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Bioactive compounds and antioxidant capacity of extruded snack-type products developed from novel formulations of lentil and nutritional yeast flours

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Pulses are well known to be gluten-free functional foods that provide a rich source of nutritional and healthy compounds with antioxidant-promoting activity. In the present study, the bioactive compounds, dietary fibre, arabinoxylans, individual phenolic compounds and tocopherols, were evaluated in different lentil flours (raw and extruded at 140 and 160 °C) formulated with nutritional yeasts, along with the changes induced by the extrusion process. The total dietary fibre and arabinoxylan content significantly ($p < 0.05$) increased after the extrusion process while a significant decrease of all tocopherol isoforms was also observed. Catechin, caffeic, kaempferol and quercetin derivatives were identified in the raw and extruded lentil flours. The decreases of total phenolic and individual phenolic compounds were directly related to the extrusion temperature; total phenolics and catechin hexoside exhibited a larger decrease in the lentil flours formulated with higher content of nutritional yeast (12 and 16%). The antioxidant activity results, determined using different assays, reflected the important effect of extrusion processing and food ingredients.

1. Introduction

Pulse grains provide a rich source of protein, dietary fibre, complex carbohydrates, resistant starch, and important bioactive micronutrients such as vitamins and minerals. Moreover, pulse seeds are generally rich in low molecular weight secondary plant metabolites with antioxidant properties, mainly hydroxybenzoics, hydroxycinnamics, catechins, procyanidins, and flavonols, among others, as well as in condensed tannins, mainly of the condensed type.[1,2]

Overwhelming evidence from epidemiological studies indicates that diets rich in pulse grains are also associated with a lower risk of several degenerative diseases, attributed to the fact that these foods supply several non-nutritive bioactive compounds/health-promoting mixture of phytochemicals, which act as natural antioxidants and protect against DNA damage.[3,4]

Pulses are particularly rich in bioactive compounds belonging to carbohydrate fractions, standing out for it dietary fibre content and its related compounds.[5] Dietary fibre is an essential nutrient in a healthy diet, contributing to health maintenance and preventing the risks of chronic diseases such as cancer, cardiovascular disease and diabetes mellitus. It is a member of the non-starch polysaccharide family of carbohydrates, not digested in the small intestine but may be fermented in the colon into short-chain fatty acids such as acetate, propionate and butyrate.[6]

Arabinoxylans (AX) are a part of dietary fibre and are made up of a backbone of a linear chain of β-D-(1,4)-xylopyranose. This chain is substituted on the hydroxyl groups (–OH) of the 2- and 3-positions by L-arabinofuranosyl residues linked by β (1,4) glycosidic bonds. Position 5 is commonly replaced with ferulic acid residues, allowing cross-link bond formation by the oxidation of ferulic acid present in adjacent arabinoxylan chains. With regard to their physiological properties, the role of these compounds in glycemic control in patients with diabetes mellitus and cardiovascular disease is noteworthy, since their intake reduces the plasma levels of glucose, cholesterol

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and triglycerides. The prebiotic effect and antioxidant activity of these compounds help in preventing colon cancer. Besides this, arabinoxylans can also act as immune system modulators.[7–10]

Extrusion, as a continuous mixing, kneading, and shaping process, has been utilized to manufacture a large variety of foods, like puffed snacks, ready-to-eat cereals, confectionery products, pet foods, textured vegetable proteins, *etc*.[11] Extrusion offers the possibility of modifying the functional properties of food ingredients and/or texturizing them. Extrusion may have beneficial effects on the nutritional value like the gelatinization of starch, destruction of antinutritional factors, increase of soluble dietary fibre, reduction of lipid-oxidation and contaminating microorganisms, and retention of the natural colors and flavors of foods.[12]

In recent years, different studies have focused on the incorporation of pulse flours to develop extruded snack-type foods rich in bioactive compounds and with acceptable quality.[3–5,13,14] Therefore, this food matrix could be included in novel formulations, for making functional and convenient extruded food products with high nutritional value as a good alternative to cereal-based snacks.

The aims of this study were to characterize the phytochemical composition and antioxidant activity of the different lentil flour formulations (raw, extruded at 140 °C and extruded at 160 °C) in terms of the yeast content (0% to 16%), as well as to evaluate the changes induced by extrusion cooking in the analyzed phytochemicals between the raw and the corresponding extruded (140° and 160 °C) formulation (see Table 1), in order to develop novel high-protein "gluten-free" snacks which may contribute to the increase in the consumption of pulses, mainly in children and adolescents.

2. Materials and methods

2.1 Standards and reagents

Fibre enzymatic kit (TDF-100A) and xylose standard were purchased from Sigma (St Louis, MO, USA). Formic and acetic acids were purchased from Prolabo (VWR International, France). Tocol in *n*-hexane (50 mg mL^{-1}) and tocopherols (α-, β-, γ-, and δ-isoforms) were purchased from Matreya (Pleasant Gap, PA, USA). Phenolic compound standards (ellagic acid, gallic acid and quercetin-3-*O*-glucoside) were obtained from Extrasynthese (Genay, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Methanol was of analytical grade purity and supplied by Pronalab (Lisbon, Portugal). Acetonitrile (99.9%), *n*-hexane (97%) and ethyl acetate (99.8%) were of HPLC grade and obtained from Fisher Scientific (Lisbon, Portugal).

Table 1 Analysis of the lentil flour formulations

	Sample	Characteristics
Raw	CF	Control raw lentil flour + 0% yeast
	CF#1	Control raw lentil flour + 4% yeast
	CF#2	Control raw lentil flour + 8% yeast
	CF#3	Control raw lentil flour + 12% yeast
	CF#4	Control raw lentil flour + 16% yeast
Extruded at 140 °C	CE140	CF flour extruded at 140 °C
	E140#1	CF#1 flour extruded at 140 °C
	E140#2	CF#2 flour extruded at 140 °C
	E140#3	CF#3 flour extruded at 140 °C
Extruded at 160 °C	CE160	CF flour extruded at 160 °C
	E160#3	CF#3 flour extruded at 160 °C
	E160#4	CF#4 flour extruded at 160 °C

2.2 Lentil flour and formulated flours

Decorticated red chief lentils (*Lens culinaris* L.) were purchased from a local wholesale distributor in California (USA). SaFlavorPlus, a nutritional yeast extract grown from pure strains of *Saccharomyces cerevisiae*, specifically for its nutritional value, was provided by Red Star Yeast Corporation (Milwaukee, WI, USA). Different percentages of nutritional yeast, ranging from 4 to 16 percent, were mixed with about 55–65 percent lentil flours to generate the different formulations (patent pending),[15] 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 air-tight glass jars at room temperature until analyzed.

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 the capacity to run at about 50 kg feed per 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).[16]

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^{-1} (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 about 17 percent. The pulse formulations were extruded through two circular dies each with a 3.5 mm diameter opening. The 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 the Intouch software (FITSYS PLUS ver. 1.23) was used to collect the extruder parameter data at 1 s intervals. The data were collected approximately 10 min after

the operation conditions of torque and pressure reached at a steady state.

2.4 Analysis of bioactive compounds

2.4.1. Total, soluble and insoluble dietary fibre. Total, soluble and insoluble dietary fibre was determined according to AOAC enzymatic–gravimetric methods 993.19 and 991.42.[17] 0.4 gram of the sample is weighed and placed in a 250 mL Erlenmeyer flask. The samples are incubated at ~100 °C with shaking for 15 min in 50 mL of phosphate buffer (pH = 6.0 ± 0.2) solution containing 0.1 mL of α-amylase. The pH is then adjusted by the addition of 10 mL of 0.275 N NaOH (pH = 7.5 ± 0.2), and 5 mg of protease is added. After incubation at 60 °C with shaking for 30 min, the pH is adjusted by the addition of 10 mL of 0.325 N HCl (pH = 4–4.6), and 0.1 mL of amyloglucosidase is added. The samples are incubated at 60 °C with shaking for 30 min. The mixture is filtered (insoluble fibre) and the filtrate is collected in a 500 mL Erlenmeyer flask; it is precipitated by the addition of 400 mL of ethanol and filtered (soluble fibre). Duplicate samples are always processed, allowing the subtraction of protein and ash for the calculation of the total dietary fibre (TDF) content. The values are expressed as g per 100 g flour.

2.4.2. Arabinoxylans. Arabinoxylans (TO-AX and WE-AX) were quantified using colorimetric methods.[18,19] 125 mg of pulse flours and formulated pulse flours was placed in a 50 mL graduated conical polypropylene tube to which was added 25 mL of distilled water. The tubes were subjected to shaking for 30 minutes. Immediately after that, 0.5 mL of the suspension sample was quickly removed and pipetted into a stoppered reaction tube. The sample suspension (0.5 mL) contained 2.5 mg of the sample and was used to determine the TO-AX content. The original sample suspension was centrifuged for 10 min at 2500 rpm (Universal 16 R, Genesys Instrumentation, SL, Spain). After centrifugation, 0.5 mL of the supernatant was removed and pipetted into a amber reaction tube, similar to the method above. The supernatant aliquot thus represented a 2.5 mg equivalent of the sample for the determination of the WE-AX content. From each sample, two sample suspensions were made. From each sample suspension, two 0.5 mL aliquots were removed, resulting in four sample replicates for the TO-AX content, two from the first sample suspension and two from the second sample suspension. For each centrifuged sample suspension, two 0.5 mL aliquots of the supernatant were removed, resulting in four sample replicates for the WE-AX content, two from the first sample suspension and two from the second sample suspension. Once the sample aliquots were collected, 1.5 mL of distilled water was added to bring the final volume to 2 mL. The determination of arabinoxylans (TO-AX and WE-AX) then followed a colorimetric method.[20]

The calibration curve was prepared using a stock solution of 10 mg of D-(+)-xylose in 100 ml of distilled water. Then, triplicate standard samples were prepared using different concentrations of xylose (0.005–1 mg mL^{-1}). The values were expressed as g per 100 g flour.

2.4.3. Phenolic compounds

Methanolic extract preparation. A fine dried powder (1 g) was extracted by stirring with 40 mL of methanol at 25 °C for 1 h and filtered through Whatman No. 4 filter paper. The residue was then extracted with one additional 40 mL portion of methanol. The combined methanolic extracts were evaporated at 35 °C under reduced pressure (rotary evaporator, Heidolph, Schwabach, Germany). The extracts were re-dissolved in methanol, to a final concentration of 20 mg mL^{-1}, filtered through a 0.45 μm Whatman syringe filter and transferred to an amber HPLC vial for phenolic compound identification and quantification.

Phenolic compound identification and quantification were performed using a Dionex Ultimate 3000 UPLC instrument (Thermo Scientific, San Jose, CA, USA) equipped with a diode-array detector and coupled to a mass detector.[21] The chromatogram was recorded at several wavelengths, characteristic of different classes of polyphenols, such as 280 nm for flavan-3-ol derivatives and 370 nm for flavonol derivatives.

2.4.4. Tocopherols. Tocopherols were determined using an HPLC system coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA).[4] The quantification was based on the fluorescence signal response of each standard, using the internal standard (tocol) and by using calibration curves obtained from commercial standards of each compound. The results were expressed as mg per 100 g of flour.

2.5 Evaluation of antioxidant activity

The above-described extracts were re-dissolved in methanol to a final concentration of 200 mg mL^{-1} and further diluted to different concentrations to be subjected to *in vitro* antioxidant assays.[4]

2.5.1 DPPH radical-scavenging activity. The DPPH radical-scavenging activity was evaluated using an ELX800 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The reduction of the DPPH radical was measured at 515 nm, and calculated according to the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radical-scavenging activity (EC_{50}) was calculated from the graph of the RSA percentage against the extract concentration.[4] Trolox was used as the positive control.

2.5.2 Reducing power. The reducing power was determined using the microplate reader described above and evaluated the capacity to convert Fe^{3+} into Fe^{2+}. The extract concentration providing 0.5 of absorbance (EC_{50}: 50% of the maximal absorbance) was calculated from the graph of the absorbance at 690 nm against the extract concentration. Trolox was used as the positive control.

2.5.3 Inhibition of β-carotene bleaching. The inhibition of β-carotene bleaching was evaluated through the β-carotene/linoleate assay using the following equation: (β-carotene absorbance after 2 h of essay/initial β-carotene absorbance) × 100. The extract concentration providing 50% antioxidant capacity

(EC_{50}) was calculated by interpolation from the graph of the β-carotene bleaching inhibition percentage against the extract concentration, measured at 470 nm. Trolox was used as the positive control.

2.6 Statistical analysis

Mean ± standard deviations (SD) were determined using the Statgraphics Plus 5.1 software to analyze the data at 95% confidence level. The data were statistically analyzed by analysis of variance (ANOVA), followed by Duncan's test. The statistical significance level was set at $P < 0.05$.

3. Results and discussion

3.1 Extrusion effects on bioactive compounds in formulated flours

3.1.1 Dietary fibre and arabinoxylans. Regarding lentil raw formulated flours, the total dietary fibre (TDF) fraction increased with the increase in the yeast content (with the exception of CF#4, with 16% of yeast), ranging from 13.10 to 18.39 g per 100 g in CF (without yeast) and CF#3 (with 12% of yeast), respectively, the insoluble dietary fibre (IDF) being the prevalent fraction in all the analyzed formulations, representing up to 82% of TDF (as in the case of CF#4), while soluble dietary fibre (SDF) was found in lower amounts (Table 2). These values were in accordance with those reported by the authors in other raw lentil flours.[4,5,22] Additionally, other authors have reported that the TDF content of different pulse grains ranges between 6.50–29.80% in lentils, 16.12–26.20% in chickpeas and 20.35–27.60% in common beans,[23] which are within the values reported in the present study, with the exception of common beans.

In general terms, the extrusion process should not affect the total dietary fibre (TDF) in cereals and pulses.[24] However, it has been established that this effect is highly conditioned to the food matrix, being reported to be either increased, decreased or unchanged after extrusion cooking, depending on the raw material condition and parameters of the extrusion process.[25]

In this respect, the TDF content of samples without yeast, extruded at 140 °C (CE140) and 160 °C (CE160), significantly increased ($p < 0.05$) after the process (compared with the non-extruded sample, CF). This tendency was previously reported by different authors in other pulses and cereal flours.[26] Moreover, the samples with different percentages of yeast also showed an increase of TDF after extrusion, with the exception of sample EF140#3 (12% of yeast and extruded at 140 °C). On the other hand, other authors have reported opposite trends, with a decrease in the TDF fraction, as in the case of green peas, dry beans, chickpeas and cowpeas with a decrease of 3–9%, 16%, 25.72% and 37.58%, respectively.[27,28] Regarding insoluble dietary fibre (IDF), its content also varied during the extrusion process without a consistent tendency, since the results did not show a variation after the process (E140#3, E160#3 and E160#4, which are the samples with the highest yeast content (12 and 16%)) or showed a decrease after extrusion at 140 °C, as in the case of E140#1 in comparison with its corresponding raw sample (CF#1) (Table 2).

Regarding soluble dietary fibre (SDF), which consists mainly of pectins, some hemicelluloses, gums, mucilages, inulin, as well as soluble arabinoxylans and non-digestible oligosaccharides, which are also involved in some mechanisms responsible for the health benefits of dietary fibre, after extrusion cooking, its content showed a significant increase, mainly in samples CE140 and E160#4, with an increment of 38 and 46%, respectively, in comparison with the corresponding raw

Table 2 Extrusion effect on the dietary fibre (insoluble, soluble and total) and arabinoxylan (total and water soluble) content in lentil flour formulations, g per 100 g dry weight (mean ± SD)

Sample	Insoluble dietary fibre	Soluble dietary fibre	Total dietary fibre	Water soluble arabinoxylans	Total arabinoxylans
Raw					
CF	$8.93 \pm 0.83^{a,A}$	$4.17 \pm 0.37^{b,A}$	$13.11 \pm 0.83^{a,A}$	$1.07 \pm 0.100^{a,A}$	$5.49 \pm 0.07^{b,A}$
CF#1	$13.74 \pm 0.20^{c,B}$	$2.70 \pm 0.24^{a,A}$	$16.44 \pm 0.20^{b,A}$	$1.07 \pm 0.08^{a,A}$	$5.30 \pm 0.24^{b,A}$
CF#2	$12.36 \pm 0.28^{b,A}$	$4.62 \pm 0.30^{c,A}$	$16.99 \pm 0.28^{bc,A}$	$1.44 \pm 0.08^{c,A}$	$3.67 \pm 0.12^{a,A}$
CF#3	$14.00 \pm 1.06^{c,A}$	$3.86 \pm 0.29^{b,A}$	$18.39 \pm 0.20^{d,B}$	$1.28 \pm 0.09^{b,A}$	$6.57 \pm 0.31^{c,AB}$
CF#4	$14.30 \pm 0.75^{c,A}$	$2.58 \pm 0.16^{a,A}$	$17.32 \pm 0.11^{c,A}$	$1.07 \pm 0.100^{a,A}$	$7.18 \pm 0.45^{d,A}$
Extruded at 140 °C					
CE140	$13.85 \pm 0.17^{a,C}$	$5.76 \pm 0.54^{b,A}$	$19.61 \pm 0.17^{c,C}$	$2.63 \pm 0.10^{b,B}$	$7.68 \pm 0.32^{c,C}$
E140#1	$13.15 \pm 0.43^{a,A}$	$5.52 \pm 0.31^{b,B}$	$18.66 \pm 0.43^{b,B}$	$2.61 \pm 0.13^{b,B}$	$7.26 \pm 0.68^{c,B}$
E140#2	$13.17 \pm 0.50^{a,B}$	$5.31 \pm 0.32^{b,B}$	$18.48 \pm 0.50^{b,B}$	$2.38 \pm 0.08^{a,B}$	$5.79 \pm 0.29^{a,B}$
E140#3	$12.98 \pm 0.95^{a,A}$	$3.59 \pm 0.20^{a,A}$	$16.57 \pm 0.95^{a,A}$	$2.54 \pm 0.14^{b,B}$	$6.35 \pm 0.25^{b,A}$
Extruded at 160 °C					
CE160	$12.16 \pm 0.46^{a,B}$	$4.52 \pm 0.22^{b,A}$	$16.68 \pm 0.46^{a,B}$	$2.77 \pm 0.31^{a,B}$	$6.05 \pm 0.35^{a,B}$
E160#3	$13.05 \pm 0.94^{a,A}$	$4.62 \pm 0.30^{b,B}$	$17.67 \pm 0.94^{a,AB}$	$2.62 \pm 0.06^{a,B}$	$6.76 \pm 0.12^{b,B}$
E160#4	$15.20 \pm 0.78^{b,A}$	$3.77 \pm 0.35^{a,B}$	$18.97 \pm 0.78^{b,B}$	$2.72 \pm 0.20^{a,B}$	$7.09 \pm 0.39^{b,A}$

In each column, different letters mean statistically significant differences ($p < 0.05$) compared by Duncan's test; small superscript letters mean differences between all samples of the same extrusion treatment, whereas capital superscript letters mean difference due to the extrusion treatment for the same formulation.

samples (CF and CF#4, respectively). A significant increase in the SDF content in some lentil formulated flours,[29] as well as in barley flours and other snack food products,[26,30] has also been reported. Although extrusion did not affect pectin, in extruded oatmeal and potato peels, both soluble and insoluble non-starch polysaccharides (NSP) were increased in the extruded samples.[31]

Physicochemical modifications in dietary fibre can occur due to high temperature and shear conditions during extrusion processing. It can be assumed that the increase of TDF during extrusion cooking was increased maybe mainly due to the increase of SDF,[26,30] which was higher than the decrease of IDF. Thermal or thermomechanical treatment leads to a redistribution of a portion of insoluble dietary fibre to soluble dietary fibre due to the fragmentation of the insoluble fibre components, as it has been previously reported in resistant starch.[32] Another possible mechanical process that could explain the increase in the SDF fraction is gelatinisation. While complete gelatinisation may not occur during extrusion, digestibility is often improved, resistant starch can be physically broken into smaller components, and this could increase the soluble dietary fibre and soluble sugar content, which are more digestible and may be exploited to produce dextrin and/or free glucose.[33]

As part of the soluble dietary fibre, arabinoxylans (AX) can be classified according to the physical properties of the solubility in water as extractable (WE-AX) and unextractable (WU-AX). WU-AX remain in the cell wall attached to other arabinoxylans and other cell constituents by non-covalent interactions (hydrogen bonding) and covalent interactions (ester bond). Besides this, WE-AX are weakly bound to the surface of the cell wall due to an incomplete cross-linking with other components or due to having undergone initial enzymatic degradation in the grain. The total arabinoxylan (TO-AX) content of the raw analyzed samples (Table 2) ranged from 3.67 to 7.18 g per 100 g, for CF#2 and CF#4, respectively, being higher in the sample formulated with the highest yeast content (16%), reaching values up to 40% of the total dietary fibre. Wheat grains contain 5.5 to 7.1% arabinoxylans, present in the endosperm and bran, being bran the richest portion. Regarding the content in pulses, it is reported to have lower values in beans, lentils and green peas (1.03 to 2.21 g per 100 g).[34] The water extractable arabinoxylan (WE-AX) content of the raw analyzed samples (Table 2) ranged from 1.07 (CF and CF#1) to 1.44 g per 100 g (CF#2) in the samples.

The WE-AX content was significantly ($p < 0.05$) increased after extrusion cooking in all samples. The TO-AX content was also significantly ($p < 0.05$) higher after the extrusion process with the exception of samples E140#3 and E160#4.

Slightly content of TO-AX in barley flours was reported by other authors. The TO-AX content in native flours ranged from 3.09% to 4.14% and from 3.11% to 4.48% in the extruded flours. The lower content of TO-AX in hulled barley could be due to the abrasion of the hulls along with a part of the aleurone layer (higher TO-AX concentration).[35] To the authors' best knowledge, there is a lack of information on the impact of extrusion on the arabinoxylan content in pulses and particularly in lentil flours.

3.1.2 Phenolic compounds. The phenolic profile of lentil flour recorded at 280 and 370 nm is shown in Fig. 1. The compound characteristics, tentative identities and quantitative results are presented in Tables 3 and 4. All the main compounds were identified according to their MS characteristics (molecular ion and MSn fragmentation pattern) and UV-visible spectra, and were tentatively identified by comparing these data with the information reported in the literature (Table 3). Eight compounds were detected, six of which were flavonol derivatives (peaks 3–8), a flavan-3-ol derivative (peak 1) and a phenolic acid derivative (peak 2). The phenolic composition of lentils (*Lens culinaris*) using different varieties and parts and from different locations has been largely reported by many authors, such as Pardina lentil,[2,36,37] green lentil,[37,38] red lentil,[39] lentil sprouts,[38,40] lentil seed coats,[41] different cultivars of Canadian lentils,[42,43] cooking and germination effects in lentils,[44,45] Castellana lentils from Spain,[36] 10 different commercial lentil genotypes,[46] *Lens culinaris* var. Morton,[47] *Lens culinaris* Medik. cv. Dimitra,[48] and 11 lentil cultivars from Idaho, USA.[49] Nonetheless, the aim of this study was to characterize the different yeast-enriched formulations as well as to evaluate the changes induced by extrusion cooking on the composition of bioactive molecules, such as phenolic compounds. Table 4 shows the total and individual phenolic compound content in raw and extruded formulated lentil flours. Catechin hexoside (compound 1) was the most abundant phenolic compound present in all the samples and has been reported as being a main compound of lentils. Kaempferol tetraglycoside (compound 3) and kaempferol triglycoside (compound 4 and 5) have been identified by the authors of many previous reports.

In the analyzed samples, the content of total phenolic compounds (TPC), particularly catechin hexoside, increases proportionally to the increase in the percentage of nutritional yeast incorporated into the lentil formulations; thus, CF#4 (formulated with 16% of nutritional yeast) presents a higher TPC content than CF (control raw flour, 0% of nutritional yeast).

It is usually thought that processing technologies, including mechanical treatment, thermal treatment, extrusion cooking and bioprocessing, cause substantial losses of antioxidant compounds in food products, because the high temperature causes the decomposition of heat-labile phenolic compounds and may also lead to the polymerization of some phenolic compounds under high pressure in extrusion cooking.[50] In general, the observed decrease in the TPC content and in the content of each individual phenolic compound was directly related to the temperature. In the samples without yeast, particularly in CE160 (extruded at 160 °C), an extended decrease (97%) in the TPC content was observed compared with the non-extruded sample (CF), which was higher than in CE140 (extruded at 140 °C). Also, the observed tendency indicates that the TPC and catechin hexoside content showed a higher decrease in the lentil flours formulated with higher content of nutritional yeast, with a decrease of 90 to 95% in

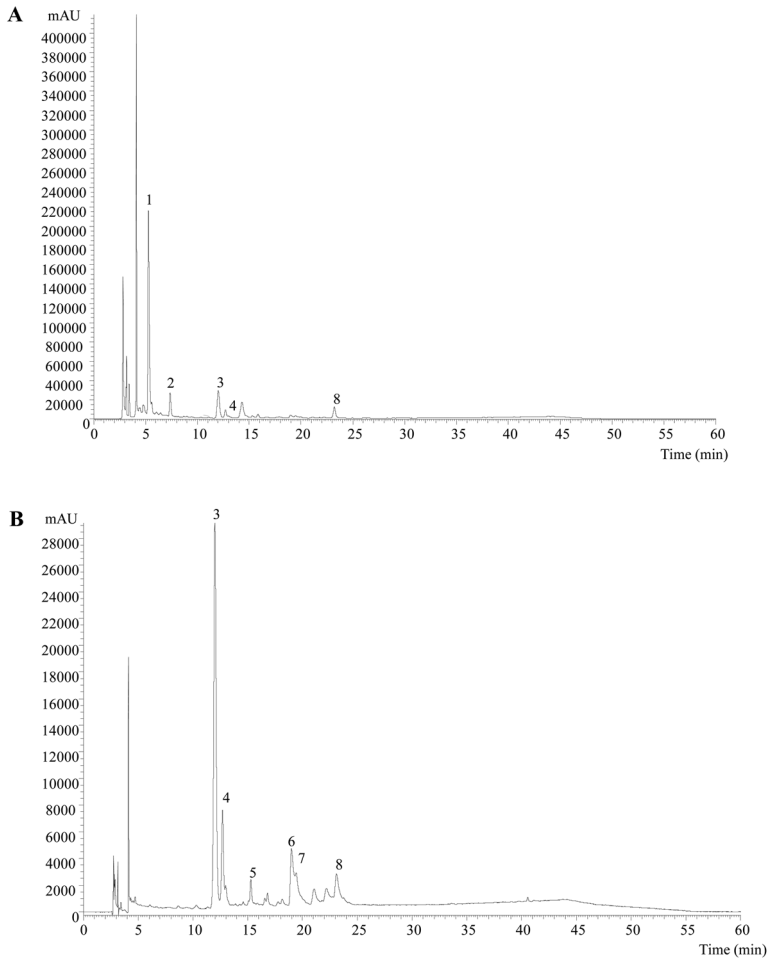

Fig. 1 Profile of the phenolic compounds of sample CF#2 recorded at 280 nm (A) and 370 nm (B).

Table 3 Identification of phenolic compounds in lentil flour formulations

Compound	*Rt* (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification	Type of identification
1	5.3	285	451	289 (100)	Catechin glucoside	DAD-MS, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
2	7.3	320	341	179 (100)	Caffeic acid hexoside	DAD-MS
3	12.0	347	901	755 (20), 593 (39), 285 (40)	Kaempferol dirutinoside	DAD-MS, 2, 4, 5, 7, 8, 9
4	12.7	350	755	609 (44), 285 (28)	Kaempferol-rhamnosyl-dihexoside	DAD-MS, 2, 4, 5, 7, 9, 10
5	15.3	348	755	593 (56), 575 (25), 285 (33)	Kaempferol-hexosyl-rutinoside	DAD-MS, 2, 4, 5, 7, 9, 10
6	19.0	350	463	301 (100)	Quercetin-3-*O*-galactoside	DAD-MS, 6
7	19.4	350	463	301 (100)	Quercetin-3-*O*-glucoside	DAD-MS, standard, 6, 7, 9, 10
8	23.2	350	433	301 (100)	Quercetin-3-*O*-xyloside	DAD-MS, 7, 9

References: 1 – Amarowicz *et al.*, 2009; 2 – Aguilera *et al.*, 2010; 3 – Amarowicz *et al.*, 2010; 4 – Troszyńska *et al.*, 2011; 5 – Zou *et al.*, 2011; 6 – Mirali *et al.*, 2014; 7 – Zhang *et al.*, 2014; 8 – Alshikh *et al.*, 2015; 9 – Zhang *et al.*, 2015; 10 – Dueñas *et al.*, 2016.

E160#4 (with 16% of yeast) and E140#3 (with 12% of yeast), respectively. The other phenolic compounds with lower molecular weight also exhibit a decrease in their content but the decrease is less severe, probably due to the partial hydrolysis of conjugated phenolics, which were covalently bound to the insoluble fibre fraction (wheat brand, legumes, *etc.*) and can only be absorbed after specific processing technologies (such as extrusion) or fermentation (*e.g.* gut fibre fermentation), which increases the bioaccessibility of polyphenols.[50,51]

It is reported that the extrusion effects on the TPC of beans depended on the cultivar. *Phaseolus vulgaris* L. Rawela cultivar extrudates showed a 14% increase in the amount of phenolic compounds compared to raw beans, corresponding the greatest rise of quercetin (84%), while Tip-Top and Toffi cultivars

Table 4 Extrusion effect on phenolic composition (mg per 100 g dry weight) in lentil flour formulations (mean±SD)

Sample	Catechin hexoside[1]	Caffeic acid hexoside[2]	Kaempferol-*O*-desoxyhexoside-*O*-hexoside-*O*-rutinoside[3]	Kaempferol-*O*-desoxyhexoside-*O*-dihexoside[3]	Kaempferol-*O*-hexoside-*O*-rutinoside[3]	Quercetin-3-*O*-glucoside[4]	Quercetin-*O*-hexoside[4]	Quercetin-*O*-pentoside[4]	Total phenolic compounds
Raw									
CF	$30.71 \pm 1.44^{a,C}$	nd	$3.19 \pm 0.11^{d,C}$	tr	tr	$3.88 \pm 0.04^{a,C}$	$3.09 \pm 0.03^{a,C}$	$4.93 \pm 0.18^{a,C}$	$45.80 \pm 1.79^{a,C}$
CF#1	$32.52 \pm 1.18^{b,B}$	tr	$2.43 \pm 0.08^{b,B}$	tr	tr	$4.17 \pm 0.08^{b,B}$	$3.26 \pm 0.04^{b,B}$	$5.11 \pm 0.11^{a,B}$	$47.50 \pm 0.94^{b,B}$
CF#2	$55.48 \pm 0.35^{c,B}$	tr	$3.45 \pm 0.02^{e,B}$	tr	tr	$4.41 \pm 0.04^{c,B}$	$3.31 \pm 0.03^{b,B}$	$5.70 \pm 0.14^{b,B}$	$72.35 \pm 0.20^{c,B}$
CF#3	$59.92 \pm 0.85^{d,C}$	tr	$2.61 \pm 0.09^{c,C}$	tr	tr	$4.78 \pm 0.14^{d,B}$	$3.74 \pm 0.19^{c,B}$	$5.85 \pm 0.15^{b,C}$	$76.89 \pm 1.04^{d,C}$
CF#4	$66.13 \pm 1.51^{e,B}$	tr	$1.40 \pm 0.01^{a,B}$	tr	tr	$4.99 \pm 0.01^{e,B}$	$4.07 \pm 0.01^{d,B}$	$6.34 \pm 0.12^{c,B}$	$82.94 \pm 1.37^{e,B}$
Extruded at 140 °C									
CE140	$4.79 \pm 0.25^{c,B}$	tr	$0.178 \pm 0.005^{a,A}$	tr	tr	$0.411 \pm 0.005^{b,B}$	$0.399 \pm 0.002^{d,B}$	$0.51 \pm 0.02^{b,B}$	$6.28 \pm 0.15^{d,B}$
E140#1	$1.76 \pm 0.02^{a,A}$	nd	$0.55 \pm 0.02^{d,A}$	tr	tr	$0.407 \pm 0.001^{b,A}$	$0.333 \pm 0.004^{a,A}$	$0.60 \pm 0.01^{d,A}$	$3.64 \pm 0.01^{a,A}$
E140#2	$3.05 \pm 0.01^{b,A}$	tr	$0.49 \pm 0.01^{c,A}$	tr	tr	$0.41 \pm 0.01^{b,A}$	$0.37 \pm 0.01^{c,A}$	$0.577 \pm 0.001^{c,A}$	$4.90 \pm 0.01^{c,A}$
E140#3	$2.92 \pm 0.15^{b,A}$	tr	$0.35 \pm 0.01^{b,A}$	tr	tr	$0.363 \pm 0.001^{a,A}$	$0.357 \pm 0.001^{b,A}$	$0.46 \pm 0.01^{a,A}$	$4.44 \pm 0.15^{b,A}$
Extruded at 160 °C									
CE160	$0.90 \pm 0.01^{a,A}$	nd	$0.86 \pm 0.01^{c,B}$	tr	tr	$0.254 \pm 0.001^{a,A}$	$0.236 \pm 0.001^{a,A}$	$0.30 \pm 0.08^{a,A}$	$2.55 \pm 0.08^{a,A}$
E160#3	$4.28 \pm 0.05^{b,B}$	tr	$0.63 \pm 0.03^{b,B}$	tr	tr	$0.45 \pm 0.01^{b,A}$	$0.41 \pm 0.01^{b,A}$	$0.68 \pm 0.01^{b,B}$	$6.45 \pm 0.02^{b,B}$
E160#4	$6.02 \pm 0.19^{c,A}$	tr	$0.48 \pm 0.02^{a,A}$	tr	tr	$0.555 \pm 0.002^{c,A}$	$0.5043 \pm 0.01^{c,A}$	$0.79 \pm 0.01^{c,A}$	$8.35 \pm 0.18^{c,A}$

Phenolic compounds used for quantification: 1 – catechin ($y = 84\,950x - 23\,200$; $R^2 = 0.999$; LOD 0.17 μg mL^{-1}; LOQ 0.72 μg mL^{-1}); 2 – caffeic acid ($y = 38\,8345x + 406\,369$; $R^2 = 0.994$; LOD 0.19 μg mL^{-1}; LOQ 0.65 μg mL^{-1}); 3 – kaempferol-3-*O*-rutinoside ($y = 37\,039x + 666\,788$; $R^2 = 0.998$; LOD 0.13 μg mL^{-1}; LOQ 0.43 μg mL^{-1}); 4 – quecetin-3-*O*-glucoside ($y = 34\,843x - 160\,173$; $R^2 = 0.999$; LOD 0.21 μg mL^{-1}; LOQ 0.71 μg mL^{-1}).

exhibited a decrease of 19 and 21%, respectively, mainly due to the decrease in the content of myricetin, quercertin and chlorogenic acids.[27] It is also reported that the effect of extrusion cooking on different sorghum phenotypes was more deleterious than that of dry heat in a conventional oven.[52] Flavones and flavanones of sorghum were more sensitive to extrusion cooking and dry heat in a conventional oven than 3-DXA and proanthocyanidins. Even though thermal degradation was a factor, part of the changes in the phenolic compound content may be attributed to the reduced extractability after processing.[52,53] On the other hand, it is reported that extruded lentil flours (enriched with different fibre sources) presented a significantly ($p < 0.05$) higher content of TPC, hydroxybenzoic and hydroxycinnamic acids, but the content of flavonols decreased significantly after treatment.[4]

3.1.3 Tocopherols. There are several factors that affect the stability of vitamins during extrusion (mixing, conditioning, cooking temperature, pressure, screw rpm, moisture, *etc*.). The vitamins that are most sensitive to the extrusion process are vitamin A and vitamin E from lipophilic vitamins, and vitamin C, B_1, and folic acid from hydrophilic vitamins.[54] Table 5 shows the effects of extrusion cooking on the different vitamin E isoforms. In all the analysed lentil flours, α-, β- and γ-tocopherols were characterized, γ-tocopherols being the major isoform, as also reported in lentil seeds by other authors.[4,45] The α-tocopherol content of the raw samples was between 0.03 and 0.06 mg per 100 g (CF#4 and CF#1, respectively). In the case of β-tocopherol, this isoform was only detected in the raw samples with a higher yeast content (12% and 16%), ranging from 0.05 to 0.09 mg per 100 g (CF#3 and CF#4, respectively). In general, α- and β-tocopherols were not detected after the extrusion process. The highest total tocopherol content was determined in raw samples formulated with 4 and 8% of nutritional yeast (CF#1 and CF#2, with 3.02 and 2.61 mg per 100 g, respectively).

As expected, there was a significant decrease in all tocopherol isoforms after the extrusion process. The results showed a reduction of 81.5–92.0% in the total tocopherol content which is in accordance with other authors.[4,55] The sensitivity of the various forms of vitamin E depends on the extrusion process variables, *e.g.* in grass peas (*Lathyrus sativus* L.), it has been reported that an increase in extrusion temperatures promote the decrease in α-tocopherol, while γ-tocopherol decreases with the increase in the moisture content during extrusion.[56] Also, a drop of 40 to 93% in total tocopherols of different extruded cereals (oat, barley, wheat, rye) is reported after a short thermal treatment (extrusion at 120 °C), the lowest decrease being observed in oat flours.[55] A 83–94% decrease of total tocopherol was observed in lentil formulated flours fortified with different fibres extruded at 160 °C.[4]

3.2 Extrusion effects on the antioxidant properties of lentil formulated flours

The extrusion conditions have an important impact on the antioxidant activity of various food materials. The antioxidant capacity of lentil flours was evaluated using three different

Table 5 Extrusion effect on tocopherol composition (mg per 100 g dry weight) in lentil flour formulations (mean± SD)

Sample	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Total tocopherols
Raw				
CF	0.05 ± 0.01^{c}	nd	$2.50 \pm 0.01^{c,B}$	$2.55 \pm 0.01^{b,B}$
CF#1	0.06 ± 0.01^{d}	nd	2.97 ± 0.01^{e}	3.02 ± 0.01^{e}
CF#2	0.04 ± 0.01^{b}	nd	$2.57 \pm 0.02^{d,B}$	$2.61 \pm 0.01^{d,B}$
CF#3	$0.05 \pm 0.01^{c,B}$	0.05 ± 0.01^{a}	$2.47 \pm 0.01^{b,C}$	$2.57 \pm 0.01^{c,C}$
CF#4	0.03 ± 0.01^{a}	0.09 ± 0.01^{b}	$1.93 \pm 0.01^{a,B}$	$2.05 \pm 0.01^{a,B}$
Extruded at 140 °C				
CE140	nd	nd	$0.30 \pm 0.01^{b,A}$	$0.30 \pm 0.01^{b,A}$
E140#1	nd	nd	nd	nd
E140#2	nd	nd	$0.17 \pm 0.01^{a,A}$	$0.17 \pm 0.01^{a,A}$
E140#3	0.01 ± 0.01^{A}	nd	$0.47 \pm 0.01^{c,B}$	$0.48 \pm 0.01^{c,B}$
Extruded at 160 °C				
CE160	nd	nd	nd	nd
E160#3	nd	nd	$0.21 \pm 0.01^{a,A}$	$0.21 \pm 0.01^{a,A}$
E160#4	nd	nd	$0.32 \pm 0.01^{b,A}$	$0.32 \pm 0.01^{b,A}$

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

in vitro assays (Table 6). In the present study, the antioxidant activity of the samples, determined using the DPPH assay, exhibited higher EC_{50} values than all the other methods for all the unprocessed (raw) and extruded samples. Additionally, all the formulated extrudates showed significantly ($p < 0.05$) lower EC_{50} values than their raw samples. The β-carotene/linoleate assay revealed that, with the exception of E140#3, all other formulated extrudates presented significantly ($p < 0.05$) lower EC_{50} values (better antioxidant properties) than their raw samples. In all cases, flours extruded at 160 °C (CE160, E160#3 and E160#4) presented the lowest EC_{50} values measured by all of the assays performed in this study. These results reflect the important effect of extrusion processing and food ingredients on the antioxidant activity determined by different assays.

Table 6 Extrusion effect on the antioxidant properties (EC_{50}, mg mL^{-1} methanolic extract) of lentil flour formulations (mean ± SD)

Sample	DPPH	Reducing power	β-Carotene bleaching inhibition
Raw			
CF	$52.99 \pm 1.91^{c,C}$	$5.22 \pm 0.04^{b,C}$	$2.39 \pm 0.02^{c,C}$
CF#1	$42.20 \pm 1.22^{b,B}$	$4.34 \pm 0.02^{a,B}$	$2.37 \pm 0.03^{c,B}$
CF#2	$42.79 \pm 0.22^{a,B}$	$5.62 \pm 0.06^{c,B}$	$2.12 \pm 0.08^{b,B}$
CF#3	$54.95 \pm 2.94^{d,C}$	$5.97 \pm 0.11^{d,C}$	$1.96 \pm 0.12^{a,A}$
CF#4	$61.42 \pm 1.14^{e,B}$	$5.62 \pm 0.02^{c,B}$	$1.94 \pm 0.15^{a,B}$
Extruded at 140 °C			
CE140	$18.33 \pm 0.65^{b,B}$	$2.24 \pm 0.01^{a,B}$	$1.50 \pm 0.02^{c,B}$
E140#1	$17.30 \pm 0.70^{a,A}$	$2.53 \pm 0.05^{c,A}$	$1.31 \pm 0.08^{b,A}$
E140#2	$31.87 \pm 1.85^{c,A}$	$2.40 \pm 0.04^{b,A}$	$0.82 \pm 0.02^{a,A}$
E140#3	$36.99 \pm 0.08^{d,B}$	$2.38 \pm 0.02^{b,B}$	$3.57 \pm 0.20^{d,B}$
Extruded at 160 °C			
CE160	$4.55 \pm 0.04^{a,A}$	$1.26 \pm 0.01^{a,A}$	$0.61 \pm 0.03^{a,A}$
E160#3	$15.96 \pm 0.57^{b,A}$	$2.09 \pm 0.01^{b,A}$	$1.85 \pm 0.14^{b,A}$
E160#4	$31.16 \pm 1.93^{c,A}$	$2.70 \pm 0.09^{c,A}$	$1.00 \pm 0.09^{c,A}$

In each column, different letters mean statistically significant differences ($p < 0.05$) compared by Duncan's test; small superscript letters mean differences between all samples of the same extrusion treatment, whereas capital superscript letters mean difference due to the extrusion treatment for the same formulation.

The antioxidant activity in lentil flour formulations was studied and it was concluded that the antioxidant activity in most of the formulated flours increased as a result of extrusion processing. Although phenolic compounds decrease after the extrusion process, different authors affirm that the total antioxidant capacity after processing was either increased or retained compared to the unheated samples because of the compensation in antioxidant activity of the degradation compounds (*e.g.* the condensed tannins could be degraded into sub-entities of lower molecular weight but with greater antioxidant activity). Simultaneously, as reported by different authors, during thermal processing, different pigments (particularly melanoidins) are produced. Some of them have antioxidant activity and the increase in the antioxidant activity could be explained by the formation of Maillard browning pigments, which enhanced the antioxidant activity.[57]

Different authors have reported that extrusion cooking significantly reduced the antioxidant activity and total phenolics in all barley flour extrudates.[57,58] This reduction was found to be 60–68% and 46–60% for barley extrudate samples when compared the unprocessed barley flour. Comparing extrusion cooking with other thermal treatments, in general terms, other authors reported lower antioxidant capacity in lentils after soaking, domestic cooking and also industrial dehydratation.[2] It is also reported that cooked lentils showed a significantly lower antioxidant activity in both the DPPH and ORAC-L assays, probably due to the water solution of extractable phenolic compounds during the traditional cooking process.[43] Moreover, a similar trend was reported in Pardina lentils after industrial dehydratation (measured by the ORAC assay); this process did not cause any further effects than those caused by ordinary soaking and cooking process.[2] However, all of them

affected to a large extent the antioxidant capacity of lentil seeds, showing that the extrusion process is a better alternative to cooking of these pulses.

4. Conclusions

Extruded lentil flours fortified with nutritional yeast appear as an interesting new snack-type product to be included in the daily diet, as a good and novel alternative to the traditional pulse stews or dishes and to the cereal-based gluten-containing snacks, showing an interesting profile of bioactive compounds.

In the analyzed samples, the total dietary fibre and arabinoxylan content significantly ($p < 0.05$) increased after extrusion cooking. It can be assumed that the increase of TDF during extrusion cooking may be mainly due to the increase of SDF, which was higher than the decrease of IDF.

In general, the observed decrease in TPC and in each individual phenolic compound was directly related to the temperature. In samples without yeast, particularly in CE160 (extruded at 160 °C), an extended decrease (97%) in the TPC content, higher than in CE140 (extruded at 140 °C), was observed compared with the non-extruded sample (CF); also, the observed tendency indicates that total phenolic and catechin hexoside showed a higher decrease in the lentil flours formulated with higher content of nutritional yeast (12 and 16%). Also, there was a significant decrease in all tocopherol isoforms after the extrusion process with a reduction of 81.5–92.0% in the total tocopherol content.

The β-carotene/linoleate assay revealed that all formulated extrudates presented significantly ($p < 0.05$) lower EC_{50} values (better antioxidant properties) than their raw samples, with the exception of E140#3. In all cases, the flours extruded at 160 °C (CE160, E160#3 and E160#4) presented the lowest EC_{50} values measured by all of the assays performed in this study.

Conflicts of interest

The authors state no conflict of interest.

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