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Water soluble feruloyl arabinoxylans from rice and ragi: Changes upon malting and their consequence on antioxidant activity

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Abstract

The objective of this study is to determine the changes brought about by germination on water soluble feruloyl arabinoxylans (feraxans), one of the major components of soluble fibre from rice and ragi and their consequence on antioxidant activity. Soluble feraxans, isolated from native and malted rice and ragi were fractionated on DEAE-cellulose. Ferulic acid content of the major [0.1 molar ammonium carbonate (AC) eluted] fraction was higher in malts (rice: 1045 μg/g; ragi: 1404 μg/g) than in native (rice: 119 μg/g; ragi: 147 μg/g) and this fraction was separated by Sephacryl S-300 chromatography into two peaks each in rice (native: 232 and 24.4 kDa; malt: 75.4 and 39.6 kDa) and ragi (native: 140 and 15.4 kDa; malt: 38.9 and 15.4 kDa). 0.1 molar AC eluted fractions showed very strong antioxidant activity in vitro as determined by β-carotene–linoleate emulsion (IC_{50}: 0.16–0.24 mg), DPPH* (IC_{50}: 4.1–11.4 mg) and Ferric reducing/antioxidant power, FRAP (EC_1: 0.76–3.1 mg) assays. Antioxidant activity of feraxans was several (4.9–1400) folds higher than the expected activity due to their bound ferulic acid content. Apart from ferulic acid, presence of sugars with >C=O (uronyl/acetyl) groups and degree/nature of glycan-polymerization were observed to influence antioxidant activity of the polysaccharides. Malting resulted in many dynamic changes in the ferulic acid content in different feraxan types and affected their antioxidant activity.

Keywords: Antioxidant; Arabinoxylan; Cereals; Dietary fibre; Ferulic acid; Non-starch polysaccharide; Ragi

1. Introduction

Cereals, the staple food for millions of people across the world, are the chief source of soluble dietary fibre (SDF) (Plaami, 1997). Arabinoxylans, along with some amount of β-D-glucans, are the major components of SDF (Rao and Muralikrishna, 2004). These water soluble non-starch polysaccharides are known to have many beneficial roles in human nutrition and health such as lowering cholesterol and fat availability, reducing the disease symptoms of constipation and reducing the risk of diabetes, atherosclerosis and colorectal cancer (Morris et al., 1977; Plaami, 1997; Willett, 1994). They are also known to influence the quality of bakery products due to their physicochemical properties like viscosity and water holding capacity (Izydorczyk and Biliaderis, 1995). Being potent natural immunomodulators and prebiotic, of late, they are considered as functional food ingredients (Charalampopoulos et al., 2002).

Ferulic acid, a major bound phenolic acid, is known to exist ester linked mainly to arabinoxylans and influence

Abbreviations: A:X, arabinose:xylose; AA, antiradical activity; AAC, antioxidant activity coefficient; AC, ammonium carbonate; BHA, butyrated hydroxy anisole; BHT, butyrated hydroxy toluene; BSA, bovine serum albumin; DEAE, diethyl amino ethyl; DPPH*, 1,1-diphenyl-2-picryl-hydrazyl; EC_1, equivalent concentration 1; FRAP, ferric reducing antioxidant power; GLC, gas liquid chromatography; HPLC, high performance liquid chromatography; IC_{50}, 50% inhibition concentration; IR, infra red; kDa, kilo Dalton; M, malt; N, native; NSP, non-starch polysaccharides; P:H, pentose:hexose; SDF, soluble dietary fibre; TPTZ, 2,4,6-tri (2-pyridyl)-triazine; UV, ultra violet; w/v, weight/volume.

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their physicochemical properties (Ishii, 1997). Feruloyl polysaccharides can interlink each other and form gel in aqueous solutions in the presence of peroxidase. Ferulic acid is supposed to have a number of health benefits. It is known to decrease total cholesterol and increase vitamin-E bioavailability, increase vitality of sperms and a good protective agent against UV radiation–induced skin damage. Ferulic acid exhibits very strong antioxidant, free radical scavenging and anti-inflammatory activity (Castelluccio et al., 1995; Shahidi et al., 1992). It is known to have anti-tumor and anti-cancer effects (Mori et al., 1999).

Apart from dietary fibre, several reports have proved that ferulic acid is a potential chemo-preventive agent for colorectal cancer (Kawabata et al., 2000; Mori et al., 1999). Colorectal cancer is a major cause for concern in the developed and developing nations' health program. For example, it is the third leading cause of cancer death and malignancy in the United States. The 5-year survival rate for colon cancer is only 60%. Epidemiological studies have shown that consumption of whole grain and grain-based diet is associated with reduced risk of chronic diseases including colorectal cancer (Jacobs et al., 1995). This has been linked to the phytochemical profile and antioxidant activity of the grains (Adom and Liu, 2002; Adom et al., 2003; Charalampopoulos et al., 2002; Mori et al., 1999). Although antioxidants can prevent oxidative stress caused by amines and nitroso-compounds, delivery of enough amount of antioxidant to the colon is essential for its good health. However, being small molecules, most antioxidants, including free ferulic acid and feruloyl oligosaccharides, are absorbed in the small intestine and do not enter entero-hepatic circulation (Bourne and Rice-Evans, 1998; Zhao et al., 2003). Thus, oral or intravenous free ferulic acid administration does not reach the colon.

Recently, efforts are made to synthesize enzyme-resistant starch-ferulate to deliver enough ferulic acid to the colon and shown to release ferulic acid by microbial fermentation (Ou et al., 2001). On the other hand, cereal fibre – bound ferulic acid can get into the colon and is partly released by colon microorganisms. However, as complex dietary fibre resists complete fermentation, the concentration of released ferulic acid may be too low to act as a chemo-preventive agent. Although free ferulic acid (Subba Rao and Muralikrishna, 2002) and feruloyl oligosaccharides are known to exhibit antioxidant activity in vitro (Ohta et al., 1994, 1997), it is not shown if feruloyl polysaccharides as such exhibit any antioxidant activity. In case, they may be the better candidates as chemopreventive agents.

The aim of the present study is to observe the biochemical changes in the water soluble feraxans brought about by malting (controlled germination – a process known to be nutritionally beneficial) of cereal grains – rice and ragi, and also to determine the possible antioxidant activity of feraxans, a ferulic acid reservoir and a parent molecule to feruloyl oligosaccharides.

2. Results and discussion

2.1. Isolation, fractionation and characterization of NSP

Water soluble non-starch polysaccharides (NSP) were obtained from native (N, ungerminated) and malted (M, germinated for 96 h) rice and ragi. The yield of water soluble NSP increased by around 3 and 5 folds upon malting of rice and ragi, respectively (Table 1). A similar pattern of increase was observed in water extractable non-starch polysaccharides (WEP) (Rao and Muralikrishna, 2004). However, the yield of water soluble NSP was low compared to WEP (rice: N, 1.2%, M, 2.2%; ragi: N, 0.6%, M, 2.1%). WEP contained small amount of starch contamination (less than 5%) as degraded starch might get extracted with cold water, and was only partially soluble in water. This might be due to the altered physicochemical and hydration characteristics of polysaccharides during processing (Fig. 1). Similar to WEP, water soluble NSP has over 98% sugar and less than 1% protein. The uronic acid content of malt (rice, 4.0%; ragi, 6.1%) NSP was higher than native (rice, 2.6%; ragi, 4.8%) NSP (Table 1). This could be due to the mobilization of high uronic acid con-

Table 1
Yield, ferulic acid and uronic acid contents, and antioxidant activity (IC_{50}, as determined by emulsion assay) of water soluble non-starch polysaccharides obtained from rice and ragi

	Yield (%)	Ferulic acid (μg/g)	Uronic acid (%)	Activity, IC_{50} (mg)	Expected[a] activity, IC_{50} (mg)
Rice					
N	0.15	510.6	2.6 ± 0.1	1.14	54.8
M	0.44	492.5	4.0 ± 0.2	1.24	56.9
Ragi					
N	0.13	528.0	4.8 ± 0.2	0.92	53.0
M	0.61	503.1	6.1 ± 0.3	1.05	55.7

N, native; M, malt.
IC_{50} (mg), the concentration of polysaccharides at which 50% inhibition of β-carotene oxidation was attained.
[a] The amount of polysaccharides containing ferulic acid equivalent to IC_{50} (mg) of free ferulic acid.

Native/malted (96 h) rice/ragi flours (100 g)
↓ Water extraction (200 ml x 4) & centrifugation (3000 x g/20 min)
Supernatant | Residue
↓ Ethanol precipitation (1:3), dialysis (~8 kDa) & lyophilization
Water Extractable Non-starch Polysaccharides (WEP)
↓ Dissolved (10%, w/v) in water, heating (95°C/10 min), centrifugation, dialysis & lyophilization
Water Soluble Non-starch Polysaccharides (NSP)
↓ DEAE-cellulose fractionation
0.1 molar AC fractions (Feraxans)

Fig. 1. Scheme for obtaining water soluble NSP/feraxans from native and malted rice and ragi.