Chapter 3

Resistant starch development from pulp and its application in food model

The Chapter 3 has been discussed under two sub-heads as follows:

A) Effect of modified resistant starch of culinary banana on physicochemical, functional, morphological, diffraction and thermal properties

3.1 Introduction

Starch is the storage polysaccharides of green plants and a major dietary component in all human populations.[1] It is composed of a number of monosaccharides or sugar (glucose) linked together with α-D-(1-4) and/or α-D-(1-6) linkages.[2] Starch is deposited in the fruit in the form of granules, partially crystalline, whose morphology, chemical composition, and super molecular structure are characteristic of each particular plant species. Starch owes much of its functionality to two major high-molecular-weight carbohydrate components, amylose and amylopectin. Amylose is essentially linear polymer in which glucose residues are α-D-(1-4) linked typically constituting 15 to 20% of starch. Amylopectin the major component of starch is a larger branched molecule with α-D-(1-4) and α-D-(1-6) linkages. This biopolymer constitutes an excellent raw material to modify food texture and consistency.[3] The amount of starch is not only important for the texture of a given food product, but starch type is equally critical.[4]

Starch, on the basis of its digestibility, has been classified into three groups, such as readily digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS).[5] Readily digestible starch is the starch fraction that causes an increase in blood glucose level after ingestion immediately, whereas SDS is the starch fraction that is digested completely in the small intestine at a lower rate as compared to RDS. RS is the portion of starch and/or starch hydrolysis products that escape digestion in the small intestine, and enter the colon for fermentation.[2] Extensive studies have shown RS to have physiological functions similar to those of dietary fibre.[6] RS appears to be highly resistant to mammalian enzyme and may be classified as a component of fiber on the basis of the definitions of dietary fibre given by AACC.[7]

The diversity of the modern food industry and the enormous variety of food products being produced require starches that can tolerate a wide range of processing techniques and preparation conditions.[8] These demands are met by modifying native starches with chemical, physical and enzymatic methods which may lead to the formation of indigestible residues. The

availability of such starches therefore deserves consideration. Four forms of RS are distinguished: RS type I is defined as physically inaccessible starch for instance in grains; type II is granular starch in raw potato and bananas; type III is retrograded starch, arising after hydrothermal treatment of starch; and type IV is considered to be a chemically modified starch.[9] Among these four types, RS type III seems to be particularly interesting because it preserved its nutritional characteristics when it is added as ingredient for cooked food. RS type III is produced by gelatinization, which is a disruption of granular structure by heating starch with excess water, and then retrogradation occurs.

The generation of RS after hydrothermal treatment is mainly due to increase interactions between starch polymers. The degree of formation of RS in foods depends not only on the type of incorporated starch and the processing conditions but is also influenced by the duration and conditions of storage.[10] As stated by Fuentes-Zaragoza et al.[11] unripe banana is considered as RS-richest non-processed food. The content of RS in unripe banana ranges between 47-57%. Because of this fact several studies have suggested that consumption of unripe banana results in beneficial effects to human health. Rodriguez-Ambriz et al.[12] studied on unripe banana flour where they found total starch content of 73.4%, RS content 17.5% and concluded that unripe banana is an alternative source of indigestible carbohydrates, mainly RS and dietary fibre. Therefore, when the unripe fruit is cooked, its native RS is rendered digestible.

The global trends in rising levels of obesity, diabetes and cardiovascular disease has renewed research interest in the dietary intake of fat, protein and carbohydrate to maintain good health. The World Health Organization (WHO) and Food and Agricultural Organization (FAO) of the United Nations stated that globally, overweight populations are a bigger problem than under nourishment and recommended people in industrialized countries base diet on low GI foods to prevent most common disease of affluence.[13] One of the most important objectives in the dietary treatment of diabetes patients is to maintain their blood glucose level, avoid obesity and achieve optimal lipids level. Foods containing resistant starch (RS) generally give a low glycaemic response because RS is not digested in the small intestine and instead RS passes into the large intestine where it is fermented.[2]

Many tropical countries have plant species which can be used as a good source of starch; unfortunately some of them have not been exploited. One such plant species is *kachkal* (*Musa* ABB), the only culinary banana found in the entire Assam and North-East India.[14] Starch being

the principal component of culinary bananas can be considered as a resource for production of modern forms of consumption like processed snacks and precooked products. Therefore, in the present study an effort has been made to isolate starch from culinary banana and modifying the isolated starch into RS in order to find an alternative route to increase value addition of culinary banana by providing RS of high quality characteristics necessary for a well balanced diet. The utilization of RS from culinary banana should not only expand the market of it but also provide a solution to those who want to consume food with low glycaemic index (GI) value.

3.2 Materials and methods

3.2.1 Raw materials

Unripe culinary bananas at optimum harvesting stage (50 days after emergence of flower) was harvested and collected from Tezpur University campus, Tezpur, Assam. Prior to the starch isolation the samples were cleaned thoroughly under running tap water followed by rinsing with distilled water and pat dried using a clean cloth. All the chemicals required for present study was high purity analytical grade supplied by Sigma-Aldrich, USA, HiMedia, India and Merck, India.

3.2.2 Starch isolation

The starch was isolated following the method described by Bello-Perez et al.[15] In brief, the fruits were peeled and cut into 5-6 cm cubes and immediately rinsed in sodium sulfite solution (1.22 gram/l) and then macerated at low speed in a blender (500 gram fruit : 500 gram solution) for 2 min. The homogenate was consecutively sieved through screens numbers 50 and 100 mesh until the washing water was clean; then the starch milk was centrifuged at 10000 rpm for 30 min. The white-starch sediments were dried in a convection oven at 40°C for 24 h, ground with a mortar-pestle and passed through 100 mesh sieve and stored at ambient temperature in sealed containers.

3.2.3 Chemical analysis

The chemical analysis was carried out where moisture content, ash, crude fiber fat and protein content were determined according to AOAC methods.[16] The pH of starch dispersion (8% w/v) was measured by using a pH meter. Total amylose content was determined as per the method described by McGrance et al.[17] The dried defated starch (20 mg) was dissolved in 8 ml 90% dimethylsulfoxide in screw cap vials. The suspension vigorously homogenized for 20 min followed by heating at 85^{o}C for 15 min. The mixture was cooled and volume was made to 25 ml with distilled water and 1 ml of diluted solution was further mixed with 40 ml distilled water. To which 5 ml of potassium iodide solution (0.0025 M iodine and 0.0065 M potassium iodide) was added and finally the volume was adjusted to 50 ml. the absorbance was taken at 600 nm using spectrophotometer after allowing the samples to stand for 15 min in dark at room temperature. The amylose content was calculated from standard curve prepared using pure potato amylose type III.

The RS content in the culinary banana starch was determined by an enzymatic method.[16] The samples were incubated with pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37°C, during this time the non-resistant starch was solubilised and hydrolyzed to glucose by the combined action of the two enzymes. The reaction was terminated by the addition of an equal volume of ethanol and the RS was recovered as a pellet on centrifugation. This was then washed twice with ethanol (50 % v/v) and centrifuged. The RS in the pellet was dissolved in 2 M KOH by vigorously stirring on an ice-water bath. This solution was neutralized with acetate buffer and the starch was quantitatively hydrolyzed to glucose with AMG. The glucose was quantified with glucose oxidase/peroxidase reagent (GOPOD), which gave a measure of the RS content of the sample.

3.2.4 Functional Properties

3.2.4.1 Water holding capacity, starch swelling power and solubility

Water holding capacity was determined as described by Hallgren.[18] Starch pastes 5% (w/v) were heated to 60, 70, 80, and 90ºC for 15 min with shaking every 5 min period. Tubes

were centrifuged at 3000g for 15 min, the supernatant was decanted, and the tubes were then weighed, and the gain in weight was used to calculate the water holding capacity. Starch suspension (40 ml of 1% w/v) was taken in previously weighed 50 ml flask and was heated from 50 to 90ºC for 30 min .The flask was removed and left for cooling to room temperature and centrifuged for 15 min at 3000 rpm. The supernatant decanted and the swollen granules weighed. A 10 ml sample was taken from the supernatant, placed in a crucible and dried in a convection oven at 120°C for 4 h to constant weight. Percentage solubility and swelling power were calculated using the Eq. (3.1) and (3.2)

$$\%\, Solubility = \frac{Weight\ of\ dried\ starch}{Sample\ weight} X100 \qquad \text{Eq. (3.1)}$$

$$Swelling\ power\ (\%) = \frac{weight\ of\ swollen\ granules}{sample\ weight - weight\ of\ dissolved\ starch} X100 \qquad \text{Eq. (3.2)}$$

3.2.4.2 Freeze-thaw stability and paste clarity

The 5% starch was dissolved at 95^{o}C for 30 min with continuous stirring and the freeze-thaw stability of the starch paste was studied by four alternate freezing and thawing of 5 ml of 5% starch pastes (freezing for 18h at -22ºC and 6 h thawing at room temperature respectively) following the method of Jeong and Lim [19] followed by centrifugation at 50 for 10 min. The percentage of water separated after the freeze thaw cycle was measured as weight of exudates to the weight of paste.

Following the method described by Bello-Perez et al.[15] starch sample (0.2 g) was suspended in 5 ml of water in screw cap tubes and placed in a boiling water bath for 30 min. The tubes were thoroughly shaken every 5 min. After cooling the tubes to room temperature, the % transmittance at 650 nm was determined against a water blank in a spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). Stability and clarity of starch pastes were determined at both room temperature (30± 2^{o}C) and at 4^{o}C at 24, 48 and 72 h.

3.2.4.3 Pasting Properties

Pasting properties of starches were evaluated in Rapid Visco-Analyser (RVA-4, Newport Scientific, Sydney, Australia). An 8% slurry was given a programmed heating and cooling cycle

set for 23 min, where the sample was held at 30ºC for 1 min, heated to 95ºC in7.5 min, further held at 95ºC for 5 min before cooling to 50ºC within 7.5 min, and holding at 50ºC for 2 min. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. Peak viscosity (PV), hold viscosity (HV), final viscosity (FV), Breakdown viscosity (BV), setback viscosity (SV) and pasting temperature (PT) of starches were measured and measurements were replicated four times.

3.2.5 Structural analysis of starch

3.2.5.1 X- ray diffraction

The X-ray diffraction was obtained from a D/max 2500 X-ray difractometer (Rigaku Miniflex, Japan) at room temperature using CuKα radiation (λ = 0.15418 nm). A conventional X-ray tube was set to 30 kv acceleration potential and 15 mA current. Data were collected from 2θ of 5 to 50º (θ being the angle of diffraction) with a scan speed of 8º 2θ/min. The starch sample was dried at 50ºC to constant moisture (10%) in a vacuum oven and 50 mg samples were added into the slide for packing prior to X-ray scanning.

3.2.5.2 Fourier transforms infrared (FT-IR) spectra

An infrared spectrum of starch was measured using KBr disk method. The dry sample was blended with KBr in a ratio starch/KBr 1:4. The blend was pressed to obtain a pellet and introduced in the spectrometer (Nicolet Instruments 410 FT-IR equipped with KBr optics and a DTGS detector). Each spectrum was analyzed in the range of resolution from 400-4000 cm^{-1} with a resolution of 4 cm^{-1} and total of 64 scans were collected

3.2.5.3 Morphological analysis by SEM

Starch granules were observed under a scanning electron microscope (JEOL JSM 6390 LV, USA) operating at an accelerating voltage of 15 kv. A small portion of starch sample was assembled on metallic stubs with double sided tape and coated with a thin layer of gold. Magnification was taken at 500X and shape and size of the starch granules were observed.

3.2.6 Thermal characteristics by thermogravimetric analysis (TGA)

Thermal degradation behaviour of starch was evaluated using TGA (Shimadzu, TGA-50, North America). The thermal stability of each sample was conducted at 25 to 600^{o}C with constant heating rate of 10^{o}C/min under nitrogen atmosphere.

3.2.7 Development of type III resistant starch (RS) from culinary banana starch

3.2.7.1 Autoclaving and cooling method

The RS samples were prepared following the method of Berry[20] with slight modification by suspending 10% and 20% (w/v) of culinary banana starch in 250 ml of water and autoclaved using 15 psi pressure at 120°C for 30 min followed by cooling at 4°C for 24 h. After three repetitions of the autoclaving and cooling cycles, the samples were freeze-dried (Model NO. LDF-5512, Daihan Lab Tech Co., Ltd, South Korea) and ground into fine particles by using mechanical grinder (Fritsch, Germany) and passed through 100 mesh screen sieve and stored until further analysis.

3.2.7.2 Enzyme debranching method

Following the method of RS production by enzyme debranching[21] RS was developed by suspending culinary banana starch (20%) in sodium acetate buffer (pH 5.0) and slurry was subjected to repeated thermal and enzymatic treatment. The samples were given thermal treatment at 100^{o}C for 15 min and diluted further using 10% sodium acetate buffer and mixed thoroughly. For the enzyme debranching process, pullulanase (EC 3.2.1.41) from *Klebsiella pneumoniae* (5%) was added to the homogenized suspension of starch gel and the hydrolysis reaction was maintained for 24 h at 60^{o}C. The thermal treatment at 100^{o}C for 15 min was repeated in between enzyme debranching process. The developed RS was freeze dried ground and stored for further analysis.

3.2.7.3 Chemical analysis of RS

Moisture content, ash, crude fiber fat and protein content were determined according to AOAC methods.[16] The pH of modified starch dispersion (8% w/v) was measured by using a pH meter. The amylose content in developed RS was measured following the method described by McGrance et al.[17] and the RS content in the starch modifications was determined by an enzymatic method.[16]

3.2.8 Statistical analysis

Experiments were carried out in 3 replicates. The data analysis tool 'Microsoft Excel' was used for statistical analysis. Data were subjected to ANOVA and Fisher's Least Significant Difference (LSD) was used to separate means.

3.3 Results and discussion

3.3.1 Chemical composition of culinary banana starch

The yield of starch isolated from culinary banana was 16.00% with a high purity of 96.00% (Table 3.1). Waliszewski et al.[22] isolated starch from *valery* variety of bananas and the yield of starch reported by author was much higher (33.8%). Bello-Perez et al.[23] reported two yields of starch from macho and criollo banana 43.8 and 11.8% respectively. The high difference in the starch yield between two varieties may be due to the texture of banana fruit and its maturity stages of the fruit used.[24] The moisture content of culinary banana starch was found to be 10.90%. According to the reports of Mweta et al.[25] the moisture content of the starches ranged from 8.96 to 11.93% and falls within the acceptable range for storage and marketing without deterioration in quality of starches. The data of the present study (Table 3.1) revealed that the culinary banana starch contained 0.35% ash, 0.31% protein, 0.27% fiber and 0.50% fat. The content of ash reported in present study was higher than that of plantain starch (0.02%) reported by Perez-Sira.[24] The high ash content in culinary banana starch may be indicative of presence of more minerals like potassium and magnesium. A result of protein content is comparable with

results of Mweta et al.[25] in case of cassava starch. The higher fat content (0.50%) reported in the present study is perhaps the reason for its resistance to amylolysis due to the formation of amylose-lipid complex.[26] The pH value obtained for the culinary banana starch was recorded to be 6.70 which are within the pH range of 3-9 obtained for most starches used in the pharmaceutical, cosmetics, and food industries.[27] As most normal starches contain 20-30% of amylose, 34.10% amylose content of culinary banana starch revealed a non-waxy starch type. Amylose content in *valery* banana starch (40.7%)[22] and was different for cavendish banana starch (19.5%).[28] The high amylose starch is much more resistant to digestive enzymes then the low amylose starch. The resistant starch (RS) content in the starch sample studied was 18.88% which was on the higher side as compared to the content of RS (17.5%) in banana flour reported by Ovando-Martinez et al.[29] The photograph of starch obtained from culinary banana is presented at Fig. 3.1.

Table 3.1 Chemical compositions (%) of culinary banana starch

Starch yield	Starch purity	Ash	Crude fiber	Fat	Protein	pH	Amylose	Resistant starch content
16.00± 0.12	96.00± 0.08	0.35± 0.01	0.27± 0.09	0.50± 0.02	0.31± 0.03	6.70± 0.03	34.10± 0.02	18.88± 0.05

Results are mean of three replicates±SD

Fig. 3.1a) Culinary banana at matured edible stage and, **b)** starch from culinary banana

3.3.2 Functional properties of culinary banana starch

3.3.2.1 Water holding capacity, starch swelling power and solubility

The present study revealed that water holding capacity of culinary banana starch (Table 3.2) increased with rise in temperature. The maximum water holding capacity (42.24%) was observed at 90ºC. Water holding capacity of culinary banana starch was in accordance with the findings of Bello-Perez et al.[30] for "criollo" and "macho" starches. The swelling behaviour of starch is an indication of the water absorption characteristics of the granules during heating[31]. Generally, the solubility and swelling profiles show a general trend of increase with increase in temperature and expansion of starch is mainly depends on degree of gelatinization.[32] The swelling and solubility profile of culinary banana starch are presented in Table 3.2. The starch sample swelled slowly up to 70ºC and above it the starch granules swelled rapidly due to the breakage of intermolecular hydrogen bonds in amorphous region.[23] However, the starch exhibited a restricted swelling pattern, which may be attributed to the fat content, as fats are known to inhibit swelling by forming insoluble complexes with the linear fraction of starch.[33] Similar range of swelling power was also reported for kernel starch[34] and white and yellow plantain starches.[35] Lower swelling power of culinary banana starch is also reflection of more stable granular structure within the starch molecule. A similar pattern was also observed for solubility (Table 3.2). This might be result of the swollen starch granules allowing amylose exudation. The maximum solubility (9.00%) was observed at 90ºC which is lower than corn starch (15.80%) at the same temperature.[36] The lower values of solubility of starches at low temperatures might be due to the semicrystalline structure of the starch granules and the hydrogen bonds formed between hydroxyl groups within the starch molecules. As the temperature increased, the solubility increased due to breaking of starch granules and exposure of hydrophilic groups to water.[37]

Table 3.2 Water holding capacity, swelling and solubility profile of culinary banana starch

Parameters	Temperature (ºC)			
	60	70	80	90
Water holding capacity (%)	12.64±0.09	20.42±0.07	36.26±0.01	42.24±0.01
Swelling (gH_2O/g dry samples)	2.30±0.12	4.50±0.04	8.90±0.00	12.80±0.06
Solubility (%)	1.55±0.01	3.51±0.52	7.11±0.08	9.00±0.17

Results are mean of three replicates ±SD

3.3.2.2 Freeze-thaw stability and paste clarity

Exudation of water from frozen gels (syneresis) indicates the retrogradation behaviour of the cooked starch pastes. Freeze-thaw stability measures the amount of water released from the gels during storage by degree of syneresis and is an important factor to be considered when formulating refrigerated and frozen foods.[38] Culinary banana starch gel was unstable during different freezing and thawing cycles releasing 24.13-42.58% of the water (Fig. 3.2a). The amount of water separated from the gels during freezing increased with storage time. This result suggests that banana starch is not desirable for frozen products. The low freeze-thaw stability of culinary banana starch might have been affected by the amylose and amylopectin content. Baker and Rayas-Duarte [38] have reported this behaviour for corn starches, mentioning low freezing-thawing gel stability for corn and amaranth starches.

Starch gel clarity is a much desirable functionality of starches for its utilization in food industries since it directly influences brightness and opacity in foods. The starch sample experienced low paste clarity (3.2-1.2% light transmittance) during storage time (Fig. 3.2b) and the results are in line with previous reports of Bello-Pérez et al.[30] that banana starch forms an opaque gel and with increase in storage period the transmittance value decreases. This reduction in transmittance is due to retrogradation tendency of starch pastes which means that under refrigerated condition banana starch have tendency to retrograde. Opaqueness of starch paste has been attributed to various factors such as granule swelling, granule remnants, leached amylose and amylopectin, amylose-amylopectin chain-length, intra-or intermolecular bonding and presence of lipids.[39] Since the paste clarity of culinary starch is very low, therefore it could be used in food products that do not required transparency.

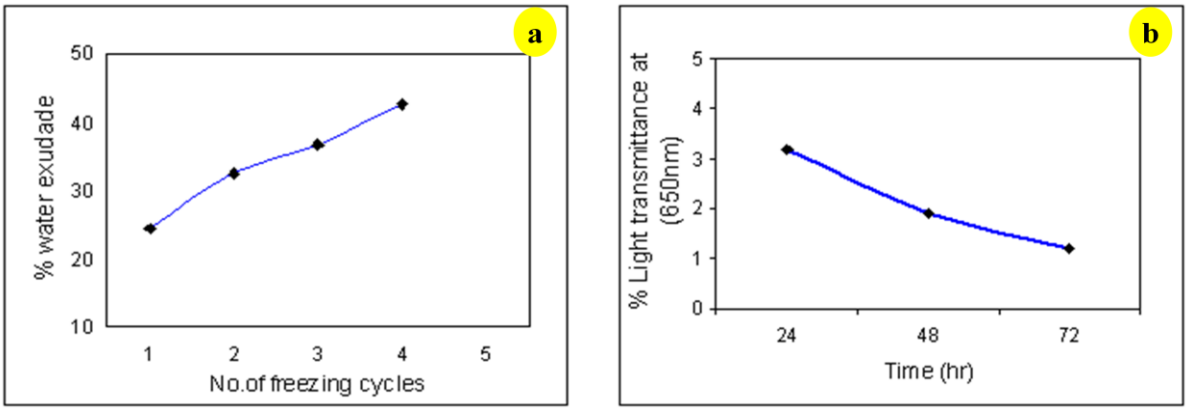

Fig.3.2 a) Freeze-thaw stability of culinary banana starch and, **b)** Paste-clarity of culinary banana starch

3.3.2.3 Pasting properties

Pasting properties of culinary banana starch are presented in (Fig. 3.3). The starch sample exhibited a high (81.80ºC) pasting temperature which indicates the starch is highly resistant towards swelling and it favourably supports our findings for swelling power. The pasting temperature is an indication of the gelatinization temperature of the starches; which indicates culinary banana starches have high gelatinization temperature. The culinary banana starch showed a peak viscosity of 4507 cP and reflects the ability of starch granules to swell freely before their physical breakdown.[40] Peak viscosity occurs at the equilibrium point between granule swelling and polymer leaching, which causes an increase in viscosity and granule rupture and polymer alignment because of mechanical shear. The starch exhibited high setback viscosity during cooling indicating it retrograded highly which might be due to the effect of amylose and amylopectin contents. Since the starch with high amylose could undergo the retrogradation process faster than the starch with low amylose content.[41] During the holding temperature at 95ºC accompanied with shear, a decrease in the viscosity of the starch pastes was observed, resulting the breakdown of some swollen starch granules. The culinary banana starch also experienced a low breakdown (447cP) viscosity which is also indicative of lower degree of swelling and subsequent disintegration. Kayitsu et al.[40] reported that the high pasting temperature, as high peak viscosity of the starches as shown are highly resistant to swelling and rupturing and exhibiting restricted swelling.

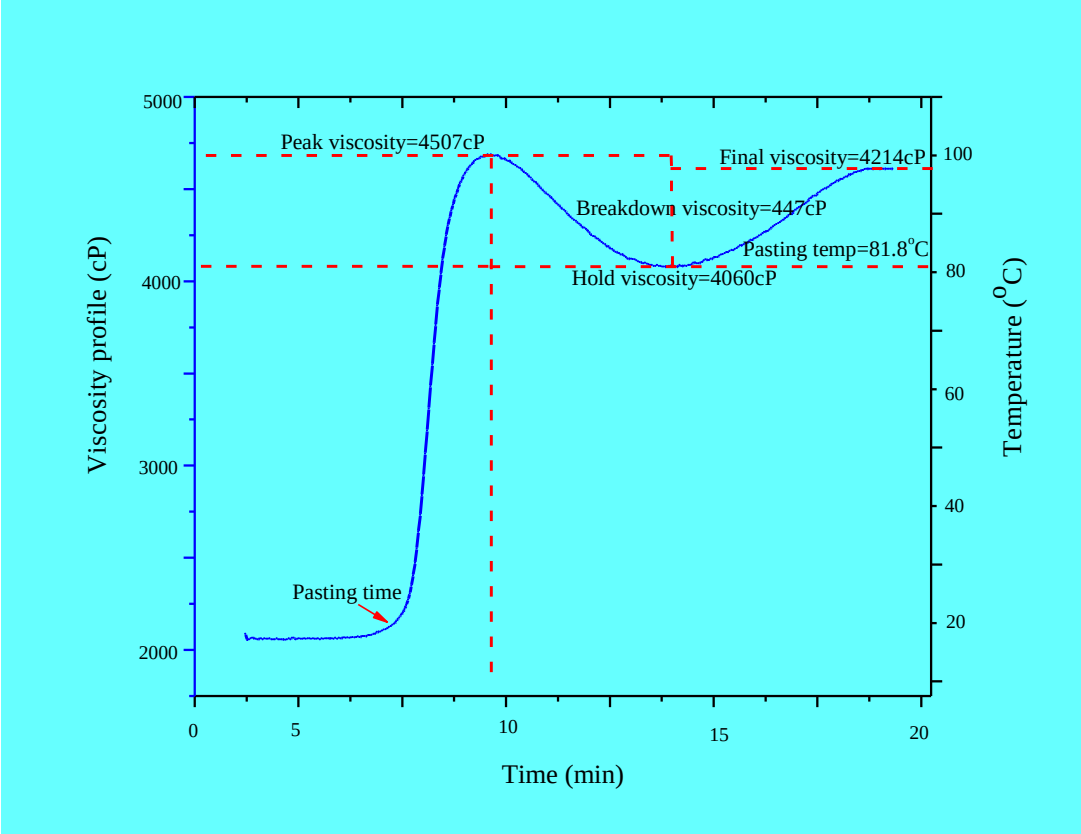

Fig. 3.3 Pasting properties of culinary banana starch

3.3.3 Structural analysis

3.3.3.1 X- Ray diffraction

X-ray diffraction is one of the most effective methods for evaluating the structure of starch and determining the crystalline form of starch.[42] X-ray diffraction provides an elucidation of the long range molecular order, typically termed as crystalline, which is due to ordered arrays of double helices formed by the amylopectin side chains.[43] The culinary banana starch exhibited strong diffraction peaks at 15.0^{o} and 17.09^{o} (2θ) and one very broad peak at 23^{o} (2θ). The wide angle x-ray diffractogram revealed that culinary banana starch (Fig. 3.4) is a mixture between the A and B-type polymorphs. According to the report of Yu et al.[44] C-type starch pattern has been considered a mixture of both A and B-types because its x-ray diffraction pattern can be resolved as a combination of the previous two. The results of XRD reported in the present study are in agreement with Chang et al.[45] and Waliszewski et al.[22] as they have also assigned a C type

diffraction pattern for banana starches. The % crystallinity of starch sample was recorded to be 27.45% which is comparatively higher than the reported values of crystallinity index of different verities of banana starches studied by Soares et al.[46] The higher crystallinity index of culinary banana starch may be attributed to the ability of enzyme penetration inside the granule and this penetration is dependent on the presence of pores as well as the lamellar organization of starch.

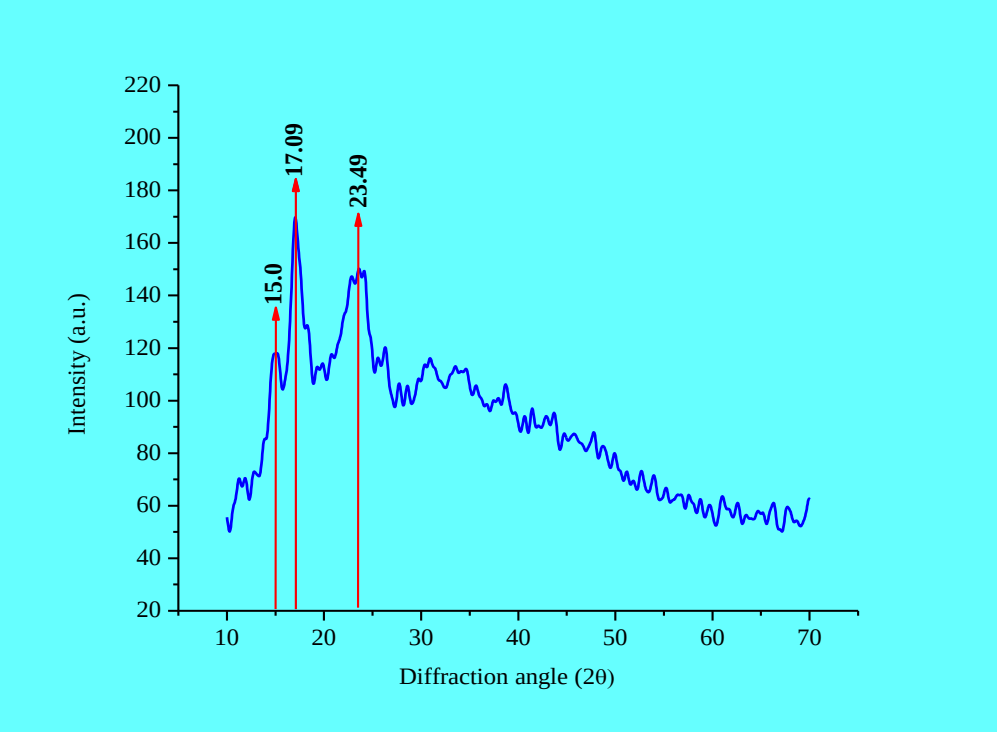

Fig. 3.4 X-ray diffractogram of culinary banana starch

3.3.3.2 Fourier transforms infrared (FT-IR) spectra

The FT-IR spectrum of culinary banana starch is illustrated in Fig. 3.5. The characteristics absorption bands appeared at 575, 785, 855, 929 cm^{-1} may be attributed to anhydroglucose ring stretching vibrations. The carbohydrate nature of the starch sample was confirmed by the spectra observed near the wave numbers 1156, 1369, 1417, 1646, 2925 and 3429 cm^{-1}.[47] An extremely broad band appeared at 3429 cm^{-1} is attributed to hydrogen bonded hydroxyl groups. The sharp band observed 2925 cm^{-1} is characteristics of O-H and H-C-H bond stretching associated with the methine ring hydrogen atom.[48] The band at 1646 cm^{-1} is related to COO- stretching vibration in a carbohydrate group.[49,50] Peaks observed at 1417 cm^{-1} and 1369

cm^{-1} were attributable to the bending modes of H–C–H and C–H symmetric bending of CH_3.[49, 51] The signal at 1156 cm^{-1} could be attributed to C-O bond stretching.[47] The additional characteristics absorption bands at 929, 855, 785, 707 and 575 cm^{-1} are due to the entire anhydroglucose ring stretching vibrations.[47] The peak observed at around 1002 cm^{-1} is associated with the crystalline and amorphous structure of starch.[49, 50] The FT-IR spectra observed in the present study resembles the spectra obtained for plantain and banana starches studied by various authors.[24, 47]

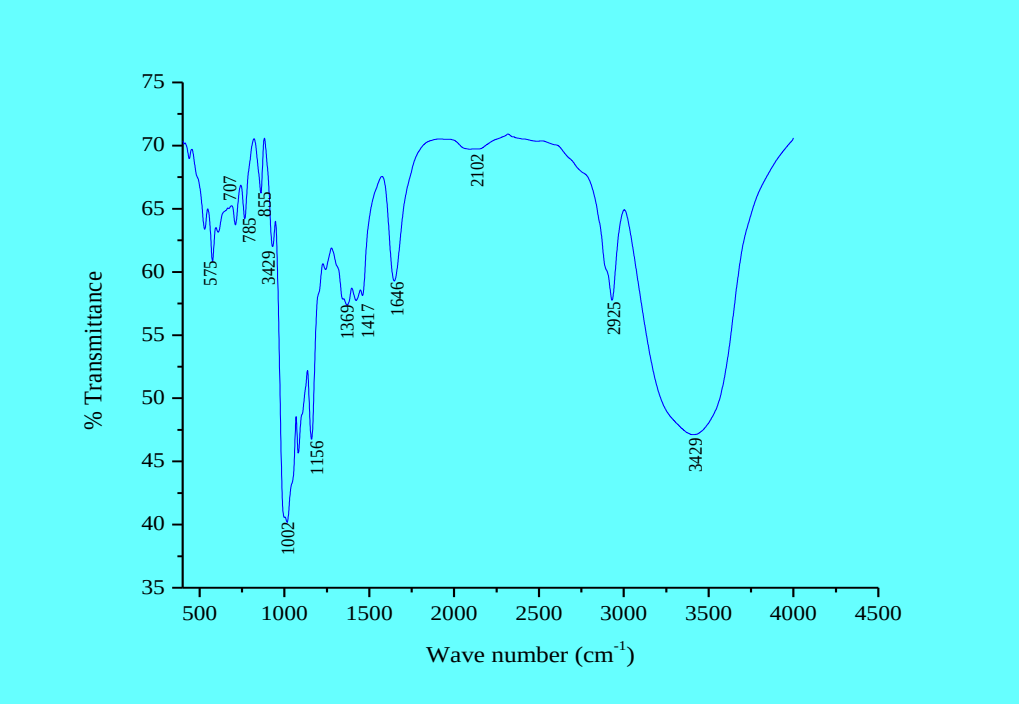

Fig. 3.5 FT-IR spectra of culinary banana starch

3.3.3.3 Morphological analysis by SEM

The scanning electron micrographs (Fig 3.6) of the starch revealed that the culinary banana starch granules appeared as a mixture of spherical and elliptical shaped with granule size ranged from 7.55µm-68.00µm. Eggleston et al.[52] reported that the plantain starch had a broad range of granule size (7.8-61.3 µm) for plantain and is, lower than our findings for culinary banana starch granules. The surface of the starch sample appeared to be smooth and as reported by Kayiasu et al.[40] it could be indicated that the isolation process was efficient and it did not cause damage to starch granules.

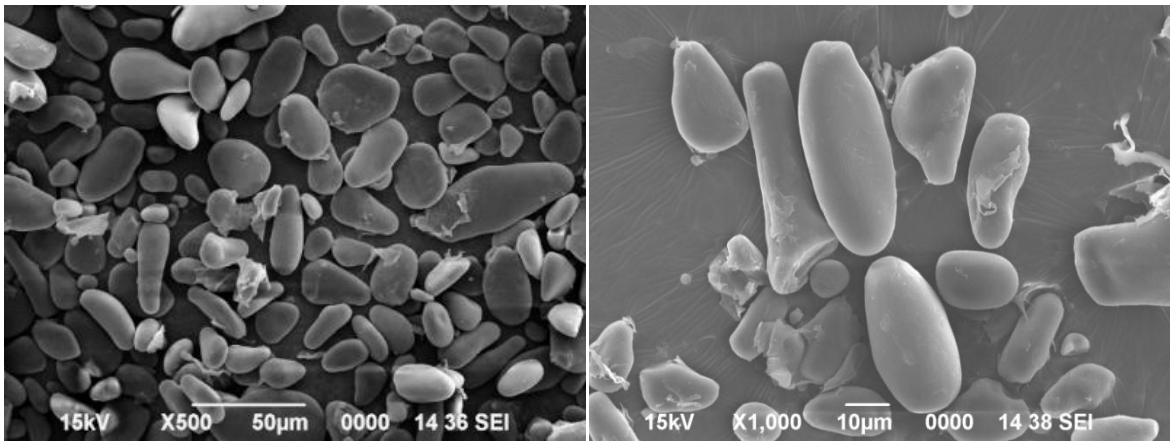

Fig. 3.6 Scanning electron micrograph of culinary banana starch

3.3.4 Thermal stability by thermogravimetric analysis (TGA)

The thermal stability of culinary banana starch with increasing temperature at 10^{o}C/min was evaluated by thermogravimetric (TG) and the corresponding derivative (DTG) curves (Fig. 3.7). From the TGA curve it is revealed that thermal degradation of starch showed three distinct weight losses, the first weight loss initiated at 25.15-247.35^{o}C corresponds to the initial vaporization of residual water. The second weight loss at around 265.39-331.34^{o}C and is due to the pyrolysis of starch and the weight loss observed at the temperature range of 360 to 480^{o}C may be due to decomposition of cellulose, hemicelluloses and lignin present in the starch sample.[53] The region at where second degradation or pyrolysis of starch occurred a sharp and well defined peak of DTG curve (Fig. 3.7) was observed which on further increase in

temperature showed asymmetric appearance (shoulder formation). The plausible reason may be the second degradation stage is quite complex and probably that may be divided into sub stages which was specifically defined by the formation of shoulder at the end of second decomposition process. The temperature and degradation rate increased with increase in heating rate which is a characteristic behaviour for the thermally stimulated processes in the solid state.[54]

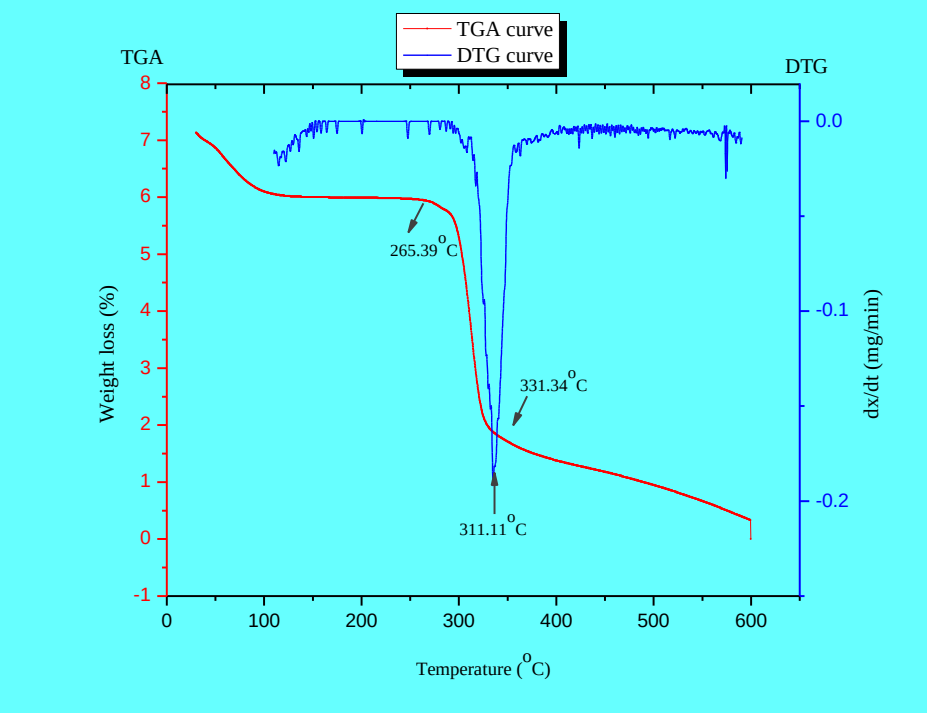

Fig. 3.7 TGA and DTG curves of culinary banana starch

3.4 Development of type III resistant starch (RS) from culinary banana starch

3.4.1 RS production by hydrothermal method

The production of RS type III by autoclaving and cooling is mainly due to the retrogradation of the amylose fraction (Fig. 3.8a). The four sets of RS obtained after autoclaving cooling cycle were coded as $RS_{10\text{-}A}$ (10% starch, 4°C storage), $RS_{10\text{-}B}$ (10% starch, -20°C storage), $RS_{20\text{-}A}$ (20% starch, 4°C storage) and $RS_{20\text{-}B}$ (10% starch, -20°C storage). The generation of RS type III by hydrothermal treatment depends mainly on the number of heating and cooling cycles, starch type, storage time and temperature etc. The amylose content and the

amount of water are directly correlated to the yield of RS.[55] The effect of starch concentration and the number of autoclaving and cooling cycles on the yield of RS type III are presented in Table 3.3. The present study showed that the yield of RS increased with the increase starch concentration. Similarly, the yield of RS content also increased with the number of cycles. However, the effect of starch concentration RS yield was considerably lower than the number of autoclaving and cooling cycles. After the third autoclaving and cooling cycle the RS content increased to 23.31%. This gradual rise in the yield of RS after repeated heating and cooling cycles might be associated with a decrease in the hydrolysis limit of pancreatic α-amylase and the increased RS type III formation. Haralampu[55] determined RS values between 2.5 and 21.3% for diverse starch sources and with the Berry method[20] the RS values ranged between 2.8-31%[56] which is in favourably supports our findings.

3.4.2 Effect of enzyme concentration on starch debranching

An increased degree of debranching enables the chains to align and aggregate and hence form perfectly crystalline structures, thereby leading to the formation of more RS.[57] Two sets of RS obtained after enzyme debraching were coded as $RS_{Ez\text{-}A}$ (4^oC storage) and $RS_{Ez\text{-}B}$ (-20^oC storage). Berry[20] reported that debranching of potato amylopectin with pullulanase before subjecting it to heating and cooling cycles substantially increased the RS type III content; and was attributed to an increase in the content of linear starch chains resulting from debranching. This pattern demonstrated that the substrate level needed higher enzyme concentration for starch debranching and maximum RS (31.17%) yield was obtained when debranched with 5% enzyme concentration.

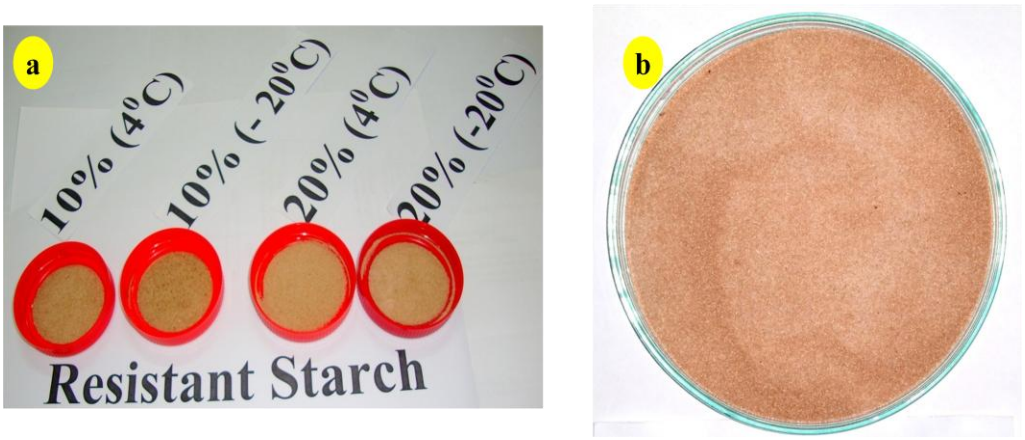

Fig. 3.8 Resistant starch (RS) obtained by **a)** Hydrothermal process and, **b)** Enzyme debranching

Table 3.3 Effect of starch concentration and number of autoclaving and cooling cycles on RS content

Starch concentration [% (w/v)]	No. of cycles	RS content (%)
10	1	9.45 ± 0.75^{a}
10	2	10.23 ± 0.06^{b}
10	3	12.30 ± 0.09^{d}
20	1	11.98 ± 0.14^{c}
20	2	14.00 ± 0.01^{e}
20	3	23.31 ± 0.67^{f}

Results are mean of three replicates±SD; mean followed by same superscript small letters within a column are not significantly different ($p>0.05$)

3.4.3 Effect of storage temperature on RS content

The RS obtained from hydrothermal treatment after third cycle of cooling and autoclaving as well as RS obtained after enzyme debranching were subjected to two different storage temperatures (4ºC and -20ºC) and their effect on the yield of RS type III are presented in Table 3.4. The results revealed that low temperature storage enhanced the formation of RS. The % of RS content gradually increased at lower level of storage condition. The RS obtained from 10% starch increased from 12.30% (stored at 4°C) to 15.43% (stored at -20°C). Similarly, the RS obtained from 20% starch increased to 26.42% when stored at -20°C. The RS obtained after

enzyme debranching showed very little increase in the RS content from 30.21 to 31.17% with respect to storage condition. This supports the general behaviour that RS yield increases during storage, especially during low-temperature storage.

Table 3.4 Effect of storage temperature on RS content

Resistant starch (RS)	Sample code	Storage (ºC)	RS (%)
Autoclaving and cooling (10%)	$RS_{10\text{-}A}$	4	12.30 ± 0.05^{a}
Autoclaving and cooling (10%)	$RS_{10\text{-}B}$	-20	15.43 ± 0.07^{b}
Autoclaving and cooling (20%)	$RS_{20\text{-}A}$	4	23.79 ± 0.0^{c}
Autoclaving and cooling (20%)	$RS_{20\text{-}B}$	-20	26.42 ± 0.12^{d}
Enzyme debranched(24h)	$RS_{Ez\text{-}A}$	4	30.20 ± 0.0^{e}
Enzyme debranched(24 h)	$RS_{Ez\text{-}B}$	-20	31.17 ± 0.01^{f}

Results are mean of three replicates±SD; mean followed by same superscript small letters within a column are not significantly different ($p>0.05$)

3.4.5 Chemical analysis

The chemical analysis of RS obtained after undergoing various treatments was evaluated and presented in Table 3.5. There was a significant difference in the moisture content of RS obtained at various isolating conditions. The moisture content was in the range of 4.65 to 5.97% in all the samples which are in the considerable range for long term storage of product without any microbial decomposition. The variation in the moisture content might be attributed to the linear chains produced during the different treatments which sometimes increased water binding properties.[58] The ash content varied from 0.14 to 0.22% in all the samples obtained and not much significant difference among the treatments given. The reason behind may be possibly the temperature used during autoclaving process is not high enough to digest the minerals present in the banana starch.[59] The content of crude fiber in all the RS obtained showed significant difference among the samples. The highest crude fiber recorded was 6.15% in case of enzymatically debranched RS stored at -20^{o}C and the minimum was 4.23% in case of RS obtained by autoclaving and cooling method followed by storage at 4^{o}C.

The fat content varied from 0.11-0.78% and did not have significant difference among the samples obtained by autoclaving and cooling cycle stored at 10^{o}C, but samples stored at -20^{o}C showed the significant difference. On the other hand enzymatically debranched RS showed statistical difference among them. Protein varied from 0.21-0.46% with significant difference among samples. The protein content obtained after the autoclaving and cooling cycle was comparatively lesser than that of RS obtained from enzyme debranching. Since the heat treatment given during autoclave cycle denatures proteins and saponifies lipids which may become solubilized.[59] The results of chemical analysis obtained in the present study corroborates with the findings of Aparicio-Saguilan et al.[59] in case of RS obtained from linterized banana.

Table 3.5 Chemical compositions (%) of culinary banana RS

Sample code	Moisture content (wb)	Ash	Crude fiber	Fat	Protein
$RS_{10\text{-}A}$	5.63±0.07^{e}	0.16±0.95^{c}	4.23±0.07^{a}	0.13±0.21^{a}	0.26±0.75^{d}
$RS_{10\text{-}B}$	4.65±0.09^{a}	0.18±0.09$^{a, b}$	5.26±0.56^{b}	0.11±0.09^{a}	0.35±0.08^{c}
$RS_{20\text{-}A}$	5.97±1.03^{f}	0.15±0.32^{a}	5.79±0.23^{c}	0.15±0.05^{a}	0.24±0.09^{d}
$RS_{20\text{-}B}$	4.92±1.21^{b}	0.14±0.67^{d}	5.81±0.04^{d}	1.21±0.78^{d}	0.21±0.04^{d}
$RS_{Ez\text{-}A}$	5.33±0.99^{d}	0.22±0.09^{a}	6.01±0.01^{e}	0.78±1.07^{c}	0.41±0.12^{b}
$RS_{Ez\text{-}B}$	5.01±1.06^{c}	0.21±0.05^{a}	6.15±0.75^{f}	0.64±0.76^{b}	0.46±0.08^{a}

Results are mean of three replicates±SD; mean followed by same superscript small letters within a column are not significantly different ($p>0.05$)

3.4.6 Scanning electron microscopy (SEM)

The modified RS ($RS_{10\text{-}B}$, $RS_{20\text{-}B}$ and $RS_{Ez\text{-}B}$) were studied under electron microscope in order to evaluate the microstructure and examined how the treatment affected the microstructure (Figs. 3.9a, 3.9b, 3.9c). The RS obtained by hydrothermal treatment (autoclaving and cooling) of 10% starch sample and stored for 24 h (Fig. 3.9a) is not prominent in its structure as it did not undergo significant morphological changes compared to RS obtained from 20% starch (Fig. 3.9b). The structure of starch is hazy and there was no proper conversion of RS from starch samples. On the other hand with increase in the concentration of starch sample from 10% to 20% the structural difference was evident and the modification of starch to RS with higher percentage of 20% was

more proper and clearly visible. This could be due to partial gelatinization of starch which occurs appropriately during retrogradation process for RS with higher amount of starch.[60] The structural morphology of RS obtained after enzyme debranching is illustrated in Fig. 3.9c and the modification of starch to RS was more distinguished and composed of round to irregular shape granules. The resistance of enzymatically modified starch changes in the composition and structure of starch particle upon modification.[61]

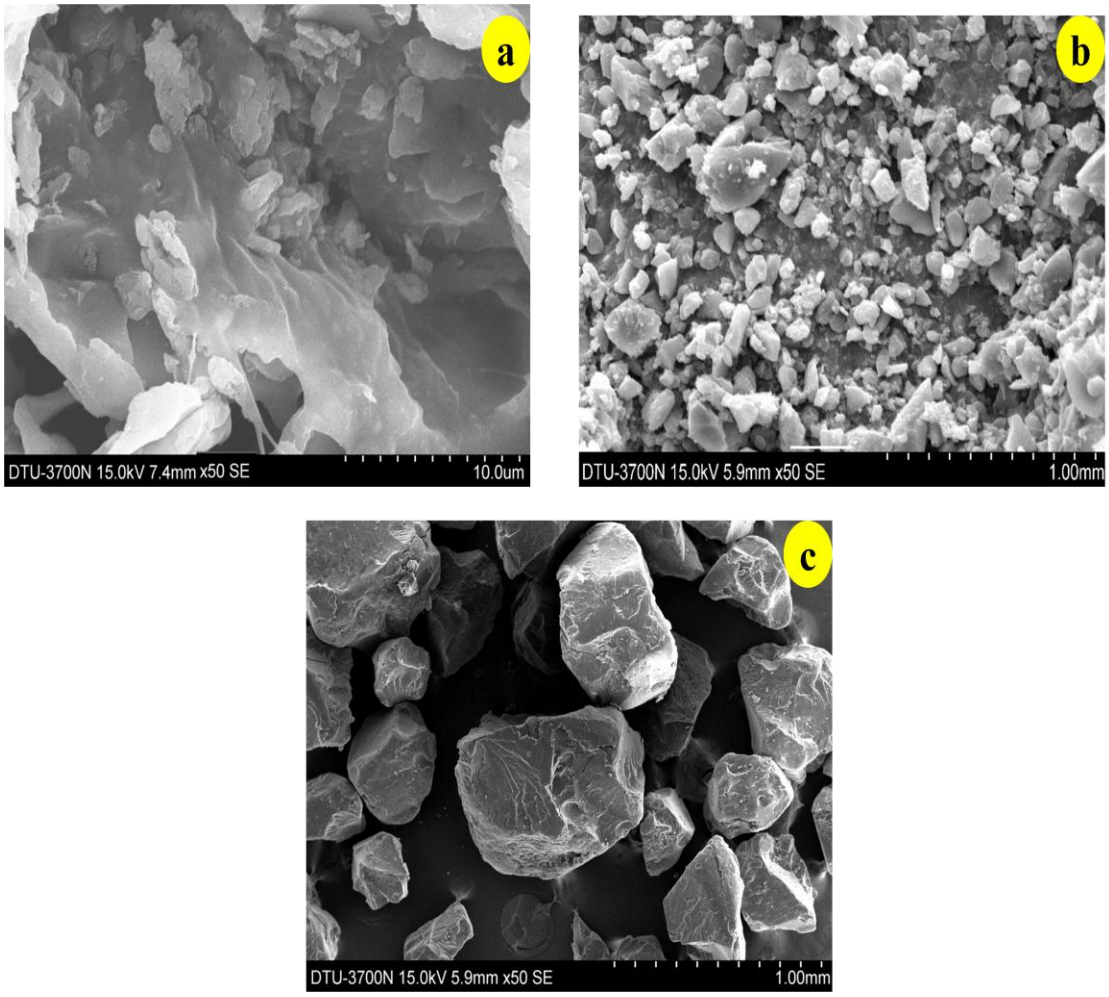

Fig. 3.9 Scanning electron micrograph of culinary banana RS **a)** ($RS_{10\text{-}B}$ = 10%, -20^{o}C); **b)** ($RS_{20\text{-}B}$ =20%, -20^{o}C) and **c)** ($RS_{Ez\text{-}B}$= 24 h, -20^{o}C)

3.4.7 FT-IR spectra of RS

The changes in molecular structure during the starch modification process were studied using FT-IR (Fig. 3.10). When FT-IR spectra of RS was compared to the spectra of culinary banana starch as illustrated in Fig. 3.5 revealed that bands in case of $RS_{10\text{-}B}$ were almost similar to the spectra of starch sample before retrogradation which suggests that the molecular structure of $RS_{10\text{-}B}$ was not altered during retrogradation process. There were seven major spectra were observed in the region of 500-3500 cm^{-1} in all the RS studied located at 3446, 2925, 2120, 1637, 1433, 1018 and 578 cm^{-1}. But the bands were sharper in case of $RS_{10\text{-}B}$ decreased in $RS_{20\text{-}B}$ and $RS_{Ez\text{-}B}$. The sharp band observed at 1018 cm^{-1} may be related to the crystalline starch and water content. Stretching vibrations of the hydrogen bonded hydroxyl groups at 3446 cm^{-1}, O-H and H-C-H bond at 2925 cm^{-1}, COO- at 1637 cm^{-1}, bending modes of H–C–H at 1433 cm^{-1} and anhydroglucose ring at 578 cm^{-1} bring the visible difference in spectral bands during retrogradation and modification of starch structurally.[62]

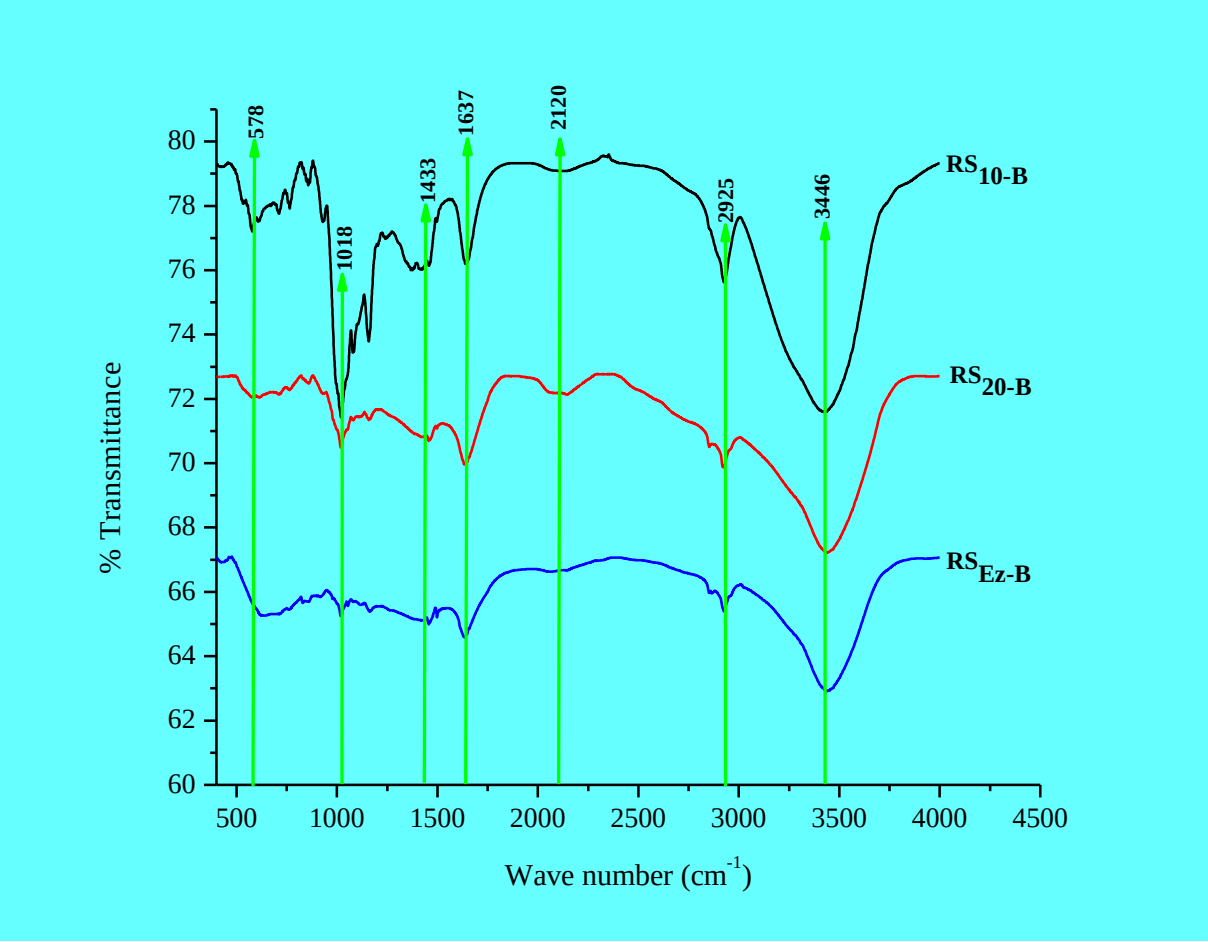

Fig. 3.10 FT-IR spectra of RS obtained after hydrothermal ($RS_{10\text{-}B}$ = 10%, -20°C and $RS_{20\text{-}B}$ =20%, -20°C) and enzyme debranched ($RS_{Ez\text{-}B}$= 24 h, -20°C)

3.4.8 Thermogravimetric analysis (TGA)

The TGA results are illustrated by a three step weight loss curves of TG and derivative DTG (Fig. 3.11). The results were compared with TGA-DTG curves of culinary banana starch where RS showed endothermic peak at around 337.91°C ($RS_{10\text{-}B}$), 334.34°C ($RS_{20\text{-}B}$) and 457.08°C ($RS_{Ez\text{-}B}$) which were comparatively higher to that of starch sample which may be because of the treatment given in order to modify the starch to RS. The different treatments (hydrothermal and enzyme debranching) given to the starch sample for modification to RS may lead to the formation of crystallites and amounts of double helices with different stabilities.[63] The endothermic transition of starch during modification are generally influenced by the interactions

between amylose-amylose, amylose-amylopectin and amylose-lipid content.[58] From the DTG curve (Fig. 3.11a, b, c) it is evident that, the sharp interval of weight loss indicates the presence of large amounts of compounds such as homopolysaccharides.[64] The RS_{EZ-B} showed faster rate of decomposition at higher temperature with higher initial degradation temperature 457.08°C with lower weight loss compared to RS_{10-B} and RS_{20-B} which may be because of crystal formation during starch modification in RS_{EZ-B} was different.[63] Our findings are in line with the reports of Zhou et al.[63] in case of RS obtained from rice starch.

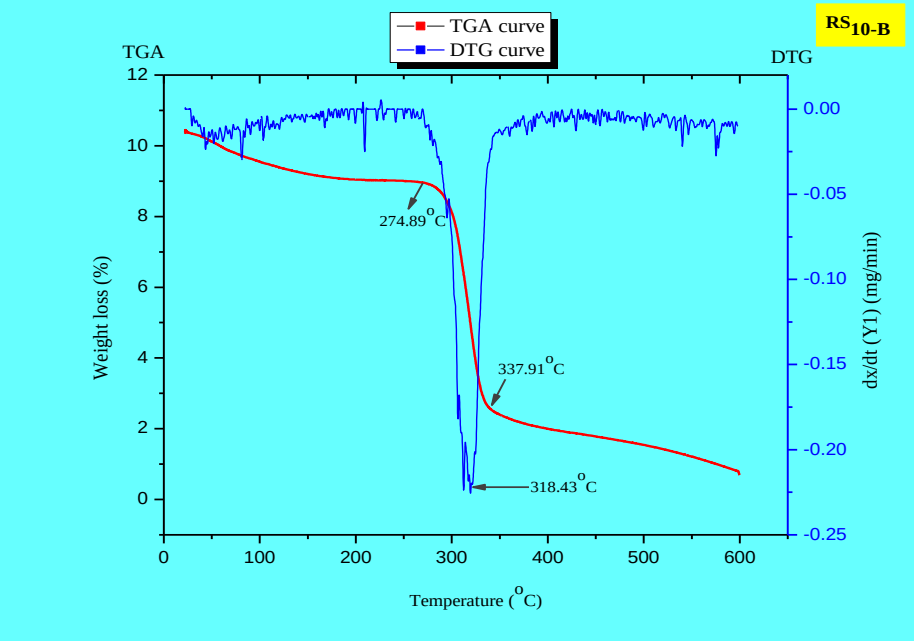

Fig. 3.11a TGA and DTG curves of RS obtained after hydrothermal (RS_{10-B} = 10%, -20°C)

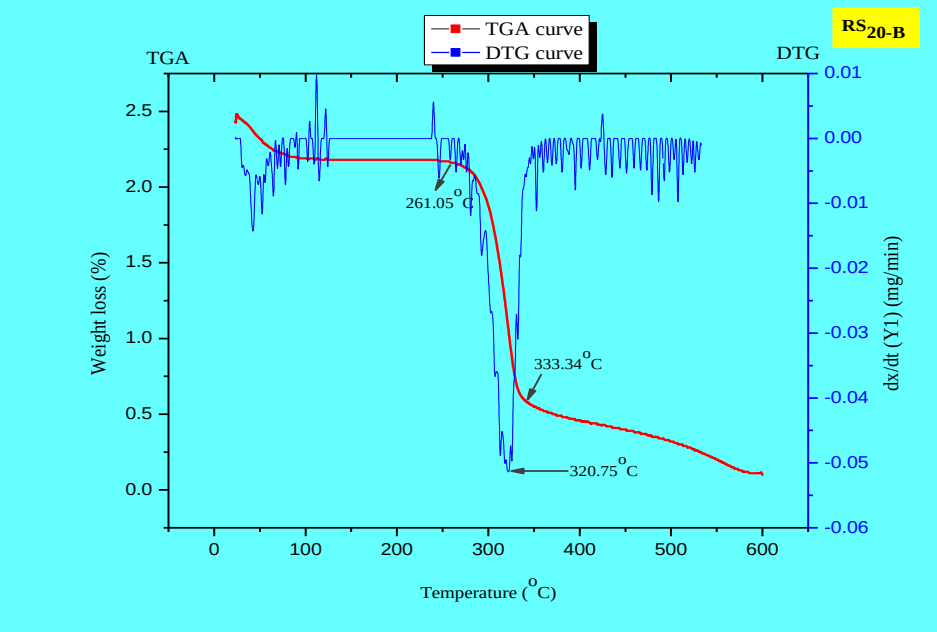

Fig. 3.11b TGA and DTG curves of RS obtained after hydrothermal ($RS_{20\text{-}B}$ =20%, -20°C)

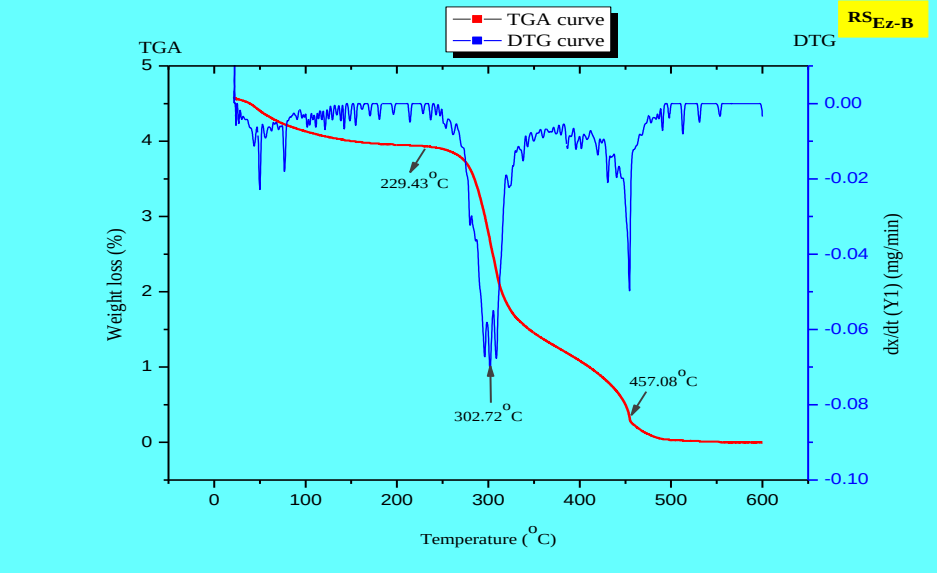

Fig. 3.11c TGA and DTG curves of RS obtained after enzyme debranched ($RS_{Ez\text{-}B}$ = 24 h, -20°C)

3.5 Conclusion

The starch isolated from culinary banana was further modified to RS type III by hydrothermal and enzyme debranching treatment and revealed marked changes in physicochemical, functional morphological and thermal properties when compared both the starches. The yield of isolated starch was 16% with a purity of 96% and the amount of RS content was 18.88 %. The isolated starch experienced a restricted swelling and solubility profile which was unstable during freezing and thawing cycles. The starch exhibited a high pasting temperature (81.80ºC) indicating high resistant towards swelling with a peak viscosity of 4507 cP reflecting the ability of starch granules to swell freely. The XRD study clearly revealed culinary banana starch is a mixture of A and B type polymorphs and FT-IR spectra evinced the functional groups present are typical bands of C-type starch with a mixture of spherical and elliptical granules. TGA behaviour showed pyrolysis of starch occurred between the temperature range of 265.39-331.34^{o}C. Further the starch was modified to RS due to retrogradation of the amylose fraction. Temperature cycling and incubation at certain temperature and storage time enhanced the formation of RS. The modified RS were analysed using SEM, FT-IR, DSC and TGA to determine the structural changes. The significant morphological changes were observed with increase in starch concentration and in enzyme debranched RS elicited more distinguished modification. The FT-IR structural changes were less in hydrothermal treatment compared to use of debranching enzyme. In addition debranching enzyme treatment revealed higher rate of initial decomposition with lower weight loss due to crystal formation and modification indicated structural changes and is dependent on type of treatment given. Therefore it is prudent to justify that culinary banana is an excellent source of RS and may be utilized as an alternative source of neutarceutical ingredient for preparing low glycemic functional foods.

B) Effect of partial replacement of wheat flour with type III resistant starch and flour of culinary banana on the chemical composition, textural properties and sensory quality of brown bread

3.6 Introduction

In today's world food is not just consumed to satisfy hunger and provide necessary nutrients but also to prevent nutrition-related diseases and improve physical and mental well-being. The concept of food has experienced radical transformation towards nutritional, sensory, health maintenance, psycho-physical well-being and prevention of diseases.[65,66] Increasing evidence shows that many of the chronic health conditions could be prevented or restrained by dietary changes. The global trends in rising levels of obesity, diabetes and cardiovascular disease has refuelled consumer and research interest in the dietary intake of fat, protein and carbohydrate to maintain good health. Taking into consideration the obese and diabetics, the choice of food in a diet should take into consideration, not only the chemical composition but also their ability to influence postpandrial glycaemia.[67] Postprandial glycemia is a normal physiological response which refers to the elevation of blood glucose concentrations after consumption of food and varies in magnitude and duration.

The treatment of people with diabetes includes the glycaemic index (GI) as a helpful additional indicator regarding the appropriate carbohydrate containing foods for inclusion in the diet.[68] In addition, choosing food according to its GI could help in metabolic activities. Foods rich in fiber are generally considered as low GI foods and its benefit includes lower postpandrial glucose and insulin response, reduce insulin resistance and improve lipid profile.[67] Foods containing resistant starch (RS) gives low GI response because RS is a type of a starch that is not digested in the small intestine instead to the large intestine where it is fermented, thus it resist digestion.[69] This is the reason, why there is no spike in the blood glucose or insulin level when we consume food enriched with RS. The RS intake of 6-12 g in a meal is being observed to beneficial effects on postprandial glucose and insulin level and in addition it has been reported that consumption of 20 g of RS in a day would promote benefits in digestive health including faecal bulking.[70]

The variety of culinary is among one of the few sources of RS. The excellent amount of starch present in it may be modified to RS for its better utilization and value addition. Keeping in view the immense importance of this crop the present study was attempted to develop a brown bread incorporated with RS modified from culinary banana. Ready to eat (RTE) foods also known as convenience food has made people's life easier in today's fast moving world and certainly baked products including breads fall under this category. Bread covers large part of daily human nutrition and also plays an important role in the diet of an ill person aiding their treatment.[71] Hence, the developed brown bread favours low GI health beneficial brown bread enriched with RS. This study will not only help in value addition but also help in identifying the hidden potential of underutilized culinary banana in order to extend the market of this fruit.

3.7 Materials and methods

3.7.1 Raw materials

Unripe culinary bananas were harvested from Tezpur University campus, Assam, India, at matured edible stage (50-55 days after emergence of flower) when the amount of starch was at its optimum level (22.66 %) Prior to the starch isolation the samples were cleaned thoroughly under running tap water followed by rinsing with distilled water and pat dried using a clean cloth in a dirt free room. All the chemicals required for present study was high purity AR grade supplied by HiMedia, India and Merck, India.

3.7.2 Starch isolation and modification in to RS

The starch was isolated following the method described by Bello-Perez et al.[30] (method described in section 3.2.2). In our preliminary experiments we studied the effect of hydrothermal and enzyme debranching treatments on modification of RS, where we found that the RS content in native culinary banana starch was 18.88% which on modification its yield increased to 26.42% in hydrothermal treatment and 31.13% in enzymatic treatment. Therefore, for development of RS enriched brown bread, we have modified RS using hydrothermal treatment to economize the production of RS (method describes in section 3.2.7.1).

3.7.3 Preparation of brown bread incorporated with RS

The materials required for preparing RS brown bread (RSB) were, commercially available whole wheat flour (Brand ITC Aashirvaad; having composition of moisture content 9.23%, protein 4%, sugar 2%, dietary fiber 5%), culinary banana flour (KF) (having moisture content 7.34%, starch 22.66%; protein 3.99%; fiber 1.66%), modified type III RS from culinary banana starch (moisture content 4.92%, fiber 5.81%, protein 1.21%), 6% moist yeast, 3% sugar, 2% salt, refined oil 3% and 4% egg white powder and luke warm water. The combinations of ingredients (wheat flour, RS and KF) are presented in Table 3.6. The selection of ingredients and quantity was determined after conducting preliminary experiments, and optimized bread making procedure as described by Tsatsaragkou et al.[72, 73] The amount of water used for mixing of dough was kept constant (150 ml/ 100 g ingredient) for all combinations and altogether 16 sets of brown bread were prepared with one control (BB-C).

TABLE 3.6 Brown bread ingredients and combination (g/100g)

Sl. No	Sample code	Wheat flour	Resistant starch (RS)	Culinary banana flour (KF)
1	BB-C	100	0	0
2	RSB-5	95	5	0
3	RSB-10	90	10	0
4	RSB-15	85	15	0
5	KFB-10	90	0	10
6	KFB-20	80	0	20
7	KFB-30	70	0	30
8	RSKFB-5-10	85	5	10
9	RSKFB-10-10	80	10	10
10	RSKFB-15-10	75	15	10
11	RSKFB-5-20	75	5	20
12	RSKFB-10-20	70	10	20
13	RSKFB-15-20	65	15	20
14	RSKFB-5-30	65	5	30
15	RSKFB-10-30	60	10	30
16	RSKFB-15-30	55	15	30

3.7.3.1 Brown bread making procedure

The procedure followed for brown bread preparation includes mixing of all dry ingredients properly followed by addition of previously active yeast. The dough was prepared using 150 ml of luke warm water and mixed properly for 5 min to obtain homogenous mixer using hand beater (Philips, HR 1459 300-Watt, India). The dough was kept for proofing at ambient temperature (28±2^{o}C) for 50 min and the bread was baked in a preheated oven at 180^{o}C for 25 min (CS aerotherm rotary rack oven-B1300, India). The bread loaves were cooled to 25^{o}C and sealed in polyethylene zip lock bags for 12 h before determination of chemical, physical and sensory properties. Photographs of the steps involved in the development of brown bread are illustrated in Fig. 3.12.

3.7.4 Chemical composition

The proximate compositions of brown breads were determined according to AOAC[16] methods. The amylose content and RS content were determined by McGrance et al.[17] and an enzymatic method described in AOAC[16] respectively.

3.7.5 Physical properties

3.7.5.1 Proofing of dough

The dough proofing was measured for each combination following the method of Cordoba[74] Dough sample 10 ml were placed in 100 ml measuring cylinder and kept for 60 min at 35^{o}C and increase in the height of dough was checked at every 10 min interval.

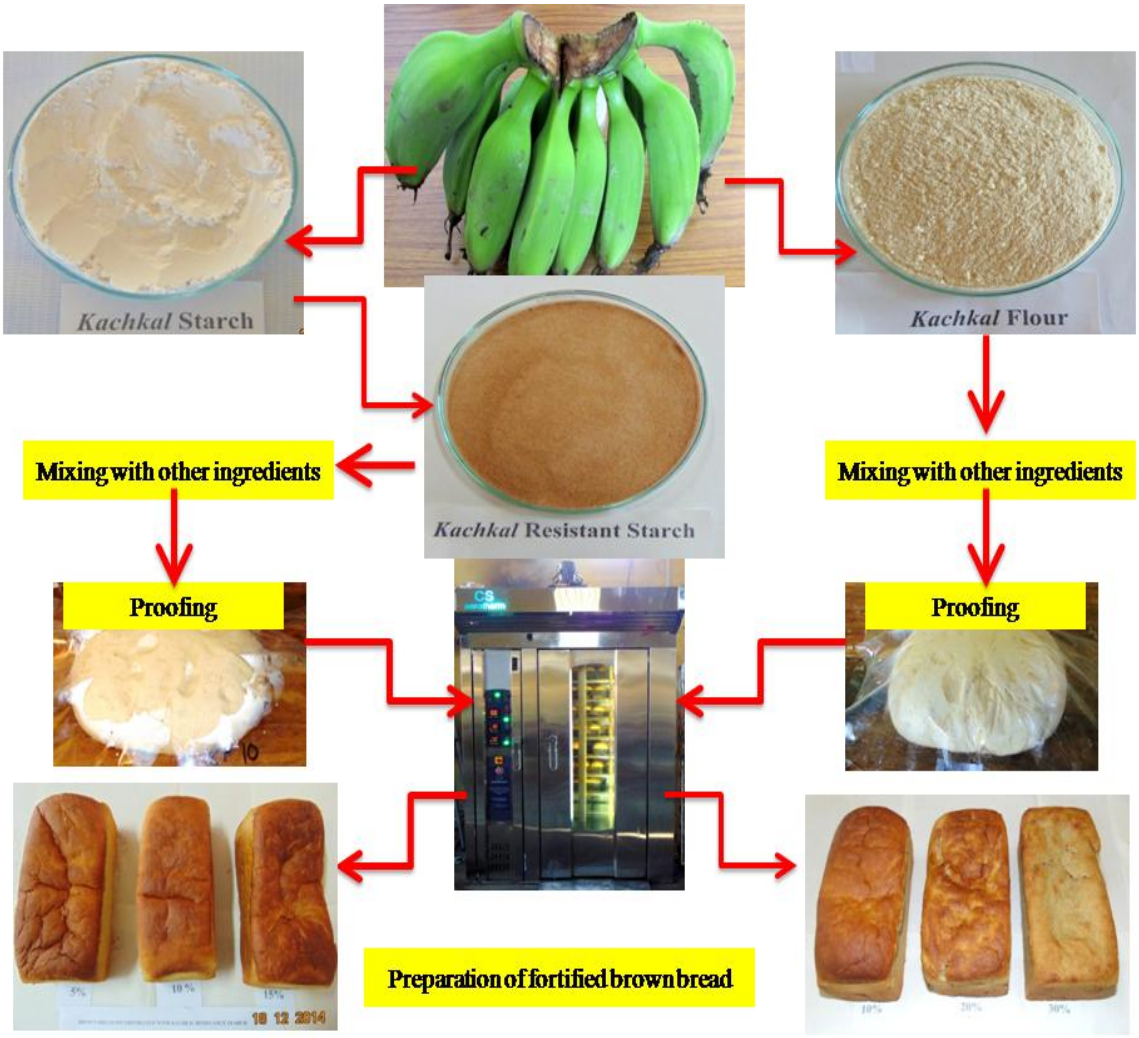

Fig. 3.12 Preparation of brown bread fortified with resistant starch (RS) and flour (KF) from culinary banana

3.7.5.2 Water retention, weight and volume of baked bread loaves

After baking process was over, bread samples were cooled to 25°C and yield of baked bread was calculated as the ratio of weight of sample before and after baking using the following formula:

$$\%\ water\ retention = \frac{W_a}{W_b} x100 \qquad \text{Eq. (3.3)}$$

Where, W_a and W_b are the weights of bread loaf after and before baking respectively. The bread samples cooled to 25°C was weighed using electrical weighing balance having 0.1 g accuracy

(Sumo Digi Tech, India). The specific volume (cm^3/g) of bread samples was evaluated following rapeseed displacement method of AACC[7] given in the following Eq. (3.4)

$$Specific\ volume = \frac{loaf\ volume}{loaf\ weight} \quad \text{Eq. (3.4)}$$

3.7.5.3 Texture profile analysis of bread crumb

Bread samples were analyzed for their extensibility and firmness using texture analyzer (TA-HD plus, Stable Micro System, UK) equipped with 50 N load cell. The sample size of thickness 15 mm was taken from the center of the loaf and compressed at 40% of its initial height with a 25 mm diameter cylindrical probe at a pre-test speed of 1 mm/s, test speed of 1.7 mm/s and post test speed of 10 mm/s. The data acquisition rate was collected at 200 pps (points per second) with relaxation time of 5 s, force 10 g expressed the resistance of crumb to the penetrating probe and represented crumb firmness. The relative elasticity of crumb was evaluated by taking a crumb cube of 2x2x2 cm^3 (length x width x height) from the center of the loaf. A uniaxial compression test with subsequent relaxation phase that lasted for 4 min was applied at 25% compression within the visco elastic region. The relative elasticity (R_{EL}) of crumb (i.e. the force with which the crumb resists the defined mechanical stress during compression) was derived from the recorded force-time diagram and calculated using Eq. (3.5).[75]

$$R_{EL}\ (\%) = \left(\frac{F_{res}}{F_{max}}\right) x100 \quad \text{Eq. (3.5)}$$

Where, F_{max} is the maximum force at 25% compression of the crumb and F_{res} is the residual force after 240 s relaxation phase (N). The textural properties evaluated were hardness, adhesiveness, cohesiveness, springiness and chewiness.

3.7.5.4 Crumb colour

The crumb colour of bread samples were evaluated using tristimulus colour parameters L* (Lightness), a* (redness to greenness) and b* (yellowness to blueness) in digital colorimeter (Ultrascan VIS, Hunterlab, USA).

3.7.6 Sensory evaluation

The sensory evaluation of brown bread was done using 9 point hedonic scale. The panel of 25 judges both trained and untrained was selected among staff members and students of Department of Food Engineering and Technology, Tezpur University, Assam. Judges were explained the definition of quality attributes selected for sensory evaluation, score sheet and method of scoring. They were advised to rinse their mouth with water between tasting the consecutive samples.[76] The major sensory parameters viz. appearance and colour was judged visually, texture was judged through sense of touch, taste and mouth feel was judged by the perceptible character and eating quality.

3.7.7 Statistical analysis

Experiments were carried out in 3 replicates. The data analysis tool 'Microsoft Excel' was used for statistical analysis. Data were subjected to ANOVA and Fisher's Least Significant Difference (LSD) was used to separate means.

3.8 Results and discussion

3.8.1 Effect of RS and KF on chemical composition of brown bread

The isolated starch from culinary banana had yield of 16% with 96% purity which was further modified to type III RS by hydrothermal treatment with 3 repeated autoclaving and cooling cycles. The yield of modified RS was 26.42% with 91.32% purity and its amylose content was 57.98%. The chemical compositions of 16 sets of bread prepared are presented in Table 3.7. Water plays very important role in bread making procedure viz. for dough formation, fluidity of dough, and also acts as a medium for food transport to the yeast through cell membrane. In addition water helps in starch and sugar hydrolysis resulting in gelatinization of starch during baking and also activates enzyme which forms macromolecules in dough responsible for rheological properties of dough. Hence, amount of water added is solely responsible for final quality of bread in terms of textural and physicochemical properties.[77] The

moisture content in all 16 sets of brown bread was ranged between 31.31-36.72% and there was not much difference among the samples. The moisture content recorded is in the general range of moisture present in bread. The moisture in the range of 35-45% in case of gluten free bread from carob flour has been reported by Tsatsaragkou et al.[72] The protein content was in the range of 8.01-11.96% and varied among the sample while fat (3.11-4.97%) did not vary significantly for all the brown bread samples. The use of 3% oil and 4% egg white powder as shortening and protein supplements not only improved the crumb appearance and flavour of brown bread but also affected the final quality and softness.[78] According to the report of Demiralp et al.[79] during dough mixing lipid entrenched in to the protein matrix interacts and resulting the viscoelastic properties of gluten network which is important for dough expansion and retention of gas during proofing. The contents of crude fiber, carbohydrates and amylose were recorded in the range of 5.54-9.79%, 7.65-20.55% and 3.82-4.01% respectively, and all these three parameters varied significantly among the samples. Crude fiber was observed higher in samples incorporated with RS which proves that RS is also considered as fiber in food.[80] The carbohydrate content was less in RS incorporated brown bread and is because of the partial replacement of wheat flour with RS and decreased in amount of carbohydrate present in the bread. The RS content varied from 0.43-13.54% among the samples and the lowest amount was recorded in control bread (BB-C) and differed significantly. The photographs of developed brown bread samples are presented in Fig. 3.13.

3.8.2 Physical properties of brown bread

3.8.2.1 Proofing of dough

The increase in volume of bread dough was measured at every 10 min interval and is illustrated in Fig. 3.14. The increase in loaf volume with respect to time was observed in all samples. As the amount of water use for dough preparation was fixed (150 ml), the similar trend in rising in dough volume in all samples was seen. The lowest increase in bread loaf volume was measured in brown bread prepared by incorporating 30% KF while highest dough proofing was recorded in brown bread prepared with the combination of 10% RS and 10% KF. Proofing of bread also termed as dough maturing or ripening is mainly attributed to the action of yeast. At

this stage starch breaks down and fermentation occurs as yeast produces carbon dioxide gas that causes the gluten network to expand which leaves an open cellular structure with the gases trapped inside loaf. Additionally, yeast also produces alcohol which influences the colloidal nature of the flour protein and alters the interfacial tension within the dough.[81] Correctly proofed dough results in optimum rheological properties of final product with absolute volume and crumb characteristics. The carbon dioxide produced during fermentation helps in dough expansion and in addition, it dissolves in the aqueous phase of dough, and carbonic acid is formed which lowers the pH of the dough.[82] The results of the present study reveal that all the dough samples absorbed water considerably and increased in fermentation capability.

3.8.2.2 Water retention, loaf weight and volume of baked product

The measure of water absorbing or holding capacity of bread is generally considered as water retention of baked products and is measured using Eq. (3.3). In the present study, the recorded water retention was in the range of 78-87% in all the 16 sets of bread samples (Fig. 3.15). Similarly, the total initial weight (W_b) of brown bread ingredients was ~500 g in all samples which after baking, weighed in the range of 393-436 g (W_a) and is illustrated in Table 3.8. It was observed that BB-C (control brown bread) was weighed heaviest with 436 g and RSKFB-15-30 (brown bread incorporated with 15% RS and 30% KF) had minimum weight of 393 g. The amount of water used for dough preparation was constant (150 ml) for all samples. The plausible reason behind this may be there was a non-uniform absorption of water in RSKFB-15-30 bread and in addition, it may also be attributed to amount of water used that helped in sufficient stretching of gluten present in wheat flour in presence of oil and yeast, thus yielding loaf with greater volume in BB-C. In case of RSB-5 RSB-10, RSKFB-5-10 and RSKFB-10-10 showed no significant difference with BB-C and had higher yield and loaf weight because of uniform absorption of water in dough. As reported by Mandala et al.[83] the water retention in baked product is usually decreased on ingredient substitution as well as if higher amount of moisture is present in dough. In our case the decrease in yield may be mainly because of the gluten dilution effect by substitution.[84]

Table 3.7 Chemical composition of fortified brown bread (g/100g)

Sample code	Moisture content	Protein	Fat	Crude fiber	Carbohydrates	Amylose	Resistant starch
BB-C	35.68±2.35^{be}	8.32±0.9^{ac}	3.51±0.01^{a}	6.32±0.45^{af}	15.73±0.97^{cdej}	3.82±0.09^{a}	0.43±0.01^{a}
RSB-5	33.46±2.44^{a}	8.01±0.18^{ab}	3.31±0.01^{a}	7.32±0.48^{def}	12.65±0.76^{c}	4.01±0.06^{ac}	4.8±0.03^{eg}
RSB-10	33.59±3.56^{a}	8.58±0.78^{ab}	3.22±0.12^{abc}	8.86±0.61^{k}	9.55±0.88^{b}	6.67±0.08^{bd}	9.65±0.08^{h}
RSB-15	34.52±3.87^{b}	8.72±0.97^{abc}	3.47±0.18^{a}	9.97±0.36^{l}	7.65±0.73^{a}	7.35±0.14^{bcef}	12.98±0.07^{lm}
KFB-10	35.67±2.49^{bd}	9.28±0.86^{d}	3.73±0.06^{a}	5.54±0.44^{abc}	17.87±0.61^{fghij}	8.32±0.47^{bcd}	1.23±0.13^{b}
KFB-20	36.72±3.28^{bf}	9.79±0.07^{e}	3.88±0.05^{a}	5.90±0.67^{a}	18.43±0.98^{ijkm}	9.28±0.49^{defh}	2.17±0.01^{e}
KFB-30	33.57±3.46^{a}	10.32±0.85^{efghj}	4.01±0.08^{abc}	6.57±0.89^{abchi}	20.55±1.03^{lm}	10.33±0.31^{ghj}	2.96±0.04^{df}
RSKFB-5-10	32.34±2.01^{ad}	9.33±0.76^{df}	3.38±0.06^{a}	6.43±0.18^{abg}	14.32±0.87^{cdghi}	8.67±0.76^{deg}	5.02±0.07^{e}
RSKFB-10-10	33.74±2.48^{a}	9.86±0.62^{egh}	3.42±0.02^{ad}	7.06±0.39^{abcdj}	13.11±0.65^{cef}	9.70±0.08^{fgi}	9.88±0.09^{hij}
RSKFB-15-10	31.37±2.11^{a}	10.27±0.99^{efg}	3.57±0.05^{a}	7.85±0.77^{fghi}	11.03±0.77^{cd}	10.91±0.65^{hik}	13.54±0.11^{mno}
RSKFB-5-20	32.89±2.38^{ae}	10.03±0.95^{ef}	3.61±0.09^{a}	6.39±0.28^{a}	15.97±0.86^{def}	11.57±0.06^{ij}	5.25±0.02^{ef}
RSKFB-10-20	33.78±1.46^{af}	10.86±0.85^{ik}	3.54±0.16^{a}	7.11±0.37^{de}	16.51±0.83^{defgk}	12.27±0.75^{jkm}	9.71±0.06^{hik}
RSKFB-15-20	34.87±2.49^{bc}	11.00±0.98^{ij}	3.76±0.27^{a}	7.97±0.29^{ghij}	12.92±0.65^{c}	12.82±0.54^{kl}	12.97±0.07^{l}
RSKFB-5-30	31.39±2.16^{abc}	11.32±0.99^{ijkm}	3.97±0.08^{ab}	6.12±0.11^{ade}	19.91±0.54^{ikl}	13.38±0.51^{lmo}	5.64±0.09^{fgij}
RSKFB-10-30	33.05±2.13^{a}	11.73±0.89^{l}	4.19±0.64^{abcd}	7.43±0.83^{defg}	17.66±0.97^{fghij}	14.66±0.73^{n}	10.46±0.18^{jkmno}
RSKFB-15-30	32.48±3.19^{a}	11.96±0.84^{lm}	4.97±0.19^{e}	7.75±0.76^{fgh}	16.93±0.39^{efgh}	15.36±0.15^{no}	13.22±0.11^{lmn}

Values reported as mean±SD of three replications; mean followed by same small letter superscripts within a column are not significantly different (p>0.05)

Fig. 3.13 Fortified brown bread samples

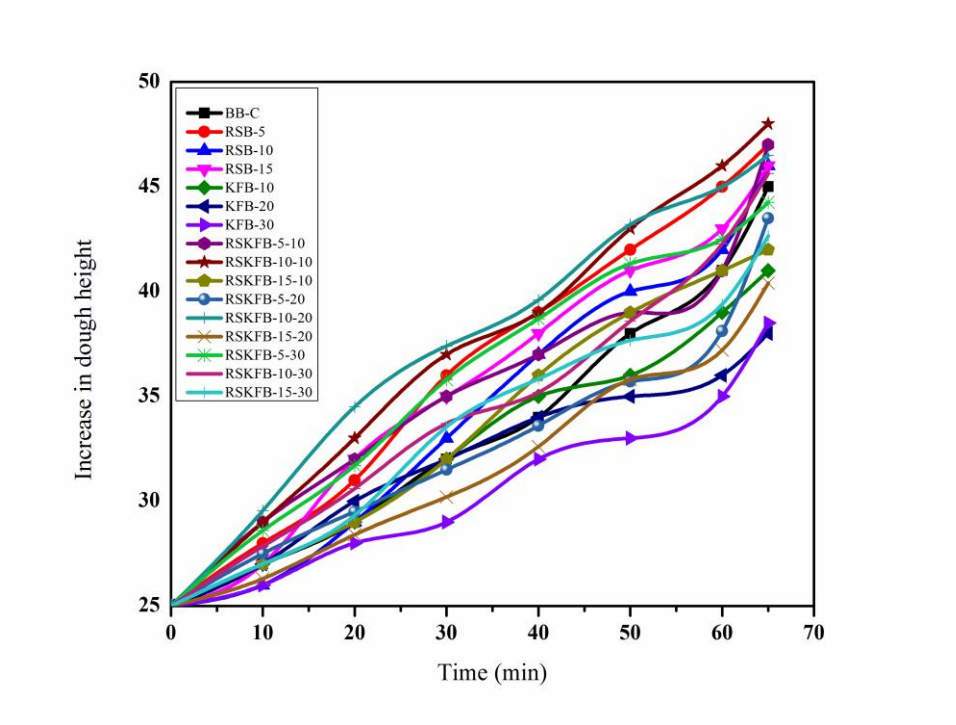

Fig. 3.14 Proofing of dough prior to baking

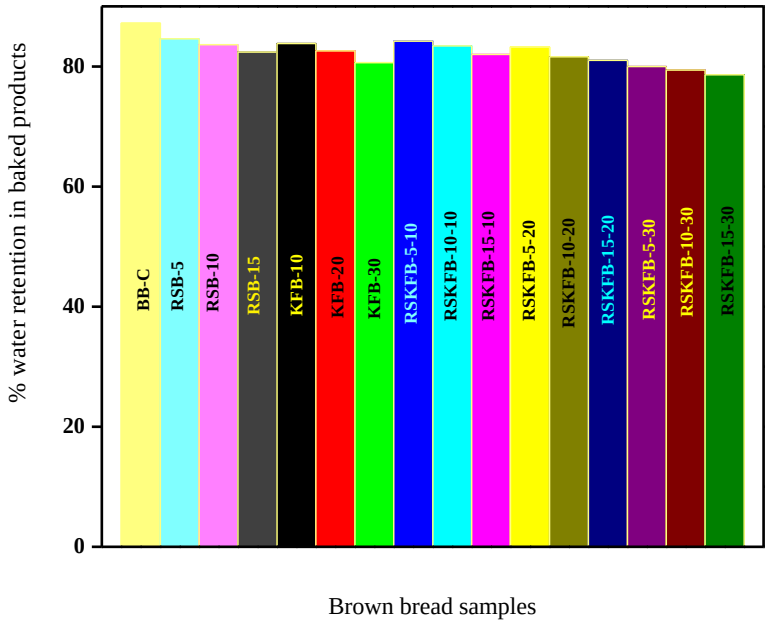

Fig. 3.15 Percentage water retention in baked products

Loaf volume of baked product provides the quantitative measurement of baking performance is one of the key parameter in bread quality. The specific volume of bread samples is a characteristic quality parameter as it indicates dough inflating ability[85] and it was evaluated using Eq. (3.4).The rheological and textural properties of bread dough are significantly affected by loaf volume and specific volume. The greater the bread volume, softer will be the bread with minimum hardness. The loaf volume was recorded in the range of 642.88-322.08 cm^3 and specific volume was 1.47-0.81 $cm^3\ g^{-1}$ in all 16 sets of brown bread samples (Table 3.8). Loaf volume and specific volume are directly related to the weight of the bread and likewise the bread samples with highest percentage of substitute ingredients (15% RS with 30% KF) showed lowest values of loaf volume and specific volume. This is also associated with gluten dilution effect as well as low protein network in the dough.[86] In the present study, as gluten (wheat flour) is substituted with starch (RS and high starch KF) which might have created weak interaction between gluten and starch.[87] On the other hand, when the gluten is substituted with high dietary fiber RS up to 10% with 10% KF, the final product was of fine quality with higher loaf weight, volume and specific volume. This may be attributed to the balance interaction between gluten and non-gluten network took place which causes sufficient production of carbon dioxide gas during proofing and thus the brown bread was of better quality.

Table 3.8 Volume of baked fortified brown bread

Sample code	Loaf weight (g)	Loaf volume (cm^3)	Specific volume ($cm^3\ g^{-1}$)
BB-C	436 ± 2.46^{p}	642.88 ± 4.56^{n}	1.47 ± 0.95^{fg}
RSB-5	423 ± 2.95^{no}	634.23 ± 4.37^{m}	1.49 ± 0.91^{g}
RSB-10	418 ± 2.67^{kl}	612.50 ± 4.42^{l}	1.46 ± 0.94^{f}
RSB-15	412 ± 2.01^{gh}	603.26 ± 4.23^{k}	1.46 ± 0.96^{f}
KFB-10	419 ± 2.55^{lmo}	428.64 ± 3.22^{h}	1.02 ± 0.86^{d}
KFB-20	413 ± 2.23^{hikl}	392.76 ± 3.38^{f}	0.95 ± 0.05^{bc}
KFB-30	403 ± 1.75^{d}	374.91 ± 3.65^{d}	0.93 ± 0.07^{b}
RSKFB-5-10	417 ± 2.17^{jk}	602.33 ± 4.29^{k}	1.41 ± 0.87^{ef}
RSKFB-10-10	421 ± 2.33^{n}	597.42 ± 4.76^{j}	1.44 ± 0.96^{egf}
RSKFB-15-10	410 ± 2.01^{fgi}	588.57 ± 4.28^{i}	1.43 ± 0.63^{e}
RSKFB-5-20	416 ± 2.22^{jm}	417.39 ± 3.87^{g}	1.00 ± 0.08^{d}

RSKFB-10-20	408±1.75^{fh}	381.75±3.85^{e}	0.93±0.05^{b}
RSKFB-15-20	405±1.65^{deg}	374.28±3.33^{d}	0.92±0.07^{bc}
RSKFB-5-30	400±1.55^{ce}	338.76±3.12^{c}	0.84±0.64^{a}
RSKFB-10-30	397±1.34^{b}	331.57±3.56^{b}	0.83±0.54^{a}
RSKFB-15-30	393±1.10^{a}	322.08±3.71^{a}	0.81±0.12^{a}

Values reported as mean±SD of three replications; mean followed by same small letter superscripts within a column are not significantly different (p>0.05)

3.8.2.3 Texture profile analysis

The bread structure is usually solid colloid, where gluten forms interconnected network with numerous pockets of carbon dioxide distributed uniformly throughout the loaf. The baking resulted in characteristic honeycomb texture of final product which can be analyzed using texture analysis. Texture analysis is one of the most important quality parameters primarily concern with measurement of mechanical, rheological and sensory properties of developed brown bread.[88] In the present study, the textural properties studied were hardness (maximum force required to compress bread in first compression cycle), adhesiveness (effort required to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes into contact e.g. tongue, teeth, palate, which can also be said as work required to pull food away from a surface), springiness (also known as elasticity is the ratio of the height the sample springs back after the first compression compared to the maximum deformation), cohesiveness (is the quality of bread that how well the sample withstand a second deformation relative to its resistance under the first deformation) and chewiness (it is the energy required to chew a bread in order to swallow it). The results presented in Table 3.9 revealed that the textural properties were affected by percentage of substituting of ingredients used. The textural quality was poor when wheat flour was substituted with 30% KF. Brown read incorporated with 10% RS and 10% KF had minimum hardness of 3.41N while maximum hardness was observed in RSKFB-15-30 (16.42N). The adhesiveness ranged -0.03 to -0.43g.s and was not observed in all samples. Hence, the minimum negative values confirm that developed brown bread is typically not adhesive.[89]

The springiness or elasticity property of developed brown breads ranged 0.81-1.08 and was minimum and maximum in sample incorporated with 30% KF and RSKFB-10-10 respectively. In order to have good quality bread, springiness should be higher, and substitution of wheat flour with RS and KF up to 10% favoured good quality of bread. Lowest springiness value in KFB-30 confirms low elasticity and expansion of dough.[90] Cohesiveness property of bread is negatively related to the hardness, which means lesser the hardness, higher will be the cohesiveness value and confirmed by our results. The minimum cohesiveness (0.36) was found in RSKFB-15-30 which recorded highest hardness value (16.42N) and on contrary RSKFB-10-10 had maximum cohesiveness value of 0.59 with minimum hardness value of 3.14N. Low cohesiveness value is indicating to loss of intermolecular attraction between bread ingredients with loss of moisture.[91] The chewiness property was directly proportional to the hardness, RSKFB-10-10 exhibited lowest chewiness (192.17N) while maximum chewiness value was observed in RSKFB-15-30 (731.84N). Chewiness is associated with moisture absorption and uniform distribution, and in developed brown bread substituted with 10% RS and KF confirmed uniform interaction of starch and gluten with uniform moisture distribution in dough.

TABLE 3.9 Texture property of brown bread fortified with RS and KF

Sample code	Hardness (N)	Adhesiveness (g.s)	Springiness	Cohesiveness	Chewiness (N)
BB-C	6.11 ± 0.01^{ce}	ND	1.00 ± 0.04^{ef}	0.47 ± 0.07^{cd}	302.45 ± 5.87^{e}
RSB-5	3.96 ± 0.37^{de}	ND	0.99 ± 0.01^{e}	0.57 ± 0.04^{gh}	310.69 ± 3.40^{h}
RSB-10	6.96 ± 0.75^{de}	-0.43 ± 0.02^{h}	1.01 ± 0.06^{fh}	0.57 ± 0.08^{gh}	206.87 ± 2.58^{b}
RSB-15	8.59 ± 0.77^{g}	-0.12 ± 0.03^{d}	0.89 ± 0.07^{bcd}	0.50 ± 0.03^{efij}	393.04 ± 3.56^{g}
KFB-10	4.71 ± 0.02^{b}	-0.17 ± 0.01^{f}	1.01 ± 0.06^{fh}	0.39 ± 0.01^{ac}	311.95 ± 4.61^{f}
KFB-20	5.70 ± 0.05^{cd}	-0.03 ± 0.01^{a}	0.95 ± 0.05^{cdf}	0.44 ± 0.01^{bce}	412.69 ± 4.88^{h}
KFB-30	8.90 ± 0.71^{ghj}	-0.05 ± 0.02^{b}	0.81 ± 0.04^{a}	0.39 ± 0.02^{ac}	290.39 ± 3.45^{d}
RSKFB-5-10	3.83 ± 0.01^{a}	-0.08 ± 0.04^{ce}	1.08 ± 0.08^{gh}	0.59 ± 0.06^{ij}	232.63 ± 2.54^{c}
RSKFB-10-10	3.41 ± 0.03^{a}	ND	1.00 ± 0.07^{ef}	0.59 ± 0.15^{ij}	192.17 ± 2.11^{a}
RSKFB-15-10	9.72 ± 0.86^{ik}	ND	0.99 ± 0.05^{e}	0.48 ± 0.02^{d}	480.58 ± 3.97^{i}
RSKFB-5-20	6.74 ± 0.54^{cd}	-0.12 ± 0.01^{de}	0.93 ± 0.07^{bc}	0.46 ± 0.01^{cf}	297.27 ± 2.34^{ef}
RSKFB-10-20	7.86 ± 0.91^{fh}	-0.40 ± 0.01^{g}	1.07 ± 0.06^{g}	0.58 ± 0.04^{hi}	509.65 ± 3.19^{j}

RSKFB-15-20	10.33±0.97^{ij}	ND	1.01±0.09^{fh}	0.43±0.07^{bd}	389.56±2.16^{g}
RSKFB-5-30	10.93±0.94^{jk}	ND	1.07±0.11^{g}	0.49±0.03^{eh}	592.69±5.29^{k}
RSKFB-10-30	13.75±1.13^{l}	ND	0.99±0.07^{e}	0.49±0.01^{de}	693.16±5.46^{l}
RSKFB-15-30	16.42±1.46^{m}	ND	0.92±0.13^{b}	0.36±0.02^{a}	731.84±6.15^{m}

Values reported as mean±SD of five replications; mean followed by same small letter superscripts within a column are not significantly different (p>0.05)

Relative elasticity is the most important sensory and textural properties of bread are mainly dependent on the quality and the proportion of different ingredients used [calculated using Eq. (3.5)]. The softer bread crumb with higher elasticity is the consumers' requirement. Sample RSBKF-10-10 showed highest relative elasticity and did not differ significantly with control (Fig. 3.16). Samples containing higher amount of culinary banana flour (30%) significantly reduced the relative elasticity of brown bread and may attributed to non-uniform distribution of water in dough because of weak interaction between gluten and starch. Our results are in line with the findings of Tsatsaragkou et al.[72] and Mandala et al.[83]

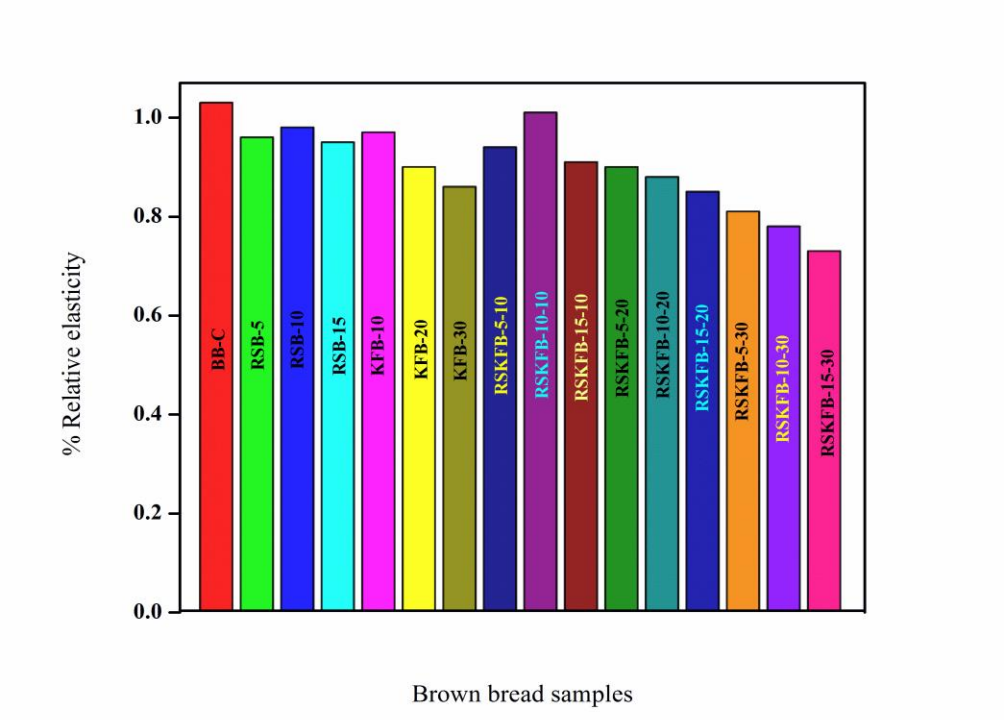

Fig. 3.16 Relative elasticity of brown bread samples

3.8.2.4 Colour attributes

One of the most important quality attributes which defines the acceptability of baked bread by consumer is the colour. During baking of breads, development of colour in crust occurs at the later stages of baking which is generally used to check the completion of baking process.[92] Therefore, follow up of crust colour during baking is necessary to check the defined colour of bread as required by consumers.[93] Colour development in bread is a function of moisture content, baking time and baking temperature. Colour of brown bread samples widely varied with respect to substituted ingredients (Table 5) and all the samples were compared to control, which showed L*, a*, b* values of 56.59, 2.45 and 12.27 respectively. The L* and b* values of bread incorporated with RS (RSB-5, 10 and 15) decreased while a* value increased with increasing percentage of RS from 5% to 15%. On the contrary, with increase in the percentage of culinary banana flour L* and b* values increased significantly and a* value decreased considerably. The colour of the RS (brown) and the KF (white) also substantially affected the crumb colour of the developed brown bread (Fig. 3.14). However, brown bread prepared by incorporating the combination of RS and KF in the balanced proportion did not affect the colour very much. The overall colour of all bread samples were found appealing from the consumer point of view which has been further confirmed by sensory analysis (Table 6).

TABLE 3.10 Colour attributes of fortified brown bread

Sample code	L*	a*	b*
BB-C	56.59 ± 2.12^{kln}	2.45 ± 0.76^{jk}	12.27 ± 1.25^{k}
RSB-5	58.39 ± 2.44^{mn}	2.07 ± 0.79^{df}	11.72 ± 1.01^{j}
RSB-10	52.16 ± 2.31^{e}	2.14 ± 0.38^{e}	9.73 ± 0.97^{fg}
RSB-15	50.12 ± 2.15^{c}	2.16 ± 0.92^{eg}	8.99 ± 0.99^{de}
KFB-10	42.44 ± 1.76^{a}	1.69 ± 0.97^{c}	5.11 ± 0.87^{a}
KFB-20	47.93 ± 2.34^{b}	1.40 ± 0.99^{b}	7.37 ± 0.91^{c}
KFB-30	48.94 ± 1.76^{bd}	1.31 ± 0.95^{a}	8.15 ± 0.97^{d}
RSKFB-5-10	52.71 ± 2.48^{ef}	2.23 ± 0.34^{fg}	9.47 ± 0.94^{f}
RSKFB-10-10	53.15 ± 3.29^{fg}	2.19 ± 0.37^{ef}	9.22 ± 1.31^{efg}
RSKFB-15-10	50.87 ± 2.88^{cdfg}	1.70 ± 0.99^{c}	6.49 ± 0.47^{b}

RSKFB-5-20	57.29±3.56^{lm}	2.39±0.87^{ik}	10.66±1.28^{i}
RSKFB-10-20	57.36±2.51^{m}	2.33±0.75^{hj}	10.17±1.97^{h}
RSKFB-15-20	54.29±1.87^{h}	2.44±0.96^{j}	8.61±0.94^{df}
RSKFB-5-30	57.96±3.76^{m}	2.65±0.97^{m}	10.86±0.99^{i}
RSKFB-10-30	55.08±2.15^{ilm}	2.47±0.29^{kl}	9.31±0.95^{f}
RSKFB-15-30	56.37±2.79^{k}	2.41±0.64^{ijl}	8.28±0.65^{de}

Values reported as mean±SD of five replications; mean followed by same small letter superscripts within a column are not significantly different (p>0.05)

3.8.3 Sensory analysis

In preparation of bread, the sensory properties are altered by role of baking as it directly relates to palatability, taste, aroma and texture. The results of sensory analysis of the developed brown bread samples are presented in Table 3.11 and the sensory attributes differed significantly among samples. The sensory attributes of RSB-5, RSB-10, RSKFB-5-10 and RSKFB-10-10 were highest in all sensory properties with good overall acceptability. The RSKFB-10-10 scored the highest overall acceptability of 8 points which was same as that of control sample. The increased percentage of KF for substituting wheat flour substantially decreased the scores of sensory attributes and overall acceptability was low. Results revealed that sensory attributes were not affected by incorporation of RS and KF up to 10%. One of the reasons of non-accepting of higher percentage incorporation of KF may be the presence of specific smell of the flour which is attributed by high amount of mineral salts present in it. Hence, from the sensory point of view, substitution of wheat flour up to 10% RS and 10% KF can be used successfully for making brown bread without affecting the consumers' acceptability.

TABLE 3.11 Sensory attributes of fortified brown breads

Sample code	Appearance	Colour	Texture	Taste	Mouth feel	Overall acceptability
BB-C	8.75±0.87^{jk}	8.88±0.05^{m}	8.75±0.14^{m}	8.0±0.07^{gh}	8.55±0.85^{j}	8.0±0.57^{h}
RSB-5	8.35±0.75^{g}	8.35±0.07^{k}	7.55±0.07^{i}	8.17±0.05^{gh}	8.0±0.71^{h}	7.85±0.21^{gh}
RSB-10	8.55±0.86^{hi}	8.55±0.08^{l}	7.75±0.31^{k}	8.55±0.16^{i}	8.25±0.44^{i}	7.65±0.45^{g}
RSB-15	8.0±0.76^{f}	7.85±0.07^{h}	6.75±0.03^{e}	8.12±0.07^{g}	7.89±0.58^{hi}	7.0±0.33^{f}
KFB-10	8.0±0.75^{f}	7.15±0.05^{e}	7.44±0.07^{h}	7.55±0.04^{f}	8.15±0.18^{hi}	7.50±0.36^{gh}
KFB-20	6.65±0.71^{d}	6.57±0.03^{d}	7.0±0.16^{f}	5.32±0.01^{d}	4.25±0.36^{d}	5.0±0.05^{d}
KFB-30	5.34±0.90^{b}	4.50±0.06^{a}	5.15±0.11^{c}	4.58±0.03^{c}	3.55±0.06^{b}	3.55±0.07^{c}
RSKFB-5-10	8.56±0.99^{hi}	8.0±0.76^{i}	7.65±0.85^{j}	8.48±0.19^{i}	8.25±0.59^{i}	7.85±0.27^{gh}
RSKFB-10-10	8.85±0.79^{jk}	8.15±0.17^{j}	7.85±0.56^{l}	8.55±0.42^{i}	8.31±0.17^{i}	8.0±0.95^{h}
RSKFB-15-10	8.0±0.54^{f}	7.50±0.11^{g}	7.0±0.09^{f}	8.18±0.65^{h}	7.45±0.08^{g}	6.55±0.38^{e}
RSKFB-5-20	7.67±0.78^{e}	7.45±0.07^{g}	7.15±0.18^{g}	6.45±0.19^{e}	6.57±0.16^{ef}	5.0±0.02^{d}
RSKFB-10-20	7.66±0.43^{e}	7.50±0.52^{g}	7.26±0.05^{h}	6.56±0.18^{e}	6.85±0.04^{ef}	5.21±0.07^{d}
RSKFB-15-20	7.75±0.56^{e}	7.25±0.13^{f}	6.35±0.01^{d}	6.55±0.25^{e}	6.70±0.08^{e}	5.0±0.05^{d}
RSKFB-5-30	5.61±0.58^{c}	5.90±0.02^{c}	4.15±0.07^{a}	3.35±0.02^{a}	3.00±0.01^{a}	2.55±0.01^{b}
RSKFB-10-30	5.35±0.55^{b}	5.58±0.04^{b}	4.56±0.05^{b}	4.15±0.06^{b}	4.25±0.02^{d}	2.00±0.01^{a}
RSKFB-15-30	5.1±0.75^{a}	5.55±0.01^{b}	4.17±0.02^{a}	3.25±0.01^{a}	3.85±0.05^{c}	2.25±0.04^{a}

Values reported as Mean±SD of twenty five replications (judges); mean followed by same small letter superscripts within a column are not significantly different (p>0.05)

3.9 Conclusion

Results of the present study revealed that replacement of wheat flour with 10% resistant starch (RS) and 10% culinary banana flour (KF) is the best combination from the standpoint of various quality parameters for making brown bread. Addition of RS and KF up to 10% significantly improved the quality characteristics of brown bread with balanced nutrient composition, soft texture, better chewiness, high water retention and loaf volume and higher consumer acceptance in terms of appearance, colour, texture and taste. Though samples viz.

RSB-5, RSB-10, KFB-10, RSKFB-5-10 and RSKF-10-10 showed uniform distribution and absorption of water in bread loaf with balance interaction between gluten and non-gluten network, however, RSKFB-10-10 was the best from the consumers' acceptability.

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