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Cereal Non-Cellulosic Polysaccharides: Structure and Function Relationship—An Overview

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The non-cellulosic polysaccharides present in cereals (2–8%) are mostly arabinoxylans, (1→3),(1→4)-β-glucans, pectins and arabinogalactans. Of these, the arabinoxylans are known to absorb large amounts of water and influence significantly the water balance, rheological properties of dough, and the retrogradation of starch and bread quality. (1→3),(1→4)-β-glucans are known as biological response modifiers (BMS) as they are believed to modulate the immune response. Cereal Pectins and arabinogalactans form a very small amount and do not contribute substantially to the functionality of noncellulosic polysaccharides. Detailed structural investigations on cereal hetero xylans using modern techniques were initiated in the 1990s and still pose a challenge to carbohydrate chemists because of their structural complexity. Nutritionally, they are classified under "unavailable carbohydrates" (dietary fiber) along with lignin and cellulose and are known to have beneficial effects in alleviating disease symptoms such as diabetes, atherosclerosis, and colon cancer. In this review isolation, purification, characterization, structural elucidation, functional, and nutritional attributes of cereal heteroxylans are covered with particular emphasis on recently characterized finger millet arabinoxylans.

Keywords cereal noncellulosic polysaccharides, arabinoxylans, structure, function, cereals, finger millet (ragi)

INTRODUCTION

Schulze first used the term "hemicellulose" for a group of polysaccharides that were obtained by extracting materials containing cell walls with alkali (Schulze, 1892). He considered these cell-wall components to be chemically and structurally related to cellulose and to serve as a food reserve. Because of their presumed relation to cellulose, he referred to these polysaccharides as "hemicelluloses." They are usually associated with various other cell-wall components such as cellulose, cell-wall proteins, lignins, and other phenolic compounds by covalent and hydrogen bonds, and by ionic and hydrophobic interactions (Sun et al., 2000). They are comprised of several polysaccharides such as arabinoxylans, (1→3),(1→4)-β-glucans, xyloglucans, pectins, and gluco- and galacto-mannans. In this review major emphasis is laid on heteroxylans of non cellulosic polysaccharides, and literature pertaining to (1→3),(1→4)-β-glucans and minor components such as xyloglucans, pectins, gluco-, and galacto- mannans is not covered.

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Various methods have been developed to extract hemicelluloses from plant cell walls. These methods include extraction with concentrated solutions of sodium or potassium hydroxide (Dupont and Selvendran, 1987), with alkaline hydrogen peroxide solution (Doner and Hicks, 1997), or with solutions of barium or calcium hydroxides at elevated temperatures (Bergmann et al., 1996). The main advantages of the alkali extractions are the fact that they are simple to perform and cost-effective.(Gruppen et al., 1991). Hemicellulose-A fraction, a mixture of (1→3),(1→4)-β-D-glucans and arabinoxylans, is insoluble in water, and is precipitated when alkaline extracts are adjusted to pH 4.5-5.0 with 50% acetic acid. Whereas hemicellulose-B fraction, which is rich in pentosans (Doner and Hicks, 1997; Woolard et al., 28, 1977), is soluble in water and is precipitated by adding three volumes of ethanol to the resultant supernatant (Blake et al., 1971; Salimath and Tharanathan, 1982).

Cereal noncellulosic polyscaccharides are highly heterogeneous in their molecular weight, physical properties, and chemical characteristics, mainly because their biosynthesis is controlled indirectly by glycosyltransferase genes. (The enzymes with individual specificities responsible for the transfer of sugar residues from particular glycosyl donors are synthesized under

genetic control). These variations may result from a) departure from absolute specificity of the transferases; b) incomplete formation of segments (side chains); or c) post-polymerization changes. If there is continuous variation in parameters, such as molecular size, proportions of sugar constituents, and linkage type, the polysaccharide is said to be polydispersed. If the heterogeneity lies in the molecular size but not in chemical composition, the polysaccharides are said to be "polymolecular."

Several methods have been used to show the absence of heterogeneity in a noncellulosic polysaccharide sample. Some of them are (a) consistency in chemical composition; (b) physical properties such as optical rotation, viscosity; (c) ion exchange, gel permeation, affinity chromatographic methods; (d) ultra centrifugation pattern; (e) cellulose acetate and capillary electrophoresis; and (f) spectroscopic methods (nuclear magnatic resonance, infrared spectroscopy).

STRUCTURAL ELUCIDATION

A structural analysis of plant polysaccharides is often difficult because unlike many microbial polysaccharides they usually have non-periodic repeating units. Elucidation of the complete covalent or primary structure of a polysaccharide includes determination of a) molecular size; b) constituent monosaccharides and their ring size and arrangement; c) configuration of the glycosidic linkages and linkage type; and d) degree of branching and their pattern of distribution. Several methods have been developed for elucidating the structures of polysaccharides, which can be categorized into three main classes 1) chemical 2) enzymatic and 3) spectroscopic methods (Aspinall, 1982).

Chemical Methods

Determination of Molecular Size

Since cereal non-cellulosic polysaccharides are polydisperse, their molecular size and molecular weights vary and the latter can be obtained as averages (number average M_n or weight average M_w). Determining both types of average molecular weight gives an indication of the molecular weight distribution, since the greater the difference between M_n and M_w, the greater is the polydispersity of the sample. Number average molecular weights can be obtained by membrane osmometry ($M_n >$ 20,000 Da) and vapor pressure osmometry (Van Dam and Prins, 1965), and weight average molecular weights by light scattering (Manley, 1963).

Ultra centrifugation gives molecular weight averages, depending upon the type of method used (sedimentation equilibrium or approach to equilibrium). The above-mentioned methods are mainly based on theoretically based average values. However, there are other methods of determining molecular weight that require pre-calibration with known molecular-weight markers. One such method is gel permeation chromatography, which is a simple and widely used method for determining the relative molecular weight of a sample.

Determining the Monosaccharide Composition

Determining the monosaccharide composition of a cereal non-cellulosic polysaccharide involves identifying and quantitatively estimating the monosaccharide constituents. After complete acid hydrolysis, the liberated monosaccharides can be identified by HPLC or GLC.

HPLC: It is not necessary to derivatize the sample and it is non-destructive, so the sample can be recovered. Separation is based on either cation/anion exchange or partition chromatography (acetonitrile-water) and identification of the monosaccharides can be done by using a refractive index detector (Mc Ginnis and Fang, 1980). The sensitivity of RI detectors is low and requires large amounts (micrograms) of the sample. High performance size exclusion chromatography (HPSEC, Eremeeva 2003) and high pH anion exchange chromatography with pulsed ampereometric detection (HPAEC-PAD, Broberg et al., 2000) are the most common procedures adopted to separate the polysaccharides.

GLC: This requires only small amounts of sample (nanograms), which need to be derivatized, and usually cannot be recovered. The derivatives must have adequate thermal stability and sufficient volatility. Trimethyl silyl (TMS) ethers, trifluoroacetyl (TFA) esters, and alditol acetates are the most commonly used derivatives. To produce alditol acetates, the monosaccharides are converted to alditols using reducing agents such as sodium borohydride or deuteride, followed by acetylation using acetic anhydride and pyridine (1:1) (Sawardekar et al., 1965). In recent times N-methyl imidazole has replaced pyridine as a catalyst in carrying out acetylation (Englyst and Cummings, 1984; Thomas and Thibault, 2002). The alditol acetates can be identified by their retention times and if necessary by their mass spectra. Only one alditol acetate, is formed for each monosaccharide in contrast to the multiple derivatives resulting from the different ring forms (pyranose and furanose) and anomers (α and β), due to TMS derivatization.

The methods described above do not distinguish between the enantiomeric forms of a monosaccharide. Many monosaccharides are present naturally in their d-form. However, some polysaccharides contain monosaccharides in their l-form. For instance, l-rhamnose occurs in pectins and l-arabinose in arabinoxylans. These enantiomeric forms can be distinguished by converting them into equilibrium mixtures of glycosides of chiral alcohols ($+/-$2-butanol or $+/-$2-octanol) which can be separated by capillary GC columns (Leontein et al., 1978). Capillary columns are the most extensively used compared to the packed columns (Andrews, 1989).

Linkage Analysis

Methylation is the oldest and most widely used method for determining the positions of the linkages in polysaccharides. The

method is based on the complete etherification of free hydroxyl groups, i.e. those not involved in ring formation, inter sugar glycosidic linkages, or carrying substituents which are stable in the conditions used for the methylation of the polysaccharide and for the subsequent hydrolysis of the methylated derivative (Lindberg, 1973). Methylation analysis is the most utilized method for the determination of the carbohydrate structure of monosaccharide units in oilgo-,polysaccharides and in glycoconjugates. Several methods have been described to give fully methylated carbohydrates. (Haworth, 1915; Kuhn et al.,1955; Hakomori, 1964). The most extensively used method is of Ciucanuand and Kerek (1989) which was further modified (Carpita and Shea, 1989). Methylation also gives information about the ring size (pyranose or furanose), non-reducing end groups, chain units, and branch points in the polysaccharide. For example, the presence of a methoxyl group at C-4 or C-5 indicates whether the ring is pyranose or furanose, respectively.

Characterization and Analysis of Methylated Monosaccharides

The complete characterization of a permethylated cereal noncellulosic polysaccharides requires the identification and quantitative analysis of all the monosaccharide derivatives formed on depolymerization. This can be done by GLC and confirmed by GC–MS (Dutton, 1973). The methyl ethers of most of the commonly occurring monosaccharides can be separated by using different liquid phases and by choosing appropriate column conditions. The depolymerization of methylated noncellulosic polysaccharides is effected by hydrolysis with subsequent formation of acyclic derivatives, thus avoiding the formation of multiple derivatives from the same monosaccharide. Since, the methylated polysaccharides are much less soluble in hot than in cold aqueous solvents, it is usually convenient to carry out partial hydrolysis in an organic solvent such as formic acid and complete the hydrolysis in a dilute aqueous acid. Permethylated alditol acetates, formed by reduction followed by acetylation are the most widely used derivatives for the characterization of methylated monosaccharides. The mass spectra of these compounds are normally simple to interpret. However, with this method the anomeric configurations of the glycosidic linkages (α or β) cannot be determined (Lonngren and Svensson, 1974).

Primary fragment ions from permethylated ions arise by α-cleavage with preferred formation of (a) ions of lower molecular weight; (b) ions from cleavage between two methoxyl bearing carbon atoms; (c) ions from cleavage between methoxyl bearing and an acetoxyl bearing carbon atoms with marked preference for the methoxyl bearing species to carry the positive charge. A very low abundance of scission takes place between two acetoxyl bearing carbon atoms. Primary fragment ions undergo a series of subsequent elimination reactions to give secondary fragment ions which include losses by (a) β-elimination of acetic acid (m/e 60) or methanol (m/e 32); (b) α-elimination of acetic acid but not of methanol; and (c) via cyclic transition states of formaldehyde, methoxymethyl acetate, or acetoxy methyl acetate (Jansson, 1976).

Oxidation Methods

Oxidative cleavage of polysaccharides and the characterization of the liberated products give details about the mode of linkage, substitution pattern, and the anomeric configuration of the linkage (α or β).

The anomeric configuration of glycosidic linkages of polysaccharides can be determined by chromium trioxide oxidation. With model compounds it was demonstrated that CrO_3 oxidizes β-linked polysaccharides preferentially to α-linked polysaccharides. The difference can be attributed to the ease of formation of a keto ester by cleavage at the bridge oxygen of β-anomeric compounds (Lindberg et al., 1975).

Periodate oxidation is one of the most widely used techniques for the determination of linkage and of substituents arrangement. Glycol cleavage via oxidation by sodium metaperiodate gives formic acid (usually from triol cleavage in pyranose) or formaldehyde from exocyclic diol (CHOH-CH_2OH) groups and the oxidant is reduced to iodate. The liberated products can be determined by various methods such as titrimetric, spectrophotometric methods to estimate the oxidant reduced, or by acid-base titration to measure the formic acid liberated or by colorimetric methods for formaldehyde (Hay et al., 1965).

The monosaccharide residues resistant to periodate oxidation and the liberated aldehydes are reduced with sodium borohydride, followed by hydrolysis to give the unattacked monosaccharide together with residual stubs of oxidized units (either glycerol from pentitol, erythreitol or threitol from 1→4 and 1→6 linked hexitol) (Goldstein et al., 1965).

Oligosaccharide Analysis

Analysis of oligosaccharides liberated upon partial deploymerization of noncellulosic polysaccharides give details about the distribution of side chains (random or uniform), and their linkage, which in turn substantiates structural information obtained about the parent polysaccharide. Oligosaccharides can be liberated using several chemical methods such as partial acid hydrolysis, acetolysis, trifluoroacetolysis, mercaptolysis, and methanolysis. The liberated oligosaccharides can be purified by conventional methods such as paper chromatography, HPLC, or GPC. They can be characterized by methylation analysis (as for polysaccharides) to determine the positions of the linkages, by optical rotation, and proton NMR to determine the anomeric proton arrangements, and by MALDI-TOF-MS and FAB-MS to determine the molecular mass and sequence of the constituent monosaccharide residues (York et al., 1990).

ENZYMATIC METHODS

The structure of non-cellulosic polysaccharides can also be determined by using substrate-specific enzymes, which cleave them into mono- or oligosaccharides with varying degrees of polymerization. By characterizing the liberated

oligosaccharides and the residual material, the structure of the parent polysaccharide can be elucidated. Enzymes that degrade non-cellulosic polysaccharides can be classified into two groups, exo- and endo-acting, depending on their mode of action. Exo-glycosidases remove mono- or disaccharide units from the non-reducing termini of polysaccharides, whereas endoglycosidases cleave randomly (unbranched regions of both internal and external chains), resulting in the liberation of oligosaccharides with varying degrees of polymerization. Enzymatic methods have several advantages over chemical methods: a) their specificity, which often includes the positions and anomeric configurations of the glycosidic links and the types and positions of substituents; b) lack of byproducts; c) high reaction rates; and d) reproducibility. Various cell wall polysaccharides such as arabinoxylans have been characterized by enzymatic methods (Hoffman et al., 1992).

SPECTROSCOPIC METHODS

Spectroscopic methods give more details about the structure and are often much simpler to carry out. Data obtained from these methods complement data obtained from chemical and enzymatic methods. Some of the most important spectroscopic methods are nuclear magnetic resonance (NMR), infra red (IR), mass spectrometry, optical rotatory dispersion (ORD), circular dichroism (CD), and X-ray diffraction.

NMR

This is a rapid and nondestructive method for studying polysaccharides without modification or degradation. ^{13}C and ^{1}H NMR spectra are very useful for elucidating the structures of poly- and oligosaccharides. ^{13}C-NMR gives details about the monosaccharide composition, sequence, and conformation of polysaccharides (Jennings and Smith, 1978) and can also be used to ascertain the purity of polysaccharide preparations. However, it cannot differentiate the enantiomeric configurations of sugars. High resolution C and H NMR(gCOSY, NOESY, TOCSY, gHSOQC, HMBC) are nowadays used to elucidate completely the polysaccharide structural features (Hoffmann et al., 2005; Brober et al., 2000).

IR Spectroscopy

Infrared spectra originate from the absorption of IR frequencies by vibrating chemical bonds, hence it is a vibrational spectroscopy. An infrared spectrum is a plot of absorption or emission, as a function of frequency in the range of ν 4000-50 cm^{-1}. IR spectra of non-cellulosic polysaccharides can be obtained with solid samples (1–10 mg). The sample is prepared either as a pellet, by mixing with dry KBr, or as a smear, by mixing with nujol (a paraffin oil). In general, IR can be used to detect functional groups, and to determine anomeric configurations and substitution patterns. IR spectral data have been used to characterize arabinoxylans and their oligosaccharides (Kacurakova et al., 1994).

Mass Spectrometry

Mass spectrometry is based on the principle that ions of different mass:charge (m/z) ratios are separated because they are differentially deflected by magnetic and electrostatic fields. There are 3 important steps to analyze a compound by mass spectrometry: a) sample introduction; b) volatilization; and c) ionization. Using the older ionization methods, samples must first be vaporized if they are not volatile by converting them into volatile acetylated or alkylated products.

The two important ionization methods, which are in general use, are a) electron ionization (EI); and b) chemical ionization (CI). In the electron ionization, the ions originally formed may fragment before entering the analyzer, because of the high energy transferred by the bombarding electrons, and give a complicated spectrum. Whereas, in chemical ionization, the molecular ions remain intact and give a simple and easily interpretable mass spectrum.

Due to the advent of recent techniques such as matrix assisted laser desorption/ionization-time of flight spectrometry (MALDI-TOF MS) and fast atom bombardment mass spectrometry (FAB-MS) it is very easy to analyze samples without any derivatization. These methods have been used to determine the molecular mass and sequence of various biomolecules such as peptides, small proteins, glycolipids and oligosaccharides (Harrison and Cotter, 1990). Electron spray ionization tandem mass spectroscopy (ESI-MS) has become a very powerful tool to differentiate isomeric oligosaccharides (Fernadez et al., 2004).

STRUCTURAL FEATURES OF NON-CELLULOSIC POLYSACCHARIDES FROM DIFFERENT CEREALS

Barley (Hordeum Vulgare)

In barley, arabinoxylans comprise 20–25% of the endosperm (Balance and Manners., 1978) and 85% of the aleurone (McNeil et al., 1975) cell-wall polysaccharides. Excess arabinoxylans in barley malt or adjuncts can cause processing problems such as poor filtration rates or the formation of hazes and precipitates. Structural studies of these polymers indicated that the back bone is made up of a (1→4)-β-D-xylan, substituted to varying extents with single arabinofuranose residues at O-2 or O-3 or at both O-2 and O-3. The polymer is made up of two types of sequences, the major one consists of blocks of isolated, substituted residues separated by one or two unsubstituted residues (Region-A) and the minor one, which separates these blocks, consists of two or more unsubstituted xylose residues (Region-B) (Vietor et al., 1994 and Han, 2000) (Fig. 1).

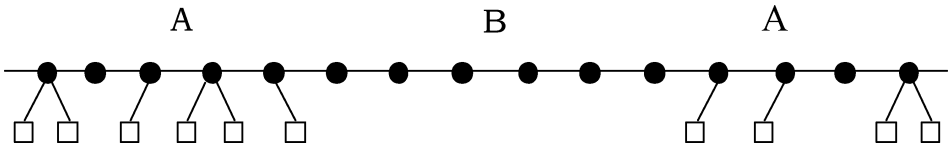

Figure 1 Schematic representation of arabinoxylans from barley endosperm

Wheat (Triticum Aestivum)

Structural analysis of water unextractable arabinoxylan from wheat indicated two different regions (regions A and B). Region A is constant, whereas region B varies in composition depending on the Ara/Xyl ratio. Region A is highly branched, consisting of tetrameric repeating units of an unsubstituted and a double arabinofuranosylated xylose residues (both O-2,3). Region B is less branched, and includes contiguous unsubstituted xylose residues. The substituted xylose residues are either isolated or present in pairs (Gruppen et al., 1993) (Fig. 2).

Rice (Oryza Sativa)

The main non-cellulosic polysaccharide in the endosperm cell walls of rice is a highly branched arabinoxylan, in which the arabinofuranose residues are attached at the C3 position of approximately 6 out of 7 of the d-xylose residues of the back bone (1→4)-β-D-xylan (Shibuya, 1984). The presence of O-2 linked arabinofuranosyl residues was also confirmed in the arabinoxylans of rice endosperm and bran. Many of the (1→4)-β-D-xylose residues (74–79%) of acidic arabinoxylans of endosperm are doubly branched. Bran arabinoxylans contain appreciable amounts of non-reducing end xylose and galactose, and (1→2)-, (1→3)-, (1→5)-linked arabinose residues, indicating more complicated structural features of their side chains compared with endosperm arabinoxylans in which most of the side chains are single monosaccharide residues. Uronic acids are known to be linked to xylose residues at the O-2 position as indicated by the presence of 2-O-(4-methyl-D-glucopyranosylurono)-D-xylose (Figs. 3, 4) (Shibuya et al., 1983; Shibuya 1984; Shibuya and Iwasaki, 1985)

Sorghum (Sorghum bicolor)

It is used in the preparation of lager-type beers. Sorghum flour contains 5–6 % (w/w) cell-wall material, which consists of non-starch polysaccharides, and protein (Verbruggen et al., 1993). These cell walls are resistant to enzymatic degradation during malting, and impair the filtration of the wort (Aisien and Muts, 1987). The major constituents of the water extractable NSP are (1→3),(1→4)-β-D-glucan and arabinoxylans (Verbruggen et al., 1995), whereas the material insoluble in water is mainly glucuronoarabinoxylans (Woolard et al., 1976). Sorghum glucuronoarabinoxylans are insoluble in water and were extracted with aqueous solutions of $Ba(OH)_2$ and KOH, resulting in nine fractions Among these fractions, BE-I was shown to be a pure arabinoxylan with a very high degree of substitution (45%) at O-3. It also contained a large amount of O-2, O-3 disubstituted (17%) and unsubstituted (28%) xylose units. Most of the arabinose units (88%) were present as terminal furanosyl units. The uronic acids were assumed to be attached to the (1→4) xylan at the O-2 position of xylose (Verbruggen et al., 1995). Pure glucuronoarabinoxylans were also isolated from the husk B fractions and consisted of l-arabinose, d-xylose, glucuronic acid, and 4-O-methylglucuronic acid in the molar ratios of 15:18:2:1. The arabinose residues were attached at the O-3, O-2, and O-3 positions of the xylose residues. Uronic acid residues (glucuronic acid, and 4-0-methylglucuronic acid) were attached to some of the xylose residues at the O-2 position (Fig. 5).

Maize (Zea Mays)

Maize bran contains about 40% (w/w) heteroxylans. Sequential extraction of purified maize bran with 0.5 M NaOH

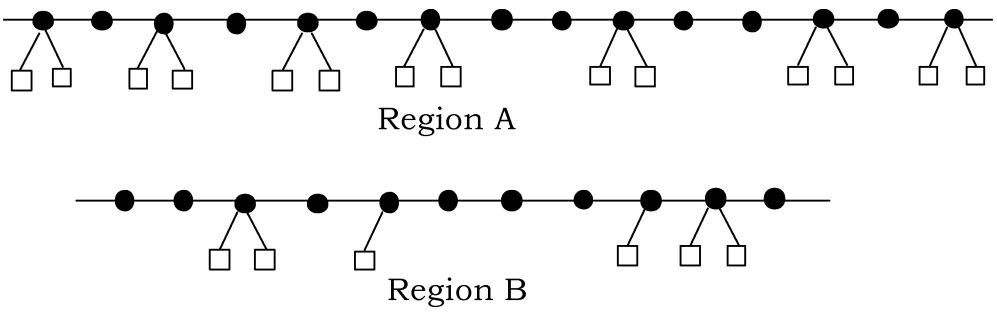

Figure 2 Schematic representation of wheat endosperm water unextractable arabinoxylans

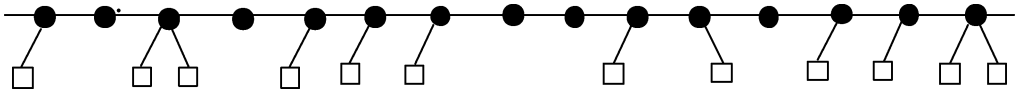

Figure 3 Rice endosperm arabinoxylans

followed by 1.5 M KOH yielded three fractions (S_1, S_2, and R_2) in different amounts (Chanliaud et al., 1995). The S_1 and S_2 fractions were shown to be heteroxylans with the monosaccharide compositions Xyl:Ara:Gal:GlcA 1.62:1.00:0.21: 0.15 and 1.61:1.00:0.22:0.16, respectively. The R_2 fraction was shown to be more of the cellulosic type polymer with a high amount of β- (1→4) linkages. Structural details obtained by methylation analysis of S_1 and S_2 indicated that they have similar types of linkages and similar distributions of side chains. The xylan backbone was highly substituted (only 23% of the xylose residues were unbranched) with oligomeric side chains, containing xylose, arabinose, and galactose. The substitutions were at O-2 (22%) or O-3 (15%) by single units of arabinose residues. Unsubstituted (15%) and disubstituted (20%) xylose residues were also found and Galactose residues (84%) were present mainly in the terminal position (Montgomery et al., 1957 and Chanliaud et al., 1995). Since the substitution of the xylan chain is very high the possibility of hydrogen bonding with other cell-wall components, particularly cellulose, is excluded (Fig. 6).

Rye (Secale Cereale)

Rye grains contains high dietary fiber content (17%) out of which (1→3),(1→4)-β-D-glucans (2–3%), arabinoxylans (6–7%), cellulose (2–3%), and lignin (3%) (Bengtsson and Aman, 1990). The concentration of arabinoxylans ranges from 6.5–12.2%, depending on the cultivar and environmental factors.

Rye arabinoxylans that are insoluble in water have been divided into three classes, differing in extractability, solubility, and structure (Vinkx et al., 1995). The first class had a low Ara/Xyl ratio (0.2) and was insoluble in water after neutralization of the alkaline extract (1M KOH). The second class had an intermediate Ara/Xyl ratio and remained in solution after neutralization of the alkaline extract (saturated $Ba(OH)_2$containing 1% $NaBH_4$), but precipitated upon saturation of the solution with ammonium sulphate. Classes one and two had some similarities in structure (terminal arabinoses, unsubstituted, 3-mono, 2-mono and disubstituted xyloses). The third class was soluble in both water and ammonia solution and had a high Ara/Xyl ratio (1.1). Their structural analysis indicated the presence of substituted arabinoses (40% of the arabinose), terminal xyloses (26% of xyloses), and terminal galactose (Vinkx and Delcour, 1996).

Ragi (Eleusine Coracana)

Finger millet also known as ragi, is an indigenous minor millet, rich in calcium and dietary fiber. It is extensively used by the South Indian rural population and is used both in native and processed (malted) forms. Hemicellulose fractions-A and B were isolated from native ragi (ungerminated, unkilined powdered grain flour) and its malt which was obtained by germinating the seeds at 25°C for 48, 96 h in a B.O.D incubator followed by kilning (Table 1). During controlled germination (malting) the concentration of "hemicellulose-A" fraction in the grain decreased with a concomitant increase in the concentration of the hemicellulose-B fraction. However, the solubility of the hemicellulose-A fraction increased three-fold during malting. The viscosity of the hemicellulose-B fraction decreased with malting time. From the monosaccharide compositions, the hemicellulose-A and B fractions probably consisted mostly of (1→3),(1→4)-β-D-glucans and heteroxylans; the hemicellulose-B fraction being rich in heteroxylans, whereas the hemicellulose-A fraction was rich in (1→3), (1→4)-β-D-glucans (Table 2).

FRACTIONATION OF HEMICELLULOSE

The hemicellulose–A fraction was sparingly soluble in water. Only 10 and 30% of the fraction obtained from native and malted grains, respectively, was water soluble. Both soluble and

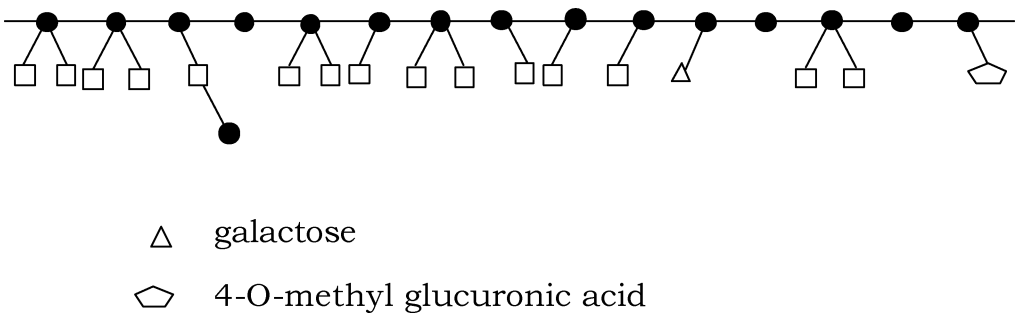

Figure 4 Rice bran arabinoxylans

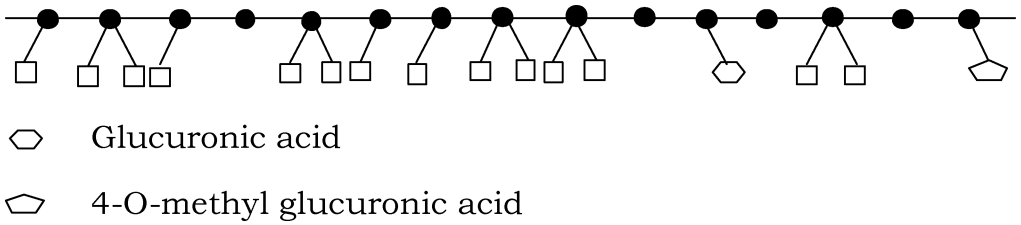

Figure 5 Structure of sorghum husk arabinoxylans

insoluble portions of hemicellulose-A consisted of arabinose, xylose, galactose, glucose, and small amounts of uronic acid in different ratios. The glucose content of the soluble fraction of fraction of hemicellulose –A decreased from 71.8 to 61.8% and from 76.3 to 70.50% in the insoluble fraction after 96 h of malting, indicating the degradation of (1→3),(1→4)-β-D-glucans by 1,3-β-D-glucanases, which are known to be induced in ragi during malting (Nirmala et al., 2000).

The water soluble portion of the hemicellulose fraction was fractionated on DEAE-cellulose (carbonate form) and five different fractions were obtained by successive elutions with water, 0.1 and 0.2 M ammonium carbonate and 0.1 and 0.2 M NaOH. The 0.1 M ammonium carbonate eluted fractions, gave the highest yields (64 and 65% from native and malted samples respectively) and were found to be heterogeneous by HPSEC and by cellulose acetate electrophoresis; they were not studied further.

FRACTIONATION OF THE HEMICELLULOSE–B FRACTION

Unlike the "hemicellulose"-A fraction, the "hemicellulose"-B fraction was found to be highly soluble in water (>96%) and rich in pentosans and was subjected to detailed fractionation and purification studies.

Fractionation by Ammonium Sulphate

In order to isolate arabinoxylans in pure form, the "hemicellulose"-B fraction was fractionated using ammonium sulphate into four fractions: F-60 (0–60%) F-70 (60–70%), F-80 (70–80%), F-100 (80-100%) and a non-precipitable fraction (N.P.). The F-70 fraction, obtained in very high yields (∼45%), was completely soluble in water and was used for further studies. The F-60 fraction, was the least soluble fraction, and was not studied further. However, the other fractions (F-80, F-100, and N.P.), which were completely water soluble, were not studied further because of their low yield (Table 3). The neutral monosaccharide compositions of these fractions showed that they all contained arabinose, xylose, galactose, and glucose but in different ratios (Table 4). As the concentration of ammonium sulphate increased, the proportion of arabinose increased from 12 (F-60) to 42% (N.P.) in native and 14 (F-60) to 32% (N.P.) in malted samples. In a similar manner, the proportion of galactose also increased from 8.1% in F-60 to 28.5% in the supernatant in native and 6.4% to 35% in malted samples, indicating the likely presence of an arabinogalactan in the non-precipitable fractions.

F-70, the major fraction obtained by graded ammonium sulphate precipitation from native and malted hemicellulose-B fractions, was further fractionated into 7 sub-fractions on a DEAE-cellulose column by eluting with water, ammonium carbonate (0.1 and 0.2 M) and sodium hydroxide (0.1 and 0.2 M). Neutral monosaccharide analyses of these fractions showed that they all contained arabinose, xylose, galactose, and glucose, but in different ratios. The fraction eluted with 0.1 M ammonium carbonate from native (N) and malted (M) hemicellulose-B fractions was obtained in maximum yield (30%). This fraction was completely water soluble, rich in arabinose and xylose and its purity was determined by GPC on Sephacryl S-400, Sepharose CL-4B, by HPSEC, and by cellulose acetate and capillary electrophoresis (Subba Rao and Muralikrishna, 2006).

Structural studies on the purified polysaccharides by methylation analysis, followed by GLC-MS, periodate oxidation, Smith degradation, ^{1}HNMR and IR spectroscopy, optical rotation, and oligosaccharide analysis by enzymatic and chemical methods (2N acetic acid partial hydrolysis for 10 h) indicated that the backbone comprised (1→4)-β-D-xylose residues, with the majority of the arabinose residues present as side chains substituted at the O-3 position of xylose (Fig. 7) (Subba Rao and Muralikrishna, 2004)

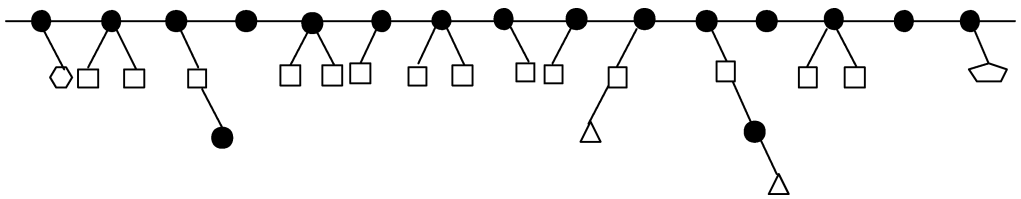

Figure 6 Arabinoxylans from maize bran

Table 1 Changes in physico-chemical properties of ragi non-cellulosic polysaccharides during malting

Fraction	Duration of malting (h)	Yield (%)	Solubility in water (%)	TC in soluble portion (%)	UA in soluble portion (%)	Relative viscosity (η_γ)
Hemi-A	Control	1.40	10.0	90.0	04.0	1.10
	48	1.30	26.0	92.2	04.6	1.12
	96	0.50	30.0	96.0	10.0	1.23
Hemi-B ‘	Control	1.90	94.0	94.0	10.0	3.04
	48	1.80	95.0	95.0	10.0	2.15
	96	3.00	96.0	96.0	10.0	1.98

Yield of the alkali-insoluble residue (AIR) increased from 7% in native flour to 8.2% in 48 h and 10% in 96 h malted flours.
UA: Uronic acid.; η_γ: relative viscosity.
This material was published in “Nonstarch polysaccharides and bound phenolic acids from native and malted finger millet (Ragi, Eleusine comcana, Indaf-15) authored by M.V.S.S.T. Subba Rao and G. Muralikrishna in *Food Chemistry* 72 (2001), 187–192. Copyright Elsevier Science Limited, 2001. Reprinted with permission from Elsevier.

The presence of 4-0-methyl glucuronic acid was identified by carboxyl reduction of the native polysaccharide followed by its acid hydrolysis, $NaBH_4$ reduction, acetylation, and identification of the resultant alditol(s) by GLC-MS. Characteristic mass fragments of 129, 189, 201, and 261 were assigned to 1, 2, 3, 5, 6 penta O-acetyl-4-O -methyl- D-glucose. The presence of 4-O-methyl glucuronic acid was also confirmed by a prominent signal at 178.0 ppm in the NMR spectrum, by a band at 178.4 cm^{-1} in the IR spectrum, and by a substantial increase in 2, 3, 4, 6-Me_4–glucose (10%) upon methylation of carboxyl reduced polysaccharides.

The major oligosaccharide obtained by endoxylanase treatment of 0.1 M ammonium carbonate eluted fraction orginated from native and malted flours was found to be pure and had a molecular weight of 1865 Da as determined by MALDI-TOF-MS and gel filtration on Biogel P-2; this molecular weight corresponds to 14 pentose residues. The structure of this oligosaccharide was elucidated by methylation analysis followed by GC-MS and ^{1}HNMR. The results indicated that it was made up of 8 xylose residues and 6 arabinose residues substituted at O-3 (monosubstituted) and at both O-2 and O-3 positions (disubstituted) (Subba Rao and Muralikrishna, 2004).

PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF NON-CELLULOSIC POLYSACCHARIDES

Arabinoxylans from cereals have been characterized thoroughly because of their important functional properties such as water absorption and water holding capacity (hold 3.5 to 7 times their weight) (Meuser and Suckow, 1986), protein foam stabilization (Izydorczyk and Biliaderis, 1992), and gas retention. These properties affect various technological processes such as bread-making and dough rheology. Recently, arabinoxylan oligosaccharides have been found to have some important beneficial effects on health and on physiological functions (Yamada et al., 1994). Although arabinoxylans are minor components of total cell-wall preparations from cereal grains, they are highly concentrated (60-70%) in the aleurone layers of cereals such as wheat, sorghum, oat, and rye. However, barley and rice have smaller proportions of arabinoxylans (20%) and (40%), respectively, in their aleurone layers (Fincher and Stone, 1986).

The fine structural features of arabinoxylans are of fundamental importance to understand their properties in food science and technology.

Molecular Weight

Estimates of the molecular weights of cereal arabinoxylans vary depending on the method used. Water-soluble arabinoxylans from wheat were shown to have molecular weight in the range of 65,000–66,000 Da by sedimentation analysis (Girhammar and Nair, 1992), but 800,000–5,000,000 Da by gel permeation chromatography (Fincher and Stone, 1986). The molecular weights of purified arabinoxylans from ragi grains and malts were in the range 1,120–1,200 KDa, as determined by gel permeation chromatography (Subba Rao and Muralikrishna, 2004).

Table 2 Changes in the neutral monosaccharide composition (%) of ragi hemicellulose-A and B fractions during malting

Fraction	Duration of malting (h)	Rha	Ara	Xyl	Man	Gal	Glc	A:X	P:H
Hemi-A	Control	2.20	33.2	9.0	6.60	3.40	45.50	1:0.27	0.74:1
	48	1.10	20.6	10.7	4.50	4.60	58.50	1:0.52	0.46:1
	96	1.50	11.0	7.00	0.00	4.60	75.90	1:0.63	0.22:1
Hemi-B	Control	0.70	49.5	25.7	3.10	5.60	15.40	1:0.52	3.10:1
	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

Hemi-A: Hemicellulose-A.; Hemi-B: Hemicellulose-B.; A:X : Arabinose to Xylose ratio, P:H : Pentose to Hexose ratio.

Table 3 Proximate composition (%) of ammonium sulphate fractionated samples

Sample	Fraction	Yield	Solubility	T.C.	U.A.	Protein
Native	F-60	19.00	42.00	90.00	5.00	3.50
	F-70	44.50	96.00	92.00	10.00	6.00
	F-80	10.00	100.00	98.00	10.50	1.20
	F-100	7.50	100.00	98.60	9.60	0.50
	N.P.	19.00	100.00	90.00	9.00	3.00
Malted	F-60	11.50	46.00	92.00	4.00	4.00
	F-70	38.50	94.00	96.00	9.00	4.00
	F-80	16.00	100.00	96.8	10.00	3.20
	F-100	9.40	100.00	98.00	9.20	2.00
	N.P.	24.6	100.00	92.00	9.00	2.50

T.C. Total carbohydrate; U.A. Uronic acid; N.P. Non-precipitable fraction.

Conformation

The conformation of arabinoxylans has been determined by X-ray diffraction analysis, and they have been shown to have a 3-fold, left-handed helix in solution and in the solid state they appear as an extended, twisted ribbon if the xylan backbone is unsubstituted (Fincher and Stone, 1986). The xylan backbone is relatively flexible, since it is supported by only one hydrogen-bond between two adjacent xylose residues, and it aggregates into insoluble complexes, stabilized by intermolecular hydrogen-bonding. The presence of arabinosyl substituents appears to prevent this aggregation, and stiffen the molecules by maintaining the xylan backbone in a more extended conformation. The asymmetry of arabinoxylans was confirmed by a very high axial ratio (140) for wheat endosperm arabinoxylans (Andrawartha et al., 1979) and by a very high Simha shape factor (507) for rye arabinoxylans (Girhammar and Nair, 1992). Conformation analysis of rice endosperm arabinoxylans by X-ray diffraction suggested a three-fold helical confirmation (Yui et al., 1995).

Viscosity

Because of their rather stiff conformation, arabinoxylans have very high intrinsic viscosities in aqueous solutions compared with those of other non-cereal polysaccharides such as dextrans, arabinans, or gum arabic (Fincher and Stone, 1986). Several factors such as overall asymmetrical confirmation, degree of polymerization, and spacial arrangement of the arabinose side chains along the xylan backbone will influence the behavior of arabinoxylans in solution. Arabinoxylans with high $[\eta]$ values have high Xyl/Ara ratios, feruloyl concentrations, and low proportions of doubly substituted xylopyranose residues. The relative proportions of singly substituted Xyl$p$ at C(O)-2 vs C(O)-3 increased with decreasing $[\eta]$, and side chains consisting mostly of arabinose residues are more common in fractions of low limiting viscosities (Izydorczyk and Biliaderis, 1993). In contrast to the above, more highly substituted arabinoxylans were shown to have lower viscosities, and this indicated that the high intrinsic viscosity of arabinoxylans is not solely governed by their asymmetrical confirmation, but also by the length of the backbone and by the arrangement of the arabinose residues along the chain. Arabinoxylans in solution were found to behave like Newtonian fluids at low shear rate; however, with increasing shear rate, they exhibit substantial shear thinning, typical of pseudoplastic materials (Izydorczyk and Biliaderis, 1992a).

Changes in the relative viscosity (η_r) of the noncellulosic polysaccharide fractions (rich in pentosans) obtained from native and malted ragi flours have been studied in relation to concentration (0.1–1.0%), pH (2.0–10.0), and temperature (28–80°C). The viscosity increased as the concentration increased, decreased as the temperature increased, and reached a maximum at pH 6.0. The relative viscosities (relative to water) of the noncellulosic polysaccharides obtained both from native and malted

Table 4 Neutral monosaccharide compositions (%) of ammonium sulphate fractionated samples obtained from native and malted ragi hemicellulose-B fractions

Sample	Fraction	Ara	Xyl	Gal	Glc	A:X
Native	F-60	12.10	17.50	8.10	62.30	1.0:1.45
	F-70	13.30	19.85	9.00	57.85	1.0:1.50
	F-80	24.80	33.80	4.80	36.60	1.0:1.36
	F-100	26.30	33.00	6.50	34.20	1.0:1.25
	N.P.	42.60	23.40	28.50	5.50	1.0:0.54
Malted	F-60	14.00	15.60	6.40	64.00	1.0:1.11
	F-70	23.00	26.00	6.00	45.00	1.0:1.13
	F-80	26.50	34.70	8.20	30.60	1.0:1.30
	F-100	26.70	29.60	11.40	32.30	1.0:1.10
	N.P.	32.00	21.00	35.00	12.00	1.0:0.65

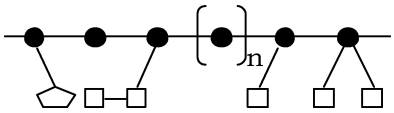

Figure 7 Probable structure of purified arabinoxylans obtained from native (N) and malted (M) ragi

flours at a concentration of 1%, were less than the viscosity of guar gum at a concentration of 0.5%. However, they were more than the viscosity of a solution of citrus pectin (1%). These results are consistent with results reported for cowpea, black gram and linseed mucilaginous polysaccharides (Muralikrishna et al., 1987a). The viscosity of any polysaccharide solution is governed not only by its overall conformation but also by the specific arrangement of substituent residues along the backbone (Izydorczyk and Biliaderis, 1995).

Gelling

Arabinoxylans, particularly those that are water extractable, are capable of forming three-dimensional gels or viscous solutions, upon the addition of free radical-generating agents (hydrogen peroxide/peroxidase, ammonium persulphate, ferric chloride, linoleic acid/lipoxygenase) resulting mainly from the presence of feruloyl residues. This process is known as oxidative gelation. Numerous hypotheses concerning the mechanism of this reaction have been proposed, one of which involves the formation of diferuloyl cross-links between arabinoxylans and other cell wall polymers such as proteins (Neukom and Markwalder, 1978; Schooneveld-Bergmans et al., 1999). Arabinoxylans containing high proportions of ferulic acid, high molecular weights, and relatively unsubstituted xylan backbones were capable of extensive cross-linking, and yielded well-developed gels. Since the presence of feruloyl residues was pivotal for cross-linking, their relative amounts and distribution along the back bone influence the gelling potential of arabinoxylans (Rattan et al., 1995) .

Oxidative gelation experiments (Vinkx et al., 1991) were carried out using a H_2O_2/peroxidase system with cold water soluble polysaccharides (CWSP) and hemicellulose-B fraction obtained from native and malted finger millet. Only negligible effects on the viscosity were found even after adding high concentrations of $H_2O_2$and peroxidase to the polysaccharide fractions, which could be due to the very low concentrations of ferulic acid present in the soluble portion of CWSP. Ferulic acid, a known antioxidant (Subba Rao and Muralikrishna, 2002), is known to play an important role in the oxidative gelation of various water-soluble polysaccharides of wheat and other cereals (Dervilly-Pinel et al., 2001) and its concentration is critical in increasing the viscosity of the solutions or in forming stable gels by cross-linking arabinoxylans.

Water Absorption

This property plays an important role in bread-making, since when added exogenously, arabinoxylans compete with other constituents of dough for water. This was shown by an increase in the farinograph water absorption and dough development time, when pentosans were added to wheat flour (Biliaderis et al., 1995). When arabinoxylans were subjected to oxidative gelation, their hydration capacity greatly increased and they held 100 g of water per gram of polymer (Izydorczyk et al., 1990).

Incorporation at different concentrations (0.25 and 0.5% on a 14% moisture basis) of CWSP and hemicellulose-B fraction isolated from both native and malted ragi to wheat dough resulted in changes in water absorption, DDT (Dough development time), DS (Dough strength), TI (Tolerance index) values. Water absorption of the dough increased by 3 and 5% on adding CWSP and hemicellulose-B fraction, respectively. Malting did not affect the water absorption capacity of these polysaccharides. The hemicellulose-B fraction, from native and malted grains, had more water absorption capacity than the CWSP, and which could be due to its high content of arabinoxylans (Subba Rao et al., 2004). The water absorption values found in the present study were less than those reported previously for wheat arabinoxylans (Jelaca and Hlynka, 1971).

Arabinoxylans in Bread Making

The addition of arabinoxylans to wheat flour is known to increase dough development time and the loaf volume of breads (Biliaderis et al., 1995). The beneficial effects of arabinoxylans on loaf volume depends on the concentrations of these polysaccharides, on the nature of the base flour, and the molecular weight of the arabinoxylan preparation. Concentrations of arabinoxylans that are higher than the optimum result in excessive viscosity of the dough and decrease the volume of the bread. If the added arabinoxylans are degraded by enzymes such as xylanases, the dough becomes sticky, yielding soggy bread with low loaf volume (Delcour et al., 1991).

Breads to which arabinoxylans had been added had less crumb firmness after storage for seven days compared with controls. This positive effect of arabinoxylans on the texture of breadcrumbs was related to increased moisture content. Since water acts as a plasticizer for the gluten-starch complex matrix, it lowers the rigidity of the products. This antifirming action of arabinoxylans depends on the amount added, and on their molecular weight, (Biliaderis and Izydorczyk, 1992). The staling of bread, which is mainly due to the retrogradation of starch, is influenced by the addition of arabinoxylans. Since arabinoxylans have been shown to increase the rate of starch retrogradation, as monitored by calorimetry, the enthalphy values of the staling endotherm of fortified bread crumbs were higher than those of control breads during storage (Biliaderis et al., 1995)

Gas Retention

Doughs fortified with arabinoxylans were found to retain gas effectively (Hoseney, 1984). This could be due to the viscosity of arabinoxylans, which adds strength and elasticity to the gluten-starch network, surrounding the gas bubbles, and thus

slows the diffusion of carbon dioxide from dough during baking. Breadcrumb texture, fineness, and homogeneity are directly related to the extent to which gas cells combine and collapse during heating.

NUTRITIONAL ATTRIBUTES OF NON-CELLULOSIC POLYSACCHARIDES

The physiological effects of non-cellulosic polysaccharides depend on their physicochemical properties. The main physicochemical properties, which determine their physiological effects, are water solubility, ion exchange, hydration capacity (water holding and water binding), and adsorption of organic compounds. All of these play an important role in the flow of digesta and nutrient availability and absorption. Increasing fecal bulk, improving large bowel function, reducing plasma cholesterol concentrations and the glycemic index, and preventing colon cancer are the major health benefits of dietary fiber. Cereals, such as wheat, are rich in insoluble dietary fibers, which increase fecal weight, bulk and softness, increase frequency of defecation, and reduce intestinal transit times. These effects probably play a role in preventing colon cancer and other bowel disorders. Soluble fibers such as oats (3–4%) and barley (4–5%) slow down glucose absorption, reduce plasma cholesterol concentrations, and are useful in the management of diabetes as well as heart diseases (Plaami, 1997).

FUTURE PERSPECTIVES

Even though non-cellulosic polysaccharides of cereals are reasonably well-characterized, the nutritional effects of individual polysaccharides is not well-documented. Future research should emphasize the health benefits of individual polysaccharides. Some of the health benefits of feruloylated arabinoxylans could be due to the ferulic acid, which is known to be an antioxidant and antimutagen. Since non-cellulosic polysaccharides are predominant in cereals, attempts should be made to incorporate them in various food preparations in different concentrations to investigate their potential and appropriate health benefits. Future research should also emphasize the possible advantages of under utilized and unexplored minor millets such as sanwa (Echinochloa frumentaceae), samai (Panicum miliare), varagu (Paspalum scrobiculatum), in terms of their nutritional and functional properties.

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