Assessment of Maillard reaction evolution, prebiotic carbohydrates, antioxidant activity and $\alpha$-amylase inhibition in pulse flours

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Abstract In this paper, the quality of bean, chickpea, fava, lentil and pea flours from Algeria has been evaluated. Maillard reaction (MR) indicators, modifications in the carbohydrate and protein fractions, antioxidant activity and α-amylase inhibitor of raw, toasted and stored samples were evaluated. Fava, beans and peas showed higher content of raffinose family oligosaccharides while chickpeas and lentils showed higher polyol content. Toasting and storage caused slightly change in pulse quality; MR showed slight losses of lysine but increased antioxidant activity. Moreover, inhibition of α-amylase was slightly augmented during processing; this could increase the undigested carbohydrates that reach the colon, modulating the glycaemic response. These results point out the suitability of these flours for preparing high-quality foodstuffs intended for a wide spectrum of the population, including hyperglycaemic and gluten intolerant individuals.

Keywords: Pulses - Antioxidant activity · Maillard reaction · Carbohydrates · Proteins
Introduction

Legumes or pulses are considered as important foods not only for their nutritional value (Tharanathan and Mahadevamma 2003), but also for their functional ingredients, including bioactive components with putative positive effects such as reduction of heart and kidney diseases, decrease of sugar indices in diabetics and increase of satiety (Mathres, 2002). Legumes present slowly digestible carbohydrates and a high amount of compounds that may interfere with the metabolism of glucose, α-amylase inhibitor being one of the most important. Although in the past this compound was considered as an anti-nutritional factor, currently, α-amylase inhibitor has aroused a growing interest as a bioactive compound to avoid rapid glucose release (Singhal et al. 2014). Other important constituents in pulses are α-galactosides, also known as the raffinose family oligosaccharides (RFOs) with a recognized prebiotic effect at a maximum daily dose of 3 g due to their indigestible α(1-6) links; however, at higher doses, RFOs could give rise to flatulence and interference with the digestion of other nutrients (Martínez-Villaluenga et al. 2008). Galactosyl-cyclitols, present in legume seeds, are also considered as important phytochemicals related to disease prevention (Ruiz-Aceituno et al. 2013). Moreover, the in vitro antioxidant activity of legume extracts has been mainly ascribed to phenolic compounds, underlying their benefits in prevention of cancer, ageing and chronic diseases (Zhao et al. 2014).

The use of pulses for their phytotherapeutic applications comes from the Mediterranean regions, where people have been eating this food for centuries as a basic element of the Mediterranean diet (Duranti 2006). In countries from the North of Africa, such as Algeria, legumes are also consumed as processed seed or flour in the elaboration of some foodstuffs because of their technological properties. According to Halila et al. (2000) the consumption of pulses in Algeria is 19 g/person/day. In addition,
the increase of pathologies related to gastrointestinal function in developed countries has promoted, in some cases, the removal of gluten; thus, the disposal of other flour sources such as those derived from legumes could open new ways to diversify the market for this type of products. Moreover, pulse flours can constitute an important ingredient in the fortification of widely-consumed cereal-based food products.

During processing and storage of pulse flours, different physical and chemical changes can take place modifying their functionality and nutritional value. RFOs can be hydrolysed during treatments at elevated temperatures or prolonged storage at ambient conditions. Because of this, hydrolysis products or RFOs, together with monosaccharides, are liable to react with proteins by Maillard reaction (MR), decreasing their nutritional value caused by the participation of the essential amino acid lysine (Voragen 1998). However, the MR may also give rise to the formation of products with antioxidant properties, mainly at the advanced stages of the reaction (Gu et al. 2010). To the best of our knowledge, limited literature is available on this issue, namely in the case of flours. Therefore, the current study is aimed at evaluating the quality of raw and processed flours of some of the most widely consumed legumes in Algeria (beans, chickpeas, fava beans, lentils and peas), and special attention is given to the MR evolution and modifications in the carbohydrate and protein fractions, antioxidant activity and α-amylase inhibitor during the toasting and storage under forced conditions.
Materials and Methods

Samples

Pulse grains lentils (*Lens culinary* L.), favas (*Vicia faba* L.), chickpeas (*Cicer arietinum* L.), field beans (*Phaseolus vulgaris* L.), and yellow peas (*Pisum sativum* L.) as dry seeds were obtained from three locations: lentils and yellow peas were obtained from ITGC (Institut Technologique des Grandes Cultures) of Setif, Algeria, chickpeas and faba from Merdj Ouaman, Wilaya of Bejaia, Algeria, and field beans from Jijel, Algeria. Fava seeds were manually separated into hulls and cotyledons. Before analyses, all samples were initially crushed in a coffee grinder followed by an analytical mill (IKA A11 basic; IKA Werke GmbH & Co. KG, Staufen, Germany), then sieved to pass through a 0.5 mm mesh sieve. These pulse flours were used in all determinations.

Taking into account the normal use of flour and the content of protein and monosaccharides of these seeds, we selected lentils and peas for toasting and beans and peas for storage under forced conditions. Lentil and pea flours were toasted in a stirring hot plate (RCT basic IKAMAG, IKA) in duplicate near to 300 °C for 10 min. The samples were stored in duplicate in open vials under vacuum and stored in the saturated salt solution of 0.44 water activity (*aw*) (*K₂CO₃*) at 40 °C. Stored samples were withdrawn after 2, 8, 19 and 29 days and were kept at -20 °C to analyse all of them at the same time.

General characterization

The moisture content and crude protein (N×6.25) of pulses were determined in accordance with the standard methods of AOAC (1990). *aw* of the samples was determined by an AW Sprint Novasina TH-500 instrument (Novasina, Pfäffikon, Switzerland).
Switzerland). The pH values were measured in samples, resuspended in water at 10% w/v, using a Mettler Toledo Five Easy Plus pH Meter.

Carbohydrate analysis by gas chromatography (GC)

Fat and protein pulses were removed by precipitation using Carrez reagents (Rada-Mendoza et al. 2004). Flours (400 mg) were gently mixed with 3 mL water and equal volumes (0.4 mL) of Carrez I (7.2% w/v K₄Fe(CN)₆·3H₂O in water) and Carrez II (14% w/v ZnSO₄·7H₂O in water) in a 10-mL volumetric flask and diluted to volume with water. Then, supernatant was collected, centrifuged at 10,000 g for 5 min at room temperature and stored at 4 °C for further analysis. All preparations were done in duplicate. 0.5 mL of supernatant (2-3 mg of carbohydrates) was added to 0.2 mg of phenyl-β-D-glucoside (Sigma–Aldrich Chemical, St. Louis, Missouri, USA) as internal standard (I.S.). Afterwards, the mixture was dried at 40 °C in a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland).

Sugar oximes were formed by adding 250 µL of hydroxylamine chloride (2.5%) in pyridine and heating the mixture at 70 °C for 30 min and then silylated with hexamethyldisilazane (250 µL) and trifluoroacetic acid (25 µL) and kept at 50 °C for 30 min. Reaction mixtures were centrifuged at 9,600 g for 2 min at room temperature. Supernatants were collected in a vial and injected or stored at 4 °C prior to analysis.

GC analysis of trimethyl silylated oximes (TMSO) of carbohydrates was performed following the method of Gamboa-Santos et al. (2012). For quantitation, 3 extractions with Carrez were made and the recovery factors were applied. The response factors were calculated after the analysis of standard solutions (fructose, glucose, myo-inositol, sucrose, raffinose, stachyose and verbascose (quantification of saccharides with DP ≥ 5))
over the expected concentration range in samples, (0.01-2 mg) and 0.2 mg I.S. All analyses were done in duplicate and data were expressed as mean ± standard deviation (SD).

GC-MS analyses were carried out using a 25 m x 0.25 mm i.d. x 0.25 μm film thickness fused silica column coated with SPB-1 (crosslinked methyl silicone) from Supelco (Bellefonte, PA, USA) installed in a Hewlett-Packard 5890 gas chromatograph with a 5971 quadrupole mass detector operating in EI mode at 70 eV (both from Hewlett-Packard, Palo Alto, CA, USA). Helium was used as carrier gas, and injections were made in the split mode, with a split flow of 40 mL/min. Oven temperature was held at 200 ºC for 20 min, then programmed to 270 ºC at a heating rate of 15 ºC/min, then programmed to 290 ºC at 1 ºC/min and finally programmed to 300 ºC at 15 ºC/min and held for 40 min. Injector temperature was 300 ºC. Acquisition was done using an HPChem Station software (Hewlett-Packard, Palo Alto, CA, USA). Identification of TMSO derivatives of carbohydrates present in pulses was carried out, when possible, by comparison of their retention times and mass spectral data with those of standard compounds. When standards were not available, identities were given as tentative.

Determination of furosine

Furosine (2-furoilmethyl-lysine) content in pulse flours was performed by ion-pair reverse phase (RP) HPLC. Before analysis, samples (0.25 g) were hydrolysed with 4 mL of 8 N HCl at 110 ºC for 24 h under inert conditions. RP-HPLC analysis was carried out following the method used by Rada-Mendoza et al. (2004). Identification and quantitation of furosine were done by using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France). All the samples were analysed in
duplicate, and data shown are the average values expressed as milligrams of furosine per 100 g of protein ± SD.

Determination of hydroxymethyl furfural (HMF)

The analysis of HMF was carried out by HPLC using a ACE 5 C18 column (ACE, UK) (250 mm x 4.6 mm, 5 µm) thermostated at 25 °C and a linear gradient from methanol:water (5:95) to methanol:water (80:20) in 6 min, isocratic elution was then continued for 6 min and, finally, initial conditions were re-established in 1 min and held for 10 min. The flow rate was 1 mL/min and injection volume 50 µL. The UV detector was set at 283 nm (Rada-Mendoza et al. 2004).

Sample preparation was performed with Carrez, according to sample preparation for analysis of carbohydrates by GC and the supernatant was passed through a filter of 0.45 mm (Waters) and then injected. Quantitation was carried out by the external standard method using a commercial standard of HMF (Sigma, St. Louis, MO, USA). Data were the mean values of duplicate expressed as mg/100 g of product.

Protein analysis by SDS-PAGE

For protein extraction, 100 mg of fresh, processed and stored pea and bean flours were mixed with 2 mL of 1% sodium metabisulfite (Merck, Darmstadt, Germany) aqueous solution. Next, samples were stirred thoroughly for 2 h and centrifuged at 3,000 ×g for 15 min. The supernatants were finally analysed by SDS-PAGE under non-reducing conditions.
Protein analysis was carried out using the method followed by Gamboa-Santos et al. (2012). Briefly, for SDS-PAGE analysis, 32.5 μL of sample supernatant was added to 12.5 μL of 4X NuPAGE LDS sample buffer (Invitrogen, Carlsbad, California, USA) and 5 μL of 0.5 M dithiothreitol (DTT, Sigma-Aldrich) and heated at 70 °C for 10 min. Samples (20 μL) were loaded on a 12% polyacrylamide Novex NuPAGE Bis-Tris precast gel; a continuous MES SDS running buffer was used. Gels were run for 40 min at 120 mA/gel and 200 V and stained using the Colloidal Blue Staining Kit (Invitrogen). The molecular weight of proteins was estimated by using a mixture of standard proteins with relative molecular weights ranging from 2.5 to 200 kDa (Invitrogen): myosin, 200 kDa; β-galactosidase, 116.3 kDa; phosphorylase B, 97.4 kDa; bovine serum albumin, 66 kDa; glutamic dehydrogenase, 55.4 kDa; lactate dehydrogenase, 36.5 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa; aprotinin, 6 kDa; insulin B chain, 3.5 kDa; and insulin A chain, 2.5 kDa.

Determination of antioxidant activity

The free-radical-scavenging activity of the different extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the procedure described by method of Huang et al. (2010). A volume of 0.3 mL of methanolic extract was mixed with a solution of 0.2 mM DPPH in methanol (2.7 mL). The mixture was shaken vigorously and allowed to stand for 1 h before the absorbance was measured at 517 nm. Radical-scavenging activity was calculated as the following percentage:

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\text{Radical scavenging activity} = \frac{As - Ai}{As} \times 100
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\(Ai = \text{absorbance of DPPH in the presence of various extracts.}\)
Analysis of α-amylase inhibitor

Amylase inhibitor activity was determined according to Dephande et al. (1982). Triplicate samples, weighing 1 g, were extracted in 10 mL distilled water for 12 h at 4 °C in a shaker. The extract was centrifuged at 5000 g for 10 min. The supernatant was incubated with α-amylase for 15 min at 37 °C, then starch was added to the mixture and this was incubated for a further 3 min at 37 °C. The reaction was stopped by adding 1 ml of dinitrosalicylic acid solution after 10 min. The contents were heated for 10 min in a boiling water bath. The absorbance of the colour developed was measured after cooling the test tubes against the blank at 540 nm using UV/VIS spectrophotometer (Power Wave XS Microplate, BIO-TEK) and the KC Junior Data Reduction software. The final results were compared with the activity of the same amount of enzyme without the inhibitor.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistic 22.0 for Windows. Univariate analysis of variance (ANOVA) and Tuckey test was also used to determine significant differences among the samples. The differences were considered significant when p < 0.05.

Results and discussion

Table 1 presents the values of dry matter (DM), aw, pH and protein, which were similar to those earlier for these pulses (Aguilera et al. 2009). The highest values of protein
were found in fava beans; cotyledons presented the highest contribution since fibre is the most important component in the hull. The pulses are important source of proteins, being one of the most valuable components ranged from 17 to 40 g/100 g. In fact, in many regions of the world, legumes are the main and cheapest protein supply in the diet, representing, after cereals, the second largest source of human food (Kaur et al. 2009).

Changes in the soluble fraction of carbohydrates

Taking into account the qualitative analysis of carbohydrates (Fig. 1), fructose, galactose, glucose, myo-inositol, sucrose, raffinose, stachyose and verbascose were present in all the pulses analysed. In addition, pinitol, a methyl-inositol with characteristic m/z 260, 305 and 318 ions was detected in all pulses. A small peak eluting after fructose (retention time 4.4 min) identified as chiro-inositol by comparison of its retention time and mass spectra with those of the commercial standard was also detected in lentils, chickpeas, fava beans and yellow peas. A chromatographic peak with similar mass spectra to pinitol but co-eluting with fructose (retention time 5.0 min) was detected in chickpeas and lentils. This peak was tentatively assigned to bornesitol as previously identified by Ruiz-Aceituno et al. (2013) in the latter. Moreover, different galactosyl-inositol (with characteristic m/z 129, 147, 204, 305, 361), galactosyl-methyl inositol (such as galactosyl-pinitol, with characteristic m/z 129, 133, 147, 204, 305, 361) and di-galactosyl-methyl-inositol (such as ciceritol) were detected in chickpeas and lentils, in agreement with Aguilera et al. (2009) and Ruiz-Aceituno et al. (2013). Only a glycosyl-inositol was found in fava beans. Citric acid (retention time 2.9 min, with characteristic m/z 273, 347, 363 and 375 ions) was also present in chickpeas, lentils and
beans. Fig. 1 depicts the carbohydrate profile obtained by GC-FID of the corresponding TMSO in chickpeas (A) and peas (B).

Table 2 shows the quantitative data of the main soluble carbohydrates obtained for the different pulse flours analysed: raw (all samples, including fava cotyledons and hull), toasted (lentils, peas) and after 19 days of storage under forced conditions, at 40 °C and 0.44 a_w (beans, peas). These conditions were selected to discover the possible evolution of the Maillard reaction (MR) and its effect on proteins and soluble carbohydrates resembling longer storage, because these products can be stored for more than one year at ambient temperature. In fava beans, as expected, most of the soluble carbohydrates were present in the cotyledons. Considering the data of raw materials, the highest value of total soluble carbohydrates was found in chickpeas (164.1 mg/g DM) and the lowest in lentils (101.3 mg/g DM). Total monosaccharides (fructose, glucose and galactose) were up to 10% of total soluble sugars, similarly to the data reported earlier (Slupski and Gebczynski 2014). Quantitatively, the most abundant was sucrose that occurred between 18.9 and 42.7 mg/g DM, and was the main carbohydrate in peas. According to Ekvall et al. (2006), a high concentration of sucrose in immature pulse seeds is desirable, giving a sweeter flavour. In the case of RFOs, the highest concentrations were observed in peas, beans and fava beans; in these, the quantitation of ajugose, the hexasaccharide derived from raffinose was also possible (Table 2). In beans, chickpeas and lentils, stachyose was the main oligosaccharide among RFOs, whereas in fava beans and peas the most abundant was verbascose.

Regarding cyclitols, different profiles and concentrations were detected. The highest value of pinitol (4.2 mg/g DM) was found in chickpea followed by lentils and bean. The amount of myo-inositol was near to 1 mg/g DM, with the exception of chickpeas and lentils. In these samples, myo-inositol exceeded 3 mg/g DM. Ciceritol
was the most abundant carbohydrate in chickpeas (44.2 mg/g DM) and was higher than that observed by Aguilera et al. (2009) for Spanish chickpea (27.6 mg/g DM).

Pulses with lower values of polyalcohols showed higher amount of RFOs. Obendorf and Górecki (2012) analysed the soluble fraction of pulse seeds and a negative correlation between verbascose and ciceritol was attributed to a metabolic competition between the synthesis pathways of RFOs and galactosyl cyclitols.

The levels of individual oligosaccharides and their proportion in the total content depend not only on type, species and cultivar, but also on the maturity of seeds and environmental conditions. According to Ekvall et al. (2006), in green peas, verbascose was the most abundant at the beginning of maturity and raffinose in more mature seeds. In immature seeds of peas, lentils, pigeon peas, chickpeas and cowpea, stachyose occurred in larger amounts than the remaining galactosides (Aguilera et al. 2009). Moreover, as mentioned by Obendorf and Górecki (2012), pulse seeds could accumulate sucrose early in development; whereas seeds tend to accumulate RFOs and/or galactosyl cyclitols during maturation and desiccation. Thus, according to all these hypotheses, it is presumable that the seeds used to produce the flours analysed mentioned herein were at the initial steps of maturation. In agreement with this, sorbitol was not detected in any of the pulse analysed. It has been claimed that this compound can be accumulated during maturation of stored pea seeds (Lahuta et al. 2007).

The effect of processing and storage of pulse grain on the carbohydrate composition is shown in Table 2. The most striking feature was the increase in the total content of RFOs, mainly raffinose, after toasting of lentil and pea (by 20%). This may be caused by the release of oligosaccharides bound to proteins and other macromolecules or to the hydrolysis of high molecular weight polysaccharides.
(Martinez-Villaluenga et al. 2008). Toasted lentil presented a similar increase for galactosyl cyclitols, although it was only significant for galactosyl-pinitol.

During storage, no significant change observed in total RFOs and polyalcohols, only peas seemed to be more susceptible to sucrose hydrolysis; this change was also observed in toasted peas. With respect to fructose, galactose and glucose content after toasting of lentil and pea flours, the three monosaccharides almost disappeared by their interaction with amino-groups of amino acids, peptides and proteins during the MR. Bean and pea decreased in fructose, galactose and glucose content during storage, although in peas the decrease in glucose was not significant. This may be attributed to hydrolysis of sucrose to glucose and fructose, both likely to participate in the MR. Moreover, glucose may be released from starch, as indicated by Martin-Cabrejas et al. (2006).

Assessment of Maillard reaction evolution

With the aim of discovering the implication of lysine in the MR during processing and storage of pulse flours, an evaluation of initial steps of MR was carried out throughout the determination of furosine (formed after acid hydrolysis of Amadori products), the main quality indicator of the severity of thermal treatments and inadequate storage conditions in powder products. Table 1 and Fig. 2 show the corresponding results for the determination of furosine in raw, processed and stored samples. All raw flours had formation of furosine which ranged 4.2 to 18.8 mg furosine/100 g protein. Chickpeas presented the highest value, probably due to the high content of carbohydrates (164.1 mg/g DM). Furosine content in chickpea flour was lower than that reported by Bignardi, et al. (2012) for a similar commercial product (108 mg/100 g protein).
Rodriguez et al. (2007) found 21.5 and 6.3 mg furosine/100 g protein in Spanish bean and lentil seeds, respectively, and non-detectable amounts in peas. These differences may be ascribed to the variability of the samples and the different analytical methods used for the determination of furosine. According to Murthy et al. (2003) the content of Amadori products in raw seed axes increased during early stages of storage, whereas the content of more advanced MR products increases steadily throughout storage, indicating a decline in seed vigour. Since the amount of furosine found in the pulse flours analysed herein were not high, it is presumable that the seeds used for their preparation were in the first period after collecting, in agreement with the data of carbohydrates mentioned above.

Significant differences in furosine content were observed as a result of processing and storage. Toasting gave rise to the highest advance of MR, in particular in peas (240% and 160%), probably ascribable to having the highest content of monosaccharides (17.8 mg/g DM), as compared to lentil and bean (≤14 mg/g DM). This is in concordance with the significant decrease in monosaccharides observed after toasting (Table 2) and the diffuse protein profile (Fig. 3B) indicated below. To the best of our knowledge, hardly any information is available in regard to the effect of processing and storage of pulse flour on the loss of lysine due to the MR by means of furosine determination. Arnoldi et al. (2007) reported values in the range of 10.2-110.5 mg furosine/100 g protein in different lupin protein isolates produced in a spray drying pilot plant.

MR was also related to hydroxymethyl furfural (HMF), formed during the intermediate steps of the reaction and/or derived from the dehydration of hexoses (Rada-Mendoza et al. 2004). The results clearly showed that HMF was formed only during toasting (lentil 28.3 mg/100 g DM and peas 50.7 mg/100 g DM) since, in the
other pulses, the amount of HMF was lower than 1 mg/100 g. Lower values of furosine, HMF formation was probably caused by the dehydration of hexoses; thus, in peas (15 mg of hexoses/g DM), the HMF concentration was higher than in lentils (10 mg of hexoses/g DM). In soy flour heated in an oven at 180 °C for 10 min, HMF and furosine contents were reported to be 1.25 mg/100 g and 45 mg/100 g protein, respectively (Rufian-Henares et al. 2009). This fact could be explained by the type of toasting at temperatures close to 300 °C, and as HMF can be a dehydration product, its formation might be better developed in dried samples at high temperatures.

Evaluation of the protein profile in raw, toasted and storage samples

In order to evaluate the changes occurring in pulse protein profiles during storage and processing, non-treated pea and bean flours, stored for 2, 8 and 19 days and toasted (in the case of peas) were analysed by SDS-PAGE (Fig. 3).

As observed in Fig. 3A, fresh bean flour (lane 1) presented a profile very similar to that found by other authors (Rui et al. 2011), consisting of three predominant bands, with an estimated molecular weight (Mw) of 97, 49 and 31 kDa (bands A, C and E, respectively), and several minor bands around 60, 47, 25, 21 and 17 kDa (bands B, D, F, G and H, respectively). Bands C, D, F and G were identified as 7S globulins (phaseolins), which were previously confirmed to be one of the main protein fractions in different varieties of field beans (Meng and Ma 2001; Rui et al. 2011). Other proteins also largely present in beans are the anti-nutritional proteins phytohaemagglutinins. They have a Mw ranging from 27 to 37 kDa (Makri and Doxastakis 2006), so band E might be identified as one of them. Likewise, according to protein profiles obtained by Rui et al. (2011), band H could be assigned to the β-subunit of α-amylase inhibitor.
Band A, with the highest Mw (97 kDa), could correspond to 7S globulins, based on protein profiles of other pulses such as peas. Likewise, the 60 kDa fraction has been described as a possible breakdown product of 11S legumins in other beans (Meng and Ma 2001). With respect to the stored flours (lanes 2-4), similar electrophoretic profiles in comparison to the initial sample (lane 1) were found and no protein aggregates of high molecular weight were observed. This is indicative of the scant degree of protein degradation during storage.

Similarly, to fresh bean flour, the protein profile obtained for fresh pea flour (Fig. 3B, lane 1) mainly consisted of 7S globulins (vicilins) (bands C, E, F, G), according to that found by Rubio et al. (2014), similarly to other pulses as lentils (Ghumman et al. 2016a). Moreover, band H was identified as a protease inhibitor. With the storage time, an increase in intensity of bands A-D and F is observed, which reached a maximum after 19 days, likely due to vicilin accumulation in seeds during storage. However, no important changes in the protein profile were observed, indicating no protein degradation occurred during storage. The most striking result was observed for toasted pea flour (lane 5), which showed a variety of diffuse bands with different molecular weights corresponding to a wide range of glycated species of proteins resulting from unfolding, cross-linking, and aggregation of proteins taking place during the advanced stages of the MR. Other authors (Ghumman et al. 2016b) observed that proteins present in lentil and horsegram disappeared or decreased with the increase of temperature (100 -125 °C) during the extrusion process.

Antioxidant activity
Table 1 and Fig. 2 present the results of the antioxidant activity of the raw and processed flours measured as the scavenging activity against DPPH radicals. As observed, the percent of inhibition in the raw material was in a wide range (4.8-41.9%), beans and fava beans had the highest values, probably due to the plant source and environmental factors, among others, which can affect the composition of the seeds. Flour made from hulls of fava beans presented the highest value of antioxidant activity (96.4%). As is known, pulse seeds contain a wide variety of bioactive non-nutrient components, including phenols, dominantly located in the seed coat since they play a relevant role in the defence system of the pulse seed (Troszynska and Ciska 2002).

Different studies have been carried out on the antioxidant activity of extracts from common pulses. Zhao et al. (2014) found that the DPPH free radical scavenging activity of ethanolic extract of lentil flour was 38.5%, similar to our result, and this value was significantly higher than that of peas, beans and chickpeas. These authors also have studied the positive correlation \( r = 0.84 \) between this bioactive property and the total polyphenolic content.

Toasting of lentil and pea flours caused a significant increase in the % of inhibition of the scavenging activity. Although less pronounced, storage under controlled conditions of bean and pea flours also gave rise to more antioxidant activity. This may be attributed to a better extraction of phenolic compounds after processing of legumes. Stanisavljevic et al. (2013) found that the processing of pea flour by cooking at 100 °C for 45 min strongly enhances the release of phenolic compounds and the methanolic extracts were more efficient in scavenging DPPH free radicals than the corresponding the raw material. Moreover, Huber et al. (2014) found that cooking at 121 °C for 10 min increased the antioxidant activity and the concentration of phenolic compounds in white beans. Roasting of fava beans initially lowered the bean
antioxidant capacity, whereas prolonged roasting at 150 °C for 60 min caused generation of new phenolic compounds and increased antioxidant activity (Siah et al. 2014). In addition to this, it was necessary to consider that the highest increase in the scavenging activity against DPPH radicals was detected after processing and storage of peas, which was the sample with the highest MR advance, highlighted by the content of furosine and HMF. Therefore, the contribution of this reaction to the increase in the antioxidant activity should not be ruled out. Zhao et al. (2013) found that HMF exhibited antioxidant activity by scavenging the DPPH free radicals in a dose-dependent manner. Fares and Menga (2012) observed an increase in the antioxidant activity in toasted chickpea flour and they ascribed this result not only to the total polyphenol content but also to Amadori and Heyn’s products between reducing sugars and proteins.

α-Amylase inhibitor

Pulses rich in α-amylase inhibitors have received more attention since this enzyme inhibits the digestion and absorption of dietary carbohydrates controlling the postprandial hyperglycaemia. However, it is necessary to consider that an excessive inhibition of α-amylase could result in abnormal bacterial fermentation of undigested carbohydrates in the colon (Oboh et al. 2012).

In this study, we also evaluated the inhibition of α-amylase in the different pulse flours analysed. As can be observed in Table 1 and Fig. 2, values of α-amylase inhibition were in a quite narrow range (34.0-42.1%). After toasting of lentil flour (Fig. 2), a significant decrease of inhibition was detected, however, in peas and bean flours the toasting and storage did not give rise to this effect, and a higher (not always significant) activity of α-amylase inhibitor was observed as a result of processing. This
could be ascribed to the different nature of sources, among other factors. α-Amylase inhibition can be due to polyphenolic compounds and/or peptides or proteins with different isoforms. In some cases, after thermal treatments and, depending on the intensity of the processing, the α-amylase inhibition can be increased or decrease. Thus, the autoclaving of velvet bean seeds was very efficient in reducing this activity due to the denaturation of the inhibitor (Vadivel and Pugalenthi 2009). However, in black and pinto beans the α-amylase inhibition increased with microwave treatment (Oomah et al. 2014), and this result was attributed to the presence of heat-stable amylase inhibitors. These authors also demonstrated that the amount and inhibition potency of α-amylase in beans are influenced by heat treatment depending on cultivar/market class.

**Conclusions**

In view of the results obtained, and taking into consideration the parameters studied (protein, carbohydrates, MR indicators, antioxidant activity, α-amylase inhibition), it can be concluded that the raw pulse flours (lentils, peas, beans, chickpeas, fava beans) from Algeria analysed in this study present a very good quality, similar to that of legumes from other origins. Considering carbohydrates and, more specifically RFOs, it seems that the pulses were at the beginning of the maturity period. Toasting and storage under controlled conditions did not modify to any great extent pulse quality, including loss of lysine due to the MR, but rather had a beneficial effect on the increase of antioxidant activity, perhaps also caused by HMF formation. The characterization carried out in this study points out the suitability of the flours studied for preparation of different foodstuffs intended for a broad spectrum of the population, including people who are hyperglycaemic and intolerant to gluten.
Acknowledgements

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References


**Figure caption:**


**Fig. 2.** Values of furosine (mg/100 g protein), antioxidant activity (AA, %) and α-amylase inhibitor (AAI, %) in flour flours of lentils, peas and beans raw, after toasting and during the storage at 40°C and 0.44 aw for 29 days. a–d Different case letters indicate statistically significant (p < 0.01) differences for each parameter considering rows. (St: storage).

**Fig. 3.** SDS-PAGE analysis of (A) bean samples, including raw (lane 1) and stored flours for 2 (lane 2), 8 (lane 3) and 19 (lane 4) days; and (B) pea samples, including raw (lane 1), stored for 2 (lane 2), 8 (lane 3) and 19 (lane 4), and roasted flours (lane 5). M, markers of molecular weight. Letters (A-I) represent electrophoretic bands corresponding to different bean and pea proteins.
Figure 2.
Figure 3.
Table 1. Physicochemical characterization and furosine content (mg/100 g protein), antioxidant activity (AA, %) and α-amylase inhibitor (AAI, %) of raw pulse flours from Algeria.

<table>
<thead>
<tr>
<th>Samples</th>
<th>DM (^1)</th>
<th>(a_v) (^1)</th>
<th>pH (^1)</th>
<th>Protein (^1)</th>
<th>Furosine (^2)</th>
<th>AA (^2)</th>
<th>AAI (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickpeas</td>
<td>90.4</td>
<td>0.55</td>
<td>6.15</td>
<td>20.1</td>
<td>18.8 (0.5)</td>
<td>26.3 (0.6)</td>
<td>34.0 (2.0)</td>
</tr>
<tr>
<td>Faba</td>
<td>89.7</td>
<td>0.57</td>
<td>6.13</td>
<td>26.3</td>
<td>9.4 (1.0)</td>
<td>40.6 (0.9)</td>
<td>41.7 (1.4)</td>
</tr>
<tr>
<td>Faba cotyledons</td>
<td>89.9</td>
<td>0.54</td>
<td>6.42</td>
<td>29.3</td>
<td>7.7 (0.2)</td>
<td>13.4 (0.5)</td>
<td>25.9 (1.6)</td>
</tr>
<tr>
<td>Faba hull</td>
<td>91.2</td>
<td>0.46</td>
<td>5.94</td>
<td>4.9</td>
<td></td>
<td>96.4 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Beans</td>
<td>90.1</td>
<td>0.52</td>
<td>6.12</td>
<td>21.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentils</td>
<td>89.6</td>
<td>0.54</td>
<td>6.08</td>
<td>20.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peas</td>
<td>90.0</td>
<td>0.52</td>
<td>6.10</td>
<td>20.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Values are expressed as mean, relative standard deviations were lower than 5%. \(^2\) Values are expressed as mean (standard deviations) and were calculated from 2 extract and 2 analyses (n=4).
Table 2. Soluble carbohydrate composition of pulse flours (raw, toasted or stored). Values are expressed as mean concentrations (mg/g of dry matter ± standard deviations) and were calculated from 2 extract and 2 analyses (n=4).

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Chickpeas</th>
<th>Faba Beans</th>
<th>Lentils</th>
<th>Peas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed</td>
<td>Cotyledon</td>
<td>Hull</td>
<td>Raw</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Stored (29 days)</td>
</tr>
<tr>
<td>Citric acid</td>
<td>4.21 ± 0.05</td>
<td>2.66 ± 0.23</td>
<td>3.74 ± 0.22</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>Pinitol</td>
<td>3.91 ± 0.09</td>
<td>2.91 ± 0.32</td>
<td>1.88 ± 0.15</td>
<td>5.55 ± 0.27</td>
</tr>
<tr>
<td>Fructose</td>
<td>40.2 ± 0.24</td>
<td>5.67 ± 0.13</td>
<td>5.85 ± 0.21</td>
<td>3.23 ± 0.20</td>
</tr>
<tr>
<td>Galactose</td>
<td>9.14 ± 0.70</td>
<td>5.76 ± 0.18</td>
<td>4.67 ± 0.18</td>
<td>1.96 ± 0.18</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.67 ± 0.18</td>
<td>9.63 ± 0.32</td>
<td>9.51 ± 0.36</td>
<td>5.39 ± 0.04</td>
</tr>
<tr>
<td>Myo1</td>
<td>3.30 ± 0.37</td>
<td>1.10 ± 0.06</td>
<td>1.08 ± 0.02</td>
<td>0.69 ± 0.03</td>
</tr>
<tr>
<td>Sucrose</td>
<td>42.70 ± 1.98</td>
<td>22.76 ± 0.38</td>
<td>34.30 ± 0.29</td>
<td>1.08 ± 0.03</td>
</tr>
<tr>
<td>Ga-Pn2</td>
<td>4.45 ± 0.29</td>
<td>10.5 ± 0.25</td>
<td>10.48 ± 0.09</td>
<td>2.3 ± 0.12</td>
</tr>
<tr>
<td>Dic3</td>
<td>6.92 ± 0.31</td>
<td>1.10 ± 0.05</td>
<td>1.48 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Raffinose</td>
<td>6.92 ± 0.31</td>
<td>1.10 ± 0.05</td>
<td>1.48 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Cic4</td>
<td>44.17 ± 3.68</td>
<td>13.69 ± 0.86</td>
<td>17.26 ± 1.36</td>
<td>0.75 ± 0.08</td>
</tr>
<tr>
<td>2Ga-In5</td>
<td>3.18 ± 0.31</td>
<td>1.62 ± 0.08</td>
<td>2.13 ± 0.04</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Stachyose</td>
<td>29.36 ± 2.76</td>
<td>16.9 ± 0.86</td>
<td>17.26 ± 1.36</td>
<td>0.75 ± 0.08</td>
</tr>
<tr>
<td>Ga-Ci6</td>
<td>6.15 ± 0.76</td>
<td>36.71 ± 3.53</td>
<td>40.43 ± 4.63</td>
<td>1.56 ± 0.35</td>
</tr>
<tr>
<td>Verbacose</td>
<td>1.21 ± 0.12</td>
<td>1.25 ± 0.15</td>
<td>0.14 ± 0.03</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Ajugose</td>
<td>0.53 ± 0.03</td>
<td>1.25 ± 0.15</td>
<td>0.14 ± 0.03</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Total8</td>
<td>164.1</td>
<td>112.2</td>
<td>127.3</td>
<td>9.7</td>
</tr>
<tr>
<td>RFOs8</td>
<td>37.5</td>
<td>52.0a</td>
<td>60.4a</td>
<td>2.5b</td>
</tr>
<tr>
<td>Polyol9</td>
<td>61</td>
<td>3.8a</td>
<td>4.8a</td>
<td>1.1b</td>
</tr>
</tbody>
</table>

*Different letters indicate significant differences considering the same legume (P<0.05). ** Coelution fructose with bornesitol. 1 Myo-inositol, 2 Galactosyl-pinitol, 3 No identified disaccharides, 4 Ciceritol, 5 Di-galactosyl-methyl-inositol, 6 Galactosyl-ciceritol, 7 Total quantified carbohydrates, 8 Total raffinose family oligosaccharides, 9 Total polyol.