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Mineral composition of isolated fibre fractions from artichoke and the effect of phosphate buffer on its structure and mineral content

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Soluble and insoluble dietary fibre of artichoke heart were extracted in water and phosphate buffer to determine their mineral contents and to evaluate the effect of the extraction medium on fibre structure (studied by scanning electron microscopy) and mineral composition (determined by X-ray microanalysis and atomic absorption spectrophotometry). The undialysed soluble dietary fibre extracted with phosphate buffer showed a different structural organisation from that extracted with water. X-Ray microanalysis of both soluble fractions showed some differences between the Na, K, Ca and P contents, and also between the Na contents of the insoluble fractions. However, when the samples were dialysed, differences in morphology and structure between the soluble fractions were not observed. A study of the mineral composition by atomic absorption showed differences between both soluble fractions and both insoluble fractions as regards Na, Ca, Mg and Fe. © 1997 Elsevier Science Ltd

INTRODUCTION

Artichoke (*Cynara scolymus*, L.), a *Compositae* family plant, is chemically characterised by a high content dietary fibre (DF) and the absence of starch (Belitz & Grosch, 1988; Lintas & Capelloni, 1988). DF and their fractions have been isolated from wheat bran, sugar beet, laminaria digitata, pea hulls or citrus fibre using solutions with different ionic strength that cause changes in the physio-chemical properties of DF (Fleury & Lahaye, 1991; Gullion *et al.*, 1992; Auffret *et al.*, 1994). Ionic strength of solution medium determines the structure and particle size of DF, modifies the functional properties of DF and influences its effect in the delay of glucose absorption (López *et al.*, 1996). Frølich *et al.* (1984) described how the mineral binding capacity changes when different ionic solutions are used for isolating fibre, while Monro (1991) found that different ionic solutions provided variations in structure and amount of DF extracted. Furthermore, several studies have shown that the mineral binding capacity of fibre is related to the ability to adsorb bile acids and bile salts in the upper gastrointestinal tract. These studies showed that some minerals such as calcium and iron, were responsible for binding of bile acids through salt linkages (Story *et. al.*, 1982; Hoagland, 1989; Pandolf & Clydesdale, 1992). DF acts in the human body through complex mechanisms which require the development of *in-vivo* studies to explain its biological effects. However, these studies should be preceded by characterisation of DF sources used to obtain information about its action in the human organic (Adiomtore *et al.*, 1990; Edwards *et al.*, 1992; Stevenson *et al.*, 1994)

Literature is short about the artichoke DF and its mineral composition. In order to add to the information, the present study had as its objectives: (1) the determination of mineral composition of isolated fibre fractions from artichoke, using X-ray microanalysis and atomic absorption spectrophotometry; and (2) observations of the modifications that occur in the mineral binding to fibre and fibre structure due to ionic strength of the extraction medium used to isolate both fibre fractions.

The extraction of DF fractions was undertaken in deionized hot water using a modification of Li and Cardozo's method for foods without starch (Li & Cardozo, 1992). To determine the effect of the medium ionic strength, DF fractions were extracted in phosphate buffer, 0.05 M, since this is a common buffer used by most enzymatic-gravimetric methods to determine DF, and this concentration has been shown to be the most effective with pectic substance extraction of several

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vegetables (cabbage, carrot and celery) (Monro, 1991) in a range of pH similar to that used by us.

MATERIALS AND METHODS

Materials

Artichoke hearts (*Cynara scolymus*, L.) ($n = 150$) were provided by HERO SPAIN, S.A. (Murcia, Spain). The reason to select artichoke heart instead of artichoke globe was that artichoke heart is the form in which this vegetable is consumed. Another reason was the possibility of obtaining both DF fractions in a high yield (25 and 21% for soluble and insoluble DF, respectively, in artichoke heart vs 3 and 48% for soluble and insoluble DF, respectively, in artichoke globe) (López, 1995). The samples (40–45 mm of diameter) were freeze-dried in a Virtis Freeze-Drier, model Bench Top 3 (Virtis Co, Gardiner, NY) and ground to obtain a powder, which was then passed through a 40-mesh sieve, placed in polyethylene bottles and stored in a still-air freezer at –40°C until analysis. The artichoke flour was used to obtain both fractions of DF (soluble and insoluble) following two procedures: (1) extraction in hot water; and (2) extraction in hot phosphate buffer.

Experimental design

The sample (0.5 g) was solubilized in 100 ml of deionized water or 0.05 M phosphate buffer, and then homogenized to obtain a homogeneous mixture. The extraction conditions were equal in both extraction proceadures: temperature (60°C), pH solution (6.0) and extraction time (90 min). The insoluble and soluble fibre fractions were separated by centrifugation (3000 × *g*/30 min/4°C) in a Beckman centrifuge 2J-21 (Palo Alto, CA, US), instead of by filtration to avoid possible mineral contamination by Celite, which is used as an aid to filtration. The residues and supernatants were then lyophilized (freeze-dried) to obtain four residues: water-insoluble fibre residue (WIF), phosphate buffer-insoluble fibre residue (PIF), water-soluble fibre residue (WSF) and phosphate buffer-soluble fibre residue (PSF). Part of each fibre residue was used to study its structure and organisation by means of SEM using a JEOL 6100, which was also equipped for energy dispersive X-ray microanalysis. Energy spectra were collected and the data (element analysis) processed with a Link analytical system.

Another portion of each fibre residue was dialyzed to remove the minerals which are not bound by fibre fractions or salts formed by PO_4^{3-} groups from phosphate buffer. The fibre fractions were redissolved in 20 ml of deionized water and contained in a cellulose wet membrane (9-36/32″, Medicell Int. Ltd, England) with an exclusion limit of 12 000 daltons, before being dialyzed against 2 litre of deionized water for 36 h at 75 oscillations/min with continuous change of water. The contents of the dialysis bags were freeze-dried and analyzed for Fe, Zn, Cu, Mn, Na, K, Ca and Mg by atomic absorption spectrophotometry. The morphology and structure of the dialysed fibre residues were studied by SEM.

Scanning electron microscopy and X-ray microanalysis

The freeze-dried samples (WIF, WSF, PIF and PSF) were coated with a thin-layer of gold in a vacuum evaporator (Bio-rad Ducaron Division) and their structure and morphology were studied in a JEOL 6100 scanning electron microscope at 15 kV.

For element analysis of the fibre residues, the freeze-dried samples (WIF, WSF, PIF and PSF) were coated with carbon in the same vacuum evaporator. The SEM was equipped for energy dispersive X-ray microanalysis (Link analyser Oxford Instruments) and operated at an accelerating voltage of 15 kV. Qualitative X-ray analyses were performeed with Link Isis Oxford software.

Analysis of minerals by atomic absorption spectrophotometry

Ash was obtained from 0.5 g of artichoke flour and of each soluble and insoluble fibre residue in a Nabertherm furnace oven, model L3/P (Lilienthal, Bremen, Germany) according to Method 25.092 (AOAC, 1984) and the ash was dissolved with 2 ml of concentrated HNO_3 on a hot plate. The volume was made up to 50 ml with deionized water. Ca, Na, K, Mg, Fe, Mn, Zn and Cu were measured by flame atomic absorption spectrophotometry, using a Perkin Elmer AA spectrophotometer model 3100 (Norwalk, CT, USA) with air-acetylene flame. A monoelemental hollow cathode lamp was used for Ca, Mg, Fe, Mn, Zn and Cu, while Na and K were determined by emission. A CRM-189 wholemeal flour (Community Bureau of Reference, Brussels) was used as reference material. The criteria of the American Chemical Society (ACS, 1980) were followed to determine detection limits.

Statistical analysis

All determinations were carried out at least three times and the results were evaluated statistically using SYSTAT software, version 5.0 (Wilkinson, 1986). Tukey's test with a significance level of 5% was used to compare individual pairs of means.

RESULTS AND DISCUSSION

Fibre structure

SEM images of the insoluble fibres (WIF and PIF, Figs 1a and b, respectively) showed no differences as regards structure and organisation. When the same microscopic technique was used to examine the soluble

fibre (WSF and PSF, Figs 1c and d, respectively), differences were detected in the structural organisation depending on the extraction method used (deionized water or phosphate buffer). The main structural difference was the PSF developed a network but WSF did not. This is contrary to the findings of Monro (1993) for whom the ionic strength of the extraction method (acetate and phosphate buffer) used to solubilize and extract polysaccharides led to the fragmentation of the pectins. However, in the present study, the minerals or salts left over from the buffer produce a differing organisation of the soluble particles of the fibres.

The energy spectra obtained by X-ray microanalysis of each sample allowed us to observe the different mineral contents of the four fibre fractions analysed. When the energy spectra of WIF and PIF (Figs 2a and b) were compared, the Na content of PIF was seen to be greater than that of WIF. This difference in mineral

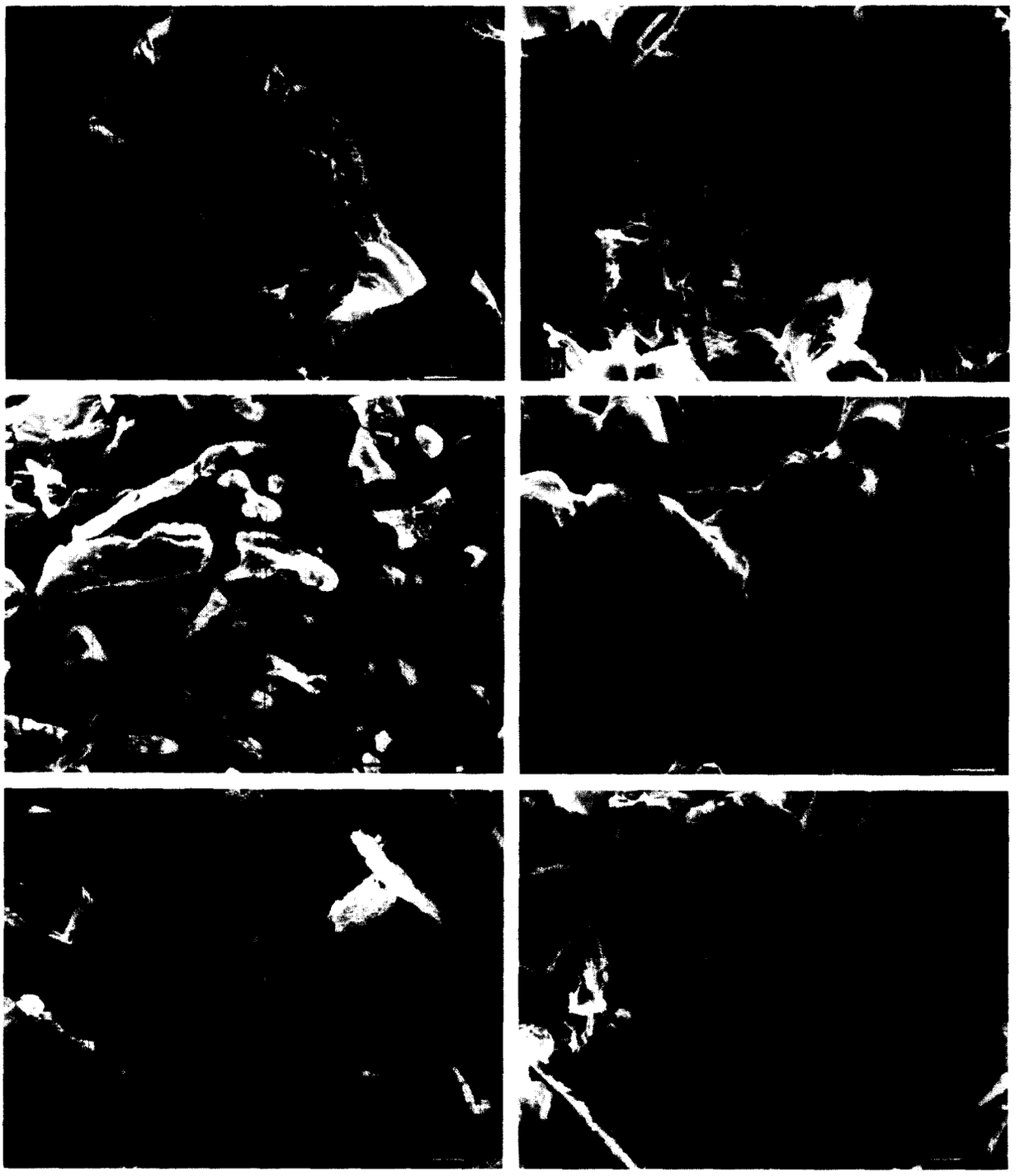

Fig. 1. Scanning electron micrographs showing different particles of artichoke dietary fibre. (1a) and (1b) particles of WIF and PIF, respectively. Magnification ×700. (1c) particles of WSF. Magnification ×370. (1d) particles of PSF. The nodes (A) are formed by salts of phosphate buffer that join the fibre particles (B)/ Magnification ×85. (1e) and (1f) particles of WSF and PSF after dialysis, respectively. Magnification ×700.

composition was more pronounced between WSF and PSF (Figs 2c and d), since their energy spectra showed that the differences in mineral composition affected P, K, and Ca more than Na. Thus, it may be assumed that the salts from the phosphate buffer are responsible for the network of fibre soluble particles, and that these salts are probably located in places in the fibre that allow the bonding between fibre fragments.

In the image obtained by SEM (Fig. 1d), some nodes (A) that act as junction zones between fibre particles (B) can be observed. A mineral study performed by line scan X-ray microanalysis (Fig. 3) showed the evolution of Na, P, Ca, K and Mg across the line established between point 'a' and point 'b' in the image of Fig. 4. Note the higher amount of minerals next to point 'b' than point 'a', suggesting that this accumulation of minerals or salts makes the association among different fractions of soluble fibre possible.

Figures 1e and f show that there were no differences between the structures of WSF and PSF after dialysis. This fact confirmed that phosphate buffer incorporates minerals and salts which may be bound to fibre by weak links, producing a network that will disappear when the salts are removed from the fibre after dialysis.

MINERAL COMPOSITION

Table 1 shows the mineral and electrolyte content of artichoke flour, WIF, PIF, WSF and PSF. In artichoke

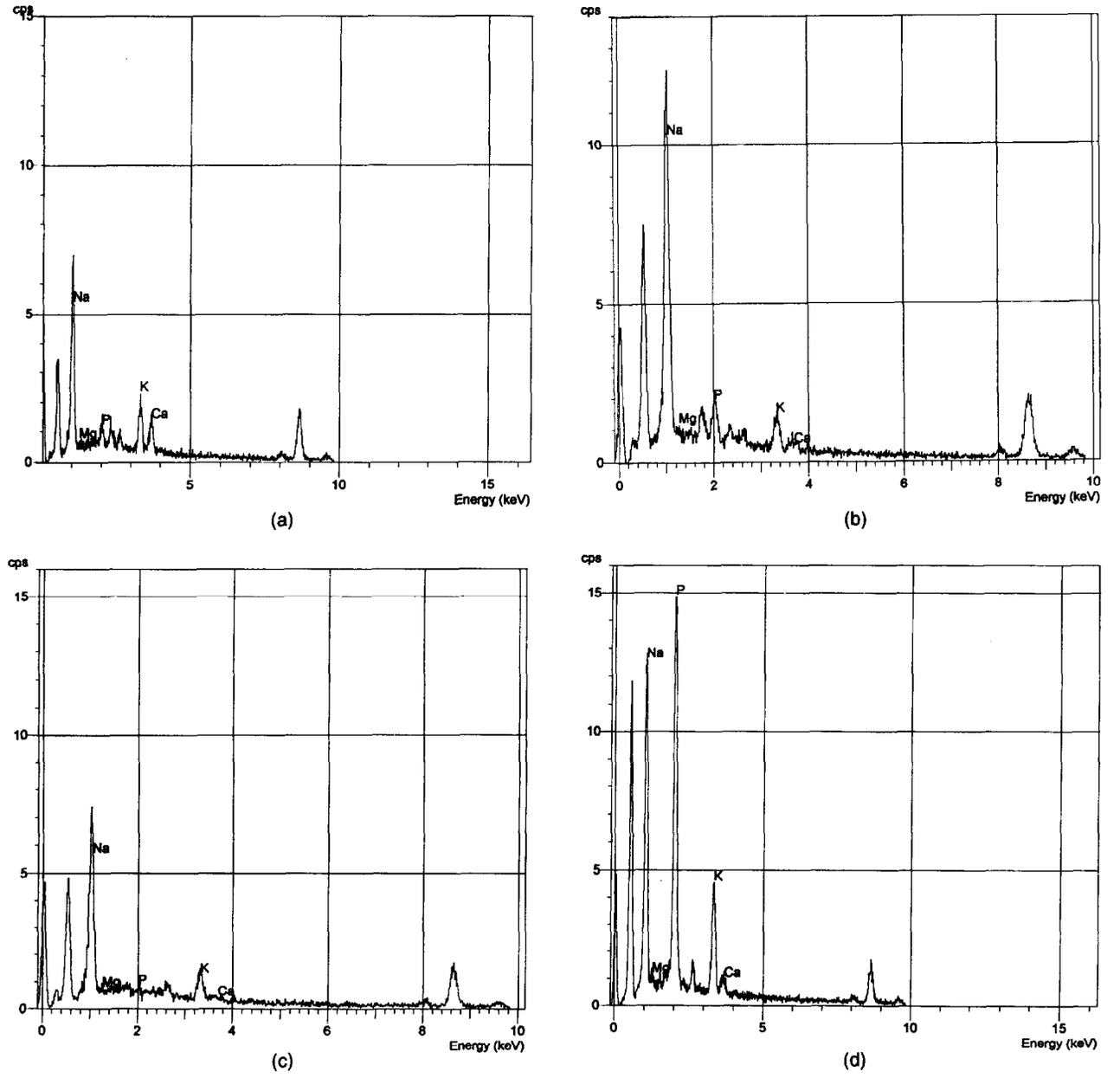

Fig. 2. Energy spectra obtained by X-ray mocrianalysis showing the P, K, Ca, Na and Mg peaks of WIF (2a), PIF (2b), WSF (2c) and PSF (2d).

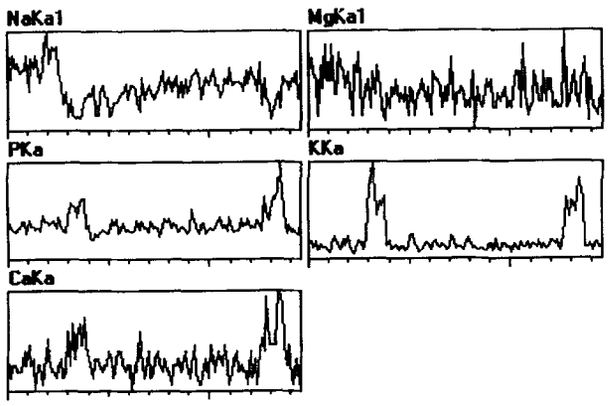

Fig. 3. Representations of the evolution of Na, Mg, P, K and Ca across the line established between points 'a' and 'b' in the image of Fig. 4.

Fig. 4. Scanning electron micrograph of PSF. Magnification 100. Across the line established between points 'a' and 'b' was obtained the evolution of Na, Mg, Ca, K and P represented in Fig. 3.

flour, K was the most abundant element, followed by Na. With respect to their concentration in artichoke flour, the amounts of Ca and Mg on WIF and WSF were comparatively higher than the amounts of Na and K. In vegetables, both electrolytes are mainly in the phloem and play an important role in the maintenance of the membrane potential. However, high proportions of Ca and Mg have a structural role as components of membranes and cell wall (Barceló *et al.*, 1992). This may explain the results obtained in both fibre fractions.

The results presented in Table 1 show that, for the fractions extracted in water, Ca was the most abundant mineral in insoluble dietary fibre (WIF), while K was in soluble dietary fibre (WSF). The K content of soluble fractions (384 and 366 mg 100 g^{-1} for WSF and PSF, respectively) was higher than in the insoluble fractions (222 and 231 mg 100 g^{-1} for WIF and PIF, respectively), with no statistical differences between them. The values of Ca oscillated between 295 mg 100 g^{-1} in WIF, and 213 mg 100 g^{-1} in PSF. The differences were only significant ($p < 0.05$) between WIF and PIF. Another divalent cation, Mg, showed the highest values in WSF (209 mg 100 g^{-1}) and the lowest in PIF (125 mg 100 g^{-1}), the differences being statistically significant ($p < 0.05$), as they were between WSF and PSF. Na content ranged from 137 mg 100 g^{-1} (WIF) to 465 mg 100 g^{-1} (PSF). The fractions obtained in phosphate buffer (PIF and PSF) retained the highest amount of electrolytes. The Na content of PSF was over four times that of WSF, while the Na content of PIF was double that of WIF. The extraction medium was a source of variation in the Na content, since statistically significant differences ($p < 0.05$) were found between WSF and PSF, and between PSF and PIF, but not between WSF and WIF (Table 1).

These results confirm that the Na incorporated by sodium phosphate buffer is bound in both fractions of fibre, mainly in SDF. Monro (1991) explained this by the competition between Ca (present in carboxylic groups in chains of uronic acids) and Na (incorporated by phosphate buffer). This competition is established by the groups of uronic acids present in DF. Due to the Ca^{2+} sequestering capacity of PO_4^{3-} (Monro, 1991) and the weak link between cation Ca^{2+} and the carboxyl group (electrostatic force) (Kohn, 1987), the Ca^{2+} bound to phosphate groups will form calcium phosphate, that will precipiate and free the Ca binding sites of uronide carboxyl groups. These zones will be occupied by Na cation, which is univalent and cannot form bridges between adjacent polyuronide chains. There are two factors that determine cation binding to

Table 1. Electrolyte and mineral content in artichoke and the different fractions of fibre analysed. The results are expressed as g 100 g^{-1}, dry weight basis (mean ± SD)[a]

Samples[b]	Na	K	Ca	Mg
Artichoke	1030 ± 20.0	2596 ± 23.0	280 ± 15.4	295 ± 3.9
WIF	137 ± 15.9^c	222 ± 13.6^b	295 ± 5.0^a	188 ± 11.0^{ab}
WSF	167 ± 24.0^c	384 ± 10.7^a	218 ± 10.7^c	209 ± 0.4^a
PIF	310 ± 7.0^b	231 ± 17.1^b	257 ± 11.4^b	125 ± 11.6^c
PSF	465 ± 16.7^a	366 ± 29.5^a	213 ± 18.5^c	187 ± 1.2^b
CRM189 Wholemeal flour	0.3 10^{-2}	69 10^{-2}	4.9 10^{-2}	19 10^{-2}

[a]Means within the same column with different letters are significantly different at $p < 0.05$.
[b]WIF: water insoluble fibre; WSF: water soluble fibre; PIF: phosphate buffer insoluble fibre; PSF: phosphate buffer soluble fibre.

fibre: (1) the difference in ionic concentration between the cell wall and the medium; and (2) the intrinsic affinity shown by each cation present in the medium for the carboxyl groups fixed to the cell wall (Lee & Garcia-Lopez, 1985; Laszlo, 1987). Thus, this phenomenon is increased when the Na concentration in the medium is increased, because of the competition between Ca and Na for carboxyl groups. This competition weakens the link among uronic acids, and increases the probability of delivering Ca to the cell wall (Monro, 1991)

Although this reasoning might be valid to explain the greater values of Na found in PIF than in WIF, it is not valid to explain the differences in the amount of Na fixed by soluble fractions. One possible explanation of these differences could be displacement of the Mg (divalent cation) joined to fibre by the Na, similar to the Ca-Na competition previously explained. There is little information concerning the mechanism that mediates the link of this mineral to fibre. Nevertheless, Laszlo (1987) described a dissociation constant for Mg in soy hull greater than for Zn and Ca; so, at a high ionic strength, Mg will be released more easily than the other two minerals. Lee and Garcia-Lopez (1985) did not find any capacity to bind Mg in the fibre of cooked pinto beans. The results shown in Table 1 indicate that the extraction medium (water or phosphate buffer) influenced the amount of Mg bound to SDF and insoluble dietary fibre (IDF), since both fractions obtained in a medium of higher ionic strength (PIF and PSF) showed lower amounts of Mg than WSF and WIF. On the basis of these results, we may assume that Mg was removed from DF by the ionic strength of the phosphate buffer, replacing its binding sites by Na, which is a monovalent cation, and which may bind two places left by Mg (divalent cation).

These two possible exchange mechanisms between the cations of fibre and phosphate buffer would explain the binding of some Na in DF. However, the total amount of this mineral bound to SDF cannot be explained by a process of Ca and Mg exchange. Since there was no significant difference in the K content between WIF and PIF, or between WSF and PSF, the Na incorporated by phosphate buffer might bind to components of fibre with a cation-exchange capacity, such as carboxylic groups of uronic acids, hydroxy groups or other groups with this capacity (Amadò, 1994).

Table 2 shows trace element content in artichoke flour and the four fibre fractions isolated. In artichoke flour, Fe and Zn were the oligoelements detected in higher content (4.9 and 4.7 g 100 g^{-1}, respectively). Cu and Mn contents were similar (around 1.5 mg 100 g^{-1}). As was described with Ca and Mg, Fe is a trace element that is bound to the cell wall structure (Barceló *et al.*, 1992), and thus the two processes to isolate fibre increased the Fe levels in all samples. The content of Fe in both insoluble fractions (WIF and PIF) were high compared with that detected in WSF and PSF. The highest amounts of iron were found in the insoluble fractions, mainly in PIF, as has been shown by other authors (Frølich & Nyman, 1988; Frølich *et al.*, 1988, Periago *et. al.*, 1995). For Zn, no difference was found between any of the fibre fractions analysed, the values ranging between 6 mg 100 g^{-1} for WSF and 8 mg 100 g^{-1} for PIF. The Cu contents in WSF and PSF (1.2 and 1.7 mg 100 g^{-1}, respectively) were higher than in WIF and PIF (0.4 and 0.6 mg 100 g^{-1}, respectively). The ionic strength due to phosphate buffer had no influence on the Cu and Zn bound to SDF and IDF. A high affinity of these minerals has been described for fibre in some of its sources (Lee & Garcia-Lopez, 1983; Laszlo, 1987; Person *et al.*, 1991), and thus external factors such as pH or ionic strength will have less influence on these minerals than on others. However, Mn was not found in any fraction analysed. This may be because it is absent in artichoke fibre or because of the presence of very weak binding types that allow the liberation of Mn when samples are dialysed. The function of Mn in the plant cell is mainly related to the photosynthesis (Barceló *et al.*, 1992) and no structural function has been described. However, this mineral was found in a high percentage in the SDF of pea (Periago *et al.*, 1995).

This work shows the mineral composition of artichoke heart and isolated fibre fractions from artichoke, and the effect of phosphate buffer on structure and mineral binding to dietary fibre fractions *in vitro*. Ca and K were the most abundant minerals in insoluble and soluble fibre fractions, respectively. This study

Table 2. Oligoelement content in artichoke and the different fractions of fibre analysed. The results are expressed as g 100 g^{-1} of dry weight basis (mean ± SD)[a]

Samples[b]	Fe	Zn	Cu	Mn
Artichoke	4.9 ± 0.56	4.7 ± 0.34	1.6 ± 0.15	1.4 ± 0.25
WIF	9.9 ± 0.42[b]	6.0 ± 0.90[a]	0.4 ± 0.09[b]	
WSF	3.7 ± 0.22[c]	6.0 ± 1.10[a]	1.2 ± 0.04[a]	
PIF	11.6 ± 1.30[a]	8.0 ± 1.80[a]	0.6 ± 0.04[b]	
PSF	3.1 ± 0.13[c]	6.0 ± 1.00[a]	1.7 ± 0.29[a]	
CRM 189 Wholemeal flour	70.4 10^{-4}	55.3 10^{-4}	6.9 10^{-4}	64.0 10^{-4}

[a]Means within the same column with different letters are significantly different at $p < 0.05$.
[b]WIF: water insoluble fibre; WSF: water soluble fibre; PIF: phosphate buffer insoluble fibre; PSF: phosphate buffer soluble fibre.

confirmed that phosphate buffer modifies the structure of the artichoke soluble fibre, forming a network that disappears after dialysis. The mineral composition of the fibre fractions was also changed by phosphate buffer, the Na content of the soluble and insoluble fractions increasing and the Ca content of the insoluble and the Mg content of both fractions decreasing.

Although the experimental conditions employed were not selected to mimic *in-vivo* conditions and it is important to perform *in-vivo* experiments before making definite conclusions it is clear from the present *in vitro* research that phosphate buffer used in the methods to isolate dietary fibre modifies its mineral binding to isolated fibre fractions, and could impair the results obtained in *in-vivo* studies when these samples are used.

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