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Non-starch polysaccharide–phenolic acid complexes from native and germinated cereals and millet

R. Shyama Prasad Rao, G. Muralikrishna*

Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore 570 013, Karnataka, India

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Abstract

Non-starch polysaccharide–phenolic acid complexes were isolated from native and germinated (96 h) cereals, such as rice, maize, wheat and millet-ragi. They were designated as water extractable (WEPs, yield: 0.60–3.56%) and water-unextractable (WUPs, yield: 7.49–37.8%) non-starch polysaccharides, which consisted mainly of arabinose, xylose and glucose in different ratios and differed in their bound phenolic acid contents (43.7–4023 μg/g). Ferulic and coumaric acids, the main bound phenolic acids, were predominantly bound (~90%) to WUPs. The amount of ferulic acid is several-fold higher than that of coumaric acid and their contents decreased markedly upon germination.

Keywords: Non-starch polysaccharides; Phenolic acids; Cereals; Millet; Germination; Ferulic acid

1. Introduction

Cereals are the predominant staple food for millions across the world. The major constituent of cereals is starch. Apart from starch, cereals also contain other polysaccharides, known as non-starch polysaccharides, which include arabinoxylans, 1-3/1-4 β D-glucans, pectins and arabinogalactans (Fincher, & Stone, 1986; Izydorczyk, & Biliaderis, 1995).

Non-starch polysaccharides from cereals and millets form the quantitatively most important source of both soluble and insoluble dietary fibres (Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001). Arabinoxylans and β D-glucans, the most important cereal non-starch polysaccharides, are partially water-soluble and have an impact on various food preparations. They are also known to alleviate disease symptoms, such as diabetes, atherosclerosis and colon cancer (Karppinen, Liukkonen, Aura, Forssell, & Poutanen, 2000; Plaami, 1997). Phenolic acids, such as coumaric and ferulic acids, mainly bound to arabinoxylans, further influence these properties, in addition to their strong antioxidant properties. (Dervilly, Saulnier, Roger, & Thibault, 2000; Dervilly-Pinel, Rimsten, Saulnier, Andersson, & Åman, 2001; Subba Rao, & Muralikrishna, 2002). There are many individual reports on the overall sugar composition of cereal fibres (Cyran, Izydorczyk, & MacGregor, 2002; Dervilly et al., 2000; Shibuya, 1984) and the amount of bound phenolic acids (Durkee, & Thivierge, 1977; Hahn, Faubion, & Rooney, 1983; Harukaze, Murata, & Homma, 1999; Huang, Johanning, & O'Dell, 1986; Nordkvist, Salomonsson, & Åman, 1984). However, comparative information on different non-starch polysaccharides and bound phenolic acids and their changes, brought about by malting (controlled germination) of cereals, is very limited (Dervilly-Pinel et al., 2001; Glennie, 1983; Salomonsson, Theander, & Åman, 1978; Subba Rao, & Muralikrishna, 2001; Voragen, Schols, Marijs, Rombouts, & Angelino, 1987).

Initial studies on native and malted ragi from our laboratory indicated that 96 h of malting resulted in maximum changes in non-starch polysaccharide composition and bound phenolic acid contents (Subba Rao & Muralikrishna, 2001). This is perhaps due to the induction of cell wall degrading enzymes, i.e., xylanase, arabinase and 1-3/1-4 β D-glucanase, whose activities were maximum in 96 h malts (Nirmala, Subba Rao, & Muralikrishna, 2000). Hence this condition was chosen to isolate water-extractable (WEPs) and

* Corresponding author. Tel.: +91-821-2514876; fax: +91-821-2517233.

E-mail address: gmk@cscftri.ren.nic.in (G. Muralikrishna).

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water-unextractable (WUPs) non-starch polysaccharide–phenolic acid complexes from native and germinated cereals and a comparative study was undertaken.

2. Materials and methods

2.1. Materials

Cereals such as rice (*Oryza sativa* Var Jaya), maize (*Zea mays* Var NAC 6002) and finger millet-ragi (*Eleusine coracana* Var Indaf- 15) were obtained from the V.C. farm of the University of Agricultural Sciences, located at Mandya, Karnataka and wheat (*Triticum aestivum* Var DWR 195) was obtained from the National Seed Corporation, Dharwad, Karnataka. 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

Rice, maize, wheat and ragi seeds (200 g each) were cleaned, steeped for 16 h and germinated under controlled conditions in a B.O.D. incubator, as reported earlier (Nirmala et al., 2000).

2.2.2. Isolation and characterization of WEPs and WUPs

WEPs were isolated by extracting cereal/millet flours (100 g each) with water and centrifuged; the supernatant was precipitated with three volumes of ethanol, dialyzed and lyophilized. The residue obtained after centrifugation was digested with glucoamylase (1 g/100 g of material) for 48 h at 55 °C to remove the starch completely and the undigested material was dried by solvent exchange and designated as WUPs (Fig. 1).

WEPs and WUPs (10 mg each) were suspended in water (0.2 ml), solubilised with concentrated sulphuric acid (0.6 ml) and hydrolyzed as described earlier (Subba Rao & Muralikrishna, 2001). Alditol acetates were prepared according to the method of Sawardekar, Slonekar, & Jeanes (1965) and the component sugars were separated, identified and quantified 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. Nitrogen (40 ml/min) was used as carrier gas. A sugar mixture, consisting of rhamnose, arabinose, xylose, mannose, galactose and glucose, was used as reference and inositol as internal standard.

2.2.3. Isolation and quantification of bound phenolic acids

Bound phenolic acids were extracted according to the method of Nordkvist et al. (1984). WEPs and WUPs (1 g each), from native and malted cereals and millet, were stirred with 1 M NaOH (2×100 ml) containing 0.5% sodium borohydride under nitrogen atmosphere for 2 h each and the supernatants were collected upon centrifugation and processed as described earlier (Subba Rao & Muralikrishna, 2001) and analysed on a C_{18} HPLC column (4.6×250 mm), using a solvent system of water, acetic acid and methanol (isocratic—80:5:15) and a UV detector (320 nm). For identification of phenolic acids present in the samples, standards, such as protocatechuic, syringic, gentisic, vanillic, gallic, caffeic, coumaric and ferulic acids, were used.

3. Results and discussion

3.1. Malting loss and yields of WEPs and WUPs

Cereals such as rice, maize, and wheat and millet-ragi were malted for 96 h. The malting loss at 96 h was found to be lowest for rice (17.1%) and highest for wheat (40.2%), which is due to the low and high vegetative growth rates, respectively.

WEPs and WUPs were obtained from native and malted flours, as shown in Fig. 1. WEP contents increased upon malting, except in maize (Table 1), which may be due to the preferential degradation of high amounts of water-soluble mannan-type polysaccharides during malting, as indicated by the sugar composition (Table 2). In general, WEP contents might have increased due to the loosening of cell walls during malting, which facilitates improved solubility of various non-starch polysaccharides (Palmer & Duffus, 1986).

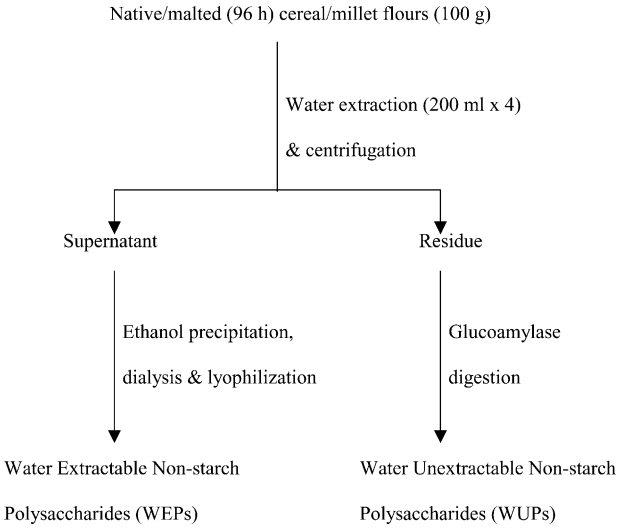

Fig. 1. Steps in obtaining WEPs and WUPs.

Table 1
Malting loss (%) and yields (%) of WEPs and WUPs from native and germinated cereals (rice, maize and wheat) and millet (ragi)

		Malting loss	WEPs[a]	WUPs
Rice	N[b]	–	1.16	8.19
	M	17.1	2.24	7.49
Maize	N	–	3.56	20.4
	M	24.7	1.57	20.0
Wheat	N	–	2.35	13.6
	M	40.2	3.35	37.8
Ragi	N	–	0.60	20.3
	M	24.7	2.06	20.3

[a] WEPs, Water extractable non-starch polysaccharides, WUPs, water unextractable non-starch polysaccharides.
[b] N, native; M, malt.

Results emanating from a previous study indicate an apparent increase in non-starch polysaccharide contents upon malting of ragi (Subba Rao & Muralikrishna, 2001). The yields of WUPs did not change much upon malting. However, in wheat, content increased 2.5-fold, which may be correlated with high malting loss. Some studies have been carried out previously, on changes in the extraction/solubility of non-starch polysaccharides upon malting (Malleshi, Desikachar, & Tharanathan, 1986).

3.2. Neutral sugar composition of WEPs and WUPs

WEPs, from all the native and malted cereals and millet, consisted mainly of arabinose, xylose and glucose in different proportions (Table 2), which accords with a previous report (Voragen et al., 1987). In general, glucose is the most predominant sugar in WEPs and hexoses are in higher concentrations than pentoses. Upon malting, a significant change in the ratio of pentose to hexose (P:H) was observed, which decreased in rice, maize and ragi, indicating higher rates of degradation of hexoses such as mannose and galactose. This may be due to the induction of hydrolytic enzymes, such as mannosidase and galactosidase. The change in the content of rhamnose is not significant in WEPs of rice and maize. However, it increased ~2.5 fold in ragi WEPs. In contrast to these, native wheat WEPs showed higher amounts of pentoses and, upon malting, the P:H ratio increased in favour of hexoses, indicating the degradation of soluble arabinoxylans. There was about a three-fold decrease in the amount of rhamnose and a concomitant increase in the galactose content. Malting resulted in increases of glucose, arabinose and xylose in rice, maize and ragi. The xylose content (compared to arabinose) decreased upon malting of rice, maize and wheat, but increased slightly in the case of ragi WEPs, which is in accordance with the earlier study (Subba Rao & Muralikrishna, 2001). Mannose present in native WEPs disappeared upon malting. This may be due to the degradation of mannan/glucomannan-type polysaccharides, which are present in small amounts in cereals (Fincher, 1975; Voragen et al., 1987). Disappearance of mannose in WEPs can be taken as an index of malting. Further studies are warranted to investigate the biochemical basis for quantitative variation of sugar composition of WEPs, especially with respect to cell wall-degrading enzymes, such as xylanase, arabinase, 1-3/1-4 β D-glucanase, mannanase and cellulase.

WUPs of all the cereals and millet consisted mainly of arabinose, xylose and glucose with small amounts of other sugars (Table 3). The pentose content is greatest in all WUPs except native rice. The P:H ratio of rice, maize and wheat WUPs increased upon malting in favour of pentoses. The P:H ratio of ragi WUPs decreased upon malting in favour of hexoses, which indicates pentosan degradation (Okokon, 1992; Subba Rao & Muralikrishna, 2001). The xylose content of rice and maize WUPs increased upon malting but decreased slightly in the case of wheat and ragi. The mannose content of WUPs disappeared upon malting, as observed with respect to WEPs.

3.3. Bound phenolic acids from WEPs and WUPs

Ferulic acid is the major bound phenolic acid identified in WEPs (Table 4), which is in accord with earlier reports on cereals (Durkee & Thivierge, 1977; Hahn et al., 1983; Harukaze et al., 1999; Salomonsson et al.,

Table 2
Neutral sugar composition (%) of WEPs from native and malted cereals and millet

		Rha[a]	Ara	Xyl	Man	Gal	Glc	A:X	P:H
Rice	N	6.90	3.4	16.3	13.1	1.42	58.9	1:4.79	1:3.72
	M	5.88	22.1	9.32	0.00	1.78	60.9	1:0.42	1:1.98
Maize	N	5.43	10.3	13.4	43.0	2.02	25.9	1:1.30	1:3.00
	M	4.43	22.0	15.5	0.00	2.96	55.2	1:0.70	1:1.55
Wheat	N	15.3	30.6	18.3	2.94	7.47	25.5	1:0.60	1:0.73
	M	5.24	10.2	3.86	0.00	24.3	56.4	1:0.38	1:5.75
Ragi	N	5.40	23.7	11.9	3.60	12.0	43.4	1:0.50	1:1.66
	M	13.0	21.8	14.6	0.00	0.00	50.6	1:0.67	1:1.39

[a] Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; A:X, arabinose:xylose; P:H, pentose:hexose.

Table 3
Neutral sugar composition (%) of WUPs from native and malted cereals and millet

		Rha	Ara	Xyl	Man	Gal	Glc	A:X	P:H
Rice	N	0.00	28.8	15.4	0.00	3.24	52.6	1:0.53	1:1.26
	M	5.08	33.5	43.6	0.00	0.00	17.9	1:1.30	1:0.23
Maize	N	0.76	34.2	28.7	4.36	6.45	25.6	1:0.84	1:0.58
	M	4.01	36.6	32.4	0.00	3.54	23.5	1:0.89	1:0.39
Wheat	N	6.88	32.9	23.7	1.30	0.77	34.4	1:0.72	1:0.64
	M	2.16	40.2	25.7	0.00	5.83	26.2	1:0.64	1:0.49
Ragi	N	0.00	34.1	22.0	1.45	3.63	38.9	1:0.65	1:0.78
	M	4.21	29.7	17.8	0.00	0.00	48.3	1:0.60	1:1.01

Table 4
Bound phenolic acids (μg/g) of WEPs and WUPs from native and malted cereals and millet

		WEPs		WUPs	
		Coumaric acid	Ferulic acid	Coumaric acid	Ferulic acid
Rice	N	9.43	104	388	1426
	M	2.98	68.0	360	915
Maize	N	22.75	140	243	3780
	M	8.36	35.4	32.5	263
Wheat	N	0.28	98.0	39.6	1836
	M	0.89	49.3	90.0	800
Ragi	N	5.91	209	77.5	1519
	M	0.88	86.8	75.6	891

1978; Shibuya, 1984; Subba Rao & Muralikrishna, 2001). Ferulic acid content was maximum in native ragi WEPs. Native maize WEPs contained relatively higher amount of coumaric acid. Both these phenolic acids underwent several-fold degradation upon malting, which is in accordance to the earlier report on ragi (Subba Rao & Muralikrishna, 2001). This may be due to the induction of phenolic acid esterases during germination (Humberstone & Briggs, 2000; Maillard, Soum, Biovin, & Berset, 1996; Sancho, Bartolomé, Gómez-Cordovés, Williamson, & Faulds, 2001).

As with the WEPs, ferulic and coumaric acids were the main bound phenolic acids identified in WUPs (Table 4). However, the ratio of ferulic:coumaric acid is less in WUPs, especially in rice, which had the highest amount of coumaric acid, in agreement with the earlier reports (Harukaze et al., 1999; Shibuya, 1984). Even in WUPs, the phenolic acid content decreased several-fold upon malting. Ferulic acid degradation was maximum in maize, followed by wheat, ragi and rice. However, coumaric acid did not undergo much degradation upon malting except in maize. The coumaric acid content of wheat WUPs (and WEPs) increased upon malting. This may be due to the degradation of ferulic acid by selective removal the of *O*-methyl group by wheat malt enzymes. About 90% of the phenolic acids were bound to WUPs of cereals and millet.

As reported in the literature, major amounts of phenolic acids are present in the bran portion, whereas the cell walls of endosperm contain very much less (Nordkvist et al., 1984). However, there is no report on the distribution of phenolic acids based on the solubility of non-starch polysaccharides. Comparative studies in the present investigation clearly show that most phenolic acids are bound to WUPs rather than to WEPs. This finding can also be supported by the fact that diferulates are 8–39 times higher in cereal insoluble dietary fibre than the soluble dietary fibre (Bunzel et al., 2001).

4. Conclusions

The above studies clearly indicate both qualitative and quantitative variations in WEPs and WUPs with respect to their sugar composition. Glucose, arabinose and xylose are the major sugars and P:H ratios change considerably and mannose content disappears upon malting.

Ferulic and coumaric acids are the major phenolic acids preponderantly bound to WUPs and these underwent extensive degradation upon malting, both in WEPs and WUPs. The degradation of phenolic acid and loosening of the cell wall materials resulted in increased yields of WEPs. Cereal/millet malts can be used as better sources of soluble dietary fibres than the native cereals, which can be utilized in various fibre-depleted foods for a possible alleviation tool in combatting disease symptoms such as diabetes, atherosclerosis and colon cancer.

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References

Bunzel, M., Ralph, J., Marita, J. M., Hatfield, R. D., & Steinhart, H. (2001). Diferulates as structural components in soluble and insoluble cereal dietary fibre. *Journal of the Science of Food and Agriculture*, *81*, 653–660.

Cyran, M., Izydorczyk, M. S., & MacGregor, A. W. (2002). Structural characteristics of water-extractable nonstarch polysaccharides from barley malt. *Cereal Chemistry*, *79*(3), 359–366.

Dervilly, G., Saulnier, L., Roger, P., & Thibault, J. F. (2000). Isolation of homogeneous fractions from wheat water-soluble arabinoxylans. Influence of the structure in their macromolecular characteristics. *Journal of Agricultural and Food Chemistry*, *48*, 270–278.

Dervilly-Pinel, G., Rimsten, L., Saulnier, L., Andersson, R., & Åman, P. (2001). Water-extractable arabinoxylans from pearled flours of wheat, barley, rye and triticale. Evidence for the presence of ferulic acid dimers and their involvement in gel formation. *Journal of Cereal Science*, *34*, 207–214.

Durkee, A. B., & Thivierge, P. A. (1977). Ferulic acid and other phenolics in oat seeds (*Avena sativa* L. Var Hinoat). *Journal of Food Science*, *42*(2), 551–552.

Fincher, G. B. (1975). Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brewing*, *81*, 116–122.

Fincher, G. B., & Stone, B. A.. In Y. Pomeranz (Ed.), *Advances in cereal science and technology* (pp. 207–296). St Paul: American Association of Cereal Chemistry.

Glennie, C. W. (1983). Polyphenol changes in sorghum grain during malting. *Journal of Agricultural and Food Chemistry*, *31*, 1295–1299.

Hahn, D. H., Faubion, J. M., & Rooney, L. W. (1983). Sorghum phenolic acids, their high performance liquid chromatography separation and their relation to fungal resistance. *Cereal Chemistry*, *60*(4), 255–259.

Harukaze, A., Murata, M., & Homma, S. (1999). Analyses of free and bound phenolics in rice. *Food Science and Technology Research*, *5*(1), 74–79.

Huang, H., Johanning, G. L., & O'Dell, B. L. (1986). Phenolic acid content of food plants and possible nutritional implications. *Journal of Agricultural and Food Chemistry*, *34*, 48–51.

Humberstone, F. J., & Briggs, D. E. (2000). Extraction and Assay of ferulic acid esterase from malted barley. *Journal of the Institute of Brewing*, *106*(1), 21–29.

Izydorczyk, M. S., & Biliaderis, C. G. (1995). Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers*, *28*, 33–48.

Karppinen, S., Liukkonen, K., Aura, A., Forssell, P., & Poutanen, K. (2000). *In vitro* fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. *Journal of the Science of Food and Agriculture*, *80*, 1469–1476.

Maillard, M. N., Soum, M. H., Biovin, P., & Berset, C. (1996). Antioxidant activity of barley and malt: relationship with phenolic content. *Lebensmittel-Wissenschaft Und-Technologic*, *29*(3), 238–244.

Malleshi, N. G., Desikachar, H. S. R., & Tharanathan, R. N. (1986). Free sugars and non-starch polysaccharides of finger millet (*Eleusine coracana*), pearl millet (*Pennisetum typhoideum*), foxtail millet (*Setaria italica*) and their malts. *Food Chemistry*, *20*, 253–261.

Nirmala, M., Subba Rao, M. V. S. S. T., & Muralikrishna, G. (2000). Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf- 15). *Food Chemistry*, *69*, 175–180.

Nordkvist, E., Salomonsson, A., & Åman, P. (1984). Distribution of insoluble bound phenolic acids in barley grain. *Journal of the Science of Food and Agriculture*, *35*, 657–661.

Okokon, U. E. A. (1992). Comparative studies of the degradation of non-starch polysaccharides by sorghum and barley during malting. *Journal of the Science of Food and Agriculture*, *58*, 129–134.

Palmer, G. H., & Duffus, J. H. (1986). Aleuron and scutellum hydrolytic enzymes in malting. *Journal of the Institute of Brewing*, *92*, 512–513.

Plaami, S. P. (1997). Content of dietary fibre in foods and its physiological effects. *Food Reviews International*, *13*(1), 29–76.

Salomonsson, A., Theander, O., & Åman, P. (1978). Quantitative determination by GLC of phenolic acids as ethyl derivatives in cereal straws. *Journal of Agricultural and Food Chemistry*, *26*(4), 830–835.

Sancho, A. I., Bartolomé, B., Gómez-Cordovés, C., Williamson, G., & Faulds, C. B. (2001). Release of ferulic acid from cereal residues by barley enzymatic extracts. *Journal of Cereal Science*, *34*, 173–179.

Sawardekar, J. S., Slonekar, J. M., & Jeanes, A. (1965). Quantitative determination of monosaccharides as their alditol acetates by gas-liquid chromatography. *Analytical Chemistry*, *37*, 1602–1604.

Shibuya, N. (1984). Phenolic acids and their carbohydrate esters in rice endosperm cell walls. *Phytochemistry*, *23*(10), 2233–2237.

Subba Rao, M. V. S. S.T., & Muralikrishna, G. (2001). Non-starch polysaccharides and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana*, Indaf- 15). *Food Chemistry*, *72*, 187–192.

Subba Rao, M. V. S. S.T., & Muralikrishna, G. (2002). Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, *Eleusine coracana* Indaf- 15). *Journal of Agricultural and Food Chemistry*, *50*, 889–892.

Voragen, A. G. I., Schols, H. A., Marijs, J., Rombouts, F. M., & Angelino, S. A. G. F. (1987). Non-starch polysaccharides from barley: structural features and breakdown during malting. *Journal of the Institute of Brewing*, *93*, 202–208.