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In vitro digestibility studies of cereal flours and starches using purified finger millet (*Eleusine coracana*, ragi, Indaf-15) amylases☆

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Abstract

Purified finger millet α-amylases designated as α-1, α-2 and α-3 were evaluated for their efficiency to digest pregelatinized cereal and finger millet flours as well as starches derived from them. Ragi and rice flours were hydrolyzed to the same extent by all the three amylases (~60%), whereas % hydrolysis in case of wheat and maize flours was found to be around ~50% (α-1), ~45% (α-2) and ~70% (α-3). Cereal starches were hydrolyzed to varied extent [60–65% (α-1), 50–55% (α-2) and 60–70% (α-3)]. Maltotetraose was the major oligosaccharide produced by these enzymes from the aforementioned flours as well as starches after 15 min hydrolysis. Higher oligosaccharides (DP-8 and above) have undergone further degradation after 120 min. α-3 was found to be the most efficient followed by α-1 and α-2 in their hydrolyzing capacity of various cereal starches as well as flours.

Keywords: Amylases; Cereal flours; Starches; Finger millet; In vitro digestibility

1. Introduction

Starch is the principle reserve polysaccharide in cereals and pulses amounting to 55–70% and an important dietary polymer providing energy in the form of glucose. It is a high molecular weight α-D-glucan polymer consisting mainly of a linear molecule namely amylose in α,1,4 linkages and a branched polymer known as amylopectin having branches of α-1,6 linkages (~5%). It contains both crystalline as well as amorphous regions (Banks & Greenwood, 1975).

Amylases are starch degrading enzymes and the degradation of various starches and products released depend upon the nature of the enzyme (α and β) and the complexity of the starch granule with respect to its size, shape, branching, amylose content, and the duration of amylase treatment. Cereal flours and starch digestibility by amylases is an important factor for their use as adjuncts in the brewing industry as well as in the preparation of wheat based products, especially bread. A good deal of research has gone into the study of the kinetics of amylolysis of cereal/pulse/minor millet starch granules. In all these, enzymatic attack was by exocorrosion, i.e. attack from the exterior portion of the granule inward as observed by scanning electron microscopy (SEM) (Bhat, Paramahans, & Tharanathan, 1983; Dronzek, Huwang, & Bushuk, 1972; Evers & McDermott, 1970; Nikuni, 1978; Palmer, 1972).

Finger millet commonly known as ragi (*Eleusine coracana*) is one of the important tropical crops cultivated mostly in parts of Asia and Africa and is consumed by the lower income groups due to its low cost. Ragi is a rich source of dietary fibre in addition to high amounts of calcium, iron and phosphorus compared to most of the other cereals and is used in developing malted, weaning and geriatric food formulations (Subbarao & Muralikrishna, 2001). Malted ragi is a rich source of α-amylases (Nirmala, Subbarao, & Muralikrishna, 2000). Ragi malt has a potential use in bread making and it may be a possible substitute for barley and wheat malts due to its low cost.

Several studies were carried out on cereal/pulse/starches and flours using purified microbial and animal amylases as quoted in the review article on plant carbohydrates

Abbreviations: DP, degree of polymerization; SEM, scanning electron microscopy; DNS, 3,5-dinitro salicylic acid; HPLC, high performance liquid chromatography.

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(Tharanathan, Muralikrishna, Salimath, & Raghavendra Rao, 1987). However, studies were not carried out on a comparative basis of various cereal flours and starches, using purified amylases from minor millet amylases especially from finger millet with respect to their digestibility and their degradation products. Keeping this in perspective, three alpha amylases were purified to apparent homogeneity from finger millet malt and their pH and temperature optima were found to be 4.5–5.0 and 45–50 °C, respectively (Nirmala and Muralikrishna, unpublished results). These enzymes were used to study the degradation pattern of various cereal flours and starches and the results obtained are presented in this communication with respect to their % digestibility, qualitative and quantitative oligosaccharide pattern as analyzed by HPLC along with the morphological characteristics of cereals starches and flours as viewed by SEM.

2. Materials and methods

2.1. Materials

Finger millet (Indaf-15) seeds were procured from V.C. Farm, University of Agricultural Sciences, Bangalore, located at Mandya, Karnataka and used for the entire studies. Wheat, rice and maize were purchased from the local market, Mysore.

General chemicals used in this study were all of analytical grade and obtained from E-Merck, BDH, SRL or SD fine Chemicals. The following special chemicals/items were obtained from various agencies.

Sugar standards; maltooligosaccharides; glucoamylase (*Rhizopus species*), peroxidase (Horse radish), glucose oxidase (*Aspergillus niger*), were from Sigma Chemical Company, St. Louis, USA.

Column for HPLC analysis (μ-Bondapak-NH_2 column) was obtained from Waters Associates, Milford, Massachusetts, USA.

2.2. Malting

Ragi seeds (100 g each) were cleaned and steeped for 24 h and germinated under controlled conditions on moist cloth at 25 °C in a BOD incubator up to 96 h. Germinated seeds were taken out every 24 h and dried at 50 °C in an air oven for 12 h and vegetative portions were removed by gentle brushing (manually). Devegetated seeds were weighed, powdered by using udy cyclone sample mill (Udy Corporation, Colorado, USA) fitted with 0.5 mm screen. 72 h malt was used for amylase extractions as it was found to have maximum amylase activity compared to 24, 48 and 96 h malts (Nirmala et al., 2000).

2.3. Analytical methods

Reducing sugar was estimated by dinitro salicylic acid (Miller, 1959) method. Starch was estimated as glucose oxidase method (Dahlqvist, 1964) after digesting with glucoamylase. Amylose was estimated by iodine binding method (McGrance, Cornell, & Rix, 1998). All the values obtained from the colorimetric estimations are average of three independent experiments.

2.4. Isolation and purification of starch

Rice, wheat, ragi and maize seeds were powdered by using a udy cyclone sample mill (Udy corporation, Colorado, USA) fitted with 0.5 mm screen (cereal flours). Starches were isolated from these flours (100 g each) as described earlier (Muralikrishna, Paramahans, & Tharanathan, 1982).

2.5. Isolation and purification of α-amylases from malted ragi

72 h malted ragi flour (100 g) was extracted with 0.05 M sodium phosphate buffer (w/v 1:4, pH 6.0) containing 1% PVPP for 2 h at 4 °C and supernatant was collected by centrifugation (5850*g*, 4 °C) using refrigerated centrifuge, dialyzed against the extraction buffer and was precipitated with acetone (pre cooled at − 10 °C for 12 h) at 4 °C up to 20% (v/v) saturation. The precipitate was removed by centrifugation and the supernatant was subjected to 75% acetone (v/v) precipitation. Precipitate obtained was redissolved in extraction buffer and loaded on to a DEAE-Sephacel glass column (2 × 40 cm) pre-equilibrated with sodium phosphate buffer (500 ml, 20 mM, pH 6.8) at a flow rate of 12 ml/h and washed with the same buffer to remove unbound proteins. A linear NaCl gradient (0–0.4 M) in equilibrating buffer was used to elute the bound components, which were collected (3 ml each) and monitored for protein (280 nm) as well as amylase activity. The three amylase activity peaks were labeled as A-1, A-2 and A-3 in the order of their elution. A1, A2 and A3 were concentrated and individually loaded on Sephacryl S-200 glass column (1 × 100 cm), pre-equilibrated with sodium acetate buffer (50 mM, pH 5.0) and fractions (1.5 ml) were collected, monitored for protein and amylase activity. Their homogeneity was ascertained by PAGE, SDS-PAGE and Gel filtration. The α-nature of these enzymes was ascertained by various criteria such as their ability to rapidly reduce the viscosity of starch solution and also determining the optical rotation and the nature of the resultant products (Nirmala and Muralikrishna, unpublished results).

2.6. Amylase assay

Amylases (α-1, α-2 and α-3) were assayed using gelatinized soluble starch (1%, 1 ml) as substrate in sodium acetate buffer (50 mM, pH 5.0) Incubating with

appropriately diluted enzyme (50 μl) at 45 °C for 30 min. The reaction was stopped by adding DNS reagent (1 ml) (Bernfeld, 1955). One unit of enzyme activity was defined as 1 μmol maltose equivalent released per minute under the assay conditions.

2.7. Scanning electron microscopic studies

The sample (cereal starch/flour, ragi flour/ragi starch) was spread on a double-sided conducting adhesive tape and pasted on a metallic stub. It was coated with (100 μ) gold in a sputter coating unit for 5 min and observed in the SEM-LEO-435-VP, LEO Electron Microscopy Ltd. Cambridge, UK at 20 kV (Hadimani, Muralikrishna, Tharanathan, & Malleshi, 2001).

2.8. In vitro digestibility of cereal flours and starches

Cereal/ragi flours and starches isolated from them (20 mg/2 ml) suspended in boiling water bath (~96 °C) sodium acetate buffer (50 mM, pH.5.0) were gelatinized at boiling temperature, cooled to 45 °C and incubated with α-1, α-2 and α-3 (5 U each) and aliquots were withdrawn at time intervals of 15, 30, 60 and 120 min and centrifuged to remove insoluble portion if any and the reducing sugar released was estimated by DNS method. The reducing sugar value was taken as % hydrolysis of starch. The above experiments were repeated thrice and the average value is represented.

2.9. Separation and identification of products by HPLC

Amylase digested cereal/ragi flours as well as starches (15 and 120 min) were precipitated with three volumes of absolute ethanol and kept overnight at 4 °C and the undigested residue was separated by centrifugation at 7000 rpm. The supernatants were concentrated by vacuum evaporation and taken in ultra pure water, filtered through millipore filter and analyzed by HPLC on μ-Bonda pak amino column using acetonitrile water solvent system (70:30) as eluant keeping flow rate as 1 ml/min. Glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose served as the standards.

The relative retention time of glucose, maltose and malto oligosaccharides (G_3 - G_7) and area under each peak were taken to identify and quantify the released maltooligosaccharides (McGinnis & Fang, 1980).

3. Results and discussion

3.1. SEM of native starch granules and flours

As a prerequisite to understand the size and shape of the cereal and millet starch granules SEM was carried out both on the native flours as well as starches and the same is depicted in Figs. 1 and 2, respectively. The flour particles are embedded with small amounts of protein bodies and other impurities and as a result the surface seems to be

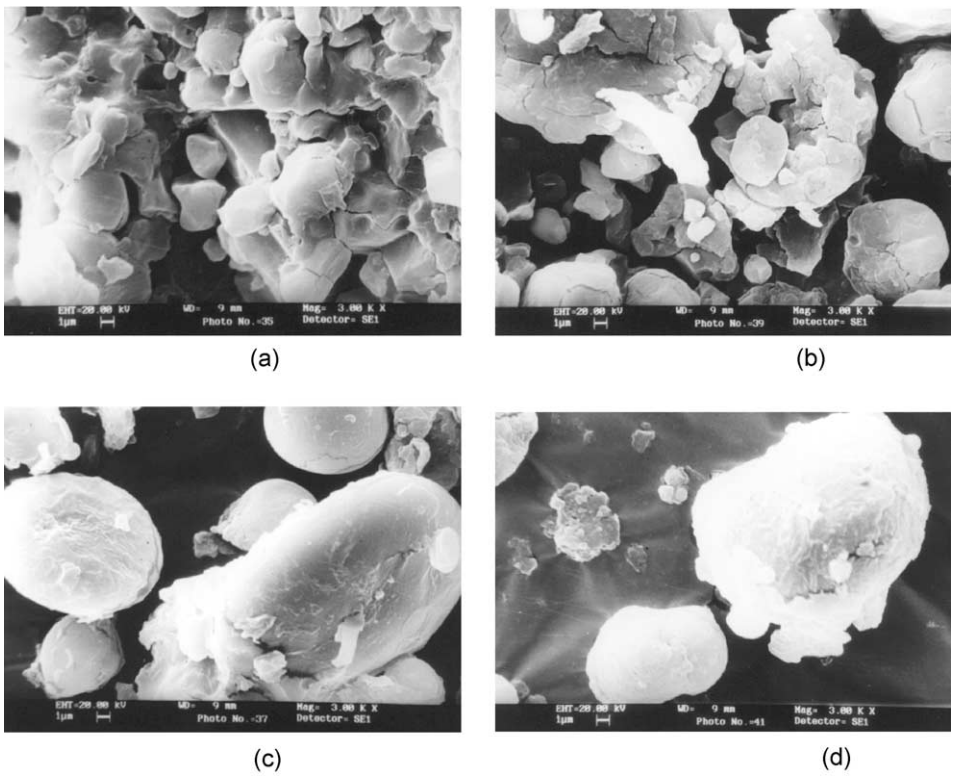

Fig. 1. SEM of cereal flours. (a) Ragi; (b) rice; (c) wheat; (d) maize.

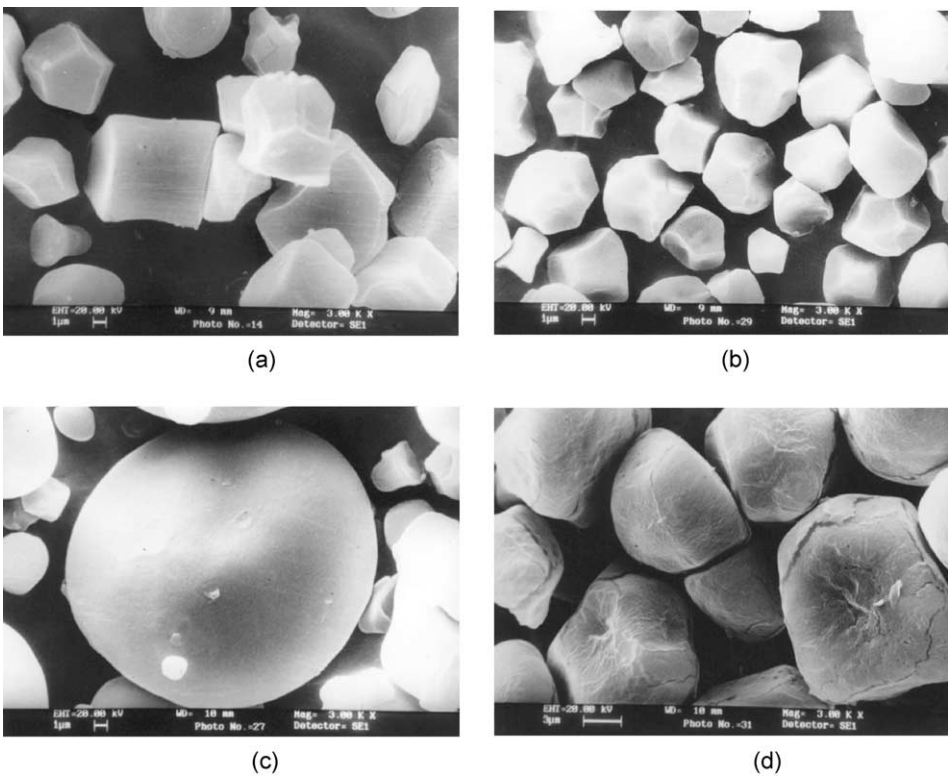

Fig. 2. SEM of cereal starches. (a) Ragi; (b) rice; (c) wheat; (d) maize.

relatively rough and patchy compared to the surface of pure starches (Figs. 1 and 2). Wheat starch consisted of both large as well as small lenticular shaped granules. Rice starch granules were angular whereas maize starch granules were spherical as well as angular in shape and some of the maize starch granules were broken due to the high voltage generated while carrying out the SEM. However, ragi granules are uneven with spherical, polygonal and rhombic shapes. Maize were granules found to be the largest ones followed by wheat, ragi and rice.

3.2. Digestibility of gelatinized cereals flours and starches

Literature reports indicated very little hydrolysis of native starch granules when subjected to hydrolysis by α-amylases isolated from pearl millet (Beleia & Marston, 1981) and wheat and barley (MacGregor, 1983). Even in the present study, the cereal/ragi starch and flour hydrolysis by finger millet amylases without pregelatinization released very negligible amounts of reducing sugar (~1%). Hence the flours as well as starches were pregelatinized and subjected for ragi α-amylases treatment and the results are described below.

3.2.1. Cereal flours

α-1, α-2 and α-3 amylases digested the cereal flours to different extents as shown in Fig. 3. At 15 min the digestion of ragi flour by α-3 was much higher (45%) than by α-2 (42%) and α-1 (33%). Ragi flour was digested by α-1, α-2 and α-3 to more or less same extent after 120 min incubation (60–63%). The % digestibility of rice flour by α-1 and α-2 was almost comparable (60%), whereas the digestibility of wheat and maize was comparatively less than that of ragi flour. However, α-3 digested wheat and maize flours to uniform extent (~70%). Initial digestibility of ragi, rice, wheat and maize flours by α-2 and α-1 at 15 min was comparatively less than that of α-3. However, α-1 and α-2 upon extended digestion (120 min) could release almost the same % of reducing sugar in case of ragi and rice flours, whereas the % of digestibility with respect to wheat and maize flours was substantially less in the case of to α-1 and α-2 compared to α-3.

3.2.2. Cereal starches

In case of cereal starches initial hydrolysis rates by α-3 were higher compared to α-1 and α-2 (Fig. 4). α-3 digested cereal starches much more rapidly at 120 min (66–70%) compared to α-1 and α-2. α-1 (60–65%) was more efficient than α-2 (50–55%). α-1 digested all the cereal starches uniformly as indicated by the % digestibility both at 15 as well as 120 min. α-2 digested cereals starches at 15 min to the extent of 35–43% and % digestibility has increased to 50–55% in all the cereal starches after 120 min. Above results are in tune with the K_m values of α-1, α-2 and α-3 for ragi, rice, wheat and maize starches [(α-1 → 0.59% (ragi), 0.71% (rice), 0.83% (wheat), 1.0% (maize); (α-2 → 1.1% (ragi), 1.33 (rice), 1.2 (wheat) 1.43 (maize); (α-3 → 0.53%

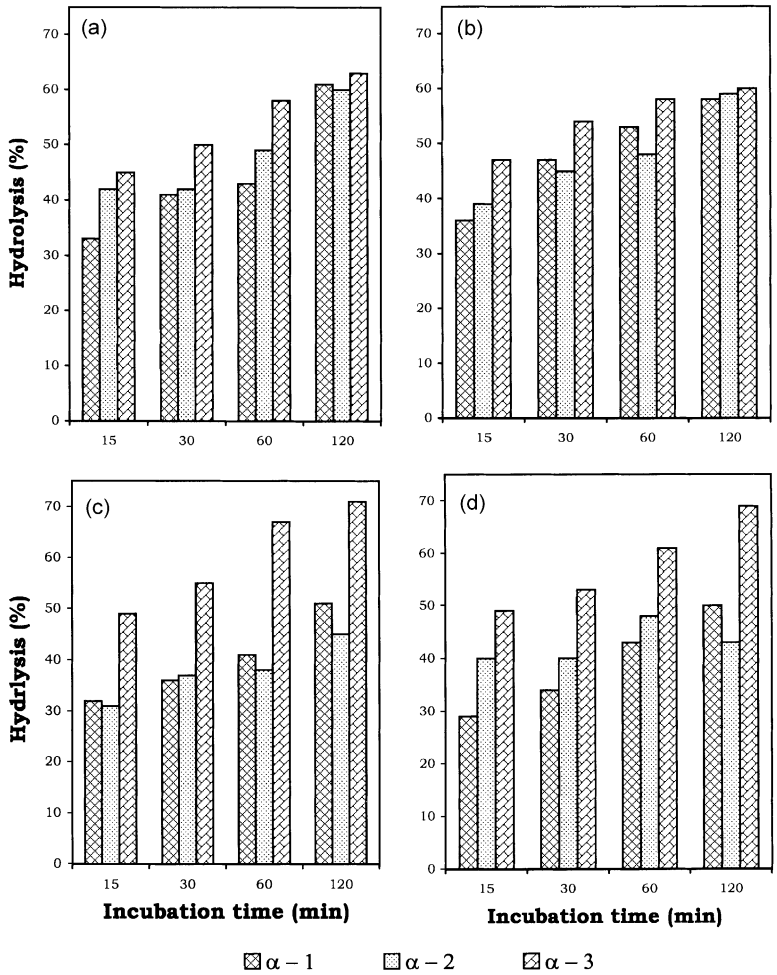

Fig. 3. Hydrolysis (%) of cereal flours by purified amylases. (a) Ragi; (b) rice; (c) wheat; (d) maize.

(ragi), 0.58% (rice), 0.67% (wheat), 1.0% (maize)] (Nirmala and Muralikrishna, unpublished results).

Many studies on hydrolysis of starch by α-amylases were carried out to obtain information on its structure. Often α-amylases from fungi and bacteria are used rather than the cereals because of their ready availability and their capacity to digest cereal starches much more rapidly (Sandstedt & Gates, 1954). However, limited information is available with respect to cereal starch hydrolysis by cereal amylases. α-Amylase from germinated wheat and barley have been studied extensively because of the importance of malted barley in the malting and brewing industries and the deleterious effects of sprouted wheat in the baking industry.

3.3. Identification and quantification of oligosaccharides by HPLC

α-1, α-2 and α-3 released mainly oligosaccharides of G2 to 7 and higher oligosaccharides (G8 and above) from cereal flours such as rice, ragi, maize and wheat and starches derived from them and in different quantities, both at 15 and 120 min. Glucose was not present indicating the absence of any contaminating α-glucosidase activity.

3.3.1. Cereal flours

The % composition of oligosaccharides obtained from cereal flours by digestion with purified ragi amylases is given in Tables 1–3. With respect to cereal flours α-1 and α-2 released mainly G4 followed by G7, G5, G3, G6 and G2. G4 was highest in maize at 15 min followed by ragi, wheat and rice with respect to α-1 whereas its quantity was found to be almost equal in all α-2 cereal flours digests. In case of α-3, initial hydrolysis resulted in high amounts of G4, G5 and G7 compared to G2, G3 and G6. However, after 120 min G4 was the major oligosaccharide accumulated in ragi, rice and wheat flours whereas G5 was much more pronounced compared to G4 and G7 in case of maize flour.

Higher oligosaccharides of G8 and above were substantially less than G2 to G7 indicating (a) the limited accessibility of interior linear chain of starch and (b) probable complexing of amylose with lipids and other

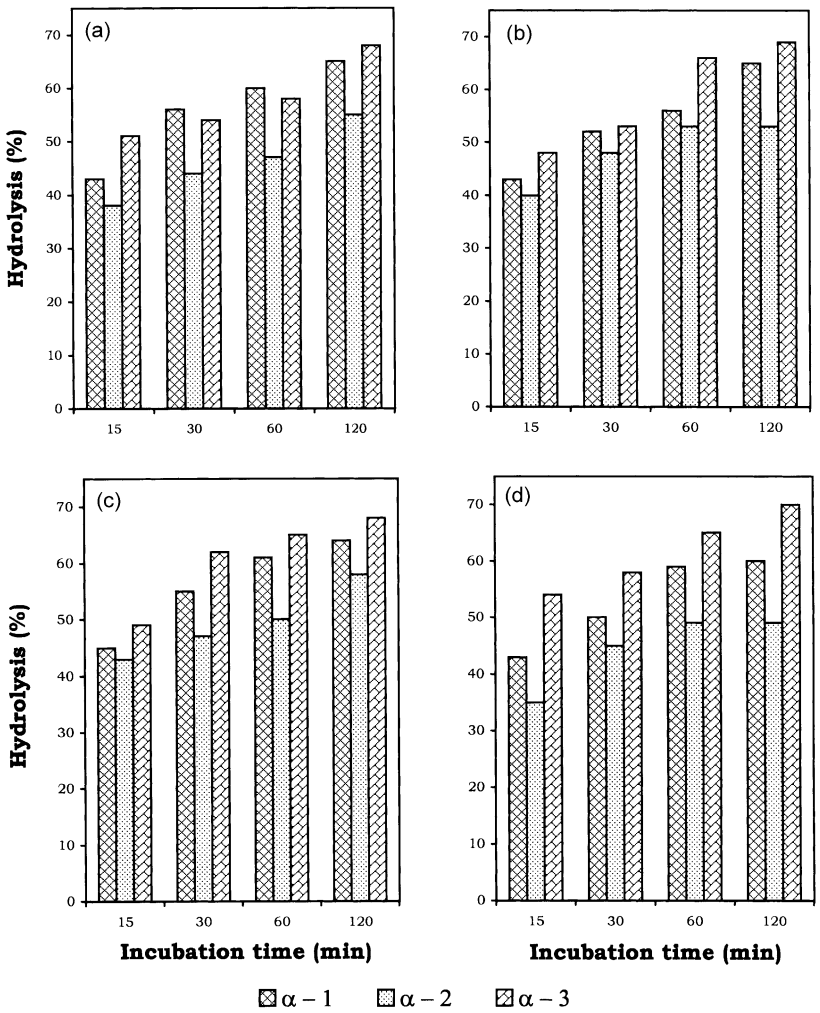

Fig. 4. Hydrolysis (%) of cereal starches by purified amylases. (a) Ragi; (b) rice; (c) wheat; (d) maize.

complex molecules such as non-starchy polysaccharides present in the flour. G8 and above have undergone further degradation resulting in increase of maltotriose and maltotetraose and maltoheptaose after 120 min digestion. In addition the degradation of G5 was also observed after 120 min with respect to α-1 and α-2, however, the content of G5 remain more or less same in case of α-3.

Table 1
Composition (%) of oligosaccharides (as determined by HPLC) obtained from the hydrolysis of cereal flours by purified α-1

Flour	Time (min)	G2	G3	G4	G5	G6	G7	HO > G7
Ragi	15	1.00	9.20	18.60	15.90	6.50	15.40	33.40
	120	2.00	10.00	26.60	9.50	6.60	23.30	22.00
Rice	15	0.90	12.70	14.00	18.60	7.40	14.80	31.60
	120	1.70	10.60	25.00	10.80	6.50	22.40	23.00
Wheat	15	1.20	13.50	18.20	24.60	6.60	13.20	22.70
	120	2.20	10.70	22.60	14.30	4.50	22.20	23.50
Maize	15	1.70	9.50	21.40	16.60	4.00	19.80	27.00
	120	0.40	25.00	21.00	27.20	25.00	–	1.40

G2—maltose; G3—maltotriose; G4—maltotetraose; G5—maltopentaose; G6—maltohexaose; G7—maltoheptaose; HO—higher oligosaccharides.

Table 2
Composition (%) of oligosaccharides (as determined by HPLC) obtained from the hydrolysis of cereal flours by purified α-2

Flour	Time (min)	G2	G3	G4	G5	G6	G7	HO > G7
Ragi	15	1.00	5.40	19.40	14.10	7.20	13.30	39.60
	120	2.50	9.00	26.50	9.30	4.10	21.20	27.40
Rice	15	1.10	5.60	19.70	20.50	7.00	12.00	34.10
	120	2.40	10.00	26.20	11.00	6.20	20.30	23.90
Wheat	15	1.00	6.10	20.10	21.80	6.20	8.70	36.10
	120	2.20	9.00	24.10	8.90	4.20	20.40	31.20
Maize	15	0.50	4.60	19.90	21.80	6.90	11.20	35.10
	120	1.90	9.90	22.20	14.30	6.00	18.20	27.50

G2—maltose; G3—maltotriose; G4—maltotetraose; G5—maltopentaose; G6—maltohexaose; G7—maltoheptaose; HO—higher oligosaccharides.

Table 3
Composition (%) of oligosaccharides (as determined by HPLC) obtained from the hydrolysis of cereal flours by purified α-3

Flour	Time (min)	G2	G3	G4	G5	G6	G7	HO > G7
Ragi	15	0.80	7.70	13.20	21.20	7.30	18.50	31.30
	120	2.90	17.70	25.40	28.00	7.00	10.40	8.60
Rice	15	0.90	8.00	12.70	17.60	6.70	19.40	34.70
	120	2.30	10.60	21.40	16.20	7.20	20.50	21.80
Wheat	15	0.70	7.10	14.70	18.70	7.00	17.50	34.30
	120	2.20	12.00	21.00	15.50	7.10	21.40	20.80
Maize	15	0.80	6.50	12.70	18.20	6.30	17.00	38.50
	120	1.80	13.50	13.70	17.50	7.20	20.30	26.00

G2—maltose; G3—maltotriose; G4—maltotetraose; G5—maltopentaose; G6—maltohexaose; G7—maltoheptaose; HO—higher oligosaccharides.

Among the three amylases, α-1 and α-3 released very high amounts of G4 and G7, whereas α-2 released considerable amounts of higher oligosaccharides (25–31%) after 120 min from all cereal flours. It can be concluded that α-1 and α-3 have more affinity towards the release of oligosaccharides G4 to G7 whereas α-2 is having preference towards higher oligosaccharides (>DP 7) both at 15 and 120 min.

3.3.2. Cereal starches

The pattern obtained from cereal starches differ especially with respect to amounts of G4 and higher oligosaccharides. α-1 and α-3 more or less have similar degradation pattern on cereal starches releasing almost comparable amounts of oligosaccharides of G3, G5 and G7 and above (Tables 4–6). Amounts of G3, G7 have increased after 120 min digestion. There is little variation with respect to G4 after 120 min digestion (2–4%). α-2 released very high amounts of G4 and higher oligosaccharides above G7, which have undergone further degradation to 15–22% extent after 120 min. α-2 released very small amounts of G7 and negligible amounts of G3 and G6, which is in contrast to the oligosaccharides released by α-1 and α-3.

Table 4
Composition (%) of oligosaccharides (as determined by HPLC) obtained from the hydrolysis cereal starches by purified α-1

Starch	Time (min)	G2	G3	G4	G5	G6	G7	HO > G7
Ragi	15	0.80	6.0	20.30	7.00	2.30	7.50	56.10
	120	2.10	10.70	18.20	8.50	3.60	11.70	45.20
Rice	15	0.90	5.70	20.10	6.40	3.10	8.60	55.20
	120	2.30	9.70	15.70	7.30	3.40	11.80	49.80
Wheat	15	0.90	5.10	18.40	6.00	2.60	7.00	60.00
	120	1.90	8.70	16.20	7.20	3.40	12.10	50.50
Maize	15	1.50	6.90	18.50	5.40	3.70	10.00	54.00
	120	3.80	9.60	15.20	7.70	3.10	10.60	50.00

G2—maltose; G3—maltotriose; G4—maltotetraose; G5—maltopentaose; G6—maltohexaose; G7—maltoheptaose; HO—higher oligosaccharides.

Table 5
Composition (%) of oligosaccharides (as determined by HPLC) obtained from the hydrolysis cereal starches by purified α-2

Starch	Time (min)	G2	G3	G4	G5	G6	G7	HO > G7
Ragi	15	0.20	1.60	28	–	–	4.20	66.00
	120	–	–	56.00	–	–	–	44.00
Rice	15	1.60	–	31.40	–	3.70	2.80	60.10
	120	–	–	56.00	–	–	4.00	40.00
Wheat	15	1.00	1.20	33.30	–	3.30	1.40	59.80
	120	–	–	58.60	–	–	1.00	40.40
Maize	15	3.30	2.80	34.10	–	3.60	1.70	54.50
	120	2.10	1.50	49.50	–	–	–	46.90

G2—maltose; G3—maltotriose; G4—maltotetraose; G5—maltopentaose; G6—maltohexaose; G7—maltoheptaose; HO—higher oligosaccharides.

The amount of higher oligosaccharides with respect to α-1 digestion ranged between 45.2 and 50% after 120 min digestion, the lowest is in the case of ragi. With respect to α-2 digestion the higher oligosaccharide contents decreased drastically with respect to ragi, rice and wheat starches after 120 min whereas ~5% decrease was observed in maize starch (54.5–47.0). However, in case of α-3, decrease of higher oligosaccharides with respect to maize and ragi starches was around 20% whereas ~10% decrease was observed in rice and wheat starches after 120 min.

The amount of maltose (G2) released is very less (0.2–3.3%) in all the cereal starch digests even after 120 min indicating the possible absence/very negligible amount of β-amylase activity in all the purified ragi amylase preparations. If α-amylase preparation is contaminated with β-amylases, invariably high amounts of maltose are formed after extended hydrolysis (MacGregor, 1983).

α-Amylase isoenzymes from germinated wheat were compared for their ability to hydrolyze a variety of starch

Table 6
Composition (%) of oligosaccharides (as determined by HPLC) obtained from the hydrolysis cereal starches by purified α-3

Starch	Time (min)	G2	G3	G4	G5	G6	G7	HO > G7
Ragi	15	1.5	7.6	18.6	6.9	3.8	10.60	51.00
	120	3.2	12.2	17.80	10.80	5.50	19.90	30.60
Rice	15	1.10	8.70	22.90	6.70	3.90	12.70	44.00
	120	3.0	10.80	18.00	9.60	5.60	19.10	33.90
Wheat	15	1.20	7.70	18.70	7.10	4.10	10.7	50.50
	120	2.3	10.2	17.1	8.70	4.90	16.30	40.50
Maize	15	1.0	7.50	20.80	7.00	4.50	10.20	49.00
	120	2.8	11.8	16.80	9.60	4.9	23.7	30.4

G2—maltose; G3—maltotriose; G4—maltotetraose; G5—maltopentaose; G6—maltohexaose; G7—maltoheptaose; HO—higher oligosaccharides.

substrates. No significant differences were found in the hydrolysis of β limit dextrin, amylopectin or amylose as indicated by product distribution. At 37 °C only the maltotriose groups adsorbed on larger and smaller granules, both groups solubilized starch, small granules were degraded faster than the larger granules (Kruger & Marchylo, 1985). Hydrolysis product of gelatinized Pearl millet starch after 30 min of digestion ranged from glucose to maltoheptaose. As the reaction progressed high molecular weight dextrin disappeared after 72 h, only glucose and maltose remained (Beleia & Marston, 1981).

α-Amylase purified from malted barley was used to hydrolyze soluble starch and resultant products ranged from glucose to maltoheptaose. However, carbohydrate fragment containing 1–3 glucose units remained after 30 min (Maeda, Kiribuchi, & Nakamura, 1978a; Maeda, Nikuni, Taniguchi, & Nakamura, 1978b). This is perhaps due to the addition of higher enzyme concentration rather than the difference in enzyme source, and hydrolysis products were essentially the same as observed in the case of pearl millet amylase digestion (Beleia & Marston, 1981).

Work carried out elsewhere indicated that the malted rye and barley enzymes hydrolyzed starches initially to G2, G4, G7 followed by the hydrolysis of G7 to larger amounts of G2 and small amounts of G1 and G3. Action of two isoenzymes I and II from germinated barley on maltodextrins was investigated. And the results showed similar actions and they hydrolyzed mainly or almost exclusively G4 into G2, G5 into G2 and G3, G6 into G2 and G4, G7 into G1 and G6, G8 into G2 and G6 and G9 into G2 and G7. They appear to form G1 more than other cereal amylases. However, these α-amylases showed slight variation with respect to the hydrolysis of G10; α amylase 1 hydrolyzing G10 into G3 and G7 while α-amylase 2 hydrolyzing G 10 in to G2 and G 8 (Manners & Marshall, 1971). Non-availability of high amounts of pure authenticated higher oligosaccharides is a limiting factor in the present study to see their degradation pattern by purified ragi amylases.

Cereal α-amylases degrade amylose or starch by the multichain attack mechanism (Banks & Greenwood, 1975; Banks, Greenwood, & Khan, 1970). During the initial phase of hydrolysis amylose is degraded into a mixture of maltodextrins in which G7 and higher oligosaccharides predominate. As hydrolysis proceeds large dextrins disappear leaving a mixture of G6 and smaller dextrins (MacGregor, Thompson, & Meredith, 1974; Okada, Kitahara, Higashihara, & Fukumoto, 1969). G6 accumulated in digests of amylose hydrolyzed by amylase from oats, rye, wheat, and malted wheat and malted barley (Greenwood & MacGregor, 1965; Greenwood & Milne, 1968a,b). Cereal α-amylases hydrolyze β-limit dextrin at a reduced rate compared to amylose resulting in branched saccharide, 6^{3-} α-D-glucosyl maltotriose (Manners, 1962; Manners & Bathgate, 1969). In the present study 4^{3-} α-D-glucosyl maltotetraose was preponderant in starch/flour digests and the amount of isomaltotriose was negligible. Evidence for higher oligosaccharides (>DP4) with α-1,6 branch points could not be documented due to the non-availability of authenticated reference standards.

The variety of oligosaccharides as the products from α-amylase digestion indicates that all the glycosidic bonds are not equally susceptible to amylase attack. This idea was refined by Greenwood and Milne (1968b) and they suggested that (a) the five bonds nearest to the non-reducing end of a saccharide molecule are resistant to amylase, (b) the bonds at the reducing end is half as likely to be attacked as other bonds, (c) the penultimate bond at the reducing end is twice as readily hydrolyzed as other bonds, and (d) all other bonds are equally readily hydrolyzed.

Amylose content and the size of the starch granules were reported to have bearing on the digestibility of some starches by microbial enzymes, i.e. higher the amylose content lower the digestibility, smaller the granule better the digestibility (Madhusudhan & Tharanathan, 1995a,b). However, results emanated from this investigation clearly indicated that, neither the size nor the amylose content of the starches (rice 25.2, wheat 28, maize 35, ragi 30%) have any visible impact on the digestibility of cereal starches using finger millet amylases as evident from the above study.

4. Conclusion

The present study has indicated the efficiency of purified ragi amylases with respect to the digestion of cereal flours and starches as revealed by the percentage hydrolysis and nature of the oligosaccharides released. It is very clear from the studies that amylase α-3 is more efficient than α-2 and α-1 with respect to its ability in digesting the cereal starches. Ragi malt due to its high amylolytic activity which efficiently digests both cereal flours as well as starches can be exploited in the bread making industry.

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