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Non-starch polysaccharides and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf - 15)☆

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Abstract

Nature of non-starch polysaccharides (NSP) and bound phenolic acids from native and malted ragi were studied using a recently-released hybrid variety of ragi, (Indaf-15). Yields of water-soluble NSP, hemicellulose-B and cellulose polysaccharides increased upon malting whereas a substantial decrease in the yield of hemicellulose-A was observed. Hemicellulose-B is the most viscogenic and its relative viscosity decreased from 3.04 to 1.98 upon 96 h of malting, whereas the solubility and viscosities of the rest of the NSP increased upon malting. The major sugars identified in all the NSP fractions were arabinose, xylose, galactose and glucose. A one- to two-fold decrease in arabinose was observed in all the NSP upon malting except for the alkali-insoluble residue wherein a decrease of glucose was observed. A progressive decrease in the pentose to hexose ratio was observed, indicating mainly pentosan degradation during malting, whereas an increase in the pentose to hexose ratio was observed in the alkaline-insoluble residue (AIR). Ferulic, caffeic and coumaric acids were identified as the major bound phenolic acids in native ragi and one- to two-fold decrease was observed in their contents after 4 days of malting.

1. Introduction

Finger millet (*Eleusine coracana*) is an important staple food in India for people in low income groups. Nutritionally its importance is well recognised because of its high content of calcium (0.38%) and dietary fibre (18%) compared to the continental cereals such as barley, rice, maize and wheat (Kamat & Belavady, 1980; Ravindran, 1991). It is consumed as a whole, thereby retaining the fibre, phenolics, minerals and vitamins present in the outer layer of the grain, which are nutritionally beneficial (Usha, Sripriya & Chandra, 1986). Traditionally ragi is processed either by malting or by fermentation and the resultant flour or extracts derived from it are extensively used in the preparation of weaning foods, beverages and other pharmaceutical preparations. The experiments carried out at CFTRI have shown that the incorporation of low-cost protein food, based on 70:30 blend of whole wheat and soybean flours, fortified with vitamins and minerals at 10 and 20% levels in poor rice or ragi diet, made up for the deficiencies in the diet and brought about a marked improvement in the growth of rats (Narayanaswamy et al., 1971).

Cereal cell walls consist of polysaccharides, such as, arabinoxylans, (1-3),(1-4)-β-D-glucans, arabinogalactoproteins, cellulose along and associated phenolics (Fincher & Stone, 1986; Izydorczyk & Biliaderis, 1995). Extensive data about the changes, brought about by malting of barley, on non-starch polysaccharides are available (Voragen, Schols, Marijs, Rombouts & Angelino, 1987). The same is not true with wheat and ragi NSP, where the experiments have been confined to preliminary investigations (Malleshi, Desikachar & Tharanathan, 1986; Suhasini, Muralikrishna & Malleshi, 1997). Phenolic acids and tannin contents of different ragi varieties have been reported (McDonough, Rooney & Earp, 1986; Virupaksha, Ramachandra & Shadaksharaswamy, 1977). Initial studies from our laboratory indicated changes of dietary fibre components and their degrading enzymes during malting of a new variety of ragi, Indaf-15 (Nirmala, Subba, Rao & Muralikrishna, 2000). Detailed studies pertaining to the changes in physicochemical properties and chemical

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composition during malting of ragi non-starch polysaccharides, and associated phenolic acids are lacking, and hence the present study is now reported.

2. Materials and methods

2.1. Materials

Finger millet (Indaf-15) seeds were procured from V.C. farm of the University of Agricultural Sciences, located at Mandya, Karantaka and used for all the studies. All the chemicals and sugar standards were of analytical grade. Phenolic acid standards and glucoamylase (E.C.3.2.1.3) were purchased from Sigma Chemical Company USA. GC (OV-225) and HPLC (Shimpack C_18) columns were obtained from Pierce Chemical Company, Rockford, IL, USA and Shimadzu Corporation, Tokyo, Japan, respectively.

2.2. Methods

2.2.1. Malting

Ragi seeds (100 g) were cleaned, steeped for 24 h and germinated under controlled conditions on moist cloth at 25°C in a B.O.D. incubator for up to 96 h. Germinated seeds were taken out at 24 h intervals and dried at 50°C in an air-oven for 12 h; growth portions were removed by gentle brushing manually. Devegetated seeds were weighed, powdered and used for the experiments, along with ungerminated ragi flour, which served as a control.

2.2.2. Analytical determinations

Total carbohydrate and uronic acid contents of different NSP samples were estimated by the phenol-sulphuric acid (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and carbazole methods (Knutson & Jeanes, 1968), respectively. Solubility (%) of NSP at 1% concentration was determined by dissolving in water. Viscosities of different NSP samples were determined by using an Ostwald viscometer and these were expressed with respect to the viscosity of deionized water (Muralikrishna, Ramadas, Bhat & Tharanathan, 1987).

2.2.3. Isolation and characterization of non-starch polysaccharides (NSP)

Different NSP were isolated from different malted flours following the method of Paramahans and Tharanathan (1982) (Fig. 1). The isolated polysaccharides 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% by the addition of water. The above mixture was refluxed in a water bath for 10–12 h; the volume was made up to 20 ml, neutralized with

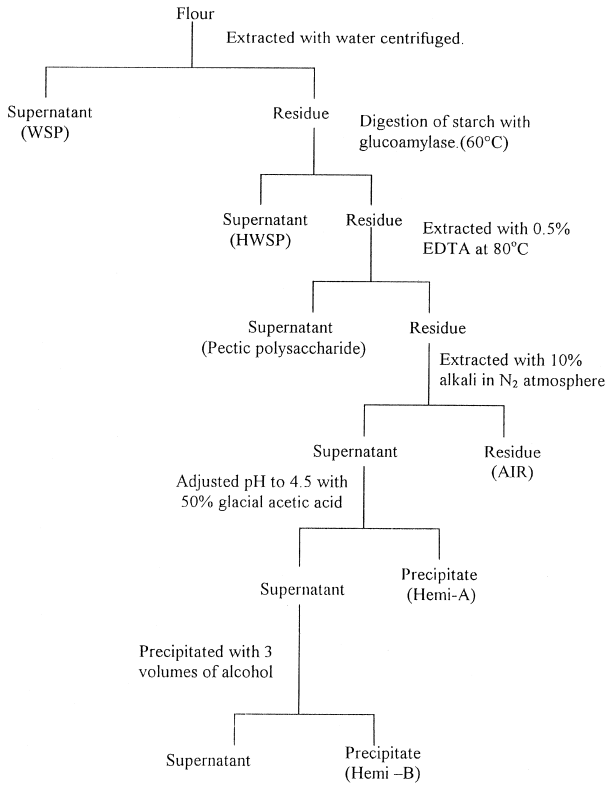

Fig. 1. Isolation scheme of non-starch polysaccharides.

barium carbonate, filtered, concentrated, deionized with Amberlite (IR 120-H+) and reduced with sodium borohydride. Alditol acetates were prepared according to the method of Sawardekar, Slonekar and James (1965) and the component sugars were separated and identified on a 3% OV-225 (1/8''×6'') column using a Shimadzu14-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 carrier gas. A sugar mixture, consisting of rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose was used as reference and inositol as internal standard.

2.2.4. Isolation and characterization of bound phenolic acids

Bound phenolics were extracted according to the method of Nordkvist et al. (1984) (Fig. 2). Native and malted flours (2 g each) were desugared and defatted with 80% ethanol (4×50 ml) and hexane (4×50 ml), respectively. The dried samples were extracted with 1 M NaOH (2×100 ml, 2 h each) containing 0.5% sodium borohydride, under nitrogen atmosphere, and the clear supernatants were collected, followed by centrifugation. The combined supernatants were acidified (4 M HCl, pH 1.5) and the phenolic acids were extracted with ethyl acetate (5×50 ml), followed by drying with anhydrous sodium sulphate, filtered evaporated to dryness, dissolved in methanol and analyzed on a C-18 HPLC

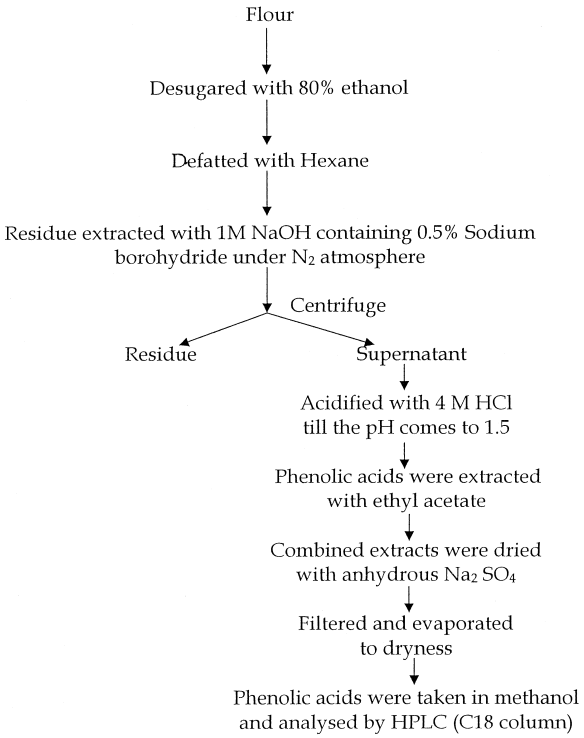

Fig. 2. Extraction of bound phenolic acids.

column (4.6×250 mm), using a UV detector (320 nm) and a solvent system of water:acetic acid:methanol (isocractic, 80:5:15). For identification and quantification of phenolic acids present in the samples, standards such as protocatechuic, syringic, gentisic, vanillic, caffeic, coumaric and ferulic acids were used.

3. Results and discussion

3.1. Non-starch polysaccharides (NSP) physicochemical properties and their changes during malting

Yields of water-soluble polysaccharides (WSP), hemicellulose-B and cellulose polysaccharides were increased upon malting. However, hemicellulose-A's yield was decreased, and very little change was noticed in hot water-soluble polysaccharides (HWSP)'s yield upon 96 h of malting (Table 1). The variation in yields of NSP can be attributed to (a) softening of tissues during malting and resulting in better extractability, and (b) precise localization of cell wall-degrading enzymes and their movement during malting between cell wall layers and their selective action on different polymers of NSP such as HWSP and hemicellulose-A (Palmer & Duffus, 1986). These changes in yields of NSP were in close agreement with the earlier report on a different variety of ragi and other minor millets (Malleshi et al., 1986).

Hemicellulose-B, in both the native and malted flours, was found to be almost completely soluble and more viscogenic than other NSP types (Table 1). This can be attributed to the presence of a relatively high percentage of pentosans in hemicellulose-B, both in native (75.6%) and malted conditions (55.6%), than other NSP types (Table 3). Cereal pentosans are known to form highly viscous solutions compared to hexosans (Fincher & Stone, 1986).

Substantial increase in the solubility and relative viscosity of most of the NSP was observed upon malting except in the hemicellulose-B. Total carbohydrate and uronic acid contents were increased upon germination in WSP, HWSP and hemicellulose-A and this could be the main reason for the better flow properties (viscosity) of these polysaccharides upon malting. In contrast the uronic acid and total carbohydrate content of hemicellulose-B remained constant. There was a visible decrease in the relative viscosity of hemicellulose-B from 3.04 to 1.98 upon malting, even though its solubility remained more or less constant (94–96%) (Table 1). Detailed investigations of the physicochemical properties and structural elucidation of hemicellulose-B are under progress to understand the anomaly in its flow properties compared to other NSP types. Solubilities, and in turn viscosities, depend on the anomeric configuration (α/β), orientation of free hydroxyl groups (axial/equatorial), substitution and nature of the sugars in the side chains (Andrewartha, Phillips & Stone, 1979). Studies were not carried out on cellulose and pectin fractions due to their poor solubility and yield, respectively.

3.2. Neutral sugar composition

The major sugars identified in all the NSP fractions in native and malted conditions were arabinose, xylose, galactose and glucose. Mannose and rhamnose were present in small quantities (Tables 2 and 3). This is in accord with the earlier reports on other cereals (Malleshi et al., 1986; Voragen et al., 1987). One- to two-fold decrease in the arabinose content was observed in all the NSP upon malting. A marginal decrease in xylose content was observed in all the NSP fractions, except WSP where a slight increase was noticed: increases in glucose and galactose contents were noticed after 96 h of malting in WSP, and hemicellulose-A and B. Mannose is present in all the NSP fractions in very small amounts (< 10%). Glucomannans are known to be present as minor constituents, along with high amounts of arabinoxylans, (1-3),(1-4)-β-D-glucans, arabinogalacto-proteins and cellulose (Fincher, 1975; Voragen et al.).

Progressive decrease in the pentose to hexose ratio indicated degradation of pentosans rather than hexosans during malting of ragi, as indicated by the substantial decrease of pentose content in hemicellulose-B fractions (from 75 to 55%). The decrease of pentose content in other NSP fractions upon malting was also

Table 1
Changes in physico-chemical properties of NSP during malting of ragi[a,b]

Fraction	Duration of malting (h)	Yield (%)	Solubility in water (%)	TC in soluble portion (%)	UA in soluble portion (%)	Relative viscosity (η_γ)
	Control	1.14	10	70.0	10.0	1.05
WSP[b]	48	1.40	24	80.0	15.8	1.20
	96	1.45	34	83.0	17.6	1.25
	Control	1.20	45	82.2	16.4	1.04
HWSP	48	1.30	50	88.0	18.0	1.20
	96	0.90	77	91.0	20.7	1.50
	Control	1.40	10	90.0	4.00	1.10
Hemi-A	48	1.30	26	92.2	4.60	1.12
	96	0.50	30	96.6	10.0	1.23
	Control	1.90	94	94.0	10.0	3.04
Hemi-B	48	1.80	95	95.0	10.0	2.15
	96	3.00	96	96.0	10.0	1.98

[a] Alkali-insoluble residue (AIR) yield was increased from 7% in native flour to 8.2% in 48 h and 10% in 96 h malted flours.
[b] WSP, water-soluble polysaccharides; HWSP, hot water-soluble polysaccharides; TC, total carbohydrate; UA, uronic acid; η_γ, relative viscosity.

Table 2
Changes in neutral sugar composition (%) of WSP and HWSP during malting of ragi[a,b]

Fraction	Duration of malting (h)	Rha[a]	Ara	Xyl	Man	Gal	Glc	A:X	P:H
	Control	8.50	26.70	10.60	05.50	17.90	30.70	1:0.40	0.69:1
WSP[b]	48	6.20	26.70	10.00	02.00	29.00	26.10	1:0.37	0.64:1
	96	8.30	16.70	12.90	04.80	20.00	37.30	1:0.76	0.48:1
	Control	4.80	30.80	15.80	06.10	20.80	21.70	1:0.51	0.96:1
HWSP	48	6.50	26.50	12.00	11.00	23.00	21.00	1:0.45	0.70:1
	96	7.00	26.40	13.00	10.00	25.60	18.00	1:0.49	0.73:1

[a] Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.
[b] WSP, water soluble polysaccharides; HWSP, hot water-soluble polysaccharides; A:X, arabinose:xylose; P:H, pentose:hexose.

substantial (Tables 2 and 3). This agrees well with our earlier work pertaining to the various levels of cell wall degrading enzymes, such as xylanase, arabinase and (1-3)-β-D-glucanase wherein the activities of the pentosanases were shown to be substantially higher than the hexosanases (Nirmala et al., 2000). Similar observations were made with respect to the changes in NSP of sorghum upon malting, including degradation of arabinoxylans rather than β-D-glucans (Okokon, 1992) and these results differ from malting studies of barley NSP, wherein a more pronounced degradation of (1-3)(1-4)-β-D-glucans was reported compared to arabinoxylans. The arabinose to xylose ratio of different NSP types gradually increased during malting, indicating the possible degradation of side chain arabinose moieties compared to xylan back bone (Tables 2 and 3). Literature reports indicate that the elimination of arabinose side chains is a prerequisite to the degradation of the xylan back bone (Schmitz, McDonald & Gilles, 1974).

Glucose was found to be the major sugar in alkali-insoluble residue (AIR), along with small amounts of arabinose and xylose, both in native and malted conditions. The presence of arabinose and xylose indicated incomplete extraction of hemicelluloses (Suhasini et al., 1997). The increase in pentose to hexose ratio in AIR unlike in the rest of the NSP, is perhaps due to the degradation of cellulose by cellulase resulting in the decrease of glucose content from 81 to 70% (Table 3).

3.3. Bound phenolic acids

The major bound phenolics in native ragi flour were found to be ferulic (18.6 mg/100 g), caffeic (1.64 mg/100 g) and coumaric (1.25 mg/100 g) acids (Table 4). Traces of syringic and protocatechuic acids were also found (not shown in Table 4). This is in accord with earlier reports of ragi phenolic acids from different varieties (McDonough, Rooney & Earp, 1986; Virupaksha et al., 1977). A two-fold decrease was observed in all the major phenolic acids after 96 h of malting. Decrease in bound phenolics may be due to the action of induced esterases which act on various phenolic acid esters linked either to arabinoxylans or other non-starch polysaccharides. Esterases are known to be induced during germination (Maillard, Soum, Biovin & Berset, 1996). Plants are known to contain phenolic acids in cell

Table 3
Changes in neutral sugar composition (%) of Hemi-A, Hemi-B and AIR during malting of ragi[a,b]

Fraction	Duration of malting (h)	Rha[a]	Ara	Xyl	Man	Gal	Glc	A:X	P:H
	Control	2.20	33.2	09.0	6.60	3.40	45.50	1:0.27	0.74:1
Hemi-A[b]	48	1.10	20.6	10.7	4.50	4.60	58.50	1:0.52	0.46:1
	96	1.50	11.0	07.0	0.00	4.60	75.90	1:0.63	0.22:1
	Control	0.70	49.5	25.7	3.10	5.60	15.40	1:0.52	3.10:1
Hemi-B	48	1.00	33.0	29.0	1.00	8.00	28.00	1:0.88	1.67:1
	96	1.00	35.0	20.5	4.50	9.00	30.00	1:0.58	1.26:1
	Control	0.60	07.3	03.3	4.20	3.20	81.40	1:0.45	0.12:1
AIR	48	0.20	13.9	04.3	1.80	3.50	76.30	1:0.31	0.22:1
	96	0.10	20.0	04.3	0.90	4.70	70.00	1:0.21	0.32:1

[a] Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.
[b] Hemi-A, hemicellulose-A; Hemi-B, hemicellulose-B; AIR, alkali-insoluble residue; A:X, arabinose:xylose; P:H, pentose:hexose.

Table 4
Changes in major bound phenolic acids during malting of ragi

Duration of malting (h)	Phenolic acids (mg/100 g total flour)		
	Caffeic acid	Coumaric acid	Ferulic acid
Control	1.64	1.23	18.60
48	1.50	0.80	14.3
96	0.90	0.72	9.6

walls (Harvey, Hartley, Harris & Curzon, 1986) and ferulic acid is the major bound phenolic acid which is ester-linked to various cell wall constituents such as arabinoxylans in various graminacious plants (Borheman, Akinand & Van Eseltine, 1986; Smith & Hartley, 1986; Smith et al. 1981).

The above studies indicate changes in solubility, viscosity and neutral sugar composition of NSP. Arabinose/xylose ratio increased and pentose to hexose ratio decreased upon malting in WSP, HWSP, Hemi-A and Hemi-B whereas, in alkali-insoluble residue, an increase of pentose to hexose was observed. Hemicellulose-B was found to be the most viscogenic and detailed investigation is warranted to see whether the structural changes of soluble NSP (hemicellulose-B) corroborate with its rheological (viscosity) changes during malting.

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