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SHORT COMMUNICATION

Determination of soluble dietary fibre content of Okara treated with high hydrostatic pressure and enzymes: a comparative evaluation of two methods (AOAC and HPLC-ELSD)

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Abstract Okara is a promising by-product from soybean and a rich source of dietary fibre, chiefly insoluble (IDF). To increase its solubility and functionality, a treatment with high hydrostatic pressure (HHP) assisted by food grade enzymes -*Ultraflo*® L or *Viscozyme*® L- has been performed. To monitor the effectiveness, an analysis of dietary fibre, mainly the soluble fraction (SDF), was accomplished by the AOAC enzymatic–gravimetric method with dialysis followed by both, spectrophotometric methods and High Performance Liquid Chromatography with Evaporative Light Scattering Detector (HPLC-ELSD) analysis of the soluble fraction. A significant increase in SDF ($\approx$1.5-times) to the expense of a decrease in IDF was shown and chromatograms revealed two peaks of 95 and 22 kDa. Thus, treated Okara possessed a more balanced and convenient ratio of soluble to insoluble dietary fibre, which could have health benefits as prebiotic. Inbuilt interferences of the official AOAC's method for dietary fibre were confirmed and the direct HPLC-ELSD approach was about twice more sensitive than spectrophotometric methods. Consequently, the direct HPLC-ELSD analysis of the supernatant is proposed as a cheaper, faster and reliable method. Combined HHP-treatments plus specific enzymes represent a promising alternative for the valorisation and preservation of agrofood by-products.

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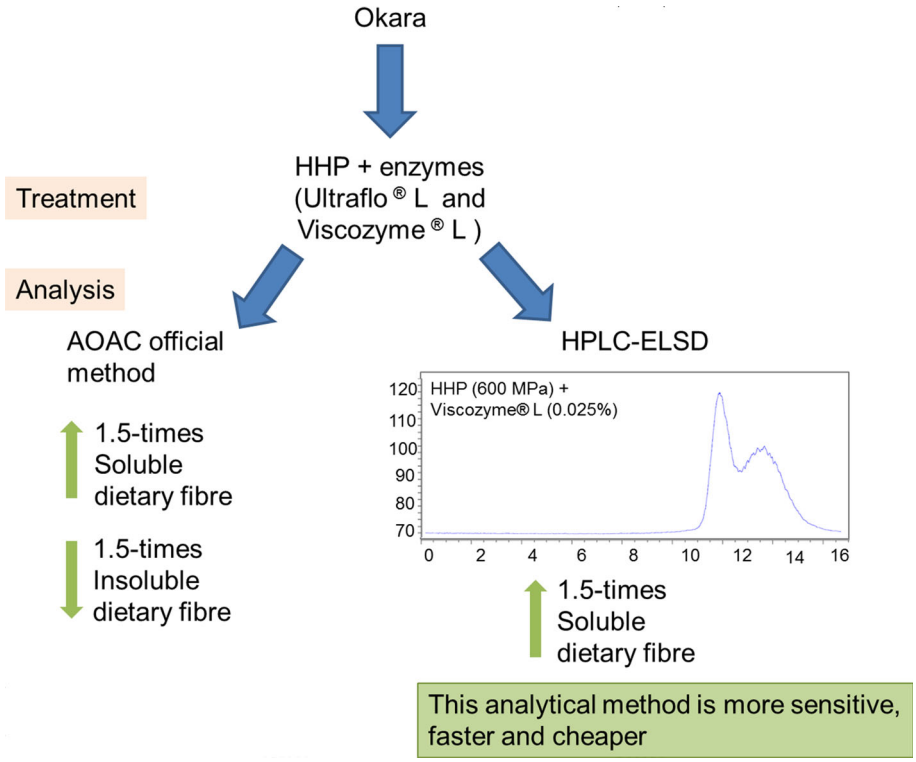
Graphical Abstract



Keywords Okara · Agrofood by-product · HHP · *Ultraflo*® L · *Viscozyme*® L · SDF analysis

Introduction

Nowadays, by-products are promising sources of bioactive compounds and prebiotics (Gullon et al. 2009; Mateos-Aparicio 2011; Mateos-Aparicio et al. 2013; Rastall and Gibson 2015). Among them, Okara from soybean, a by-product of soymilk or tofu industries (Mateos-Aparicio et al. 2010c; O'Toole 1999) is cheap, abundant and a valuable source of dietary fibre (50% insoluble dietary fibre, IDF and 4.5% soluble dietary fibre, SDF) (Mateos-Aparicio et al. 2010c), which makes it a potential prebiotic supplement (Jiménez-Escrig et al. 2008; Préstamo et al. 2007; Redondo-Cuenca et al. 2008). However, as Okara is mostly insoluble, different approaches are used to increase its SDF content, which is responsible for the prebiotic and anti-carcinogenic effects (Charalampopoulos and Rastall 2012), These include, chemical (Mateos-Aparicio et al. 2010a), enzymatic treatments with food grade hydrolytic enzymes at atmospheric pressure, such as *Ultraflo*® L or *Viscozyme*® L (Kasai et al. 2004; Rovaris et al. 2012; Rupérez et al. 2011; Villanueva et al. 2013), as well as high hydrostatic pressure (HHP) treatment (Li et al. 2012; Mateos-Aparicio et al. 2010b) are employed to release soluble carbohydrates from the complex cell wall found in Okara. Moreover, a combination of HHP and enzymatic treatment has been used, since HHP can enhance the activity of some enzymes on Okara's cell wall, achieving up to 3.5-times higher soluble content after *Ultraflo*® L hydrolysis under HHP (Pérez-López et al. 2016a), measured by a direct HPLC-ELSD method. However, SDF and IDF have not been measured by the official AOAC method, and a comparison between both methods is needed. Therefore, the aim of this work is to compare the direct HPLC-ELSD method with the official AOAC method in samples of Okara treated with HHP and enzymes and to develop the application of the optimized HPLC-ELSD method for SDF analysis after AOAC procedure.

Materials and methods

Materials, reagents, samples and equipment

A local food processing industry (Toofu-Ya S.L., Arganda del Rey, Madrid, Spain) provided fresh Okara from soybean [*Glycine max* (L.) Merr]. Once at the laboratory it was freeze-dried (Virtis Bench Top 3 L, Hucoa-Erlöss S.A., Madrid, Spain) and defatted by extraction with ethylic ether in a Soxtec System (Tecator, Höganäs, Sweden). Freeze-dried Okara has a fat content of 8% before

defatting. Before any further treatment, Okara was re-hydrated in water (1% w/v) (Pérez-López et al. 2016a). Enzymatic treatments were performed with two commercial food-grade endo-β-1,3(4)-glucanases: *Ultraflo*® L, with both xylanase and cellulase activities, and *Viscozyme*® L, with xylanase, cellulase and hemicellulase activities (Novozymes Spain, S.A., Pozuelo de Alarcón, Madrid, Spain).

All other reagents and carbohydrate standards used were of chromatography grade. All solutions, including, dilutions and mobile phases were prepared with ultrapure water (Resistivity 18.2 MΩ cm at 25 °C; Milli-Q Integral 5 Water Purification System from Millipore, Merck KGaA, Darmstadt, Germany).

HHP treatment of Okara assisted by enzyme

Re-hydrated Okara solution (1%, w/v) was treated with high hydrostatic pressure (HHP) at 400 and 600 MPa, aided by *Ultraflo*® L or *Viscozyme*® L (concentration 0.025% v/w), at 40 °C for 15 or 30 min (Pérez-López et al. 2016a). HHP treatment was performed in a Stansted SFP 7100:9/2C equipment, using water as pressure transmitting medium. After treatment, samples were freeze-dried (Virtis Bench Top 3 L, Hucoa-Erlöss S.A., Madrid, Spain) for the analysis of dietary fibre by official AOAC method.

Dietary fibre analysis of Okara treated with HHP assisted by enzyme

Soluble and insoluble dietary fibre fractions were determined in HHP + *Ultraflo*® L or HHP + *Viscozyme*® L treated samples according to the modified AOAC 991.43 (Association of Official Analytical Chemists, 1995) enzymatic–gravimetric method with dialysis (12 kDa MW cut off) (Mañas and Saura-Calixto 1993; Mateos-Aparicio et al. 2010b) to avoid ethanolic precipitation (in AOAC 991.43 and 2011.25 methods) that may cause an underestimation of SDF and overestimation of IDF. An aliquot of SDF fraction was taken for direct HPLC-ELSD analysis.

Dietary fibre analysis by spectrophotometric methods

All the spectrophotometric methods used were previously miniaturized and adapted to microplate by volume adjusting. According to AOAC method, uronic acids (UA) in SDF fraction were spectrophotometrically quantified by the Scott method (Scott 1979) (200 µL total volume and 50–200 mg L^{-1} galacturonic acid detection range, DR), and neutral sugars (NS) were determined by the anthrone method (Loewus 1952) (200 µL total volume and, 25–150 mg L^{-1} glucose DR). Moreover, SDF and IDF fractions after AOAC method were hydrolysed with H_2SO_4 (1 M) at 105 °C for 1.5 h and reducing sugars were spectrophotometrically measured by dinitrosalicylic acid method (DNS) (Miller 1959) (270 µL total volume and, 250–1500 mg L^{-1} glucose DR). Absorbance readings were measured on a Biotek PowerWawe XS spectrophotometer. Thus, SDF was calculated either as total neutral sugars plus uronic acids (NS + UA) or as reducing sugars (DNS method). IDF was calculated as reducing sugars (DNS method) and total dietary fibre (TDF) was calculated as SDF plus IDF.

Soluble carbohydrate analysis by HPLC-ELSD

The soluble fraction from the modified AOAC method for dietary fibre was filtered through 0.45 µm (cellulose acetate, 25 mm diameter, Análisis Vínicos, Tomelloso, Toledo, Spain) and directly analysed by High Performance Liquid Chromatography with Evaporative Light Scattering Detector (HPLC-ELSD) on a size-exclusion column, as previously reported for carbohydrate analysis (Condezo-Hoyos et al. 2015; Pérez-López et al. 2016a). SDF was quantified from area data and MW estimated from retention times, with log-calibration curves (Condezo-Hoyos et al. 2015).

Statistical analysis

Results were expressed as mean values ± standard deviation. At least, three different measurements were accomplished for each mean. Comparison of means was performed by one-way analysis of variance with a significance level of $P < 0.05$ according to Statgraphic version 5.1. (Statpoint Technologies, Inc. Warrenton, Virginia, USA).

Results and discussion

Results from the modified AOAC procedure are shown in Tables 1 and 2 and Fig. 1 (1.1, 1.2). This method can detect differences between samples, as can be done by direct HPLC-ELSD analysis (Pérez-López et al. 2016a, b). Thus, for each treatment, an increase in SDF was observed and 600 MPa, at 40 °C for 30 min treatment was the most effective one with both enzymes, as in a previous work (Pérez-López et al. 2016a). Also, a synergy between the HHP and the enzymatic treatment has been observed, especially in *Ultraflo*® L (Table 1), which was comparable with our previous experiments (Pérez-López et al. 2016a, b). 2.9, 1.5 and 1.5-times higher SDF content using 0.025% *Ultraflo*® L and 1.2, 1.3 and 1.2- times higher using 0.025% *Viscozyme*® L were achieved in these conditions compared to the control, by NS + UA, DNS and

Table 1 Analysis of dietary fibre in Okara treated with HHP and assisted by *Ultraflo*® L

Treatment conditions			NS + UA	DNS					SDF by HPLC-ELSD				
HHP (MPa)	min	*Ultraflo*®L %	SDF	SDF	IDF	TDF	SDF/TDF	TDF/IDF	Peak 1		Peak 2		Total CHO
			% d.w.	% d.w.	% d.w.	% d.w.			% d.w.	MW (kDa)	% d.w.	MW (kDa)	% d.w.
0.1	0	0	1.20 ± 0.08^{a}	1.57 ± 0.19^{c}	30.27 ± 2.78^{b}	31.84 ± 2.79^{b}	0.049	1.052	1.31 ± 0.09^{a}	94.3 ± 4.36	1.42 ± 0.04^{a}	22.64 ± 1.81	2.73 ± 0.09^{a}
400	15	0	2.33 ± <0.01^{c}	1.05 ± <0.01^{b}	36.04 ± <0.01^{cd}	37.09 ± 0.03^{cd}	0.028	1.029	2.84 ± 0.05^{b}	104.93 ± 2.04	0.79 ± 0.07^{b}	20.51 ± 2.81	3.63 ± 0.12^{bc}
		0.025%	2.33 ± <0.01^{c}	2.12 ± <0.01^{d}	33.35 ± 0.06^{c}	35.47 ± 0.06^{c}	0.060	1.064	3.19 ± 0.04^{cde}	99.59 ± 0.91	0.73 ± 0.06^{b}	23.29 ± 0.43	3.92 ± 0.09^{cd}
	30	0	2.21 ± <0.01^{b}	0.84 ± <0.01^{a}	37.10 ± 0.02^{d}	37.94 ± 0.02^{cde}	0.022	1.023	2.85 ± 0.18^{b}	105.08 ± 0.84	0.62 ± 0.04^{b}	20.05 ± 0.86	3.47 ± 0.14^{b}
		0.025%	2.22 ± <0.01^{b}	2.04 ± <0.01^{d}	33.36 ± 0.22^{c}	35.40 ± 0.22^{c}	0.058	1.061	3.14 ± 0.10^{bcd}	95.14 ± 1.31	0.78 ± 0.13^{b}	22.53 ± 0.74	3.92 ± 0.03^{cd}
600	15	0	2.17 ± <0.01^{b}	1.57 ± <0.01^{c}	38.53 ± 0.03^{d}	40.10 ± 0.03^{e}	0.039	1.041	3.14 ± 0.04^{bcd}	98.40 ± 1.67	0.81 ± 0.11^{b}	22.21 ± 2.19	3.95 ± 0.15^{cd}
		0.025%	2.32 ± <0.01^{c}	2.24 ± <0.01^{de}	35.89 ± 0.05 ^{cd}	38.13 ± 0.05^{cde}	0.059	1.062	3.41 ± 0.02^{de}	96.03 ± 1.12	0.75 ± 0.02^{b}	22.47 ± 0.16	4.16 ± 0.04^{d}
	30	0	3.28 ± <0.01^{d}	1.48 ± <0.01^{c}	37.09 ± 0.06^{d}	38.56 ± 0.06^{de}	0.038	1.040	3.09 ± 0.15^{bc}	99.59 ± 1.18	0.89 ± 0.14^{b}	23.81 ± 1.87	3.98 ± 0.29^{cd}
		0.025%	3.48 ± 0.01^{e}	2.34 ± <0.01^{e}	22.16 ± 0.07^{a}	24.50 ± 0.07^{a}	0.095	1.105	3.47 ± 0.08^{e}	100.64 ± 3.17	0.73 ± 0.06^{b}	19.90 ± 0.17	4.19 ± 0.02^{d}

Different letters in each column differ significantly ($P < 0.05$)

NS + UA Neutral sugars + Uronic acid, *DNS* 3,5-dinitrosalicylic acid, *SDF* Soluble Dietary Fibre, *IDF* Insoluble Dietary Fibre, *TDF* Total Dietary Fibre, *d.w.* dry weight, *MW* Molecular Weight, *CHO* Carbohydrates

Table 2 Analysis of dietary fibre in Okara treated with HHP and assisted by Viscozyme® L

Treatment conditions			NS + UA	DNS					SDF by HPLC-ELSD				
HHP (MPa)	min	*Viscozyme*®L %	SDF	SDF	IDF	TDF	SDF/TDF	TDF/IDF	Peak 1		Peak 2		Total CHO
			% d.w.	% d.w.	% d.w.	% d.w.			% d.w.	MW (kDa)	% d.w.	MW (kDa)	% d.w.
0.1	0	0	1.20 ± 0.08	1.57 ± 0.19^{a}	30.27 ± 2.78^{cd}	31.84 ± 2.79	0.049	1.052	1.31 ± 0.09^{a}	94.3 ± 4.36	1.42 ± 0.04^{cde}	22.64 ± 1.81^{b}	2.73 ± 0.09
400	15	0	1.23 ± 0.09	1.75 ± 0.1^{a}	33.58 ± 6.46^{d}	35.32 ± 6.46	0.050	1.052	1.4 ± 0.01^{ab}	84.12 ± 6.3	1.34 ± 0.05^{bcd}	13.82 ± 1^{a}	2.74 ± 0.06
		0.025%	1.32 ± 0.18	1.77 ± 0.04^{a}	23.94 ± 2.24^{b}	25.71 ± 2.24	0.069	1.074	1.43 ± 0.01^{ab}	93.18 ± 3.95	1.46 ± 0.01^{de}	15.89 ± 2.18^{a}	2.89 ± 0.01
	30	0	1.27 ± 0.07	1.75 ± 0.17^{a}	23.23 ± 1.82^{b}	24.98 ± 1.82	0.070	1.075	1.65 ± 0^{c}	91.59 ± 4.04	1.19 ± 0.2^{abc}	23.47 ± 4.5^{bc}	2.84 ± 0.2
		0.025%	1.36 ± 0.17	1.73 ± 0.07^{a}	25.52 ± 4.22^{b}	27.25 ± 4.22	0.063	1.068	1.67 ± 0.07^{c}	89.64 ± 6.35	1.16 ± 0.06^{ab}	29.51 ± 6.93^{cd}	2.83 ± 0.09
600	15	0	1.07 ± 0.05	1.74 ± 0.04^{a}	26.14 ± 2.1^{bc}	27.87 ± 2.1	0.062	1.066	1.63 ± 0.26^{bc}	92.19 ± 6.97	1.09 ± 0.18^{a}	31.31 ± 4.38^{d}	2.72 ± 0.32
		0.025%	1.3 ± 0.13	1.67 ± 0.06^{a}	22.65 ± 1.74^{ab}	24.32 ± 1.75	0.069	1.074	1.61 ± 0.04^{bc}	91.26 ± 5.1	1.2 ± 0.08^{abc}	25.36 ± 2.51^{bcd}	2.81 ± 0.09
	30	0	1.18 ± 0.11	1.71 ± 0.1^{a}	23.12 ± 4.19^{b}	24.83 ± 4.19	0.069	1.074	1.4 ± 0.01^{ab}	90.63 ± 0.14	1.19 ± 0.13^{abc}	19.15 ± 1.64^{ab}	2.59 ± 0.13
		0.025%	1.45 ± 0.08	2.11 ± 0.14^{b}	18.83 ± 1.31^{a}	20.93 ± 1.32	0.100	1.111	1.56 ± 0.22^{bc}	91.24 ± 0.72	1.66 ± 0.14^{e}	15.54 ± 2.6^{a}	3.22 ± 0.26

Different letters in each column differ significantly ($P < 0.05$)

NS + UA Neutral sugars + Uronic acid, *DNS* 3,5-dinitrosalicylic acid, *SDF* Soluble Dietary Fibre, *IDF* Insoluble Dietary Fibre, *TDF* Total Dietary Fibre, *d.w.* dry weight, *MW* Molecular Weight, *CHO* Carbohydrates

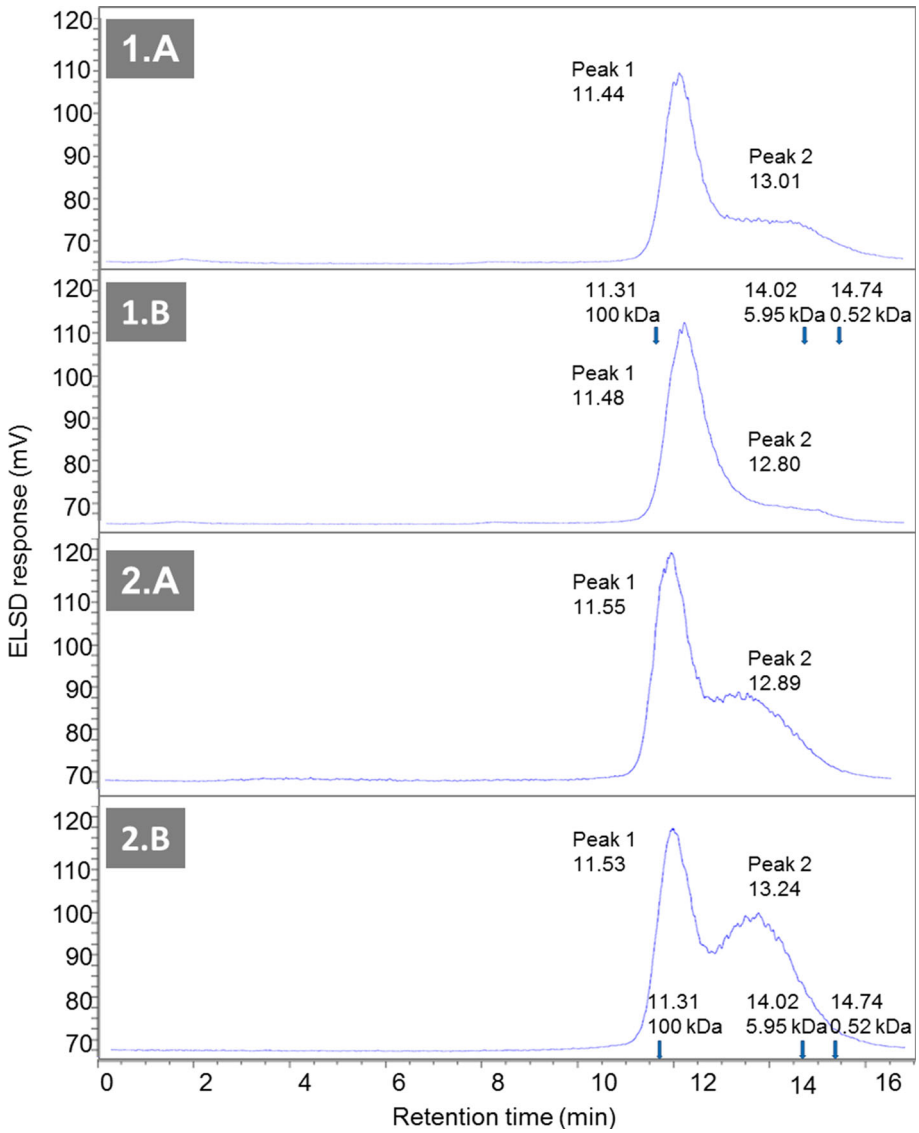


Fig. 1 HPLC-ELSD chromatograms of soluble fibre after modified AOAC method in Okara treated with HHP, assisted by *Ultraflo*® L (**1.1**) or *Viscozyme*® L (**1.2**). Treatment conditions Okara 1%, 600 MPa for 30 min, **a**: without enzyme; **b**: with 0.025% (v/w) enzyme. Retention time and MW of standards are represented on the chromatogram. ELSD response is expressed in millivolts (mV)

HPLC-ELSD methods, respectively. However, the direct HPLC-ELSD analysis was more sensitive than the AOAC method followed by spectrophotometric methods. Values were correspondingly 3.6- and 3.4-times lower than those reported for soluble polysaccharides measured by direct HPLC-ELSD method in the supernatant after treatment with 0.025% *Ultraflo*® L (Pérez-López et al. 2016a) or *Viscozyme*® L at 600 MPa and 40 °C for 30 min (unpublished results). Furthermore, SDF values were reported to be lower than in previous works on native Okara using an AOAC method (991.42) for dietary fibre without dialysis (Redondo-Cuenca et al. 2008) and in Okara treated with HHP + autoclaving using the same AOAC method as in this study but followed by gas liquid chromatography of monosaccharides (Mateos-Aparicio et al. 2010b). Changes in the IDF profile of Okara caused by the treatment with HHP and hydrolytic enzymes have not been assessed before. Results suggested that SDF increase was due to a hydrolysis of IDF polysaccharides, as this was reported to be 1.4 and 1.6-times lower in *Ultraflo*® L and *Viscozyme*® L treatment at 600 MPa for 30 min, respectively (Tables 1 and 2). This was attributable to release of CHO from Okara's intricate cell wall (Kasai et al. 2004; Mateos-Aparicio et al. 2010c; Villanueva et al. 2013). Considerable changes in SDF/TDF ratio were detected (from 0.049 to 0.100) in combined treatments, as in other previous reports (Mateos-Aparicio et al. 2010b; Pérez-López et al. 2016a).

AOAC official method is frequently used followed by spectrophotometric analysis (Mateos-Aparicio et al. 2010a,b; Redondo-Cuenca et al. 2008). Here, the use of the HPLC-ELSD method for analysing the SDF fraction has been suggested instead. HPLC-ELSD chromatogram of the SDF fraction after AOAC method revealed the existence of one peak of ≈95 kDa and a smaller one of ≈22 kDa,

which presented a gradual decrease in its MW upon treatment and it was more noticeable in *Viscozyme*® L experiment (Fig. 1.2). These low MW non-digestible carbohydrates could have beneficial prebiotic effects as they are more easily fermentable by specific bacteria than those with high MW (Charalampopoulos and Rastall 2012; Jiménez-Escrig et al. 2008; Mateos-Aparicio et al. 2010a, b; Préstamo et al. 2007; Villanueva et al. 2011). Direct HPLC-ELSD analysis of supernatant in Okara after treatment with HHP + *Ultraflo*® L (without further AOAC method) showed three peaks of approximately 24, 10 and 0.55 kDa (Pérez-López et al. 2016a). The smallest MW peak corresponds to an oligosaccharide with a degree of polymerisation of three. Non-physiological conditions for dietary fibre by AOAC method could release high MW polysaccharides from Okara's cell wall, and small MW molecules (<12 kDa) were lost during dialysis of SDF (Villanueva et al. 2013). Thus, after modified AOAC method with dialysis only two peaks were detected, with the loss of the smallest peak. Therefore, direct HPLC-ELSD analysis as reported in Pérez-López et al. (2016a) was found to be cheaper, less time consuming, more precise and sensitive for monitoring SDF after HHP + enzymes treatment of samples and could directly assess the molecular weight of the carbohydrates released without the inherent interferences of the AOAC methods for dietary fibre analysis.

Conclusion

The official AOAC enzymatic–gravimetric method for dietary fibre modified with dialysis, followed by spectrophotometric analyses, was able to detect and quantify an increase in SDF (≈ 1.5-times) and a concomitant decrease in IDF of Okara (≈1.6-times) in samples of Okara treated with HHP and assisted by food-grade enzymes (*Ultraflo*® L or *Viscozyme*® L). Analysis of the SDF fraction by HPLC-ELSD method was twice more sensitive than the spectrophotometric approaches, and revealed the presence of two carbohydrate peaks (≈95 kDa and ≈22 kDa MW) which could have improved beneficial health effects. Compared to the AOAC method for dietary fibre, direct HPLC-ELSD analysis of the supernatant after HHP + enzymatic treatment of samples was reported to be faster, cheaper and more precise as small MW carbohydrates can also be detected.

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Compliance with ethical standards

Conflict of interest All authors declare there are no conflicts of interest.

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