In vitro fermentation by human faecal bacteria of total and purified dietary fibres from brown seaweeds

BY CATHERINE MICHEL[1], MARC LAHAYE[2], CHRISTIAN BONNET[3], SERGE MABEAU[1] AND JEAN-LUC BARRY[3]*

[1] *Centres d'Etudes et de Valorisation des Algues, BP 3, F-22610 Pleubian, France*
[2] *INRA, Laboratoire de Biochimie et Technologie des Glucides, BP 527, F-44026 Nantes Cedex 03, France*
[3] *INRA, Laboratoire de Technologie Appliquée à la Nutrition, BP 527, F-44026 Nantes Cedex 03, France*

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The *in vitro* degradation of dietary fibre from three brown seaweeds (*Himanthalia elongata*, *Laminaria digitata* and *Undaria pinnatifida*) was studied, using human faecal flora. Two sets of fibre were tested: (1) total algal fibres extracted from the whole algae, mainly composed of alginates, and (2) purified fibres (sulphated fucans, Na-alginates and laminarans) representative of those contained in the whole brown algae. Mannuronate, one algal component, was also investigated. Substrate disappearance and short-chain fatty acid (SCFA) production were monitored after 6, 12 and 24 h fermentation. Gas production was followed hourly during the first 9 h and then at 12 and 24 h. Sugarbeet fibre was used as a fermentation reference substrate. According to the fermentative indices used, most of each of the total algal fibres disappeared after 24 h (range 60–76%) but, unlike the reference substrate, they were not completely metabolized to SCFA (range 47–62%). Among the purified algal fibres, disappearance of laminarans was approximately 90% and metabolism to SCFA was approximately 85% in close agreement with the fermentation pattern of reference fibres. Sulphated fucans were not degraded. Na-alginates exhibited a fermentation pattern quite similar to those of the whole algal fibres with a more pronounced discrepancy between disappearance and production of SCFA: disappearance was approximately 83% but metabolism was only approximately 57%. Mannuronate was slowly fermented but its metabolism corresponded to its disappearance from the fermentative medium. Thus, the characteristic fermentation pattern of the total fibres from the three brown algae investigated was attributed to the peculiar fermentation of alginates, and mannuronate was shown not to be directly involved.

***In vitro* fermentation: Faecal bacteria: Dietary fibre: Brown algae**

Most algal polysaccharides are resistant to hydrolysis by human endogenous digestive enzymes and thus are considered as dietary fibre (Trowell *et al.* 1976; Southgate, 1977; Salyers *et al.* 1977*a–c*). The recent approval of ten seaweeds for human consumption in France (Fleurence, 1991) led to a revival of interest in these sources of fibre, and information concerning methods of quantification, amounts and physico–chemical properties of algal dietary fibres has been published recently (Kishi *et al.* 1982; Mori, 1982; Lahaye & Thibault, 1990; Fleury & Lahaye, 1991; Lahaye, 1991; Lahaye *et al.* 1993; Lahaye & Jegou, 1993). These reports demonstrate that seaweeds are rich in dietary fibre, with a particularly high amount of the soluble forms in brown algae. In these seaweeds, soluble fibre consists of laminarans (β1–3, β1–6 glucans associated with mannitol residues;

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Percival & McDowell, 1967), fucans (sulphated polymers rich in fucose associated with xylose, galactose and glucuronic acids; Kloareg & Quatrano, 1988), and alginates (β1–4 D-mannuronic, α1–4 L-guluronic acid polymers; Gacesa, 1988), whereas the insoluble fibre is essentially cellulose (Fleury & Lahaye, 1991).

The approval of alginates for use as gelling and thickening agents in food led to toxicity and digestibility studies (Nilson & Wagner, 1951; Humphreys & Triffitt, 1968; Sharrett & Dearn, 1972; Adrian & Assouman, 1977). However, the physiological effects of the algal fibres have only begun to be investigated recently (Anderson *et al.* 1991*a*, *b*; Torsdottir *et al.* 1991) and in particular their fermentative degradation by the colonic flora (Salyers *et al.* 1977*a–c*, 1978; Gibson *et al.* 1990). Since the latter digestive event determines many of the physiological effects of fibres in the gut (Edwards & Rowland, 1992), the aim of the present study was to investigate the *in vitro* degradation of brown-algal fibres by human faecal bacteria.

MATERIALS AND METHODS

Materials

Laminaria digitata (L. Lamouroux) was collected in January at Pleubian, (Côtes d'Armor, France) and *Himanthalia elongata* (L. SF Gray) in June at Roscoff (Finistère, France). *Undaria pinnatifida* (H. Suringar) was harvested in September from a culture at Pleubian: gametophytes from free-living algae were sprayed on twine and incubated in a hatchery for 3 weeks before an off-shore cultivation period of 6 months. The algae were washed with tap-water and dried at 60° with circulating air.

Na-alginates from *Macrocystis pyrifera* (L. C. Aghard), laminarans from *Laminaria digitata* (L. Lamouroux) and mannuronic acid lactone were obtained from Sigma (La Verpillière, France). Fucans obtained from *Ascophyllum nodosum* (L. Lejolis) were kindly provided by Institut Français de recherches et d'exploitation de la mer (IFREMER, Nantes, France). Whole sugarbeet fibre (ST) was provided by Agroalimentaire Recherche et Développement (ARD, Arthenay, France).

Fibre extraction

Total dietary fibre was extracted from ground algae (particle size: 280–630 μm) according to a modification of the method described by Lahaye (1991).

Dried algal powders (10 g) were suspended in phosphate buffer (0·08 M, pH 7·5, 500 ml). Pronase solution (from *Streptomyces griseus*, Boehringer, Mannheim, Germany; 3 ml, 50 mg/ml) was added and the suspension incubated for 30 min at 50° with shaking. The total algal fibre was precipitated by 4 volumes of cold (−20°) absolute ethanol and recovered by centrifugation (9000 ***g***, 20 min), washed with absolute ethanol and dehydrated by solvent exchange with acetone and diethyl ether.

To remove the excess of salts, the total fibre was suspended again in deionized water, extensively dialysed against deionized water and then recovered and dehydrated as described above. Amounts of total dietary fibre were expressed on a dry matter basis after subtraction of ash and crude protein contents. Total dietary fibre preparations from *H. elongata*, *U. pinnatifida* and *L. digitata*, with particle sizes in the range of 200–500 μm are referred to as HT, UT and LT respectively.

Chemical characterization

Mineral contents were quantified gravimetrically in duplicate after heating overnight at 550°, and for 2 h at 900°.

Crude protein (N × 6·25) was calculated from the total N content determined in duplicate by the Kjeldahl method.

Sugars were quantified after substrate pre-hydrolysis in 13 M-H_2SO_4 for 30 min at 25° followed by 2 h in 1 M-H_2SO_4 at 100° (total acid hydrolysis, Hoebler *et al.* 1989) or after hydrolysis only in 1 M-H_2SO_4 at 100° for 2 h (partial acid hydrolysis). They were converted to alditol acetates and quantified by GC using a DB 225 (J & W, Folsom, CA, USA) fused silica capillary column operating isothermally at 220° and eluted with H_2 (Hoebler *et al.* 1989). This column was connected to a Series 300 chromatograph (Girdel Instruments, Delsi, Argenteuil, France) fitted with a flame ionization detector.

Uronic acids were quantified from the above acid hydrolysates by the automated colorimetric method of Thibault (1979) with Na-alginate as a reference.

Sulphate was quantified after HCl hydrolysis of polysaccharides (1 M, 2 h, 120°) and HPLC of the hydrolysates through a Carbopack PA 1 column (Dionex, Jouy-en-Josas, France) eluted with 40 mM-Na_2CO_3 – deionized water (50:50, v/v) at 1 ml/min. Elution was monitored by conductimetry. Short-chain fatty acids (SCFA) were quantified from aqueous samples by GC according to the method described by Jouany (1982). Modifications of the chromatographic conditions (oven, injector and detector temperatures were 105, 165 and 155° respectively) were used for the analyses of ethanolic samples.

In vitro *fermentation study*

Incubation. In vitro fermentations were conducted according to the method of Barry *et al.* (1989). The inoculum was made from fresh faeces anaerobically collected from two healthy volunteers. Faeces were mixed (1:2·25, w/v) with a CO_2-saturated nutritive solution containing (g/l): $NaHCO_3$ 9·24, $Na_2HPO_4.12H_2O$ 7·125, NaCl 0·470, KCl 0·450, Na_2SO_4 0·100, anhydrous $CaCl_2$ 0·055, anhydrous $MgCl_2$ 0·047, urea 0·400 and (mg/l): $FeSO_4.7H_2O$ 36·80, $MnSO_4.7H_2O$ 19·00, $ZnSO_4.7H_2O$ 4·40, $CoCl_2.6H_2O$ 1·20, $CuSO_4.5H_2O$ 0·98, $Mo_7(NH_4)_6O_{24}.4H_2O$ 0·174 (Durand *et al.* 1988) then filtered through six layers of surgical gauze.

Fermentations were conducted in serum bottles maintained at 37° in a water-bath. The bottles were fitted with a tube allowing for the collection of produced gases which were monitored hourly during the first 9 h and then after 12 and 24 h incubation. Blanks, without substrate, were incubated under the same conditions and for each substrate incubations were carried out twice in duplicate.

Laminarans, Na-alginates, fucans, LT, mannuronate and ST (200 mg), UT (215 mg) and HT (230 mg) were pre-hydrated with 5 ml of the nutritive solution in serum bottles. After addition of 15 ml inoculum, the bottles were closed and incubated for 24 h under N_2 at 37° with shaking.

Ethanolic precipitation. After 6, 12 or 24 h incubation, unfermented residues from total or purified algal fibres and ST were precipitated from fermentative media with 4·21 volumes of cold (−20°) ethanol (950 ml/l) and recovered by centrifugation (2400 ***g***, 20 min). They were then dehydrated by absolute ethanol and acetone and dried under vacuum at 40° in the presence of P_2O_5. Samples were taken from the ethanolic supernatant fractions.

Freeze drying of the whole media. After 6, 12 or 24 h incubation, unfermented residues from laminarans, Na-alginates, mannuronate and ST were recovered by centrifugation (2400 ***g***, 20 min) of the fermentative media. Samples were taken from supernatant fractions before recombination of the residues and the supernatant fractions. Then they were freeze-dried and dehydrated overnight under vacuum at 40° in the presence of P_2O_5.

All unfermented residues were ground (particle size 100–200 μm) before chemical characterization.

SCFA analyses were done on portions of ethanolic or aqueous supernatant fractions to which were added (100 ml/l) a solution containing mercuric chloride (1 g/l) and orthophosphoric acid (50 g/l).

Calculations

For each experiment and each substrate, gas volumes were calculated by subtracting blank values from each experimental value and SCFA amounts were calculated after subtraction of the initial amount of SCFA in inocula from each experimental value.

Substrate degradation was calculated as the disappearance coefficient (DC):

$$DC = (S+I-R) \times 100/(S+I),$$

where S, I and R are the amounts of total sugars in the initial substrate, the inoculum and the unfermented residue respectively.

Total SCFA production was calculated as theoretical fermented organic matter (TFOM) according to Demeyer & Van Nevel (1975) and was expressed as a percentage of the initial sugar content of the substrate:

$$TFOM = (C2/2+C3/2+C4+IC4+C5+IC5) \times 162 \times 100/IS,$$

where C2, C3, C4, IC4, C5 and IC5 are the amounts (mmol) of acetic, propionic, isobutyric, butyric, isovaleric and valeric acids produced respectively, and IS is the initial sugar content (mg) of the investigated substrate.

The initial sugars content of each substrate was estimated by sugar analysis (IS_A) or by calculation (IS_B):

$$IS_A = (W \times TS)/100$$

and

$$IS_B = (W \times (100-MA-CPA))/100,$$

where W is the initial weight (mg), TS is the amount of analysed total sugars (%), MA and CPA are mineral and crude protein amounts (%) of the investigated substrate.

Thus two values of TFOM ($TFOM_{(A)}$ and $TFOM_{(B)}$) were calculated for each substrate and each incubation duration.

Since not all the substrates could be studied at once in the same experiment and as ST was used as a fermentative reference in each incubation, experimental values for each variable were calibrated using the following formula:

$$P_{Fcal} = P_{Fexp} \times (M_{ST}/P_{STexp}),$$

where P_{Fcal} corresponds to the calibrated value for a given variable (gas volume, SCFA amount, DC or TFOM value); P_{Fexp} and P_{STexp} are the values for the given variables obtained from the same experiment for the fibre sample and the fermentation reference (ST) respectively, and M_{ST} is the mean value calculated for this variable for the reference (ST), from all the experiments. Calibration was done only when it was statistically checked that there was no significant interaction between experiments and substrates with respect to the considered variable.

Statistical analysis

Except for ST, for which calibration suppresses standard errors, data are expressed as means with their standard errors. Statistical tests were performed on calibrated fermentative variables by variance analysis (ANOVA) and, when effects were significant, means were compared using Student's *t* test (Statview® SE software). Homogeneity of variance was assumed. The number of observations per substrate was too low to provide information about deviations from this assumption. Therefore, some statistical significance levels may be inappropriate.

RESULTS

Total dietary fibres from *H. elongata* (HT), *L. digitata* (LT) and *U. pinnatifida* (UT) amounted to 512, 464 and 695 g/kg initial dry algal weight. Chemical compositions of these

fibres and of the purified fibres are reported in Table 1. The total algal dietary fibre contained large amounts of uronic acids (494, 313 and 587 g/kg dry weight of UT, HT and LT respectively) and neutral sugars amounted to 95, 154 and 199 g/kg dry weight of UT, HT and LT respectively. Glucose was the major neutral sugar, followed by fucose and smaller amounts of xylose, mannose and galactose. Partial acid hydrolysis was used to estimate the amounts of laminarans. The low amount of glucose measured by this method indicated that most of the glucose measured after total acid hydrolysis was derived from cellulose. Sulphate content was low (16 to 27 g/kg). Ash was in the range 91–123 g/kg dry weight. Crude protein content varied from one alga to another with the highest amount (223 g/kg) measured in HT.

Commercial Na-alginates were essentially composed of uronic acids (860 g/kg) and ash (136 g/kg). Fucans were mainly composed of fucose (365 g/kg) and sulphate (221 g/kg) with smaller amounts of xylose, uronic acids, mannose and galactose. Glucose was the major component of laminarans (877 g/kg) and was detected totally after partial hydrolysis (873 g/kg). A low amount of mannose (46 g/kg) was also detected. Uronic acids and fucose were also present and amounted to 38 and 15 g/kg dry weight respectively. Mannuronate was totally detected as uronic acid (Table 1).

Analyses of the sugarbeet fibre (Table 1) revealed that it contained similar amounts of uronic acids, glucose and arabinose (224, 221 and 172 g/kg dry weight respectively). Galactose was also present in low amounts (43 g/kg).

All these substrates were submitted to *in vitro* incubation in the presence of human faecal inocula. Since the unfermented residue recovery processes did not affect the gas production values, but modified the SCFA solvent (ethanol or water) and could affect the DC values (recovery of oligosaccharides with small degrees of polymerization), only gas production values were combined from all the experiments.

According to the gas production observed from the reference substrate (Table 2), the activity of the inoculum was high. Indeed, the gas production mainly reflects the CO_2 released from the carbonate buffer upon acidification by SCFA (Salvador *et al.* 1993) and thus can be used as an index of the intensity of fermentation. Nevertheless, when substrates are completely fermented, bacteria are able to reuse gas and thus gas production from different substrate incubations can be compared only in the ascending phase of bacterial growth.

Gas production from ST increased steadily between the first and the twelfth hour of incubation, after which it remained constant. The pattern of gas production was similar for all the total algal fibres: gas production began only after 6 h of incubation and most of the total gas was produced after 12 h. These productions were equivalent and plainly lower than for the reference substrate, during the first 9 h incubation. In contrast, purified fibres exhibited different behaviour. Fucans led to a weak gas production occurring only during the first hour of incubation. Compared with the reference substrate, incubation of laminarans led to the highest gas production between the third and the twelfth hour of incubation. This production was slightly delayed at the beginning of incubation but then increased intensively. Alginate led to an intermediate gas production very close to that of the three total algal fibres but lower during the first 12 h incubation. Gas production from mannuronate increased progressively during the first 12 h incubation, then intensively until the end of the incubation period.

Ethanolic precipitation

With regard to SCFA production (Table 3), the classification of substrates was the same as that described for gas production. SCFA production from ST increased steadily with the course of incubation. No SCFA production was observed from the total algal fibres after

Table 1. *Chemical composition (g/kg dry weight of total fibre) of total dietary fibres from* Himanthalia elongata (*HT*), Laminaria digitata (*LT*), Undaria pinnatifida (*UT*), *sodium alginates* (*alginates*), *fucans*, *laminarans*, *mannuronate and sugarbeet fibre* (*ST*)

(Mean values with their standard errors from duplicates)

	HT		LT		UT		Alginates		Fucans		Laminarans		Mannuronate		ST	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Rhamnose	1	1	2	0	1	0	n.d.		n.d.		11	1	n.d.		15	2
Fucose	18	1	24	0	9	1	1	0	365	21	15	0	4	2	1	2
Arabinose	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		172	5
Xylose	6	1	10	1	5	1	n.d.		69	3	n.d.		3	1	13	1
Mannose	5	1	8	1	4	1	2	0	24	3	46	1	1	1	11	1
Galactose	6	0	4	0	9	0	1	0	30	1	n.d.		3	2	43	1
Glucose TH*	117	6	151	15	67	3	4	0	9	0	877	6	3	1	221	7
Glucose PH†	27	1	20	2	9	1	N.D.		N.D.		873	5	N.D.		N.D.	
Glucose TH−PH‡	90	4	131	12	58	2	N.D.		N.D.		4	0	N.D.		N.D.	
Uronic acid	313	18	587	38	494	36	860	13	52	1	38	3	952	18	224	14
N × 6·25	223	1	95	0	141	2	8	1	9	0	n.d.		N.D.		75	2
Ash	91	3	123	1	121	10	136	1	225	11	33	4	N.D.		47	2
Sulphate	27	2	21	3	16	1	4	1	221	2	N.D.		N.D.		N.D.	

N.D., not determined; n.d., not detected; TH, total hydrolysis; PH, partial hydrolysis.

* Total glucose determined by alditol acetate analysis after total hydrolysis.

† Soluble glucose determined by alditol acetate analysis after partial hydrolysis.

‡ Insoluble glucose calculated by subtraction of soluble glucose from total glucose.

Table 2. *Total net gas production (ml) from total fibres of* Himanthalia elongata (*HT*), Laminaria digitata (*LT*), Undaria pinnatifida (*UT*) *and from sodium alginates (alginates), fucans, laminarans, mannuronate and sugarbeet total fibre (ST) incubated with human faecal inoculum**

(Mean values with their standard errors)

	ST	HT		LT		UT		Alginates		Fucans		Laminarans		Mannuronate		Variance ratio		
n...	9	2		2		2		9		2		4		2				
Time (h)	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Between all substrates	Between total algal fibres	Between purified algal fibres
1	4·3^{b}	0·4^{cd}	0·7	−0·4^{ad}	0·0	−0·1^{ad}	0·2	−1·6^{a}	0·6	2·2^{c}	0·4	0·4^{cd}	0·8	6·6^{e}	0·8	28·095	0·976	25·019
2	5·6^{b}	0·6^{ac}	1·2	−0·3^{a}	0·2	0·2^{ac}	0·7	−1·1^{a}	0·5	2·7^{c}	0·8	2·9^{c}	0·7	9·7^{d}	2·0	23·771	0·293	23·862
3	6·6^{bc}	0·8^{a}	1·1	0·0^{a}	0·5	0·8^{a}	0·9	−0·8^{a}	0·3	2·7^{ac}	0·8	7·7^{bd}	3·6	10·9^{d}	2·2	11·466	0·231	11·442
4	8·0^{bc}	1·2^{a}	1·0	0·5^{a}	0·5	1·3^{a}	0·9	−0·5^{a}	0·3	2·8^{ac}	0·7	14·3^{d}	6·5	12·2^{bd}	2·9	7·201	0·268	7·432
5	9·4^{b}	1·7^{a}	0·9	0·8^{a}	0·8	1·5^{a}	1·1	−0·2^{a}	0·4	3·2^{a}	1·0	19·0^{c}	5·9	12·2^{b}	2·5	12·325	0·259	12·965
6	10·8^{b}	2·5^{a}	0·9	1·9^{a}	0·5	2·8^{a}	0·6	0·4^{a}	0·6	3·4^{a}	1·0	23·1^{e}	4·4	12·6^{b}	2·4	24·252	0·464	26·474
7	12·8^{b}	4·8^{a}	0·5	5·3^{a}	1·2	5·5^{a}	0·5	1·7^{a}	0·9	3·4^{a}	1·0	25·7^{e}	3·5	13·7^{b}	2·9	26·536	0·237	30·482
8	14·7^{b}	6·5^{a}	0·4	8·5^{ad}	1·8	7·6^{a}	0·1	3·1^{a}	1·3	3·5^{a}	1·2	27·0^{e}	4·2	14·2^{bd}	2·9	18·590	0·798	21·728
9	15·8^{b}	7·7^{a}	0·9	10·1^{ab}	2·3	8·9^{ad}	0·7	4·6^{a}	1·6	3·1^{a}	1·0	26·3^{e}	3·9	15·3^{bd}	3·3	13·473	0·668	15·665
12	18·6^{bd}	10·1^{ac}	1·9	12·2^{ad}	1·9	11·8^{a}	1·1	10·0^{a}	1·9	3·0^{c}	1·1	24·1^{b}	2·2	19·2^{bd}	4·1	8·779	0·432	9·498
24	20·0^{bc}	14·8^{abc}	4·6	15·3^{abc}	0·2	13·7^{ab}	0·3	16·4^{abc}	0·6	3·3^{d}	1·7	23·3^{e}	1·3	36·8^{e}	8·3	9·724	0·087	12·478

$^{a, b, c, d, e}$ Mean values within a row bearing unlike superscript letters were significantly different, $P < 0·05$.

* For details of procedures, see pp. 264–266.

Table 3. *Total short-chain fatty acid production (mmol/l) from total fibres of* Himanthalia elongata *(HT),* Laminaria digitata *(LT) and* Undaria pinnatifida *(UT), and from sodium alginates (alginates), fucans, laminarans and total sugarbeet fibre (ST) after 6, 12 or 24 h incubation with human faecal inoculum (unfermented residues recovered by ethanolic precipitation)**

(Mean values with their standard errors)

	ST	HT		LT		UT		Alginates		Fucans		Laminarans		No fibre		Variance ratio		
n...	7	2		2		2		7		2		2		2				
	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Between all substrates	Between total algal fibres	Between purified algal fibres
6 h	$28{\cdot}5^{a}$	$4{\cdot}2^{c}$	6·7	$1{\cdot}2^{c}$	9·9	$0{\cdot}2^{c}$	2·5	$-10{\cdot}9^{b}$	2·4	$4{\cdot}7^{c}$	0·3	$48{\cdot}0^{d}$	6·0	$4{\cdot}7^{c}$	1·6	36·462	0·09	70·287
12 h	$48{\cdot}9^{a}$	$29{\cdot}6^{bc}$	2·6	$41{\cdot}0^{ac}$	0·3	$32{\cdot}1^{acf}$	0·5	$21{\cdot}6^{bf}$	6·3	$9{\cdot}2^{be}$	0·2	$65{\cdot}5^{d}$	1·1	$7{\cdot}0^{e}$	1·8	12·378	15·226	11·760
24 h	$73{\cdot}1^{a}$	$39{\cdot}5^{c}$	0·6	$54{\cdot}6^{b}$	1·0	$46{\cdot}2^{bc}$	2·7	$54{\cdot}3^{b}$	3·6	$11{\cdot}4^{d}$	1·5	$87{\cdot}6^{e}$	3·6	$10{\cdot}6^{d}$	1·7	97·800	63·037	44·322

^{a,b,c,d,e,f} Mean values within a row bearing unlike superscript letters were significantly different, $P < 0{\cdot}05$.

* For details of procedures, see pp. 264–266.

Table 4. *Short-chain fatty acid (SCFA) ratios (%) for total fibres from* Himanthalia elongata *(HT),* Laminaria digitata *(LT), and* Undaria pinnatifida *(UT) and for sodium alginates (alginates), laminarans and sugarbeet total fibre (ST) after 24 h incubation with human faecal inoculum (unfermented residues recovered by ethanolic precipitation)**

(Mean values from uncalibrated data with their standard errors)

n...	ST 7		HT 2		LT 2		UT 2		Alginates 7		Laminarans 2	
SCFA	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Acetic	65·6	0·9	73·3	7·3	68·5	2·0	74·9	4·7	79·9	1·5	60·4	1·0
Propionic	19·3	0·5	15·3	5·6	19·0	0·5	14·3	4·0	13·7	1·1	22·9	1·4
Butyric	12·2	0·4	9·6	0·6	8·9	0·0	8·6	0·2	5·4	0·5	16·2	2·3
Others	2·9	0·7	1·8	4·3	3·6	1·3	2·2	2·1	1·0	0·9	0·5	1·7

* For details of procedures, see pp. 264–266.

6 h incubation, and about 75% of the total production was obtained after 12 h. Final SCFA production from LT was higher than that from HT, and that from UT was intermediate. Laminaran incubation led to the highest SCFA production among the studied substrates and 50% of the whole production was reached after 6 h. SCFA production from fucans was similar to that in the blank during the whole incubation period. SCFA production from alginates was very close to that from the total algal fibres, and was characterized by a lack of production during the first 6 h incubation. Compared with the total algal fibres, a smaller proportion (50%) of the total production was reached after 12 h.

Except for fucans from which no SCFA were produced, acetic, propionic and butyric acids represented approximately 90% of total SCFA (Table 4). Laminarans and ST led to high propionic and butyric acid ratios whereas SCFA production from total algal fibres and alginates was typified by elevated acetic acid proportions.

Percentages of substrate disappearance are presented in Table 5. A steady increase was observed for ST disappearance. About 20% of each total algal fibre had disappeared after 6 h incubation. In this respect, DC from total algal fibres were close to that for ST and thus disagreed with the corresponding gas and SCFA productions. Among purified fibres, percentages of substrate disappearance were in close agreement with gas and SCFA results for fucans and for laminarans which exhibited a lack of disappearance and a significant substrate disappearance respectively. Degradation percentages for alginates were similar to those for ST and laminarans and, in this way, were inconsistent with SCFA and gas productions.

For HT, UT and ST, $TFOM_{(A)}$ values calculated using the analysed total sugar content were higher than $TFOM_{(B)}$ values calculated using the deduced total sugar content, whereas these two values were similar for the other substrates (Fig. 1).

Freeze-drying of the whole media

SCFA contents from aqueous supernatant fractions of ST, alginates or laminarans fermentative media (Table 6) were similar to those of ethanolic supernatant fractions from the corresponding precipitated residues: SCFA production from ST was high and increased during the whole course of incubation. In contrast, SCFA production from alginates started only after 6 h incubation and yielded the lowest total amount among the substrates

Table 5. *Disappearance coefficients* (% *of initial substrate*) *for total fibres from* Himanthalia elongata (*HT*), Laminaria digitata (*LT*) *and* Undaria pinnatifida (*UT*) *and for sodium alginates* (*alginates*), *fucans, laminarans and total sugarbeet fibre* (*ST*) *after 6, 12 or 24 h incubation with human faecal inoculum* (*unfermented residues recovered by ethanolic precipitation*)*

(Mean values with their standard errors)

	ST	HT		LT		UT		Alginates		Fucans		Laminarans		Variance ratio		
n...	7	2		2		2		7		2		2				
	Mean	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Between all substrates	Between total algal fibres	Between purified algal fibres
6 h	29·0[a]	17·7[ab]	4·4	23·3[ab]	5·1	20·9[ab]	5·6	15·1[b]	4·3	6·8[b]	12·1	64·3[c]	4·0	12·271	0·310	21·706
12 h	55·9[a]	46·4[a]	0·7	55·4[a]	0·6	59·7[a]	0·7	54·2[a]	5·6	6·8[c]	4·1	85·9[b]	6·6	11·4	95·464	18·214
24 h	77·3[ab]	60·2[d]	3·6	67·1[ad]	0·5	75·6[bd]	1·0	83·1[b]	2·0	−2·0[e]	5·5	89·9[b]	4·0	38·719	11·472	177·681

[a, b, c, d] Mean values within a row with unlike superscript letters were significantly different, $P < 0·05$.

* For details of procedures and calculations, see pp. 264–266.

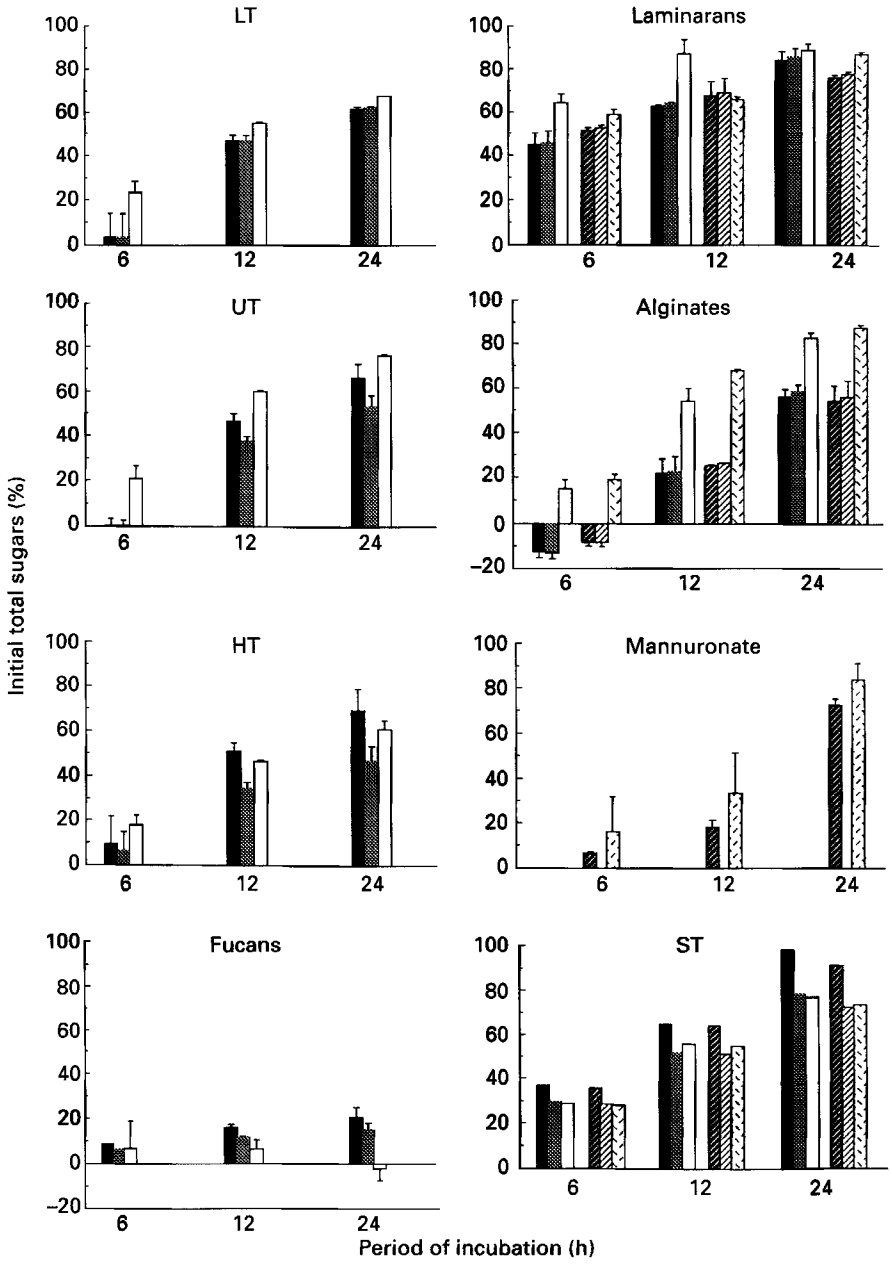

Fig. 1. Comparison of disappearance coefficients (DC) and total fermented organic matter (TFOM) values for total algal fibres from *Himanthalia elongata* (HT), *Laminaria digitata* (LT) and *Undaria pinnatifida* (UT), isolated algal fibres (fucans, laminarans and alginates), mannuronate and total sugarbeet fibre (ST). TFOM values are expressed as a percentage of the analysed substrate initial sugar content ($TFOM_A$) or as a percentage of the calculated substrate initial sugar content ($TFOM_{(B)}$). (□), DC; (■), $TFOM_{(A)}$; (▩), $TFOM_{(B)}$ when residues were recovered by ethanolic precipitation; (▨), DC; (▨), $TFOM_{(A)}$; (▨), $TFOM_{(B)}$ when residues were recovered by freeze-drying. Values are means with their standard errors indicated by vertical bars.

Table 6. *Total production of short-chain fatty acids (mmol/l) from sodium alginates (alginates), laminarans, mannuronate and total sugarbeet fibre (ST) after 6, 12 or 24 h incubation with human faecal inoculum (unfermented residues recovered by freeze-drying)**

(Mean values with their standard errors)

n...	ST 2	Alginates 2		Laminarans 2		Mannuronate 2		
	Mean	Mean	SE	Mean	SE	Mean	SE	Variance ratio
6 h	28·6[a]	−5·6[b]	0·9	57·7[c]	5·2	7·3[b]	0·6	137·903
12 h	49·7[a]	29·5[b]	1·2	70·0[c]	4·1	20·5[b]	4·0	41·409
24 h	69·7[ab]	58·5[a]	2·3	84·9[b]	4·9	83·8[b]	3·7	10·412

[a, b, c] Mean values within a row bearing unlike superscript letters were significantly different, $P < 0{\cdot}05$.
* For details of procedures, see pp. 264–266.

investigated. Laminarans led to the highest SCFA production which increased intensively during the first 6 h incubation. SCFA production from mannuronate was delayed and mainly occurred between 12 and 24 h incubation. The total production was high and close to that obtained from laminarans.

SCFA ratios calculated after aqueous fermentative media analyses were comparable with those obtained from ethanolic supernatant fractions (Table 7). As for total algal fibres and alginates, SCFA production from mannuronate was characterized by a high proportion of acetate.

DC calculated from freeze-dried ST or alginate residues were similar to DC calculated from the respective ethanol-precipitated residues (Table 8). After 6 and 12 h incubation the DC obtained from the freezer-dried laminarans were lower than those obtained from ethanol-precipitated ones. The DC from laminarans recovered by freeze-drying were similar only after 24 h incubation. Mannuronate disappeared slowly during the first 12 h incubation then rapidly until the end of the experiment. The DC obtained for mannuronate after 24 h incubation was close to those of laminarans and alginates.

Freeze-drying of the whole fermentative media as a method for recovering unfermented residues led to similar $TFOM_{(A)}$ and $TFOM_{(B)}$ values for ST, alginates and laminarans to those obtained by ethanol-precipitation (Fig. 1).

Comparison of disappearance coefficient and theoretical fermented organic matter values

For ST, after both ethanolic precipitation and freeze-drying recoveries, $TFOM_{(A)}$ values were higher than DC values for all incubation times and particularly after 24 h. In contrast, $TFOM_{(B)}$ did not differ from DC and both indicated a high substrate metabolism (73–78%) and degradation (73–77%) respectively.

After ethanolic precipitation, $TFOM_{(A)}$ and $TFOM_{(B)}$ values were always lower than DC for LT and UT. For HT, only $TFOM_{(B)}$ values were lower than DC. Total algal fibre samples incubated for 24 h disappeared to 60–76% according to the DC but were metabolized to 51–66% or 47–62% according to the $TFOM_{(A)}$ and $TFOM_{(B)}$ values respectively.

Whatever the process of unfermented residue recovery used, only alginates, among the purified fibres, led to a similar disagreement between DC and TFOM values: after 24 h incubation they disappeared to approximately 85% but were only metabolized to approximately 56%. Fucans were very slightly degraded and metabolized.

Table 7. *Short-chain fatty acid (SCFA) ratios (%) for sodium alginates (alginates), laminarans, mannuronate and total sugarbeet fibre (ST) after 24 h incubation with human faecal inoculum (unfermented residues recovered by freeze-drying)**

(Mean values from uncalibrated data with their standard errors)

n...	ST 2		Alginates 2		Laminarans 2		Mannuronate 2	
SCFA	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Acetic	64·1	0·1	77·6	0·9	58·6	0·4	85·6	3·4
Propionic	18·9	0·6	15·7	0·2	24·5	0·3	3·4	1·3
Butyric	12·6	0·9	1·5	0·3	15·9	0·6	10·0	2·5
Others	4·4	0·7	5·2	1·2	1·0	0·3	1·0	4·8

* For details of procedures, see pp. 264–266.

Table 8. *Disappearance coefficients (% of initial substrate) for sodium alginates (alginates), laminarans, mannuronate and total sugarbeet fibre (ST), after 6, 12 or 24 h incubation with human faecal inoculum (unfermented residues recovered by freeze-drying)**

(Mean values with their standard errors)

n...	ST 2	Alginates 2		Laminarans 2		Mannuronate 2		Variance ratio
	Mean	Mean	SE	Mean	SE	Mean	SE	
6 h	28·4	18·6	1·9	57·0	3·8	16·3	15·7	5·760
12 h	55·0	67·3	0·1	64·7	0·7	33·7	17·8	3·047
24 h	73·7	84·6	1·9	84·6	2·1	83·8	7·6	1·264

* For details of procedures, see pp. 264–266.

For laminarans the process of unfermented residue recovery led to differences in DC values calculated after 6 or 12 h incubation. Therefore, the differences observed between TFOM values and DC values after 6 and 12 h when unfermented residues were recovered by ethanol precipitation did not exist when freeze-drying was used. After 24 h incubation both the metabolism (approximately 82%) and the disappearance (approximately 88%) were in agreement. For mannuronate, TFOM and DC values were very close.

DISCUSSION

In Western countries, edible brown seaweeds represent new sources of dietary fibre. Although some of the chemical and physico–chemical characteristics of these fibres have been described (Lahaye & Thibault, 1990; Fleury & Lahaye, 1991; Lahaye, 1991), their physiological properties in humans are largely unknown. In order to predict some of these properties, the fermentation of these algal fibres by human faecal flora was investigated using total and representative isolated fibres and was compared with the fermentation of a well-investigated substrate, total sugarbeet fibre.

The amounts of total dietary fibre obtained in the present study for *Laminaria digitata*

were in agreement with previous results (Fleury & Lahaye, 1991) but those for *Undaria pinnatifida* and *Himanthalia elongata* were higher than those published (Lahaye, 1991). These variations may reflect differences in the harvesting time and geographical location of the algae, in their processing (e.g. drying) and in the method of dietary fibre measurement.

The chemical analysis of the total algal fibres revealed the presence of different soluble fibres: alginates, fucans and laminarans. In agreement with previous reports (Vinot *et al.* 1987; Ito & Hori, 1989), alginate predominated in these algae. In contrast, according to the fucose and sulphate contents, these three algal fibres contained low amounts of fucans. Cellulose was the second most important fibre fraction whereas contents of laminarans were low as determined by the difference in glucose quantities measured after total and partial acid hydrolysis. The chemical composition of the three algal fibres differed slightly: UT contained the highest amount of alginate whereas HT contained the highest amounts of cellulose, laminarans and fucans.

The residual proteins in the total algal fibres demonstrated the low extent of the enzymic proteolysis. This behaviour may be related to the low *in vivo* digestibility of algal proteins observed in pigs (Black, 1955) and may be due to their cellular localization and/or to their putative associations with cell-wall polysaccharides (Kloareg & Quatrano, 1988). All algal fibres except laminarans had high mineral contents which most probably represented counterions associated with ionic algal polysaccharides (alginates, fucans; Lahaye, 1991).

Reference and algal substrate fermentabilities were studied *in vitro* in the presence of inocula from fresh faeces obtained from two volunteers only. However, seven different experiments involving ST and alginates were carried out. No significant interaction was observed between the experiments and the extents of substrate fermentation; intraindividual variation of fermentation activity is higher than interindividual variation (Auffret *et al.* 1990). Thus, our results can be considered representative of the general *in vitro* fermentation of algal fibres.

Substrate disappearance was assessed in terms of DC values and metabolism by gas and SCFA production rates. The latter were converted into TFOM values to allow comparison between substrate degradation and metabolism.

The validity of DC depends mainly on the efficiency of unfermented residue recovery since it is based on the sugar content in the initial substrate and in the unfermented residues. Errors due to methods of sugar analysis were assumed to be constant for both the initial substrates and the unfermented residues and thus were not taken into account. In contrast, the process of unfermented residue recovery is of crucial importance because, according to the method used, small oligosaccharides may be lost. For the reference fibre ST, no difference was observed for the DC calculated from freeze-dried or ethanol-precipitated unfermented residues. Thus, ST was degraded and metabolized without oligosaccharide accumulation.

The calculation of TFOM values takes into account the stoichiometry of each SCFA produced from sugar metabolism following the glycolysis pathway and the initial total sugar content in the substrate. Thus, TFOM values reflect sugar metabolism (Auffret *et al.* 1991). Although some acetate can result from autotrophic acetogenesis by CO_2 reduction (Lajoie *et al.* 1988; Grivet *et al.* 1992) and some SCFA can be produced from protein fermentation (putrefaction; Macfarlane *et al.* 1992), most of the SCFA production in the present study was assumed to originate from sugar fermentation. The validity of the TFOM calculation also relies on the accuracy of the total sugar content determination. The low recovery yield obtained in the analysis of UT, HT, fucans and ST left some doubts concerning the total sugar content values. Alginates and cellulose are relatively resistant to acid hydrolysis (Dutton, 1973; Hoebler *et al.* 1989) and pentoses, methyl pentoses and guluronic acid are liable to be degraded in concentrated acid (Harris, 1975). Incomplete

acid hydrolysis or acid instability of sugar will lead to underestimation of sugar content and thus to lower recovery yields. Because of uncertainties concerning the total sugar content of the fibres, TFOM values were calculated on the basis of analysed total sugar contents ($TFOM_{(A)}$) and of the theoretical total sugar contents calculated by subtraction of the mineral and crude protein contents ($TFOM_{(B)}$). As previously reported (Cherbut *et al.* 1991; Auffret *et al.* 1993), ST incubation with human faecal bacteria yielded high gas and SCFA production values which were correlated with substrate disappearance. For this reference substrate, a closer agreement was obtained between $TFOM_{(B)}$ and DC than for $TFOM_{(A)}$.

Discrepancies were noted for all algal total fibres between disappearance (DC) and metabolism (TFOM), even though the latter value was calculated using the theoretical total sugar content ($TFOM_{(B)}$). Although the extent of this disagreement varied according to species and to incubation duration, it was more important for all algal total fibres after 6 h incubation. The fermentation of these substrates was also characterized by a 6 h latency phase in gas production. This fermentative behaviour diverged markedly from that of the reference fibre (ST).

In order to distinguish which fraction(s) of the total algal fibres is (are) responsible for such behaviour, isolated fibres characteristic of brown algae, namely laminarans, fucans and alginates, were fermented following the same protocol.

Laminarans were highly fermented by human faecal bacteria with a short delay in the gas production phase. Such a latency phase has already been reported for fermentation of laminarans by isolated *Bacteroides* strains and has been attributed to the time required for the induction of the specific β-glucanases to occur (Salyers *et al.* 1977*a*, 1978). These authors also observed that laminarans were degraded into monomeric glucose and laminaro-oligosaccharides (degree of polymerization between 2 and 6). The existence of such oligosaccharides would explain the small disagreement observed during the first hours of incubation, between disappearance (DC) and metabolism (TFOM) of laminarans calculated from ethanol-precipitated unfermented residues. Such a disagreement was not observed when using the freeze-dried residues. Indeed, the ethanol-soluble laminaro-oligomers would be lost during the ethanol-precipitation, whereas they would have been retained by freeze-drying the whole fermentative medium. The persistence of the laminaro-oligosaccharides during the first hours of fermentation indicates that these fibres were not simultaneously degraded and metabolized, probably due to the presence of the quite unusual β 1–6 sugar linkages and the mannitol residues in these polysaccharides.

These results emphasized the importance of the method used for recovery of unfermented residues since part of them may be lost during the process leading to erroneous interpretation of the results. For the algal total fibre fermentation, the disagreement between the disappearance and metabolism occurred for all the incubation times. Thus, the particular fermentation behaviour did not come from that of laminarans but from other fibre fraction(s).

Fucans resisted fermentation since very low amounts of gas and SCFA were produced and no substrate disappearance was observed. This resistance was probably not due to the lack of inducibility of particular enzymes, as this would have had time to occur during the 24 h incubation period. The low production rates of SCFA and gas were attributed to bacterial protein fermentation. Lack of fermentable substrate favours bacterial lysis which releases proteins into the fermentative medium (Krishnamoorthy *et al.* 1991).

Salyers *et al.* (1977*b*) previously observed that fucans mainly composed of fucose and sulphate were not fermented by isolated *Bacteroides* strains although these bacteria were able to grow on fucose. However, endogenous mucous glycoproteins (mucins) are highly fermented by colonic flora (Gibson *et al.* 1988; Salyers *et al.* 1988) even though these

substrates also contain both fucose and sulphate (Allen, 1981; Neutra & Forstner, 1987). Therefore, neither fucose nor sulphate *per se* would constitute a limit to the fermentation of fucans by the faecal flora. The high concentrations of fucose and sulphate as well as the particular structural arrangement in these algal fibres (Kloareg & Quatrano, 1988) are probably responsible for their resistance to bacterial degradation.

These results contradict the general assumption that water-soluble fibres are highly fermentable (Cherbut *et al.* 1991). The fermentation resistance could lead to particular physiological effects since these fibres will probably retain their physico–chemical properties in the colon. At any rate, these fibres were not responsible for the peculiar total algal fibre fermentative pattern.

Alginates exhibited a particular fermentative pattern characterized by a 6 h latency phase in the production of gas and SCFA, and discrepancies between substrate disappearance and metabolism values. An 8–10 h delay for metabolism has already been reported during alginate fermentation by isolated *Bacteroides* strains (Salyers *et al.* 1977*b*, 1978).

Fermentability studies on mannuronate, a component of alginates, showed that this uronate was very slowly fermented. Therefore, mannuronate could be involved in the 6 h latency phase observed in the fermentation of alginates and total algal fibres. Nevertheless, fermentation of mannuronate led to corresponding disappearance and metabolism values. Thus, this sugar cannot be responsible for the discrepancy observed between disappearance and metabolism of alginates.

Since the process of recovery of unfermented alginate residues did not have any influence on DC values, the disagreement observed between disappearance and metabolism of these fibres was not explained by the release of oligosaccharides. The hypothesis of metabolism producing mainly organic acids (e.g. lactate, succinate, fumarate) is refuted by the weak acidifications observed during alginate incubations (results not shown). The discrepancy corroborated the low metabolism of these fibres by colonic bacteria as concluded from the fermentative gas production (Gibson *et al.* 1990) and the high substrate disappearance observed during *in vivo* digestibility studies on rats (Nilson & Wagner, 1951).

These results pointed to the involvement of an unusual and unknown fermentative pathway in which only 60% of the degraded alginates were metabolized to SCFA. L-Guluronate, a scarcely encountered uronate in plants and animals, may be involved in this fermentation pathway. Additionally, the degradation of alginates which usually involves β-elimination (Gacesa, 1992) may be hampered by the particular sequences of the mannuronates and guluronates present in these fibres (Gacesa, 1988). The 40% of unrecovered material after fermentation of alginates may consist of products resulting from β-eliminated-product catabolism.

Thus, from the similarity between the fermentation patterns of total algal fibres and alginates, it was concluded that the latter isolated fibres, which corresponded to the main algal fibre fraction, were responsible for the discrepancy observed between total algal fibre disappearance and metabolism. The small differences in gas and SCFA productions observed between the total algal fibres were attributed to the contributions of laminarans, fucans and cellulose. The highest similarity between Na-alginates and total algal fibre fermentations was observed for UT which contained the lowest amount of neutral sugars among the three algae investigated. In contrast, fermentation of HT led to the fastest gas the SCFA productions which can be attributed to the relatively high laminarans content of this fibre.

The quite unusual fermentation behaviour of total brown algal fibres and especially the Na-alginate raises the question of the degradation, disappearance and fermentation pathways involved. Investigations of these pathways, including identification of the metabolites obtained, will be of great nutritional importance.

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