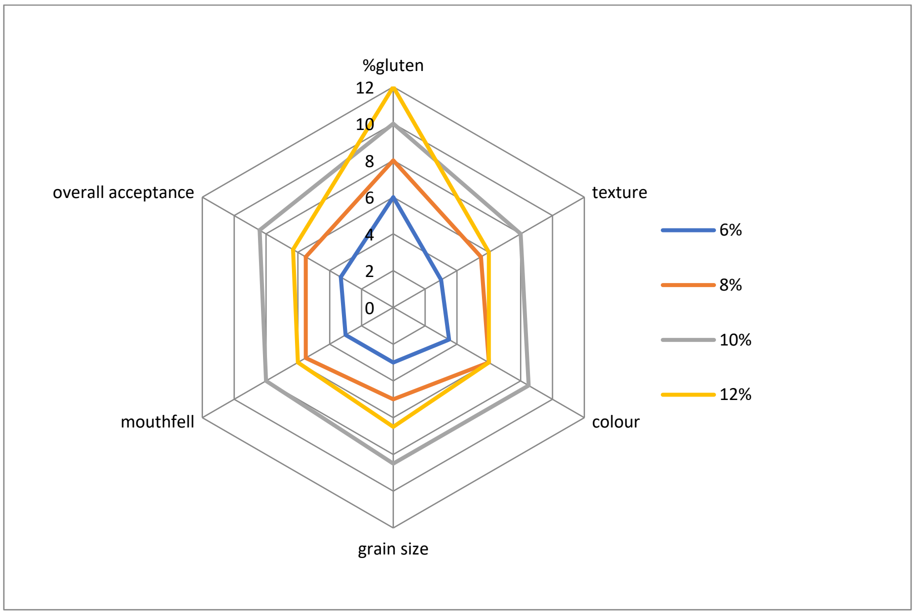


Figure 5. Sensory readings for gluten percentage selection*(n=50)*

Effect on GI of the addition of gum to starch & protein mix

Guar Gum addition was carried out in concentrations varying from 1% to 5% to check whether the interaction resulting between gum, protein, and starch has a promising effect on the GI of the flour.[12]. A decrease of up to 30%-35% in GI values of the flour was observed after the addition of gum to the starch-protein complex. As a decrease in GI value was observed for all the percentages of gum added to flour, a sensory analysis was carried out in order to select a value of gum that gives the best results on chapatti formation. (Figure 7) shows the sensory values obtained by each of the gum percentage variations, and by observing, we can say that the starch-protein complex with 2% gum gave the best results.

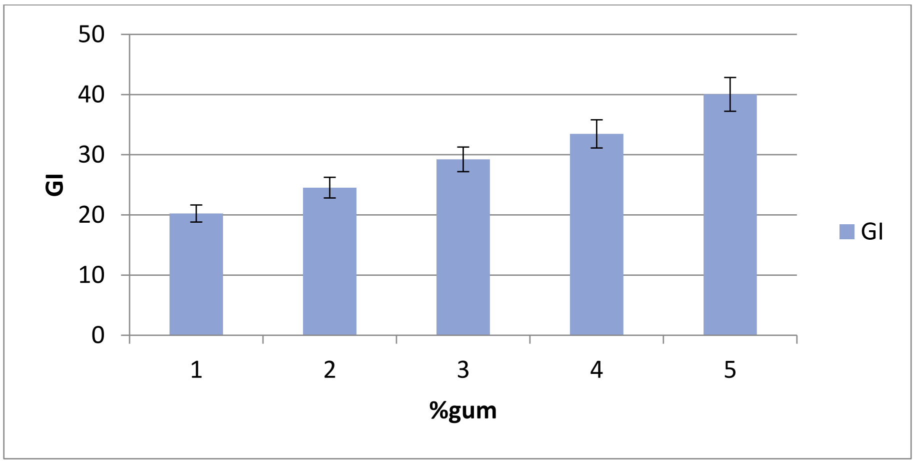


Figure 6. GI after gum addition to starch-protein complex*(n=3)*

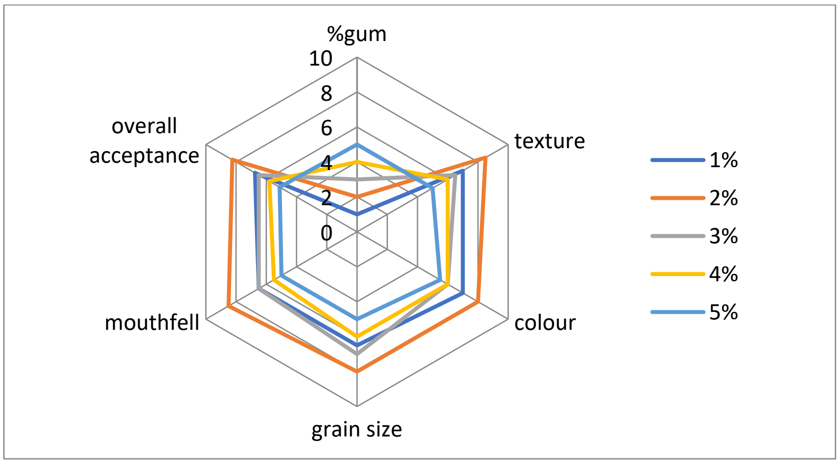


Figure 7. Sensory graph for gum percentage selection*(n=50)*

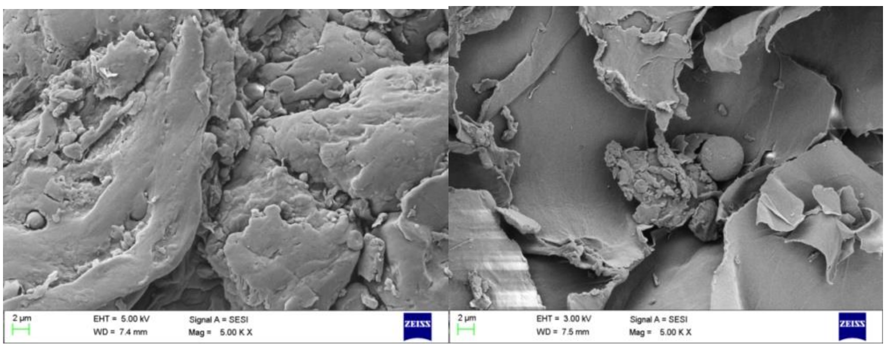


Figure 8.SEM image of treated and non-treated flour samples

Microstructure analysis of low GI flour

Electron microscopy of both raw and treated test samples was carried out (Figure.8.). A vast structural difference was observed in both the samples. Thus it can be concluded that the treatment of retrogradation of the starch gave the test sample a more smooth and compact structure. The compactness suggests the re-combining of amylose and amylopectin during retrogradation. The SEM image of starches after retrogradation shows that a prominent layer structure exists in retrograded amylopectin, which agrees with the literature (David S. Ludwig,2013).

Differential Scanning Calorimetry:

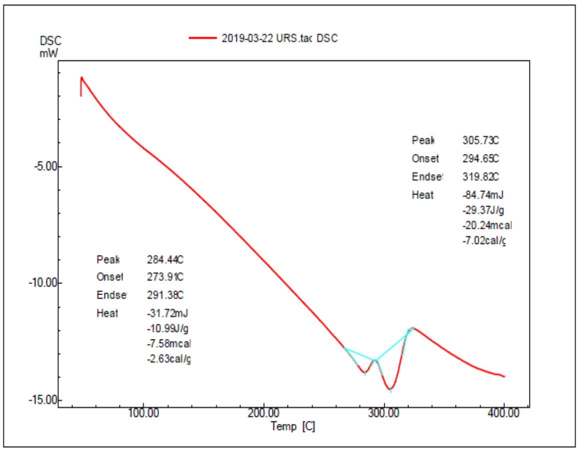
The below (Figure 9) shows the DSC curves of Raw; Final Treated low GI flour sample, respectively. On observing the curves, we see few differences that occurred due to the treatment given in order to reduce the GI.

The parameter ΔH measures the energy change due to loss of molecular order and melting of crystallites when hydrogen bonds break within the granule. The value of ΔH has been considered to be an indicator of the

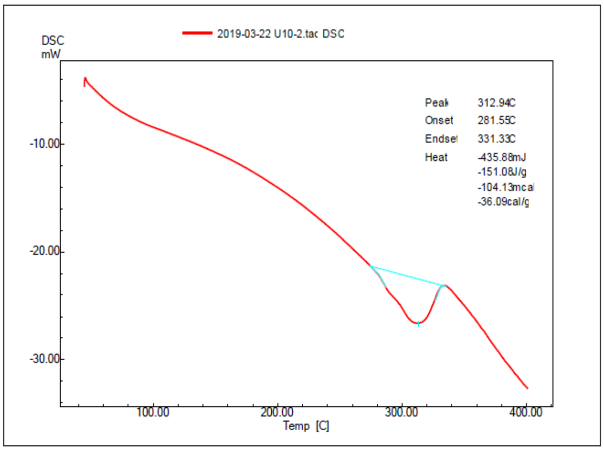
quantity and quality of the crystalline starch structure. Higher DSC transition temperatures (T_g) are thought to result from a higher degree of crystallinity, high bond strength or more ordered crystalline regions, which impart more excellent structural stability and make the granules more resistant to gelatinization as per the (Figure 9) the T_g of Low GI flour is 312.0°C, and that of wheat starch is 58.0°C. Generally, starch with less crystalline order was observed to gelatinize at a lower temperature and reach a higher degree of gelatinization at the same temperature than more crystalline starches. The same study showed that glycemic response increased with a greater degree of starch gelatinization. The higher gelatinization onset temperature of Low GI flour starch suggests that the crystalline regions of Low GI flour starch are more stable than those of the Raw sample. Hence, under the same cooking conditions, the lower glycemic response elicited by low GI flour could be because its starch was gelatinized to a lesser extent than starch from the Raw sample with a high GI value (K. M. CLEGG,1956).

Colour Analysis:

The LAB and cooking time analysis was carried out simultaneously. (Table1) shows the LAB values for wheat flour chapatti, which was considered as standard for the chapattis made from low GI flour. The cooking time used was not less than 3 minutes as a standard wheat chapatti required a minimum of 3 minutes on each side to cook at low flame. The ΔE values for whole wheat flour cook for 3 minutes was 6.92 (L=56.33, A=0.3 and B=36.33 for n=3)and for 4 minutes was 5.21(L=61.93, A=1.9 and B=36.33 for n=3) and ΔE values for Low GI flour cooked for 3 minutes 7.45±1.32(L=49.55, A=1.9 and B=39.15 for n=3) and for 4 minutes was 5.44±1.21(L=57.3, A=1.5 and B=33.7 for n=3) thus 4 minutes cooking time gave the nearest deviation.



Raw sample



Test Sample

Figure 9. DSC thermogram for A- Raw sample and B- Test Sample

CONCLUSION

The retrogradation treatment to fox nut starch showed a significant reduction in the Glycemic Index. The reduction of GI was more when gum was added along with gluten when compared to only retrogradation. Previous work has indicated a good relationship between the addition of protein and hydrocolloids and the glycemic response to foods, and the present studies confirm this. The factors proposed as influencing the digestion of starchy foods include the nature of the starch, the starch-protein interaction, fiber content. The results reported here highlight the possible importance of factors associated with a starch-protein-gum interaction. Such food demonstrates a reduced digestion rate and lower blood-glucose responses in comparison with conventional foods, in which the protein content is, in general, high. Our results indicate that 0.5-1.0% more carbohydrate presence as compared to wheat flour. This corresponded with a 60% ± 5% reduction in glycemic response with comparison to white bread. Therefore, the carbohydrate does not account fully for the percentage flattening of the blood-glucose profile. This disparity has been seen in other situations and suggests that the glycemic response is determined by the rate of luminal digestion rather than by the amount of carbohydrate mal-absorbed.

Declarations:

Manuscript Title- LOW GLYCEMIC INDEX SIMULATED FLOUR FORMULATION FROM RETROGRADED FOX NUT (*Euryale ferox*) STARCH.

The authors whose names are listed above declare that; that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. Hence we assure that there will not be a generation of conflict of interest raise in future.

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