



Chemical, physicochemical and in-vitro fermentation characteristics of dietary fibres from *Palmaria palmata* (L.) Kuntze

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The content, chemical composition and structure, some physicochemical properties and fermentation characteristics of dietary fibres from *Palmaria palmata* (dulse) were studied. The marine red algae contains 33.2–33.5% total dietary fibres measured on a dry weight basis using conditions simulating the gastric and intestinal environment (pH, temperature, ionic strength and time of incubation) and measured by an enzymatic/gravimetric method, respectively. The yield of soluble fibres by the simulated digestive tract conditions was lower (12.2%) than by the enzymatic/gravimetric method (18.9%).

All soluble fibre fractions consisted of linear β -1,4/ β 1,3 mixed linked xylans containing similar amounts of 1,4 linkages (70.5–80.2%). The insoluble fibres contained essentially 1,4 linked xylans with some 1,3 linked xylose and a small amount of 1,4-linked glucose (cellulose). Low intrinsic viscosities were measured from the soluble fibres (49.4–97.0 ml g⁻¹) and water holding capacities of dry dulse particles of 4.3 and 4.7 g g⁻¹ were measured in buffer at pH 3.0 and 7.3, respectively.

Soluble fibres are fermented within 6 h by human faecal bacteria into short chain fatty acids.

INTRODUCTION

Although land plants have been used worldwide since ancient times as food or food sources, seaweeds are consumed traditionally only in Asia and marginally in the rest of the world (Chapman & Chapman, 1980). In western countries, seaweeds are essentially used for the production of gelling, thickening and stabilizing colloids used by the food industry (Davidson, 1980). The recent authorization in France for the human consumption of 11 algae as seasonings and vegetables (Fleurance, 1991) has stimulated interest in their dietary values. Known to be particularly rich in minerals and

vitamins (Ito & Hori, 1989), recent studies showed that edible seaweeds are also rich in dietary fibres (Kishi *et al.*, 1982; Lahaye & Thibault, 1990; Fleury & Lahaye, 1991; Lahaye, 1991; Nishimune *et al.*, 1991; Lahaye & Jegou, 1993). With such characteristics, consumption of edible seaweeds could contribute to diversify and increase the content of fibre in western diets in which actual shortage has been correlated with 'civilisation diseases' (diabetes, obesity, heart diseases, some cancers, diverticular diseases, ...; Southgate, 1990).

Palmaria palmata (L.) Kuntze, also known as dulse, has been consumed by Europeans and North Americans living on coastlines since as far back as the eighth century in Iceland (Indergaard & Minsaas, 1991). Although the chemical constituents of dulse have been thoroughly reviewed (Morgan

et al., 1980), no detailed information exists in the literature regarding dietary fibre contents and characteristics. In this paper, the content, the chemistry and some physicochemical properties of dietary fibres from dulse are reported with their in-vitro fermentability by human faecal bacteria.

MATERIALS AND METHODS

Algae

Palmaria palmata (dulse) was obtained dry from a local health food store.

Determinations of dietary fibre content

Dietary fibre content determination was done on shredded dry algae (size <5 mm long). The 'standard' method for determining soluble and insoluble dietary fibre content was done as described by Lahaye (1991) except that the buffer was changed to sodium acetate (0.1 M, pH 6.0) and that pH changes were carried out with M NaOH or glacial acetic acid.

The physiological approach to determine dietary fibre content was as described by Fleury & Lahaye (1991) except that a sintered glass filter G4 (5–15 μm porosity) was used and the extraction buffers were at pH 3.0 and 7.3. The soluble fractions at pH 3.0 and pH 7.3 were treated with protease at pH 7.3 (0.1 ml of 50 mg ml⁻¹; Sigma, La Verpillière, France) followed by amyloglucosidase (0.1 ml of 50 mg ml⁻¹; Merck, Darmstadt, Germany) for 30 min at 60°C each and then extensively dialysed against deionized water and freeze-dried. The insoluble residue was resuspended in acetate buffer (100 ml, pH 6.0) and treated with Termamyl (0.1 ml, Novo Industri, Bagsvaerd, Denmark) for 30 min in a boiling water bath, then with protease and amyloglucosidase as for the soluble fractions. The insoluble material was recovered on a sintered glass filter G4, washed extensively with deionized water, dehydrated by solvent exchange (80%, absolute ethanol, acetone and diethyl ether) and the soluble enzyme digest was dialysed extensively against deionized water and freeze-dried.

Chemical analysis

All results are expressed on a dry weight basis. The protein content was determined by the micro-Kjeldhal method ($N \times 6.25$) and ashes were weighed after overnight incineration of samples at 500°C followed by 2 h at 900°C.

Monosaccharide composition and content

The water insoluble fractions were subjected to acid pre-hydrolysis (H₂SO₄, 13 M, 30 min, 20°C) prior to hydrolysis by M H₂SO₄ (2 h, 100°C); water soluble

fractions were hydrolysed by M H₂SO₄ for 2 h at 100°C (Hoebler *et al.*, 1989). Monosaccharides as alditol acetates were analysed by gas-liquid chromatography following the procedure of Blakeney *et al.* (1983). A BP225 (Scientific Glass Engineering Sarl, Villeneuve-St-Georges, France) fused silica capillary column operating isothermally at 220°C and eluted with H₂ was connected to a DI 200 chromatograph (Delsi Nermag Instruments, Argenteuil, France) equipped with a flame ionization detector.

Permethylation and gas chromatography analysis of permethylated alditol acetates

Fibre fractions (1–2 mg) in 0.5 ml anhydrous DMSO were methylated using lithium methylsulphonyl carbanion (Blakeney & Stone, 1985; 0.5 ml) and iodomethane (0.5 ml; Merck). Methylated polysaccharides were hydrolysed by 2 M TFA (0.2 ml) containing meso-inositol (0.1 mg) for 1.5 h at 120°C. The acid was evaporated under dry air at 40°C and 0.5 ml of methanol added and evaporated. Reduction and acetylation was done as described (Harris *et al.*, 1984). Methylated alditol acetates were analysed with the same gas chromatographic system used for monosaccharide composition analysis except that a gradient of temperature was used (175°C for 15 min, then 175 \rightarrow 210°C at 15°C min⁻¹ then hold at 210°C for 13 min). Methylated sugar identification was done by comparison with standards and by gas chromatography coupled to a mass spectrometer operating in EI mode at 70 eV with a transfer chamber temperature of 250°C (R10-10C, Delsi-Nermag) and using the same column and conditions as above.

Nuclear magnetic resonance spectroscopy

¹³C and ¹H NMR spectra were recorded on a Bruker AM 500 spectrometer at 80°C from 5–10% solutions in D₂O. Chemical shifts are expressed in parts-per-million referred to internal dimethyl sulphoxide and converted to values related to tetramethylsilane (conversion constant 39.6) for ¹³C and referred to the OH line (4.2 ppm) for ¹H.

Size exclusion chromatography and molecular weight determination

To determine the weight average molecular weight (M_w), xylan fractions in 50 mM NaNO₃ containing 0.02% sodium azide were chromatographed with the same solvent as the eluent by high-pressure size exclusion chromatography (HPSEC) at 40°C through 2 columns (Shodex OHPak KB-806 and Shodex OHPak KB-805 both having 8 mm \times 300 mm dimensions and a precolumn Shodex OHPak KB-800P 6 mm \times 50 mm) of exclusion limits of 2×10^7 and 4×10^6 (pullulans). Elution (1 ml min⁻¹) was monitored on-line by a multi-

angle laser light scattering (MALLS) photometer equipped with a K5 flow cell (Dawn F, Wyatt Technology Corp., Santa Barbara, CA, USA) and a differential refractometer. The peak molecular weight was determined with ASTRA software (v. 2.0) (Wyatt Technology Corp.).

Viscosimetry

The viscosity of soluble xylans fractions was determined at 37°C in 155 mM NaCl with an automatic capillary Ubbelohde Viscometer (Amtec, France) and the intrinsic viscosity was derived from Kraemer and Huggins equations by extrapolation to infinite dilutions (Billmeyer, 1984).

Water absorption capacity

The water absorption capacity of 50–100 mg algal particles of size ranging between 125 and 250 µm was measured on a Baumann apparatus (Baumann, 1967) at 20°C with the buffers at pH 3.0 and 7.3 used for extraction of fibres as solvents.

In-vitro fermentation of dietary fibres

The pooled soluble fibre fractions extracted from dulse under the 'physiological' conditions and sugar beet fibres used as a fermentation standard were incubated under nitrogen with a human faecal inoculum at 40°C with shaking (Barry *et al.*, 1989). The fermentation experiment was performed in duplicate using pooled freshly and anaerobically harvested faeces from two healthy subjects as inoculum in CO₂-saturated nutritive buffer. Gas production was monitored continuously whereas pH, short chain fatty acids (SCFA) and residual polysaccharide contents and nature were evaluated after 6, 12 and 24 h of incubation. Unfermented residues were recovered by ethanol precipitation and were dehydrated by absolute ethanol, acetone and diethyl ether. The sugar composition was determined as above for insoluble fibres. Short chain fatty acid amounts were determined from the ethanolic supernatant by gas chromatography (Jouany, 1982).

The fermentability of fibres was estimated from the bacterial utilization coefficient (BUC) of individual sugars in fibres:

$$BUC = (IF + IC - R)/(IF + IC)$$

where *IF*, *IC* and *R* represent the amount of individual sugars in the initial fibres, the inoculum and the residues, respectively, and from the theoretical fermented organic matter (TFOM) according to Van Nevel *et al.* (1970). The latter calculation is based on the hypothesis that one mole of sugar (glucose) will be fermented into two moles of acetate (C2) or two moles of propionate (C3) or one mole of butyrate (C4), isobutyrate (IC4), valerate (C5) or isovalerate (IC5):

$$TFOM = (C2/2 + C3/2 + C4 + IC4 + C5 + IC5) \times 162$$

RESULTS AND DISCUSSION

Palmaria palmata is a promising edible seaweed because of appealing organoleptic characteristics to consumers (Mabeau, Personal communication) and nutritional values (Morgan *et al.*, 1980). The present results demonstrate that these algae are also rich in dietary fibre.

Dietary fibres have been partitioned between water-soluble and water-insoluble forms because of different physiological and metabolic effects (Roehrig, 1988). Soluble fibres are more often associated with viscous characteristics implied in lower blood cholesterol and glucose levels; insoluble fibres are generally associated with the lowering of total transit time.

The amount of dietary fibres in algae has recently been determined following a modification of an AOAC method (Lahaye, 1991), and Fleury & Lahaye (1991) devised conditions to simulate gastric and intestinal phases for the dietary fibre content determination of kombu breton (*Laminaria digitata*). Using the latter method, it was possible to demonstrate a possible differential solubilization of *L. digitata* fibres in the digestive tract. The two methods were used in the present study to quantify dietary fibre in *P. palmata*. The dietary fibre contents estimated by both methods were similar (Table 1) and according to the 'standard' method about 56% of them were soluble. However, if the solubilization conditions are closer to those prevailing in the digestive tract, only 36.8% are extracted during the simulated gastric and intestinal phases of digestion. An additional 4.7% of fibre was solubilized during enzymic treatment of the insoluble fractions and arose from the hot buffer treatment (gelatinization of starch) and/or the action of enzymes (amylases and protease). Such a fraction of fibre is not expected to be solubilized *in vivo* and can be considered as insoluble fibre. It is therefore clear that the soluble dietary fibre content depends heavily on the method used (Marlett *et al.*, 1989) and may not represent exactly the amount of fibre that will

Table 1. Soluble and insoluble dietary fibre contents of *Palmaria palmata* obtained by the standard method and the 'physiological' approach

Fraction ^a	Yield ^b	Protein ^c	Ash ^c	Dietary fibre ^b
S _s	24.3 (0.8)	15.2	7.2	18.9
I _s	21.3 (0.6)	29.4	2.2	14.6
Total	45.6			33.5
S _{3.0}	6.0 (0.3)	12.9	3.8	5.0
S _{7.3}	8.4 (0.6)	10.2	4.0	7.2
I _E	8.1 (0.3)	37.3	4.5	4.7
I _P	21.4 (0.3)	22.3	1.6	16.3
Total	43.9			33.2

^a S_s, I_s refer to the soluble and insoluble fractions, respectively, recovered by the standard method of fibre determination, S_{3.0}, S_{7.3}, I_E and I_P refer to the soluble fractions in buffer at pH 3.0, pH 7.3 and in the enzymic digest, and insoluble fractions, respectively, using the 'physiological' approach of fibre content determination.

^b Yield from the dry weight of alga (standard deviation) n = 3 (%).

^c Yield from the dry weight of the fractions (%).

be solubilized in the digestive tract. At any rate, the *P. palmata* total fibre content is within the range observed for other edible seaweeds (Kishi *et al.*, 1982; Fleury & Lahaye, 1991; Lahaye, 1991; Nishimune *et al.*, 1991; Lahaye & Jegou, 1993) but further studies are required to assess the effect of processing on the content and availability of fibres *in vivo*.

Dietary fibres in food consist essentially of undigestible plant cell-wall polysaccharides (Trowell, 1974). The composition and structure of *P. palmata* cell-wall polysaccharides have been extensively studied (Percival & Chanda, 1950; Cronshaw *et al.*, 1958; Myers & Preston, 1959; Dennis & Preston, 1961; Manners & Mitchell, 1963; Björndal *et al.*, 1965; Young, 1966; Turvey & Williams, 1970). It consists essentially of matricial linear xylans composed of mixed β -1,4 and β -1,3 linkages with a degree of polymerization reported between 40 and 114, of about 2–7% of cellulose and some linear skeletal β -1,4-linked xylans. The distribution of the β -1,3 linkages in the matricial polysaccharides occurs at random and contributes to 20–38% of all the linkages in this type of xylan. Mixed xylans have also been identified by chemical means from *Chaetangium fastigiatum* (Cerezo *et al.*, 1971; Cerezo, 1972; Nunn *et al.*, 1973) but ^{13}C nuclear magnetic resonance spectroscopy has become a classical tool for the analysis of soluble xylans from red algae (Usov *et al.*, 1978, 1981; Kovac *et al.*, 1980; Adams *et al.*, 1988). It was shown by this technique that, in some species of red algae, the ratio of 1,4 to 1,3 β -linkages varied between extracts and these xylans differed in solubility.

The present study confirmed that xylose is the major sugar in *P. palmata* cell-wall polysaccharides (Table 2). Traces of mannose, galactose and glucose were also present in the soluble fractions. Methylation analysis and ^{13}C and ^1H NMR analyses of the soluble extracts confirmed literature data in that xylose occurs essentially as 1,3 and 1,4 linked residues (Fig. 1). The percentage of 1,4 linkages in the different fractions is slightly greater by methylation analysis than that calculated using ^1H NMR anomeric peaks integral for 1,4 linkages at 4.37 ppm (Bengtson & Åman, 1990) and for 1,3 linkages attributed by deduction to the resonance at 4.57 ppm (Table 3). These values are within the range of those reported in the literature for *Palmaria palmata*

xylans and do not demonstrate marked variations between fractions.

Discrepancies between the NMR and chemical measurements may arise from incomplete methylation of polysaccharides or preferential degradation of linkages leading to errors in the estimation of 1,4 linkage percentages. Trace amounts of 1,3 linked mannose and terminal galactose were also observed in the soluble polysaccharides and may have originated from glycoproteins and residual floridoside (2-*O*-glycero α -D-galactopyranoside; Percival & McDowell, 1967), respectively.

The molecular weight distribution of the fibres extracted at pH 3.0 and 7.3 was determined by high pressure size exclusion chromatography and laser light scattering detection of the eluted polymers. The chromatographic profile obtained from the fraction soluble at pH 3.0 is markedly different from that obtained for the fibres soluble at pH 7.3 (Fig. 2). The former shows at least four populations while the latter shows only one population of polymers. This fraction has an average weight molecular weight of 1.3×10^5 with a polydispersity index of 2.97. This weight average molecular weight corresponds to a mean degree of polymerization of the xylan fraction of about 980 which is markedly higher than that reported in the literature for *Palmaria palmata* xylan.

Thus, although the molecular weight distributions of the xylans extracted under the simulated gastric and intestinal conditions are different, their structures are very similar. There is no particular chemical characteristic of xylans that would direct their solubility at pH 3.0 or 7.3. It is most likely that the xylan extracted at the latter pH resulted from incomplete solubilization during the simulated gastric phase. Such solubilization behaviour of dietary fibres was also observed for green seaweed soluble dietary fibres (Lahaye & Jegou, 1993) but markedly differs from that of *Laminaria digitata* (Fleury & Lahaye, 1991) in which different polysaccharides were extracted under similar conditions.

On acid pre-hydrolysis with sulphuric acid, a method to improve cellulose hydrolysis, the insoluble fibre fractions yielded xylose, glucose and a small amount of galactose (Table 2). Methylation analysis of these insoluble polysaccharides demonstrated the presence of 1,3 and 1,4 linked xylans (Table 4) and of 1,4 linked glucose arising from cellulose. Small amounts of unmethylated xylose were also observed, particularly from

Table 2. Sugar composition of the different fibre fractions from *Palmaria palmata*

Fraction	Sugar (% molar)				Yield (% dry weight)
	Xyl	Man	Gal	Glc	
<i>S_s</i>	89.5	4.2	5.4	0.9	65.5
<i>I_s</i>	86.1		1.6	12.3	79.6
<i>S₃</i>	87.4	2.5	9.5	0.7	84.3
<i>S_{7.3}</i>	94.3	1.4	4.1	0.2	98.8
<i>I_E</i>	83.5	3.4	9.7	3.4	62.6
<i>I_P</i>	88.7		1.6	9.7	85.6

See footnotes for Table 1 for the identification of fractions.

Table 3. Percentage of 1,4 linkages in soluble xylans from *Palmaria palmata* as measured from the integral of the anomeric proton by ^1H NMR spectroscopy (see Fig. 1 and text) and by methylation analysis

Fraction	^1H NMR	Methylation analysis
<i>S_s</i>	80.2	83.9
<i>S₃</i>	73.7	85.2
<i>S_{7.3}</i>	70.5	85.2
<i>I_E</i>	nd ^a	84.9

^a Not determined.

See footnotes for Table 1 for the identification of fractions.

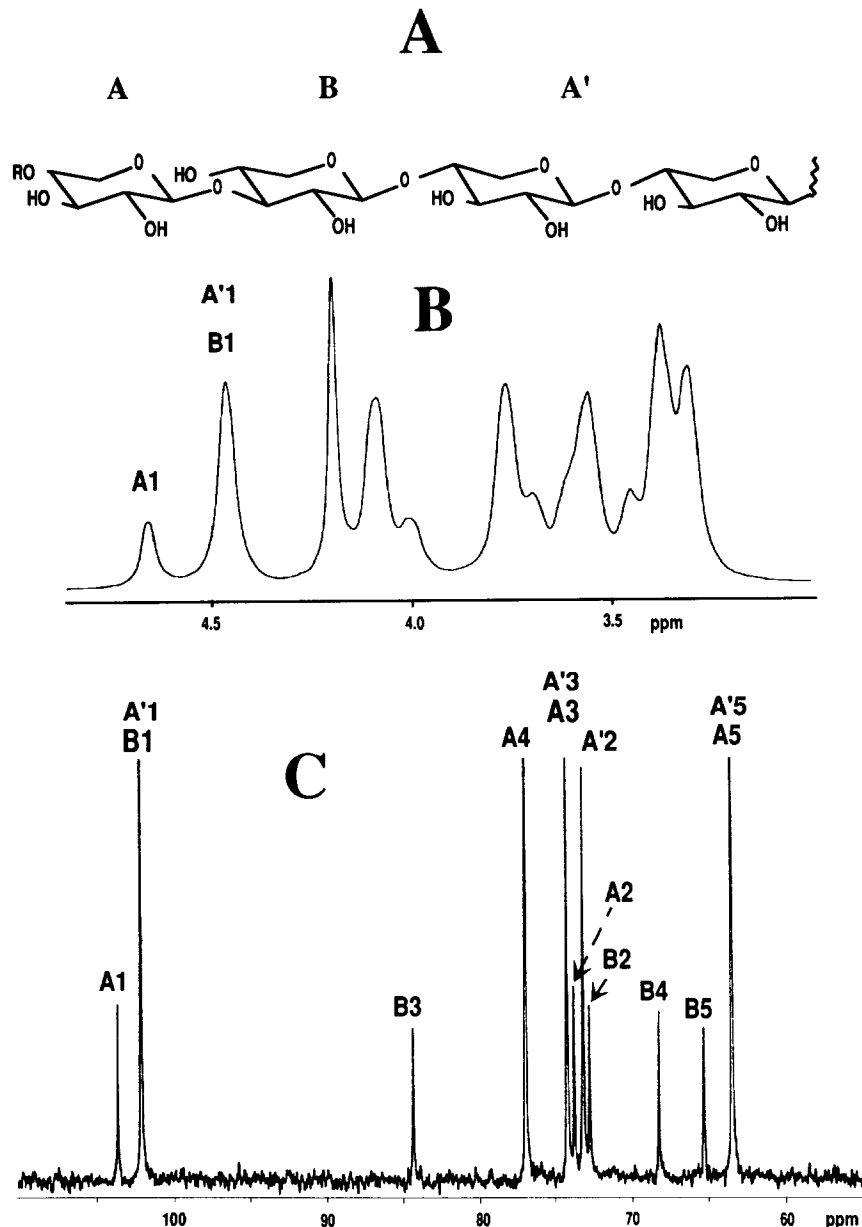


Fig. 1. (A) chemical structure, (B) ¹H and (C) ¹³C NMR spectra of *Palmaria palmata* soluble xylans. (A) Letters refer to the xylose residues 1,4 linked to a 1,3 linked xylose, A, 1,4 linked to a 1,4 linked xylose, A' and 1,3 linked to a 1,4 linked xylose, B. (B) Spectrum of the soluble fibres recovered from the standard method; A, A' and B refer to the anomeric protons of the 1,3 and 1,4 linked xylose residues, respectively. (C) Spectrum of the soluble fibres recovered from the standard method; A, A' and B with the number correspond to the different carbons of the xylose residues depicted in (A); chemical shifts were attributed by comparison to values published by Adams *et al.* (1988).

the standard insoluble fibre fraction (Is) and they most probably arise from under-methylation. All mixed-linked xylans are not solubilized either under the standard or 'physiological' conditions and the remaining xylans may represent the mixed-linked xylans and the homogeneous β -1,4 xylan fractions extracted with bases by Turvey & Williams (1970). Such homogeneous 1,4 linked xylan and cellulose were observed in the enzyme-resistant fraction after digestion of *P. palmata* with a purified endoxylanase (Lahaye & Vigouroux, 1992). Attempts to recover cellulose from the insoluble fibres by extraction with methyl morpholine N-oxide, a solvent for cellulose (Joseleau *et al.*, 1981), failed. In agreement with the results of Young (1966), cellulose does not represent a major structural polysaccharide in *P. palmata*.

The physiological effects of dietary fibres are related to their particular physicochemical properties and particularly their hydration characteristics (water holding, water binding capacity) and viscosity of the soluble fibres (Roehrig, 1988).

The water holding capacity of *P. palmata* particles was measured at 20°C with buffers used for the extractions under the 'physiological' conditions. Absorptions rapidly reaching 4.3 and 4.7 g g⁻¹ were obtained with buffer at pH 3.0 and 7.3, respectively (Fig. 3). These values are in the range of those obtained for *L. digitata*, *Ulva lactuca* and *Enteromorpha compressa* (fraction 125–250 μ m, Fleury & Lahaye, 1991; Lahaye & Jegou, 1993) and other vegetables (Thibault *et al.*, 1992) and are not markedly affected by pH nor type of ions in the different buffers.

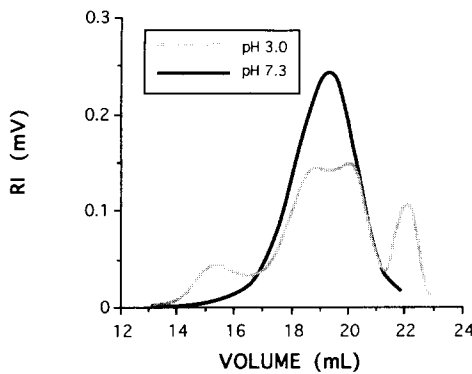


Fig. 2. High pressure size exclusion chromatograms of the soluble fibres extracted with buffer at pH 7.3 and 3.0; RI, differential refractometer response.

Intrinsic viscosities were determined in 155 mM NaCl at 37°C for all soluble fibre fractions including those polymers solubilized during the enzymic treatment of the insoluble fibres in the 'physiological' protocol. They are 49.4, 74.8, 97.0 and 90.8 ml g⁻¹ for the soluble fibres obtained with the standard method, extracted at pH 3.0, at pH 7.3 and during the enzymic treatment of the insoluble fibre under the 'physiological' conditions, respectively. These viscosities are very low and thus *P. palmata* soluble fibres are not expected to affect physiological parameters (blood glucose or cholesterol levels, for example) through viscosity.

Being undigested by the endogenous enzymes of the digestive tract, dietary fibres will reach the large bowel where they can be fermented by the bacterial flora essentially into gases and short chain fatty acids. This fermentability is as important as physicochemical properties for the physiological effects of fibres and would be involved in the regulation of the total transit time of the alimentary bowel (Read, 1986; Cherbut *et al.*, 1988). Furthermore, highly fermentative fibres will provide energy to the host through the production of short chain fatty acids (~2–4 kcal g⁻¹ for fibres fermented to 80%; McBurney & Thompson, 1989).

Fermentation of the pooled soluble xylan fractions extracted with the 'physiological' buffers from dulse was followed by production of gases, short chain fatty acids (SCFA), change in pH and bacterial utilization

Table 4. Nature and content of methylated sugars after permethylation of the insoluble fibre fractions from *Palmaria palmata*

Fraction	Methylated sugar	Total methylated sugars (%)	Linkage
I _s	2,4-Di-O-methyl xylose	26.7	1,3
	2,3-Di-O-methyl xylose	60.7	1,4
	Xylose	5.7	
	2,3,6-Tri-O-methyl glucose	6.9	1,4
I _p	2,4-Di-O-methyl xylose	17.3	1,3
	2,3-Di-O-methyl xylose	72.6	1,4
	Xylose	2.2	
	2,3,6-Tri-O-methyl glucose	7.9	1,4

See footnotes for Table 1 for identification of fractions.

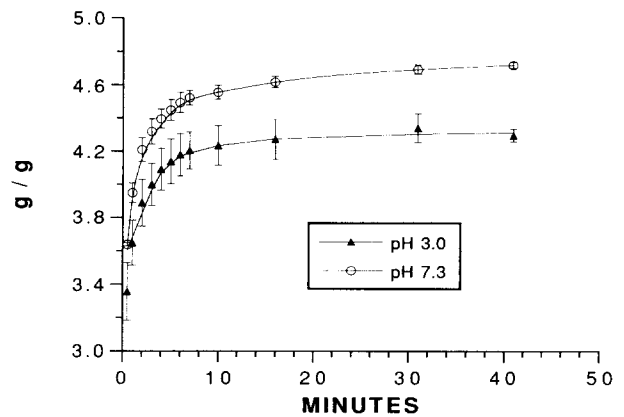


Fig. 3. Water absorption kinetics of *Palmaria palmata* particles with buffer at pH 3.0 ($n = 2$) and 7.3 ($n = 3$). Bars correspond to standard deviations.

coefficient (BUC). Sugar beet was used as a standard for the fermentation experiment and results obtained for BUC and theoretical fermented organic matter (TFOM, 77.5 and 72.0% of dry matter degraded, respectively) were in total agreement with data in the literature (Auffret *et al.*, 1991; Cherbut *et al.*, 1991). On this basis, the *in-vitro* incubation conditions used and the results obtained for the fermentation of dulse fibres were considered as valid. Values of BUC and TFOM obtained after 6, 12 and 24 h of incubations indicated that the soluble xylans were highly fermentable and their fermentation was completed within the first 6 h of incubation. Values reached after 6 h fermentation were pH: 6.7, total SCFA produced: 107 mmol litre⁻¹, BUC: 76.7 ± 1.4% and TFOM: 87.7 ± 3.6%. Throughout the incubation period, the values for BUC and TFOM were closely related ($y = 1.7x - 42.84$; $r = 0.86$) but the slope of the equation (>1) indicated that TFOM values were higher than BUC ones probably because of acetogenesis ($\text{CO}_2 + \text{H}_2 \rightarrow \text{CH}_3\text{COOH}$; Demeyer *et al.*, 1989).

BUC values for dulse soluble xylans showed that xylose was very rapidly fermented since 96% of this sugar was used within the first 6 h of incubation. Bacterial fermentation of monomeric xylose by human faecal flora is slow but complete (Barry *et al.*, 1989). However, the rate and intensity of fermentation of xylan by complex human faecal flora or isolated bacteria from human faeces depends on both their origin and solubility (Salyers *et al.*, 1981; Fleming *et al.*, 1983; Bayliss & Houston, 1984; Englyst *et al.*, 1987; Dufour-Lescoat *et al.*, 1991; Salvador *et al.*, 1992).

Total SCFA production from dulse or sugar-beet fibre was similar (107 and 97 mmol litre⁻¹, respectively) with relative percentages of C2 : C3 : C4 of 58 : 28 : 14 for dulse fibres. The high proportion of C2 is in agreement with the range of proportions obtained from the fermentation of other fibres (Cherbut *et al.*, 1991) and the relatively high proportion of C3 produced is typical of xylose fermentation (Mortensen *et al.*, 1988). The C3/C4 ratios from dulse and sugar beet were similar. Taking into account both the latter value and the total production of SCFA, dulse fibres could be expected to have a similar effect as beet fibres in increasing the

total oro-caecal time but decreasing the total oro-faecal time (Cherbut *et al.*, 1991).

CONCLUSION

Dulse is rich in dietary fibres like other edible algae and these are essentially composed of mixed-linked β -1,3/1,4 xylans. The simulation of the gastric and intestinal conditions for the extraction of fibres demonstrated that, rather than having discrete xylan solubilizations in the different compartments of the digestive tract, the soluble fibres will tend to be continuously extracted along the tract as digestion proceeds and will be chemically similar. These fibres have a low viscosity and consequently are not expected to be involved in physiological and metabolic effects related to this physicochemical property in the upper digestive tract. However, the high fermentability of these soluble fibres could be involved in physiological and metabolic effects which are chemically controlled. Such high fermentability is most likely to have a positive energetic contribution and thus, dulse fibres may provide some calories to the host. Nevertheless, this elevated fermentability should be confirmed *in vivo* since solubilization of xylans will most likely be continuous in the digestive tract.

The hydration properties of insoluble fibres can be compared to those of fibres from land vegetables. However, further studies are required to determine their fermentability by the human faecal flora and their involvement in nutritional effects (total digestive transit time, for example).

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REFERENCES

- Adams, N. M., Furnaux, R. H., Miller, I. J. & Whitehouse, L. A. (1988). Xylan from *Leptosarca simplex* and carrageenans from *Iridea*, *Cenacrum* and *Nemastoma* species from the subantarctic islands of New Zealand. *Bot. Mar.*, **31**, 9–14.
- Auffret, A., Barry, J.-L. & Thibault, J.-F. (1991). *In vitro* degradation of chemically treated sugar-beet fibres by human faecal bacteria. *Food Hydrocoll.*, **5**, 41–4.
- Barry, J.-L., Chourot, J.-M., Bonnet, C., Kozlowski, F. & David, A. (1989). *In vitro* fermentation of neutral monosaccharides by ruminal and human faecal microflora. *Acta Vet. Scand., Sup.* **86**, 93–5.
- Baumann, H. (1967). Baumann apparatus nach Baumann zerbestimmung der flüssigkeitsaufnahme von pulvigenen substanzen. Glastechnik und instrumententechnik. *Fachzeitschrift Laboratorium*, **11**, 540–2.
- Bayliss, C. E. & Houston, A. P. (1984). Characterization of plant polysaccharide- and mucin-fermenting anaerobic bacteria from human feces. *Appl. Environ. Microbiol.*, **48**, 626–32.
- Bengtsson, S. & Åman, P. (1990). Isolation and characterization of water-soluble arabinoxylans in rye grain. *Carbohydr. Polym.*, **12**, 267–77.
- Billmeyer, F. W. Jr (1984). *Textbook of Polymer Science*. John Wiley, New York.
- Björndal, H., Eriksson, K.-E., Garegg, P. J., Lindberg, B. & Swan, B. (1965). Studies on the xylan from the red seaweed *Rhodomenia palmata*. *Acta Chem. Scand.*, **19**, 2309–15.
- Blakeney, A. B. & Stone, B. A. (1985). Methylation of carbohydrates with lithium methylsulfinyl carbanion. *Carbohydr. Res.*, **140**, 319–24.
- Blakeney, A. B., Harris, P. J., Henry, R. J. & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.*, **113**, 292–9.
- Cerezo, A. S. (1972). The fine structure of *Chaetangium fastigiatum* xylan: studies of the sequence and configuration of the (1 → 3)-linkages. *Carbohydr. Res.*, **22**, 209–11.
- Cerezo, A. S., Lezeovich, A., Labriola, R. & Rees, D. A. (1971). A xylan from the red seaweed *Chaetangium fastigiatum*. *Carbohydr. Res.*, **19**, 289–96.
- Chapman, V. J. & Chapman, D. J. (1980). *Seaweeds and their Uses*. Chapman and Hall, London.
- Cherbut, C., Bonnet, C. & Delort-Laval, J. (1988). Actions of volatile fatty acids on intestinal transit. *Gastroenterology*, **94**, A66.
- Cherbut, C., Salvador, V., Barry, J.-L., Doulay, F. & Delort-Laval, J. (1991). Dietary fibre effects on intestinal transit in man: involvement of their physicochemical and fermentative properties. *Food Hydrocoll.*, **5**, 15–22.
- Cronshaw, J., Myers, A. & Preston, R. D. (1958). A chemical and physical investigation of the cell walls of some marine algae. *Biochim. Biophys. Acta*, **27**, 89–103.
- Davidson, R. L. (1980). *Handbook of Water-Soluble Gums and Resins*. McGraw-Hill, New York.
- Demeyer, D., De Graeve, K., Durand, M. & Stevani, J. (1989). Acetate: a hydrogen sink in hindgut fermentation as opposed to rumen fermentation. *Acta Vet. Scand.*, **86** Suppl., 68–75.
- Dennis, D. T. & Preston, R. D. (1961). Constitution of cellulose microfibrils. *Nature*, **191**, 667–8.
- Dufour-Lescoat, C., Le Coz, Y. & Szyllit, O. (1991). Nutritional effects of wheat bran and beet fibre in germ-free rats and in heteroxenic rats inoculated with human flora. *Sci. Alim.*, **11**, 397–408.
- Englyst, H. N., Hay, S. & MacFarlane, G. T. (1987). Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiol. Ecol.*, **95**, 163–71.
- Fleming, S. E., Marthinsen, D. & Kuhnlein, H. (1983). Colonic function and fermentation in men consuming high fiber diets. *J. Nutr.*, **113**, 2535–44.
- Fleurance, J. (1991). L'habilitation des algues en alimentation humaine. *IAA*, Juin, 501–2.
- Fleury, N. & Lahaye, M. (1991). Chemical and physicochemical characterisation of fibres from *Laminaria digitata* (Kombu breton): a physiological approach. *J. Sci. Food Agric.*, **55**, 389–400.
- Harris, P. J., Henry, R. J., Blakeney, A. B. & Stone, B. A. (1984). An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr. Res.*, **127**, 59–73.
- Hoebler, C., Barry, J.-L., David, A. & Delort-Laval, J. (1989). Rapid acid hydrolysis of plant cell wall polysaccharides and simplified quantitative determination of their neutral monosaccharides by gas-liquid chromatography. *J. Agric. Food Chem.*, **37**, 360–7.
- Indergaard, M. & Minsaas, J. (1991). Animal and human nutrition. In *Seaweed Resources in Europe: Uses and Potential*, ed. M. D. Guiry & G. Blunden. John Wiley, New York, pp. 21–64.
- Ito, K. & Hori, K. (1989). Seaweed: chemical composition and potential food uses. *Food Rev. Int.*, **5**, 101–44.

- Joseleau, J.-P., Chambat, G. & Chumpitazi-Hermoza, B. (1981). Solubilization of cellulose and other plant structural polysaccharides in 4-methylmorpholine *N*-oxide: an improved method for the study of cell-wall constituents. *Carbohydr. Res.*, **90**, 339–44.
- Jouany, J. P. (1982). Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sci. Alim.*, **2**, 131–44.
- Kishi, K., Inoue, G., Yoshida, A., Fuwa, H., Koishi, H., Koike, G., Miyoshi, T., Inoue, T., Yshida, M. & Omori, A. (1982). Digestibility and energy availability of sea vegetables and fungi in man. *Nut. Rep. Int.*, **26**, 183–92.
- Kovac, P., Hirsch, J., Shashkov, A. S., Usov, A. I. & Yarotsky, S. V. (1980). ¹³C-n.m.r. spectra of xylo-oligosaccharides and their application to the elucidation of xylan structures. *Carbohydr. Res.*, **85**, 177–85.
- Lahaye, M. (1991). Marine algae as sources of dietary fibres: determination of soluble and insoluble dietary fibre content in some 'sea vegetables'. *J. Sci. Food Agric.*, **54**, 587–94.
- Lahaye, M. & Jegou, D. (1993). Chemical and physico-chemical characteristics of dietary fibres from *Ulva lactuca* L. and *Enteromorpha compressa* (L.) Grev. *J. Appl. Phycol.* (in press).
- Lahaye, M. & Thibault, J.-F. (1990). Chemical and physico-chemical properties of fibers from algal extraction by-products. In *Dietary Fibre: Chemical and Biological Aspects*, ed. D. A. T. Southgate, K. Waldron, I. T. Johnson & G. R. Fenwick. The Royal Society of Chemistry, Cambridge, pp. 68–72.
- Lahaye, M. & Vigouroux, J. (1993). The liquefaction of dulse (*Palmaria palmata* (L.) Kuntze) by commercial enzyme preparations and a purified endo- β -1,4-D-xylanase. *J. Appl. Phycol.*, **4**, 329–37.
- Manners, D. J. & Mitchell, J. P. (1963). The fine-structure of *Rhodomenia palmata* xylan. *Biochem. J.*, **89**, 92–3.
- Marlett, J. A., Chesters, J. G., Longacre, M. J. & Bogdanske, J. J. (1989). Recovery of soluble dietary fibre is dependent on the method of analysis. *Am. J. Clin. Nutr.*, **50**, 479–85.
- McBurney, M. I. & Thompson, L. U. (1989). Dietary fiber and energy balance: integration of the human ileostomy and in vitro fermentation models. *Anim. Feed Sci. Technol.*, **23**, 261–75.
- Morgan, K. C., Wright, J. L. C. & Simpson, F. J. (1980). Review of the chemical constituents of the red alga *Palmaria palmata* (Dulse). *Econ. Bot.*, **34**, 27–50.
- Mortensen, P. B., Holtug, K. & Rasmussen, H. S. (1988). Short-chain fatty acid production from mono and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans. *J. Nutr.*, **118**, 321–5.
- Myers, A. & Preston, R. D. (1959). Fine structure in the red algae. II The structure of the cell wall of *Rhodomenia palmata*. *Proc. R. Soc. Lond.*, **B 150**, 447–55.
- Nishimune, T., Sumimoto, T., Yakusiji, T., Kunita, N., Ichikawa, T., Doguchi, M. & Nakahara, S. (1991). Determination of total dietary fiber in Japanese foods. *J. Assoc. Off. Anal. Chem.*, **74**, 350–9.
- Nunn, J. R., Parolis, H. & Russell, I. (1973). Polysaccharides of the red alga *Chaetangium erinaceum*. Part 1. Isolation and characterisation of the water-soluble xylan. *Carbohydr. Res.*, **26**, 169–80.
- Percival, E. & McDowell, R. H. (1967). *Chemistry and Enzymology of Marine Algal Polysaccharides*. Academic Press, London.
- Percival, E. G. V. & Chanda, S. K. (1950). The xylan of *Rhodomenia palmata*. *Nature*, **166**, 787–8.
- Read, N. W. (1986). Dietary fiber and bowel transit. In *Dietary Fiber: Basic and Clinical Aspects*, ed. G. Vahouny & D. Kritchevsky. Plenum Press, New York.
- Roehrig, K. L. (1988). The physiological effects of dietary fiber—a review. *Food Hydrocoll.*, **2**, 1–18.
- Salvador, V., Cherbut, C., Barry, J.-L., Bertrand, D. & Delort-Laval, J. (1992). Sugar composition of dietary fibre and short chain fatty acid production during in vitro fermentation by human bacteria. *Br. J. Nutr.* (in press).
- Salyers, A. A., Gherardinin, F. & O'Brien, M. (1981). Utilization of xylan by two species of human colonic bacteroides. *Appl. Environ. Microbiol.*, **44**, 1065–8.
- Southgate, D. A. T. (1990). Dietary fibre and health. In *Dietary Fibre: Chemical and Biological Aspects*, ed. D. A. T. Southgate, K. Waldron, I. T. Johnson & G. R. Fenwick. The Royal Society of Chemistry, Cambridge, pp. 10–19.
- Thibault, J. F., Lahaye, M. & Guillon, F. (1992). Physico-chemical properties of food plant cell walls. In *Dietary Fibre. A Component of Food*, ed. T. Schweitzer & C. Edwards. Springer, Berlin, pp. 21–39.
- Trowell, H. (1974). Definitions of fiber. *Lancet*, **1**, 503.
- Turvey, J. R. & Williams, E. L. (1970). The structures of some xylans from red algae. *Phytochemistry*, **9**, 2383–8.
- Usov, A. I., Yarotskii, S. V., Shashkov, A. S. & Tishchenko, V. P. (1978). Polysaccharides of algae XXII. Polysaccharide composition of *Rhodomenia stenogata* Perest and the application of ¹³C NMR spectroscopy to the determination of xylan structures. *Bioorg. Khim.*, **4**, 57–65.
- Usov, A. I., Yarotskii, S. V. & Esteves, M. L. (1981). Polysaccharides of algae. XXXII. Polysaccharides of the red alga *Galaxaura squalide* Kjellm. *Bioorg. Khim.*, **7**, 1261–70.
- Van Nevel, C. J., Demeyer, D. I., Cottyn, B. G. & Henderickx, H. K. (1970). Effect of sodium sulfite on methane and propionate in the rumen. *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde*, **26**, 91–100.
- Young, E. G. (1966). The chemical nature of the insoluble residue after severe extraction in some Rhodophyceae and Phaeophyceae. *Proc. Int. Seaweed Symp.*, **5**, 337–46.