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Food Chemistry 69 (2000) 175–180

Food Chemistry

www.elsevier.com/locate/foodchem

Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf-15)☆

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Received 19 April 1999; received in revised form and accepted 16 September 1999

Abstract

A recently released hybrid ragi, Indaf-15 was germinated up to 96 h at 25°C and the sprouts, drawn at 24 h intervals, were dried, devegetated, powdered and evaluated for malting loss, reducing sugar, free sugar profile, starch content, dietary fibre and an array of carbohydrate-degrading enzymes. Malting loss was maximum (32.5%) at 96 h. The total reducing sugar content increased from 1.44 to 8.36%, whereas the total carbohydrate content decreased from 81 to 58% at 96 h of germination. Analysis of 70% alcohol-soluble sugars revealed glucose, fructose and sucrose in different proportions with respect to germination time. Maltose and maltotriose were detected after 48 and 72 h, respectively. There was a linear decrease in starch content (from 65 to 43%). Activities of amylase and pullulanase were maximum at 72 h whereas those of α–D-glucosidase and 1,3–β–D-glucanase, were maximum at 48 h. Xylanase activity was maximum at 96 h with a concomitant decrease in arabinose to xylose ratio from 1:1 to 1:0.38 in the dietary fibre. α-Galactosidase activity was negligible, which is in tune with a very small amount of raffinose series oligosaccharides. The above results indicated that Indaf-15 is a potential variety for malting purposes as it develops high levels of amylases during germination, and its malt form is a rich source of reducing sugar.

1. Introduction

Finger millet (*Eleusine coracana*, popularly known as "ragi" in India) is an important millet and its malting has been practised both at household and industrial level in India and some of the African countries. Nutritionally, its beneficial role is well recognized because of its high content of calcium and dietary fibre (Ravindran, 1991). Malted ragi flour, or extracts derived from it, are extensively used in the preparation of weaning and infant foods, beverages or other pharmaceutical preparations (Narayanaswamy, Somakurien, Daniel, Rajalakshmi, Swaminathan & Parpia, 1971). Many high-yielding varieties of ragi were developed and released and evaluation of their relative malting quality, with specific reference to their use in weaning food, was reported (Malleshi & Desikachar, 1979, 1982). Studies on malting preliminary characteristics of finger millet were carried out in comparison with sorghum and barley (Nout & Davies, 1982).

The studies on malting of ragi, carried out at CFTRI, indicated variation in free sugars and non-starch polysaccharides upon germination (Malleshi, Desikachar & Tharanathan, 1986). Recently, changes in carbohydrates, free amino acids, organic acids, phytate and HCl-extractable minerals, during germination and fermentation of finger millet, have been reported (Sripriya, Antony & Chandra, 1997). However, detailed information pertaining to changes in carbohydrates and their degrading enzymes is lacking. The present investigation reports the changes in some of the carbohydrates (70% alcohol-soluble sugars, starch, and dietary fibre) and their degrading enzymes (amylase, pullulanase, α-glucosidase, xylanase, arabinase, 1,3-β-D-glucanase, sucrase and α-galactosidase) during malting of a new variety of finger millet, Indaf-15.

2. Materials and methods

2.1. Materials

Finger millet (Indaf-15) seeds were procured from V.C. farm of University of Agricultural Sciences, Bangalore, located at Mandya, Karnataka and used for the studies. All the chemicals used were of analytical reagent

☆ Presented at the 4th International Food Convention (IFCON) held at CFTRI, Mysore, India, 23–27 November 1998.

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0308-8146/00/$ - see front matter
PII: S0308-8146(99)00250-2

grade. Laminarin, larchwood xylan, pullulan, glucoamylase (E.C.3.2.1.3), D-glucose oxidase (E.C.1.1.3.4) and peroxidase (E.C.1.11.1.7) were from Sigma Chemical Co., USA. The HPLC column (μ-Bondapack carbohydrate) and GLC column (OV-225) were obtained from Waters Associates, USA and Pierce Chemical Co., Rockford IL, USA, respectively.

2.2. Methods

2.2.1. 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 growth portions were removed by gentle brushing (manually). Devegetated seeds were weighed, powdered and used for the experiments along with ungerminated ragi flour as control.

Total malting loss was calculated by using the following formula

$$\%\ \text{total malting loss} = \frac{W_1 - W_2}{W_2} \times 100$$

where W_1 = initial weight, W_2 = weight after removing growth portion.

2.2.2. Analytical determinations

Total carbohydrate content of the samples, reducing sugar content of 70% alcohol-extractable sugars, as well as the sugars liberated from different enzymatic assays were assayed by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and DNS method (Miller, 1959), respectively. Starch was determined enzymatically by digesting with glucoamylase and the resultant glucose was assayed by the glucose oxidase method (Dahlqvist, 1964).

2.2.3. Isolation and characterization of free sugars

The free sugars from native and germinated flours were extracted with 70% aqueous ethanol, concentrated and purified successively on Dowex (H^+ 50W) and (OH^- 1×8) resins to remove cationic as well as anionic contaminants. The purified sugars were concentrated and passed through a millipore filter, resolved and identified by high performance liquid chromatography (C-R4A, Shimadzu) using a μ-bondapack carbohydrate column (4.1 mm × 30 cm) using acetonitrile–water (80:20) solvent system at a flow rate of 1 ml/min (McGinnis & Fang, 1980). Glucose, fructose, galactose, maltose, sucrose, maltotriose, raffinose and stachyose were the reference sugars. The area under each peak was measured to quantify individual sugars. Under the experimental conditions, glucose and fructose were eluted together due to their close retention times.

2.2.4. Isolation and determination of composition of dietary fibre samples

Dietary fibre was isolated from native and malted flours according to the method of Asp, Schweizer, Southgate and Theander, (1992). The isolated dietary fibres were suspended in water (0.5 ml) and solubilized with concentrated sulphuric acid (0.6 ml) at ice-cold temperature, after which the concentration of sulphuric acid was brought down to 8.0% by the addition of water. The above mixture was refluxed in a water bath for 10 to 12 h, volume was made up to 20 ml, neutralized with barium carbonate, concentrated, deionized and reduced with sodium borohydride. Alditol acetates were prepared according to the method of Sawardekar, Slonekar and Jeanes (1965) and the component sugars were separated and identified on a 3% OV-225 (1/8″ × 6′) column using a Shimadzu 14-B gas-liquid chromatograph equipped with flame ionization detector at 200°C column temperature and 250°C injector and detector port temperatures, respectively. Nitrogen (40 ml/min) was used as the carrier gas. A sugar mixture consisting of rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose, was used as a reference of sugars and inositol was the internal standard.

2.2.5. Enzyme assays

Different enzymes were extracted in the following ways. Five grammes of native/germinated ragi flours were extracted either with (a) 0.05 M sodium phosphate buffer (pH 6.0) for total amylase (E.C.3.2.1.1 and 3.2.1.2), pullulanase (E.C. 3.2.1.33), α-glucosidase (E.C. 3.2.1.20), 1,3-β-D-glucanase (E.C. 3.2.1.59) and sucrase (E.C. 3.2.1.26) and (b) 0.1 M sodium acetate buffer (pH 4.8) for obtaining xylanase (E.C. 3.2.1.32), arabinase (E.C. 3.2.1.55) and α-galactosidase (E.C. 3.2.1.22) at 4°C for 2 h and dialysed against respective enzyme–assay buffers. The following substrates and conditions were employed for assaying the above enzymes.

2.2.5.1. Amylase. 1% soluble starch, 0.05 M sodium phosphate buffer pH 6.0 at 55°C for 1 h (Bernfeld, 1955). One unit of activity is defined as the amount of enzyme that catalyzes the liberation of reducing sugar equivalent to 1 μmol of maltose/min under the assay conditions.

2.2.5.2. Pullulanase. 1% pullulan, 0.1 M sodium acetate buffer pH 4.6 at 35°C for 2 h (Norman 1982). One unit of activity is defined as the amount of enzyme releasing 1 μmol maltotriose/min under the assay conditions.

2.2.5.3. α-D-Glucosidase. 2 mM maltose, 0.1 M sodium acetate buffer pH 4.6, at 35°C for 15 min (MacGregor & Lenoir, 1987). One unit of activity is defined as the amount of enzyme that catalyzes the liberation of 1 μmol of glucose/min under the assay conditions.

2.2.5.4. Xylanase. 0.5% larch wood xylan, 0.1 M sodium acetate buffer pH 4.8, at 50°C for 1 h (Cleemput, Hassing, Vanoort, Deconynck & Delcour, 1997). One unit of activity is defined as the amount of enzyme required to liberate 1 μmol of xylose/min under the assay conditions.

2.2.5.5. Arabinase. 0.25% cowpea arabinan (isolated and purified according to Muralikrishna & Tharanathan, 1986) used as the substrate to assay the enzyme by adopting the procedure followed for xylanase at 40°C. One unit of activity is defined as the amount of enzyme required to liberate 1 μmol of arabinose/min under the assay conditions.

2.2.5.6. 1,3-β-D-Glucanase. 0.2% laminarin, 0.05 M sodium acetate buffer pH 4.8, at 38°C for 4 h (Fink, Liefland & Mendgen, 1988). One unit of activity is defined as the amount of enzyme required to liberate 1 μmol of glucose/min under the assay conditions.

2.2.5.7. Sucrase. 143 mM sucrose, 0.08 M sodium acetate buffer pH 4.8, at 37°C for 1 h (Geracino & Whitaker, 1990). One unit of activity is defined as the amount of enzyme required to liberate 1 μmol of reducing sugar equivalent/min under the assay conditions.

2.2.5.8. α-Galactosidase. 0.5% raffinose, 0.1 M sodium acetate buffer pH 4.8, at 40°C for 1 h (McCleary, 1988). One unit of activity is defined as the amount of enzyme required to liberate 1 μmol of reducing sugar equivalent/min under the assay conditions.

3. Results and discussion

3.1. Malting loss

Malting loss increased with increase in time and was maximum (32.5%) at 96 h of germination (Fig. 1). This is slightly higher than the reported values for the other Indaf varieties (Malleshi & Desikachar, 1979). In the case of Imele variety (Nout & Davies, 1982) the malting loss was considerably less compared to the present study. The loss in dry weight is mainly due to the increase in metabolic activity and the partial degradation of carbohydrate material. In the present study the total carbohydrate decreased from 81 to 58% in 96 h malted flour (Fig. 1).

3.2. Starch and starch-degrading enzymes

Starch content decreased from 65 to 43% after 4 days of malting (Fig. 1). Earlier reports mentioned a decrease of 10% starch at 24 h (Sripriya et al., 1997). The reduction in starch content varied from 33 to 58.4% in 96 h-

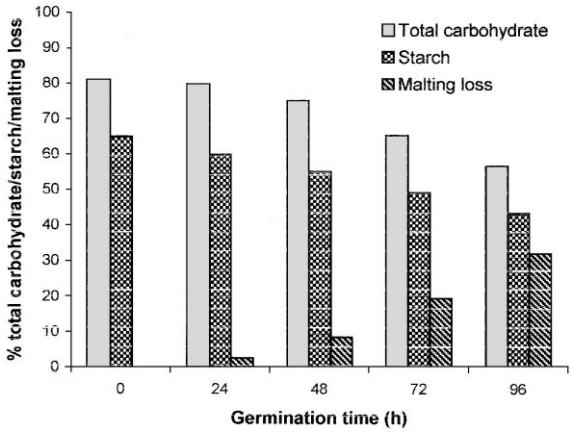

Fig. 1. Changes in total carbohydrate/starch/malting loss during germination.

germinated sorghum cultivars (*Sorghum bicolor*) as compared with the values for ungerminated grains (Subramanian, Murty, Sambasiva Rao & Jambunathan, 1992), which is slightly higher than the present study. In germinating barley, the decrease of starch content was around 65% (Morrall & Briggs, 1978) which was substantially higher than the present study. Barley is known to have the highest amylase activities among cereals. In the present case, the amylase activity was maximum at 72 h and thereafter a slight decrease was observed (Fig. 2). The maximum development of amylase activity usually occurs after 6 to 7 days in wheat, 4 days in corn and after 3 days in millets (Chavan & Kadam, 1989). Prolonged germination beyond 96 h decreased amylase activity in different sorghum cultivars (Subramanian et al.) α-Glucosidase and pullulanase activities were 20- and 600-fold less than amylase and were maximum at 48 and 72 h, respectively. In malted barley, α-glucosidase activity was 1300 times less than that of amylase (Sim & Berry, 1996).

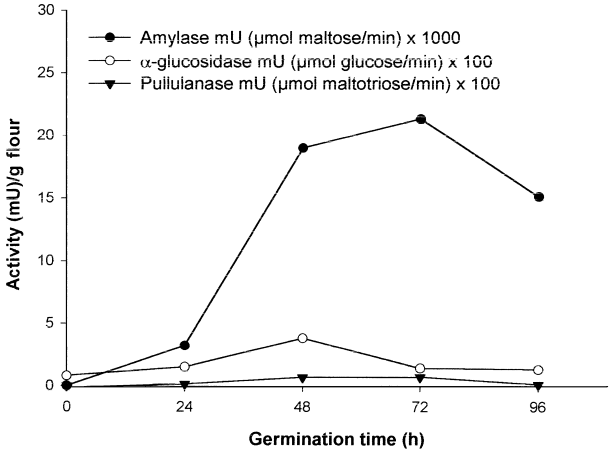

Fig. 2. Changes in starch degrading enzyme activities during germination.

3.3. Changes in soluble sugars

The total and reducing sugar contents, in 70% alcohol-extracted samples, increased from 1.50 to 16.0% and 1.44 to 8.36%, respectively, upon 96 h of germination (Fig. 3). The difference in total carbohydrate to reducing sugar is not only due to non-reducing sugars such as sucrose, but also from the non-reducing end groups of higher oligosaccharides. Upon purification, the yield of sugars was substantially decreased because of the removal of ionic compounds such as phosphorylated sugars on Dowex-ion-exchange columns. Analysis of the purified sugars by HPLC indicated glucose, fructose and sucrose in different yields (Table 1). A 95- and 378-fold increase was observed in hexoses (glucose and fructose) and sucrose, respectively. Increase in sucrose content up to 72 h, along with glucose and fructose, is not surprising since earlier reports (Nomura, Kono & Akazawa, 1969) suggested the transport of glucose to scutellum where it was converted to sucrose by sucrose synthase. But sucrose content decreased slightly at 96 h compared to 72 h of germination, which can be explained by the rise in sucrase activity (Fig. 4). Maltose was observed after 2 days whereas maltotriose was detected after 3 days of germination. Maltose and maltotriose were not present in ungerminated flour and have originated from the degradation of starch by the action of amylase and pullulanase. Raffinose series oligosaccharides and galactose were absent in the germinated flour which was substantiated by a negligible activity of α-galactosidase (data not shown). This agrees well with the earlier reports on cereals containing low amounts of raffinose series oligosaccharides, compared to the pulses (Kruger, 1989).

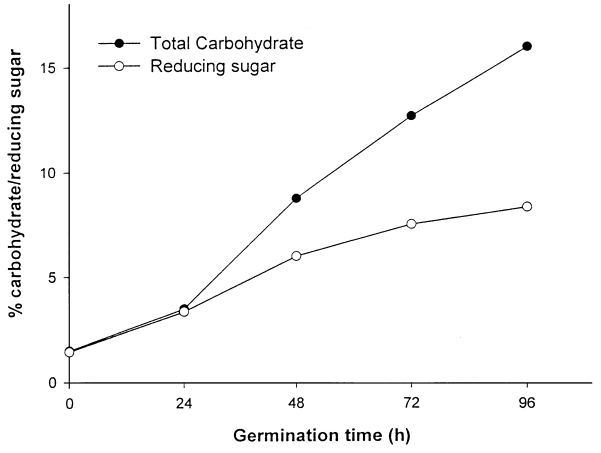

Fig. 3. Changes in 70% alcohol-soluble sugars during germination.

Table 1
70% alcohol-soluble sugars profile in native and germinated samples by HPLC (per g flour)

Germination time (h)	Purified sample (mg/g)	Glucose/ fructose	Sucrose	Maltose	Maltotrise
Control	0.32	0.29	0.03	N.D.[a]	N.D.
24	0.51	0.42	0.09	N.D.	N.D.
48	2.31	1.42	0.75	0.13	N.D.
72	25.4	10.5	12.0	1.90	0.89
96	42.8	27.1	11.7	3.00	1.00

[a] N.D. not detected.

Very few studies have been carried out on purification and characterization of free sugars by HPLC. Free sugar contents of good and poor malting varieties of wheat and their malts, analyzed by HPLC, revealed glucose, maltose, sucrose in addition to the presence of small amounts of raffinose (Suhasini, Muralikrishna & Malleshi, 1997). Increases in free sugars and loss of raffinose during germination of cereals have also been reported in wheat (Kruger & Matsuo, 1982), barley (Woollard, Rathbone & Novellie, 1977), sorghum (Aisien, 1982) and millets (Malleshi et al., 1986).

3.4. Dietary fibre and cell wall degrading enzymes

A marginal decrease in the yields of dietary fibre was noticed. Dietary fibre analysis of native and malted ragi samples revealed the presence of high amounts of arabinose, xylose and glucose (90%) and small amounts of galactose, mannose and rhamnose (10% (Table 2). Major polysaccharides present in most of the cereals are arabinoxylans, β-D-glucans and cellulose (Fincher & Stone, 1986) along with minor polysaccharides such as arabinogalactans and glucomannans.

Arabinose to xylose ratio decreased from 1:1 to 1:0.38 only at 96 h of germination, indicating the degradation of backbone of arabinoxylans, which tallies with the high xylanase activity at 96 h of malting (Fig. 5). Arabinase activity was also noticed in the malted samples; however, there was hardly any change in the arabinose content as it did not vary up to 72 h. A three-fold increase in arabinose content was noticed in 96 h-germinated samples, which was perhaps only apparent, but not actual

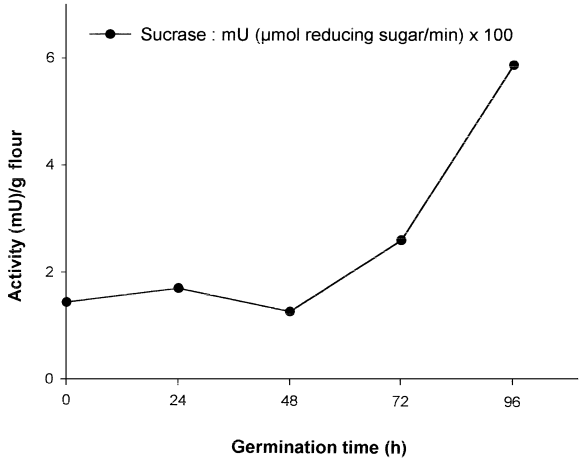

Fig. 4. Changes in sucrase activity during germination.

Table 2
Sugar composition (%) of dietary fibre samples by GLC[a]

Germination time (h)	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose	Ara:Xyl:Glc ratio
Control	00.57	08.60	08.53	02.50	03.50	76.3	1:0.99:8.8
24	02.00	11.6	12.0	03.40	05.00	66.0	1:1.03:5.7
48	00.78	11.3	12.0	01.00	03.50	71.4	1:1.06:6.3
72	01.60	10.7	10.0	01.00	05.70	71.0	1:0.93:6.63
96	04.40	36.0	14.0	00.00	05.60	40.0	1:0.38:1.12

[a] Ratio of arabinose, xylose and glucose is given as relative proportion.

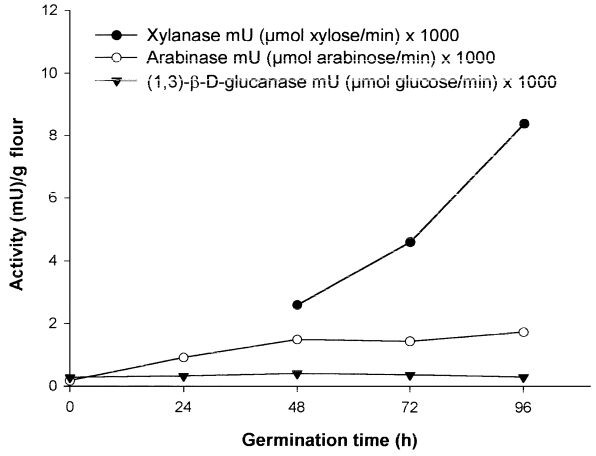

Fig. 5. Changes in cell wall degrading enzyme activities during germination.

with a concomitant decrease in xylose and glucose. Since malting is essentially a degradative process of structural and storage molecules, biosynthesis of arabinan polymer can be ruled out.

1,3-β-D-Glucanase activity showed only marginal increase during germination. However, there was a considerable decrease in glucose content of dietary fibre in the 96 h-germinated sample possibly because of the degradation of 1,3-β-D-glucan along with cellulose. These results are almost comparable with barley and its malt, wherein a complete/partial degradation of 1,3-β-D-glucan has been reported (Vietor, Voragen, Angelino & Pilmik, 1991). Linear increase in development of β-D-glucanase activity and the degradation of β-D-glucan in barley grown in Scotland and in Spain has been reported (Ellis, Swanston, Rubio, Perez-vendrell, Romagosa & Molina-cano, 1997). Carbohydrate-degrading enzymes in germinating wheat have been reported, whereby a steep increase in xylanase activity is observable at 4 days (Corder & Henry, 1989). Present results are therefore in accordance with those reported by other workers. In the present study, arabinase activity was comparatively less than xylanase activity, which is in consonance with the results obtained from wheat malts and oats (Debyser, Derdelinck & Delcour, 1997). Further detailed work pertaining to the isolation, fractionation and chemical characterization of dietary fibre components (non-starch polysaccharides) of native and germinated flours is in progress.

The above study indicated Indaf-15 variety to have potential for malting as it produces (a) high amounts of reducing sugar and (b) high enzyme activity, especially with particular reference to amylase. Slow degradation of cell wall components is also useful for the preparation of fibre-rich foods. Detailed studies pertaining to the various end-uses of the different malts obtained from this variety have to be carried out for any possible commercial exploitation.

Acknowledgements

We thank Dr. V. Prakash, Director, C.F.T.R.I, for his keen interest in the work and encouragement. M.N. and M.V.S.S.T.S.R thank the Council of Scientific and Industrial Research (C.S.I.R), New Delhi for the grant of research fellowships.

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