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Lentil flour formulations to develop new snack-type products by extrusion processing: Phytochemicals and antioxidant capacity

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ABSTRACT

The effects of extrusion processing on fibre (soluble and insoluble), total available carbohydrates, tocopherols, organic acids, total phenolics, flavonols, hydroxycinnamic and hydroxybenzoic acids, as well as on the antioxidant capacity of different fibre-enriched lentil flours, were evaluated before and after extrusion process. Total dietary fibre was partially decreased after extrusion, which correlated with a significant increase in the soluble fibre fraction. γ-tocopherol was the major isoform, before and after extrusion. Additionally, a marked decrease of 83–94% in total tocopherols content after extrusion was observed. Conversely, an increase in most polyphenolic fractions was found, probably due to the effect of extrusion in the hydrolysis of polyphenols bound to fibre and proteins, with an increase in antioxidant activity. Only flavonols presented an extensive decrease (62–82%) after treatment. The novel pulse-based flours, enriched with gluten-free soluble and insoluble fibres, provide snack-type products with a balanced nutritional and antioxidants composition.

1. Introduction

Extrusion of foods is a growing technology for the food industries that process and market a large number of products, like pasta, breakfast cereals, biscuits, crackers, baby foods, snack foods, confectionery items, chewing gum, texturised vegetable protein (TVP), pet foods, dried soups, and dry beverage mixes, among others. This technology is a high-temperature, short-time process in which food materials are plasticised and cooked by the combination of temperature under pressure and mechanical shear, resulting in molecular transformation and chemical reactions that modified food functional properties and could affect their nutrient and phytochemical composition (Ainsworth, 2011; Alam, Kaur, Khaira, & Gupta, 2015). In general, extrusion cooking can influence polyphenols content in food products (Nayak, Berrios, Powers, & Tang, 2011) but does not affect the total dietary fibre (TDF), and it is

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highly conditioned to the food matrix (Berrios, Camara, Torija, & Alonso, 2002). Furthermore, the oil-soluble vitamins, such as tocopherols, are not as severely affected by processing as water-soluble vitamins. Among the biologically active forms of Vitamin E, α-tocopherol is less resistant to temperature compared to other forms (Riaz, Asif, & Ali, 2009).

Extrusion cooking has been extensively used in the processing of cereal-based flours for the fabrication ready-to-eat snack products. In recent years, different studies were performed in order to evaluate the suitability of other promising food ingredients to snacks production, such as different fruits and vegetables, namely apple, beetroot, carrot, cranberry, blueberry, cactus fruits, etc. (Camire, Dougherty, & Briggs, 2007; Moussa-Ayoub, Youssef, El-Samahy, Kroh, & Rohn, 2015; Potter, Stojceska, & Plunkett, 2013; Stojceska, Ainsworth, Plunkett, & Ibanoglu, 2010), as well as pulses (lentil, chickpea, dry, carioca and green beans), with very few studies focusing on the incorporation of pulse flours to develop snack-type foods rich in bioactive compounds and with acceptable quality (Berrios, 2006; Berrios et al., 2002; Berrios, Morales, Cámara, & Sánchez Mata, 2010; da Silva, Ascheri, de Carvalho, Takeiti, & Berrios, 2014; Flores-Silva, Berrios, Pan, Osorio-Díaz, & Bello-Pérez, 2014; Morales et al., 2015; Nayak et al., 2011; Simons et al., 2014). Pulses are currently considered as functional gluten-free foods, since they encourage different metabolic functions, including glycaemic and cholesterol indices stabilisations, reduction of body lipids accumulation, promotion of intestinal transit, and may act in the prevention of some cancers, osteoporosis, heart disease or diabetes (Asif, Rooney, Ali, & Riaz, 2013). In this way, pulses could be included as vegetable protein sources with high-content dietary fibres and complex carbohydrates, leading to low glycaemic index in extrusion formulations, for making functional and convenient products with high nutritional value, and could be included in the daily diet, being a good alternative to cereal-based snacks.

Based on the literature reviewed, there is limited information on the effect of extrusion processing on some phytochemicals in pulses' extruded products. Therefore, the aim of this study is to evaluate the changes induced by extrusion cooking on phytochemicals and antioxidant activity in functional novel formulations fortified with fibre-rich, gluten-free or gluten-containing ingredients, in order to establish suitable formulations for the development of gluten-free snack-type products.

2. Materials and methods

2.1. Standards and reagents

Methanol was of analytical grade purity and supplied by Pronalab (Lisbon, Portugal). Formic and acetic acids were purchased from Prolabo (VWR International, France). Tocopherol standards (α, β, γ and δ-isoforms), glucose, fructose, sucrose, and organic acid standards (L (+)-ascorbic, oxalic, malic, citric and succinic acids), glucose standards and fibre enzymatic kit (TDF-100A) were purchased from Sigma (St. Louis, MO, USA). Glutamic acid, HPLC-grade acetonitrile, *n*-Hexane and ethyl acetate were purchased from Merck (Darmstadt, Germany). The 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, ascorbic acid, iron chloride, and potassium ferricyanide were obtained from Alfa Aesar (Ward Hill, MA, USA). Folin–Ciocalteu's reagent, iron sulphate, methanol, phosphate buffer, sodium carbonate, thiobarbituric acid, trichloroacetic acid and Tween 80 were acquired from Fisher Scientific (Waltham, MA, USA). Sulphuric acid, perchloric acid, hydrochloric acid, sodium hydroxide and anthrone reagent were obtained from Panreac Quimica S.L.U. (Barcelona, Spain). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Lentil flour and formulated flours

Decorticated red chief lentils (*Lens culinaris* L.) were purchased from a local wholesale distributor in California (USA). Different fibre-rich samples, based on lentil flour, were formulated. The samples with at least 68% of lentil flour were used for extrusion processing by blending the lentil flours with specific food ingredients, such as starch, soluble fibre as Nutriose®, and/or insoluble fibre from wheat bran, corn and apple, and flavouring agents (patent pending) (Berrios, Tang, & Swanson, 2008), as shown in Table 1. The pulse flours and formulated pulse flours, before and after extrusion cooking, were reduced to uniform powders using a Cyclone mill (Udy Corp., Fort Collins, CO, USA) fitted with a 0.5-mm screen, and then stored in airtight glass jars at room temperature until analysed.

2.3. Extrusion process

A Clextral EVOL HT32-H twin-screw extruder (Clextral, Inc., Tampa, FL, USA) with co-rotating and closely intermeshing screws and capacity to run at about 50 kg feed/h was used. The extruder was equipped with six barrel sections, each 128 mm in length. The temperature of the last barrel section and the die was maintained at 160 ± 1 °C. The screw diameter (D) was 32 mm and the total configured screw length (L) was 768 mm, which gave an overall L/D ratio of 24. Screws were driven by a 74.8 kW variable speed drive, Model ACS600 (ABB Automation, Inc., New Berlin, WI, USA). The screw speed was maintained constant at 500 rpm. A combination of feeding, transporting, compression and kneading elements was used to provide a moderate-shear screw configuration (patent pending) (Berrios et al., 2008).

Table 1 – Lentil flour formulations analysed.

Sample		Characteristics
CR	Control raw flour	Raw lentil flour (*Lens culinaris* L.)
CE	Control extruded flour	Extruded CR
CF#1	Control formulated 1	Raw lentil flour + wheat bran + apple fibre
EF#1	Extruded formulated 1	Extruded CF1
CF#2	Control formulated 2	Raw lentil flour + wheat bran + Nutriose®
EF#2	Extruded formulated 2	Extruded CF2
CF#3	Control formulated 3	Raw lentil flour + apple fibre + Nutriose®
EF#3	Extruded formulated 3	Extruded CF3
CF#4	Control formulated 4	Raw lentil flour + apple fibre + corn fibre
EF#4	Extruded formulated 4	Extruded CF4

The mixture was metered into the feed port by a twin-screw, loss-in-weight gravimetric feeder, Model LWFD5-20 (K-Tron Corp., Pitman, NJ, USA) at a rate of 20 kg/h (wwb). Water was supplied to the extruder by a triplex variable stroke piston pump with 12 mm plungers, Type VE-P33 (Bran and Luebbe, Wheeling, IL, USA) to provide a final moisture content of 17%. The pulse formulations were extruded through two circular dies each with a 3.5 mm diameter opening. Pressure at the die was monitored using a pressure transducer, Type PT412-5M (Dynisco Instruments, Sharon, MA, USA). A PLC + Industrial computer (Allen-Bradley, Milwaukee, WI, USA) using Intouch software (FITSYS PLUS ver. 1.23) was used to collect extruder parameter data at 1 s intervals for a total of 5 min.

2.4. Analysis of phytochemicals

Soluble and insoluble dietary fibres were determined according to AOAC enzymatic–gravimetric methods 993.19 and 991.42, respectively (Latimer, 2012).

Total available carbohydrates (TAC) was carried out by the Anthrone method as described by Osborne and Voogt (1986). Samples and glucose calibration curve (10–100 mg/mL) absorbance was measured at 630 nm on a UV/Vis Spectrometer EZ210 (Perkin Elmer, Waltham, MA, USA) equipped with Lambda software PESSW ver. 1.2.

Individual organic acids were determined based on a protocol described by Sánchez-Mata et al. (2012). The analysis was performed by HPLC (Micron Analítica, Madrid, Spain) coupled to a Thermo Scientific Spectra Series UV100 (Madrid, Spain) UV-visible detector using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths; the quantification was performed by comparison of the area of the peaks recorded at the corresponding wavelength with calibration curves.

Tocopherols were determined by HPLC-fluorescence following a procedure previously described by Morales et al. (2014), using HPLC coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) programmed for excitation at 290 nm and emission at 330 nm; the identification was performed by chromatographic comparisons with authentic standards, while the quantification was based on the fluorescence signal response of each standard, using the internal standard (tocol).

Phenolics, hydroxycinnamic acids, hydroxybenzoic acids and *flavonols* were extracted from 1 g sample powder by stirring it with 40 mL of methanol at 25 °C for 1 h and filtering the extract. The residue was then re-extracted with one additional portion of methanol. The combined methanol extracts were evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland), re-dissolved in methanol at a concentration of 100 mg/mL, and stored at 4 °C for further use.

Total phenolic compounds were determined by *Folin–Ciocalteu* assay based on the procedure described by Wolfe, Wu, and Liu (2003) with some modifications. Absorbance was measured at 765 nm (AnalytikJena 200 spectrophotometer, Jena, Germany). Gallic acid was used as standard. For determination of total hydroxycinnamic acids, hydroxybenzoic acids and flavonols, a modified version of the method described by Bonoli, Verardo, Marconi, and Caboni (2004) was used. The method consisted of placing 1 mL of sample or standard in a test tube and adding 4 mL of diluent (methanol for hydroxycinnamic acids and flavonols or 0.3% of formic acid for hydroxybenzoic acids). The solution was mixed and allowed to sit for approximately 15 min before reading the absorbance at 280, 320 and 360 to estimate hydroxybenzoic, hydroxycinnamic acids and flavonols, respectively. Gallic acid, ferulic acid and quercetin were used as their corresponding standards.

2.5. Evaluation of antioxidant activity

The *in vitro* antioxidant activity assays were performed following the previously described methodology by the authors (Morales et al., 2014). Methanol extract was further diluted to different concentrations to be used in the antioxidant activity assays. DPPH radical-scavenging activity was evaluated using an ELX800 microplate Reader (BioTek Instruments, Inc.; Winooski, VT, USA) and calculated as a percentage of DPPH discolouration after 1 hour of incubation with the antioxidant extract measuring the absorbance of the solution at 515 nm. The reducing power was evaluated by the capacity to reduce Fe^{3+} to Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated through the β-carotene/linoleate assay; the neutralisation of linoleate free radicals avoids β-carotene bleaching. In thiobarbituric acid reactive substances (TBARS) assay, the colour intensity of the malondialdehyde–thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm. The results of the antioxidant activity were expressed as EC_{50} values.

2.6. Statistical analysis

Analysis of variance (ANOVA), followed by Duncan's test, was conducted using Statgraphics Plus 5.1 software to analyse data at the 95% confidence level.

3. Results and discussion

3.1. Extrusion effects on phytochemicals of lentil formulated flours

Dry leguminous seeds, also known as pulses, represent important sources of plant proteins and carbohydrates in the human diet. Total available carbohydrates (TAC) content in raw lentil flours (Table 2) ranged from 60.15 to 69.73 g/100 g dry matter. These values were similar to those reported by Berrios et al. (2010) in other raw lentil, chickpea and dry pea flours. Regarding the effect of extrusion processing, a significant ($p < 0.05$) increase of 5–17% in TAC was observed after treatment. The resulting TAC increase at a consequence of extrusion processing was probably due to a mechanical-structure modification induced by cell rupture and higher cell wall porosity, which increases the specific surface area, improving the diffusion of solvent inside the extrudates and increasing the availability of these carbohydrates, as previously reported by different authors (Amor, Lamy, Andre, & Allaf, 2008; Pedrosa et al., 2012) for similar thermal-pressure process. Conversely, Berrios et al. (2010) reported a decrease in TAC in dry peas and in chickpea flours (3 and 17%, respectively) extruded at 160 °C, while Alonso, Rubio, Muzquiz, and Marzo (2001) reported that

Table 2 – Extrusion effect on carbohydrate composition: Fibre (soluble, insoluble and total) and total available carbohydrate, mg/100 g dry weight (mean ± SD).

Samples		Insoluble fibre (g/100 g dw)	Soluble fibre (g/100 g dw)	Total fibre (g/100 g dw)	Total available carbohydrates (g/100 g dw)
Raw formulated flours	Control	$12.31 \pm 2.54^{a,B}$	$0.11 \pm 0.00^{a,A}$	$12.42 \pm 2.54^{a,B}$	$65.80 \pm 0.65^{b,A}$
	CF#1	$9.20 \pm 0.25^{a,A}$	$0.12 \pm 0.02^{a,A}$	$9.32 \pm 0.24^{a,A}$	$61.15 \pm 2.28^{a,A}$
	CF#2	$11.12 \pm 1.11^{a,B}$	$0.27 \pm 0.02^{b,A}$	$11.39 \pm 1.19^{a,B}$	$60.15 \pm 4.28^{ab,A}$
	CF#3	$8.70 \pm 0.74^{a,A}$	$0.23 \pm 0.03^{b,A}$	$10.13 \pm 0.53^{a,B}$	$60.86 \pm 1.99^{a,A}$
	CF#4	$10.81 \pm 2.22^{a,A}$	$0.52 \pm 0.07^{c,A}$	$10.15 \pm 2.22^{a,A}$	$69.73 \pm 1.16^{c,A}$
Extrusion formulated flours	Extruded control	$7.65 \pm 0.35^{a,A}$	$0.55 \pm 0.07^{c,B}$	$8.20 \pm 0.35^{b,A}$	$68.94 \pm 2.07^{a,B}$
	EF#1	$8.64 \pm 0.26^{a,A}$	$0.75 \pm 0.05^{e,B}$	$9.39 \pm 0.26^{c,A}$	$70.04 \pm 0.73^{b,B}$
	EF#2	$7.09 \pm 0.34^{a,A}$	$0.31 \pm 0.06^{b,B}$	$7.49 \pm 0.33^{a,A}$	$69.75 \pm 0.69^{a,B}$
	EF#3	$7.50 \pm 0.65^{a,A}$	$0.25 \pm 0.02^{a,B}$	$7.75 \pm 0.64^{ab,A}$	$71.63 \pm 0.66^{b,B}$
	EF#4	$9.97 \pm 0.39^{b,A}$	$0.63 \pm 0.09^{d,B}$	$10.23 \pm 0.39^{c,A}$	$70.08 \pm 1.28^{b,A}$

In each column, different letters mean statistically significant differences ($p < 0.05$) compared by Duncan test; small superscript letter means differences between all samples analysed, whereas capital superscript letter means difference due to extrusion treatment for the same formulation. nd: non-detected.

the TAC content of unprocessed common bean flours did not significantly change after extrusion at 150–155 °C. These differences may be attributed to the difference in extrusion parameters used in the processing of the raw materials, as well as the difference in food matrix. Pulses are traditionally considered as good sources of dietary fibre. Table 2 presents that the total dietary fibre (TDF) in the studied samples ranged from 9.32 to 12.42 g/100 g within raw flours and from 7.49 to 10.23 g/100 g within extruded flours. All the extruded flours can be considered good source of dietary fibre, since a 100 g of flour can provide up to 27% and 41% of the daily amount of dietary fibre required for men and women, respectively (Trumbo, Schlicker, Yates, & Poos, 2002). Extrusion processing did not affect the TDF content in EF#1 and EF#4 formulations, both formulated with apple fibre, while there was decrease of 34% and 33% in formulations EF#2 and EF#3, respectively. Alonso et al. (2001) and Varo, Liane, and Koivistoinen (1983) reported insignificant changes in TDF content of extruded wheat flour extruded at 161–180 °C and common bean flours extruded at 150–155 °C, while Frias et al. (2011) reported a decrease in TDF from 7 to 16% in *Pisum sativum* L. flour after extrusion at 129–142 °C. Extruded beans (*Phaseolus vulgaris* L.) were reported to have TDF values comparable to those before extrusion, but a redistribution of insoluble to soluble dietary fibre occurred (Berrios et al., 2002; Martin-Cabrejas et al., 1999).

IDF was the prevalent fraction in all the analysed formulations, ranging from 8.69 to 12.31 g/100 g dw (in CF#3 and control flour, respectively; Table 2), being also the main fraction after extrusion process, with a slight decrease (6–14%) in EF#1, EF#3 and EF#4 flours, and higher decrease in the control and EF#2 flours (36–37%). Berrios et al. (2010) had previously reported a significant ($p < 0.05$) decrease in IDF for extruded lentil and dry pea-based formulations. Reduction in IDF associated with the extrusion cooking was also previously reported for black bean (Berrios et al., 2002) and cereal extrudates (Gualberto, Bergman, Kazemzadeh, & Weber, 1997). In raw samples, SDF ranged from 0.11 to 0.521 g/100 g for control and CF#4, respectively. Regarding extrusion effect, a significant increase ($p < 0.05$) was observed for EF#1 and EF#2 formulations; no significant variation was observed among the other extruded flours. Berrios et al. (2010) reported no significant variation in SDF after extrusion cooking of lentil and chickpea flours, while for dry pea flour a large increase on this fraction was observed due to processing. Moreover, Ralet, Della Valle, and Thibault (1993) reported an increase over 10% of SDF after extrusion of pea hull-based formulations.

In general, tocopherols are not as severely affected by food processing as the water-soluble vitamins; reported losses are more closely associated with lipid degradation at consequence of thermal processing (Riaz et al., 2009). Table 3 shows the effects of the extrusion processing on the various isoforms of vitamin E. In the entire analysed lentil flours, α-, γ- and δ-tocopherols were characterised. The highest total tocopherol content was determined in the control and in CF#4 (6.17 to 5.86 mg/100 g, respectively), γ-tocopherol being the predominant isoform in all the evaluated formulations. Among all the analysed phytochemicals, tocopherols in all the isoforms (α-, γ- and δ-tocopherol) suffered the most marked effect after extrusion processing, with a decrease of 83–94% in total content. CF#3 was the flour with the highest content of total and δ-tocopherols after extrusion process, which showed a reduction of 83–85%.

Grela, Jensen, and Jakobsen (1999) reported that the sensitivity of vitamin E to extrusion cooking depends on the extrusion processing variables and conditions used. The authors observed that an increase in extrusion temperatures promotes the decrease in α-tocopherol, while γ-tocopherol decreases with moisture raising during extrusion. Zielinski, Kozlowska, and Lewczuk (2001) reported a drop of 40–93% in total tocopherols of different extruded cereals after short thermal treatment (extrusion at 120 °C), α-tocopherol being the least resistant to temperature compared to other forms in cereals. The highest losses in tocopherol reported by these authors are similar to those obtained in the present study.

The profiles of organic acids in the lentil and formulated lentil-based flours are presented in Table 4. In all samples analysed, oxalic, tartaric, quinic, malic and fumaric acids were identified, except quinic acid which was not determined in the control nor in the CF#1 sample flour. The concentration of malic acid ranged from 927.64 to 1796 mg/100 g in formulations #1

Table 3 – Extrusion effect on tocopherols composition (mg/100 g dry weight) in lentil flour formulations (mean ± SD).

Samples		α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherols
Raw formulated flours	Control	$0.16 \pm 0.01^{b,B}$	$5.95 \pm 0.23^{c,B}$	0.06 ± 0.01^{a}	$6.17 \pm 0.25^{c,B}$
	CF#1	$0.09 \pm 0.01^{a,B}$	$2.92 \pm 0.09^{a,B}$	0.09 ± 0.01^{b}	$3.10 \pm 0.09^{a,B}$
	CF#2	$0.15 \pm 0.01^{b,B}$	$4.23 \pm 0.45^{b,B}$	0.12 ± 0.01^{c}	$4.50 \pm 0.48^{b,B}$
	CF#3	$0.15 \pm 0.04^{b,B}$	$4.20 \pm 0.42^{b,B}$	0.12 ± 0.02^{bc}	$4.47 \pm 0.48^{b,B}$
	CF#4	$0.27 \pm 0.02^{c,B}$	$5.46 \pm 0.35^{c,B}$	0.13 ± 0.02^{c}	$5.86 \pm 0.39^{c,B}$
Extrusion formulated flours	Extruded control	$0.03 \pm 0.01^{b,A}$	$0.99 \pm 0.16^{d,A}$	nd	$1.02 \pm 0.17^{d,A}$
	EF#1	$0.01 \pm 0.00^{a,A}$	$0.40 \pm 0.04^{b,A}$	nd	$0.41 \pm 0.04^{b,A}$
	EF#2	$0.01 \pm 0.00^{a,A}$	$0.24 \pm 0.04^{a,A}$	nd	$0.25 \pm 0.04^{a,A}$
	EF#3	$0.02 \pm 0.00^{b,A}$	$0.63 \pm 0.06^{c,A}$	nd	$0.65 \pm 0.06^{c,A}$
	EF#4	$0.02 \pm 0.00^{b,a}$	$0.47 \pm 0.02^{b,A}$	nd	$0.49 \pm 0.08^{b,A}$

In each column, different letters mean statistically significant differences ($p < 0.05$) compared by Duncan test; small superscript letter means differences between all samples analysed, whereas capital superscript letter means difference due to extrusion treatment for the same formulation. nd: non-detected.

Table 4 – Extrusion effect on organic acids composition (mg/100 g dry weight) in lentil flour formulations (mean ± SD).

Samples		Oxalic acid	Tartaric acid	Quinic acid	Malic acid	Fumaric acid
Raw formulated flours	Control	$654.45 \pm 37.73^{c,B}$	$629.36 \pm 13.54^{d,B}$	nd	$572.39 \pm 11.46^{a,B}$	$443.09 \pm 113.03^{d,B}$
	CF#1	$317.69 \pm 47.57^{b,B}$	$258.07 \pm 40.44^{c,A}$	nd	$1235.55 \pm 181.92^{b,A}$	15.23 ± 0.32^{b}
	CF#2	$297.87 \pm 52.86^{ab,B}$	$273.75 \pm 39.92^{c,B}$	196.10 ± 20.82^{b}	$1139.78 \pm 78.18^{b,B}$	$11.79 \pm 0.012^{b,A}$
	CF#3	$223.79 \pm 49.97^{a,B}$	$218.51 \pm 14.53^{b,A}$	169.87 ± 3.66^{a}	$1796.27 \pm 313.24^{c,B}$	$19.95 \pm 2.44^{c,A}$
	CF#4	$232.92 \pm 8.74^{a,B}$	$143.70 \pm 41.03^{a,A}$	285.13 ± 35.18^{c}	$927.64 \pm 62.58^{b,B}$	$8.93 \pm 0.03^{a,A}$
Extrusion formulated flours	Extruded control	$153.30 \pm 5.81^{a,A}$	$495.34 \pm 69.68^{d,A}$	nd	$226.13 \pm 9.47^{a,A}$	$44.94 \pm 1.84^{c,A}$
	EF#1	$191.72 \pm 2.51^{b,A}$	$245.53 \pm 22.43^{c,A}$	nd	$1504.69 \pm 134.67^{e,B}$	nd
	EF#2	$188.08 \pm 23.29^{b,A}$	$187.46 \pm 1.35^{a,A}$	nd	$593.99 \pm 62.73^{b,A}$	$23.41 \pm 2.77^{a,B}$
	EF#3	$204.15 \pm 13.59^{b,A}$	$203.73 \pm 3.94^{b,A}$	nd	$1199.44 \pm 154.69^{d,A}$	$21.87 \pm 1.97^{a,A}$
	EF#4	$198.46 \pm 15.67^{b,A}$	$179.63 \pm 11.51^{a,A}$	nd	$812.01 \pm 43.03^{c,A}$	$39.80 \pm 2.67^{b,B}$

In each column, different letters mean statistically significant differences ($p < 0.05$) compared by Duncan test; small superscript letter means differences between all samples analysed, whereas capital superscript letter means difference due to extrusion treatment for the same formulation. nd: non-detected.

and #3, respectively. Regarding the extruded processed flours, important differences were observed with a remarkable decrease in all the organic acids in the control flour, probably due to decarboxylation of the organic acids at a consequence of extrusion conditions of temperature and shear. Although extrusion treatment effect did not affect all the organic acids in the samples in the same way, the decrease was more relevant in quinic acid content, which was not detected in any extruded sample. In the case of oxalic acid, its content decreased in all the samples (14–77% in formulation #4 and control flour, respectively), this effect suppose an advantage of the extruded samples, since low oxalic acid food products have beneficial health effect with a lower incidence of kidney calculus formation. On the other hand, malic acid presented only a slight decrease (33–48%) in the samples formulated with apple fibre and maintained high values of this acid after extrusion treatment. These results indicate that samples formulated with apple fibre could confer desirable antioxidant properties to the extrudates.

Food polyphenols are a complex group of substances that can be found either free or protein-bound or fibre-bound, commonly known as non-extractable polyphenols (Bravo, 1998; Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007), not always considered in the measurement of total phenolics present in food products, and particularly in cereal matrix. In this regard, polyphenols bioaccessibility is highly conditioned to the food matrix and extraction method, and particularly to the cell wall hydrolysis in the case of fibre-bound polyphenols. The insoluble-bound phenolic acids have very low bioavailability being non-accessible to the necessary enzymes that contribute to their release in the human gastrointestinal tract (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Wang, He, & Chen, 2014). Conjugated phenolics, which were covalently bound to insoluble fibre fraction (wheat brand, legumes, etc.), can only be absorbed after being released from cell structures by digestive enzymes or microorganisms in intestinal lumen (Anson et al., 2011; Chandrasekara & Shahidi, 2012), or after specific processing technologies (as extrusion) or fermentation (e.g. gut fibre fermentation) could have an effect on cell wall hydrolysis and polyphenols bioaccessibility (Manach et al., 2004; Wang et al., 2014). Different authors (Chandrasekara & Shahidi, 2012; Saura-Calixto, Serrano, & Goñi, 2007) studied the polyphenol bioaccessibility diet and reported that pulses and cereals were the food group that showed the highest concentration of polyphenols in the fermentation media per gram of original sample, probably due their high non-extractable phenolics bound to fibre fraction. Moreover, Wang et al. (2014) reported that in cereal food matrix thermal treatment and extrusion cooking may positively or negatively affect the bioavailability of phenolic compounds in cereal grains because high temperature causes decomposition of heat-labile phenolic compounds and may also lead to polymerisation of some phenolic compounds under high

pressure in extrusion cooking. Apart from the health benefits of these phytochemicals, natural phenolic compounds in food and food products also act as antioxidants to improve shelf life and consumer acceptance of extruded snacks. In this work individual phenolics and their contents are not included, as it is a preliminary research on the effect of extrusion process on bioactive compounds; thus, the different families of phenolics compounds have been quantified: hydroxycinnamic acids, hydroxybenzoic acids and flavonols.

In the raw samples, total phenolics content ranged from 2.01 to 4.68 mg GAE/g in CF#2 and control flour, respectively (Table 5); hydroxybenzoic acids content range from 1.88 and 2.99 mg GAE/g in CF#3 and CF4#, respectively; hydroxycinnamic acids range from 7.51 to 9.79 mg FAE/g in CF#1 and control flour, respectively; and flavonols between 5.88 and 13.50 mg QE/g in CF#4 and control flour, respectively. These results demonstrated the influence of the different food ingredients in the mixes with respect to the control lentil flour, which showed significantly ($p < 0.05$) higher content of total phenolic, hydroxycinnamic acids and flavonols than those determined in the mixes. The content of hydroxybenzoic acids was significantly ($p < 0.05$) higher in the CF#4 sample, which contained apple fibre, as apples are a good source of these organic acids.

All extruded samples from formulated lentil flours presented significantly ($p < 0.05$) higher content of total phenolics, hydroxybenzoic and hydroxycinnamic acids. However, the control (lentil flour) sample was not significantly ($p < 0.05$) different from the control unprocessed (raw) lentil flour and the formulated lentil flours. Therefore, this indicated that the determined increase in those functional components was due to the effect of extrusion processing. Extrusion process could encourage phenolic decarboxylation due to high barrel temperature and high moisture content, which may promote polymerisation of phenols and tannins, leading to reduced extractability and antioxidant activity (Repo-Carrasco-Valencia, Pena, Kallio, & Salminen, 2009). However, in the present study, an increase in total phenolics content after extrusion process was observed in all the samples, but more pronounced in formulations #1 and #2 with an increase of three and five folds, respectively, compared to the raw samples. Hydroxybenzoic acids content presented two-fold increase after extrusion process. The hydroxycinnamic acids content in all the extruded samples, with the exception of the control sample, was significantly ($p < 0.05$) higher than the unprocessed (raw) formulations. Only the flavonols fraction suffered significant ($p < 0.05$) decrease of about 62–82% in the control and formulation #1, respectively, after the extrusion treatment. This revealed the sensibility of flavonols to the extrusion processing conditions used in this study.

The increase in the phenolic fractions mentioned above could be explained by the possible effect of extrusion process to induce hydrolysis of polyphenols bound fibre and/or proteins moieties, changing from non-extractable to extractable polyphenols. Something similar was reported by Korus, Gumul, and Czechowska (2007) in dark-red beans. They reported an increase of 14% in the amount of phenolics in extruded bean flours compared to the raw flours, mainly do to an increase in quercetin (by 84%) and ferulic acid (by 40%). They also indicated that these phenols are normally associated with plant cell walls, and extrusion treatment could hydrolyse these

Table 5 – Extrusion effect on phenolic families content and antioxidant properties (EC_{50}, mg/mL methanolic extract) of lentil flour formulations (mean ± SD).

Samples		Phenolic families				Antioxidant properties (EC_{50}, mg/mL methanolic extract)			
		Total phenolics (mg GAE/g)	Hydroxybenzoic acids (mg GAE/g)	Hydroxycinnamic acids (mg FAE/g)	Flavonols (mg QE/g)	Ferricyanide/ Prussian blue assay	DPPH assay	β-carotene/ linoleate assay	TBARS assay
Raw formulated flours	Control	$4.68 \pm 0.30^{c,A}$	$2.52 \pm 0.10^{b,A}$	$9.79 \pm 0.00^{c,A}$	$13.50 \pm 3.91^{c,B}$	$53.49 \pm 5.34^{d,B}$	$64.26 \pm 2.84^{a,A}$	$9.40 \pm 0.05^{c,B}$	$2.45 \pm 0.11^{a,B}$
	CF# 1	$3.17 \pm 0.04^{b,A}$	$2.91 \pm 0.97^{b,A}$	$7.51 \pm 0.43^{ab,A}$	$9.30 \pm 0.51^{c,B}$	$10.06 \pm 0.97^{a,B}$	$69.99 \pm 1.09^{b,B}$	$6.68 \pm 0.81^{b,B}$	$2.49 \pm 0.29^{a,A}$
	CF# 2	$2.01 \pm 0.01^{a,A}$	$1.91 \pm 0.02^{a,A}$	$7.69 \pm 0.09^{a,A}$	$7.59 \pm 0.07^{b,B}$	$39.91 \pm 0.99^{c,B}$	$74.19 \pm 1.83^{c,B}$	$7.42 \pm 0.06^{b,A}$	$5.51 \pm 0.03^{c,B}$
	CF# 3	$2.02 \pm 0.01^{a,A}$	$1.88 \pm 0.17^{a,A}$	$7.98 \pm 0.09^{b,A}$	$6.10 \pm 0.16^{a,B}$	$14.95 \pm 0.06^{b,B}$	$86.93 \pm 2.79^{d,B}$	$5.55 \pm 1.49^{ab,A}$	$9.32 \pm 0.51^{d,B}$
	CF# 4	$3.22 \pm 0.08^{b,A}$	$2.99 \pm 0.65^{b,A}$	$8.02 \pm 0.13^{b,A}$	$5.88 \pm 0.19^{a,B}$	$9.87 \pm 0.28^{a,B}$	$59.57 \pm 3.90^{a,A}$	$4.11 \pm 0.43^{a,A}$	$4.46 \pm 0.18^{b,A}$
Extrusion formulated flours	Extruded control	$5.18 \pm 0.19^{b,A}$	$4.40 \pm 0.98^{b,B}$	$12.28 \pm 2.87^{abc,A}$	$5.09 \pm 0.06^{c,A}$	$23.58 \pm 0.68^{a,A}$	$60.77 \pm 0.87^{d,A}$	$3.80 \pm 0.06^{b,A}$	$1.48 \pm 0.15^{a,A}$
	EF# 1	$9.14 \pm 0.01^{d,B}$	$4.93 \pm 0.36^{b,A}$	$11.23 \pm 0.38^{c,B}$	$1.64 \pm 0.41^{a,A}$	$4.25 \pm 0.41^{a,A}$	$43.26 \pm 1.02^{c,A}$	$2.66 \pm 0.74^{a,A}$	$3.57 \pm 0.30^{c,B}$
	EF# 2	$9.38 \pm 0.51^{d,B}$	$3.88 \pm 0.41^{c,B}$	$9.97 \pm 0.30^{a,B}$	$2.07 \pm 0.08^{ab,A}$	$7.12 \pm 0.92^{c,A}$	$6.63 \pm 0.44^{a,A}$	$9.75 \pm 0.37^{e,B}$	$1.52 \pm 0.12^{a,A}$
	EF# 3	$4.51 \pm 0.02^{a,B}$	$3.15 \pm 0.14^{a,B}$	$10.68 \pm 0.19^{b,B}$	$2.21 \pm 0.08^{ab,A}$	$10.31 \pm 0.30^{d,A}$	$37.90 \pm 4.49^{b,A}$	$8.41 \pm 0.08^{d,B}$	$2.10 \pm 0.00^{b,A}$
	EF# 4	$6.59 \pm 0.05^{c,B}$	$5.76 \pm 1.19^{b,B}$	$11.52 \pm 0.17^{c,B}$	$2.03 \pm 0.11^{ab,A}$	$5.28 \pm 0.11^{b,A}$	$63.56 \pm 0.90^{e,A}$	$6.46 \pm 0.12^{c,B}$	$4.59 \pm 0.02^{d,A}$

In each column, different letters mean statistically significant differences ($p < 0.05$) compared by Duncan test; small superscript letter means differences between all samples analysed, whereas capital superscript letter means difference due to extrusion treatment for the same formulation. GAE (gallic acid equivalents); FAE (ferulic acid equivalents); QE (quercetin equivalents).

bounds, increasing their extraction. A similar increase in total phenolic compounds after extrusion cooking was reported for sweet potato (Shih, Kuo, & Chiang, 2009) and for purple potatoes/dry peas (65%) blend flour, with significant increase of 2741–4308 μg GAE/g dw, and also in total flavonoids content, with values from 3210 to 3758 μg CE/g dw, after extrusion at die temperature of 130 °C (Nayak et al., 2011).

3.2. *Extrusion effects on antioxidant properties of lentil formulated flours*

Results from lentil flours antioxidant capacity were evaluated by four different *in vitro* assays (Table 5). The antioxidant and antiradical activities of functional components in food products are dependent not only on the level of bioactive compounds, but also on the composition of bioactive compounds. In the present study, the antioxidant activity of the samples, determined by DPPH assay, exhibited higher values than all the other methods for the all the unprocessed (raw) and extruded samples. Additionally, with the exception of extruded control sample and EF#4 sample, all other formulated extrudates were significantly ($p < 0.05$) lower than their raw samples. However, the β-carotene/linoleate assay revealed that, with the exception of extruded control sample and EF#1 sample, all other formulated extrudates were significantly ($p < 0.05$) higher than their raw samples. Moreover, samples EF#1 (with wheat bran and apple fibre) and EF#2 (with wheat bran and Nutriose®) presented the lowest EC_{50} measured by most of the assays performed in this study. Formulation EF#1 gave EC_{50} of 4.25 and 2.66 mg/mL extract under the Ferricyanide/Prussian blue and β-carotene assays, respectively, while EF#2 presented EC_{50} of 6.63 and 1.52 mg/mL extract, measured by DPPH and TBARs assays, respectively. These results reflect the important effect of extrusion processing and food ingredients on antioxidant activity determined by different assays.

4. Conclusions

Extrusion processing did not affect the content of all the studied functional compounds in the same way. Extrusion promoted an increase in soluble fibre, total phenolic, hydroxycinnamic and hydroxybenzoic acids in the lentil-based, fibre-enriched analysed flours. Also, the antioxidant activity in most of the formulated flours increased as a result of extrusion processing due to an increase in phenolic bioavailability mediated by fibre bound phenolics release. However, the content of insoluble fibre, tocopherols, flavonols, and most organic acids was reduced in the extrudates. From the results obtained, it can be concluded that the pulse-based flours formulated with gluten-free fibre ingredients present good alternative as gluten-free extruded snack-type products with a balanced nutritional and phytochemical composition and particularly good source of soluble and insoluble fibres. These flours would be a good alternative to increase pulses consumption in children and young adults, emphasising the gluten free formulations (EF#3 and EF#4) as the most appropriate for those suffering from celiac disease or gluten sensitivity-related conditions. Furthermore, due to their phytochemicals content, their consumption could prevent various diseases associated with metabolic syndrome, among others.

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