Nutrients and non-nutrients composition and *in vitro* starch digestibility of five Algerian legume seed flours

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Abstract

Lentil (*Lens culinarius* L.), faba bean (*Vicia faba* L.), chick pea (*Cicer arietinum* L.), common bean (*Phaseolus vulgaris* L.) and yellow pea (*Pisum sativum* L.) seeds produced in Algeria were evaluated for physico-chemical properties, chemical composition, *in vitro* starch digestibility and phytate and protease inhibitor content. Significant differences were observed in bulk density (0.794-1.293 g/mL), hydration capacity (0.030-0.362 g/seed), hydration index (0.704-0.937), swelling capacity (0.032-0.353 mL/seed) and swelling index (0.488-1.087). The crude protein content ranged between 20.10 and 26.37%. *V. faba* and *P. sativum* seed flours had comparatively higher total non-starch polysaccharides (NSP) contents, with mean values of 172.26 and 192.51 mg/g, respectively. Among the minerals of nutritional interest, seed meals were rich in potassium (838.56-285.43 mg/100g) and magnesium (46.20-77.33 mg/100g). Significant differences were determined in *in vitro* starch digestibility. *V. faba* flour was hydrolysed more slowly than the other legumes. The amount of slowly digestible starch (SDS) in *V. faba* flour was the highest among these legume flours, but also had the lowest resistant starch (RS) content. Trypsin and chymotrypsin inhibitor contents of the different samples ranged from 2.27 to 16.22 TIU/g, and from 1.77 to 27.15 CIU/mg, respectively. Protease inhibitor content was significantly higher in common bean, while pea and faba bean showed the lowest TIU/mg and CIU/mg content. The total amino acids content was 190.42-223.33 mg/g, and the total essential amino acids content was 74.82-84.77 mg/g. The tannin content of faba beans and peas was the lowest, while the amount in common beans was the highest. The potential nutritional implications of these results are discussed. The present work demonstrated that chick pea, lentil, faba bean, common bean and pea whole flours have a great potential as nutritious and healthy food ingredients.

Abbreviations

CIU, chymotrypsin inhibitor units;NSP, non-starch polysaccharides; RDS, rapidly digestible starch; RS, resistant starch; SDS, slowly digestible starch; TIU, trypsin inhibitor units; TS, total starch.

Introduction

Developing countries are facing an increasing demand for protein-rich foods due to increasing population, cereal-based diet and scarcity of fertile land (Seena and Sridhar, 2006), and under-exploited legumes are being explored as alternative protein crops for the future (Martín-Cabrejas *et al.*, 2008). Grain legumes are considered nutritious and healthy food ingredients for a balanced human diet, particularly in combination with cereals. In addition, legumes are also known for their properties in assisting with disease prevention, including cardiovascular diseases, type 2 diabetes, obesity and possibly colon cancer. Carbohydrates and proteins are the main macro-nutrients provided by seed legumes;

the contribution to fat intake is usually negligible, and vitamins as well as dietary fibre are usually present in substantial amounts. Grain legumes are consumed as raw vegetables after simple processing in some cases, and in salads or soups often after heat processing (Guillon and Champ, 2002).

Carbohydrates constitute the main fraction of grain legumes, accounting for up to 55-65% of the dry matter. Starch and non-starch polysaccharides (NSP) are the major constituents of the legume carbohydrate fraction, and oligosaccharides constitute a smaller but significant fraction (Knudsen, 1997; Bravo *et al.*, 1998). Pulses are among foods characterised by a relatively low glycaemic index, which is considered beneficial in reducing postprandial blood glucose and insulin responses; therefore, legumes are specially indicated in diets for people with insulin-dependent diabetes (type 2). Vegetarian diets high in grain legumes have been reported to reduce the incidence of digestive tract cancers by reducing the consumption of saturated fats and increasing the content of unavailable carbohydrates in the diet (Aranda *et al.*, 2001). The protein content of legume grains ranges from 17 to 40 g/100 g, much higher than that in cereals (7-13 g/100 g), and approximately equal to the protein content of meat (18-25 g/100 g) (De Almeida Costa *et al.*, 2006). Legumes enhance the protein content of cereal-based diets and improve their nutritional status because cereal proteins are deficient in certain essential amino acids, particularly lysine (Iqbal *et al.*, 2003). In turn, legumes are known to contain adequate amounts of lysine, but are deficient in S-containing amino acids (methionine and cysteine), which are usually higher in cereals (Farzana and Khalil, 1999).

The national production of legumes does not satisfy the needs of entire Algerian population. Increased surfaces are reserved each year to dry legumes in order to reduce imports, but yields remain too low; according to the agricultural services of the Ministry of Agriculture and Fisheries, they range between 0.4 and 1.4 ton/ha (Amir *et al.*, 2007). The objective of the present work was to quantify some physico-chemical and compositional parameters of five legume seed flours, namely chick pea, lentil, faba bean, pea and common bean grown in Algeria. In addition, information on parameters not usually reported such as *in vitro* starch digestibility, including nutritionally important starch fractions (rapidly digestible, slowly digestible, and resistant starches) and protease inhibitor determinations, were also included. The goal of legume seed production is human and animal nutrition. Therefore, it is very relevant to know not only productive parameters of these feed- or foodstuffs, but also their potential nutritional value. Accordingly, as a first step in this direction, our aim was to chemically characterise these Algerian legume seeds by using appropriate chemical analyses.

Materials and methods

Materials

Five grain legumes produced in Algeria were selected according to their consumption indices. Dry seeds were obtained from three locations: lentil (*Lens culinary* L.) and yellow peas (*Pisum sativum* L.) were from ITGC (Institut Technologique des Grandes Cultures) of Setif, Algeria; chick pea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.) were from Merdj Ouaman, Wilaya of Bejaia, Algeria, and common bean (*Phaseolus vulgaris* L.) was from Jijel, Algeria. Seed samples were divided into two equal portions; one portion was used for investigating physical characteristics, and the other was ground to a fine powder by using a coffee grinder followed by an analytic mill (IKA A11 basic; IKA Werke GmbH and Co. KG, Staufen, Germany) to pass through a 0.5 mm mesh sieve. This portion was used for chemical analysis. All samples were kept in airtight plastic containers at 4°C until further use.

Physical characteristics of the seeds

Seed weight, dimensions, volume, density, hydration capacity, hydration index, swelling capacity and swelling index were determined following the procedures described by Williams *et al.* (1983) and Bishnoi and Khetarpaul (1993).

Weight and dimensions

Seeds (100 seeds) were tested for seed weight (g) and seed dimensions (cm). Each seed was weighed on a balance with 0.001 g precision. Linear dimensions, [length (L), width (W) and thickness (T)] were measured by using a digital calliper with a sensitivity of 0.001 mm. The average of three replicates of 100 seeds each was taken as the mean for each parameter.

Volume and density

Seeds (100 g) were accurately weighed and transferred to a 200 mL graduated cylinder, into which 100 mL deionised water were added, and the volume of the displaced water was recorded. Density was calculated as the weight of seeds divided by the volume (g/mL).

Hydration capacity and hydration index

Seeds (100 g) were counted and transferred to a graduated cylinder, along with 100 mL water. The cylinder was covered with aluminium foil and left at room temperature. After 24 h, the seeds were drained, superfluous water was removed with filter paper and swollen seeds separated and reweighed. The following parameters were calculated for each seed type:

Hydration capacity per seed = (Weight of soaked seeds - Weight of seeds before soaking) / (Number of seeds)   (Eq. 1)

Hydration index = (Hydration capacity per seed) / (Mean weight)   (Eq. 2)

Swelling capacity and swelling index

Seeds (100 g) were counted, and their volume annotated after soaking in distilled water in a graduated cylinder. Seeds were soaked for 24 h in distilled water, and their volume measured again. Swelling capacity and swelling index ware calculated as follows:

Swelling capacity = (Volume after soaking - Volume before soaking) / (Number of seeds)   (Eq. 3)

Swelling index = Swelling capacity / Mean volume   (Eq. 4)

Chemical analyses

The contents of moisture, ash, crude protein (N × 6.25) and crude fat were determined following the methods of Association of Official Analytical Chemists (AOAC, 1990). The total N content of the samples was determined by using a combustion N analysis procedure (N analyser Flash EA® 1122; Thermo Scientific, West Palm Beach, FL, USA) with EDTA as standard. Amino acids were determined as in Rubio (2003). Briefly, flour samples were subjected to protein hydrolysis in 6 M HCl plus 1% phenol in sealed tubes at 110°C for 24 h, by HPLC according to the Waters Pico Tag method, using pre-column derivatisation with phenylisothiocianate and a Waters 2695 separation module (Waters Cromatografía, S. A., Madrid, Spain). A Millenium 32 chromatography manager system (Waters Cromatografía, S. A., Madrid, Spain) was used for gradient control and data processing. Cysteine and methionine were determined as cysteic acid and methionine sulphone, respectively, obtained by oxidation with performic acid before 6 M HCl hydrolysis. Tryptophan was

not determined. Total sugars were extracted in $HClO_4$ (52%, w:v) based on the procedure described by Osborne and Voogt (1978). Anthrone solution (5 mL, 0.1% in concentrated H2SO4) was added to 2 mL of sample extract. Samples were kept in a boiling water bath for 12 min, and absorbance was measured at 630 nm on a UV/Vis Spectrometer. The gross energy (GE) of the legume flours was determined in a bomb calorimeter (Parr 1356 bomb calorimeter, Parr Instruments Co., Moline, IL) using benzoic acid as a calibration standard. Total starch content was determined in accordance to AACC Approved Method 76-13 (American Association of Cereal Chemists) (AACC, 2000). Starch was firstly solubilised and partially hydrolysed to dextrins. In the second phase, the resulting starch dextrins were quantitatively hydrolysed to glucose by amyloglucosidase, and total glucose measured by using the glucose-oxidase/peroxidase procedure and UV-visible spectrophotometer at 510 nm. NSP analysis was carried out by GLC following the method of Englyst *et al.* (1992b). Samples for mineral analysis were digested by using concentrated nitric acid and perchloric acid (1:1, v/v) according to Thompson and Wagstaff (1980). Sodium, potassium, calcium, magnesium, copper, iron, zinc and manganese were determined by atomic absorption spectrophotometry (Perkin-Elmer). Phosphorus content of the digest was determined calorimetrically based on the method described by Nahapetian and Bassiri (1975).

In vitro starch digestibility

In vitro starch digestibility was determined following the AACC (2000) method 32-40 with minor modifications. Legume flours (100 g) were incubated with porcine pancreatic amylase (10 mg) (No. 7545, Sigma-Aldrich, St. Louis, MO) and amyloglucosidase (12 U) (EC 3.2.1.3) in 4 mL of 0.1 M sodium maleate buffer (pH 6.0) in a shaking water bath (200 strokes/min) at 37°C (0.5-16 h). Following incubation, ethanol (95%) was added and the sample was centrifuged at 2,000 rpm for 10 min. Total glucose (TG) content was measured by using a glucose oxidase peroxidase (GOPOD) diagnostic kit (Megazyme International, Ireland). The rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and total starch (TS) were calculated according to Englyst *et al.* (1992a) according to the expressions:

RDS = G0.5 × 0.9   (Eq. 5)
SDS= (G15.5 - G0.5) × 0.9   (Eq. 6)
TS = TG × 0.9   (Eq. 7)
RS = TS - (RDS + SDS)   (Eq. 8)

where G0.5 and G15.5 = amounts of glucose liberated after incubation with amylase and amyloglucosidase for 0.5 h and a further 15.5 h, respectively.

Determinations of protease inhibitors and condensed tannins

Trypsin inhibitor was determined using a-N-benzoyl-DL-arginine p nitroanilide hydrochloride (BAPNA) as the trypsin substrate. Trypsin inhibitor activity (TIA), expressed as TIU/mg protein, was calculated from the absorbance read at 410 nm against the reagent blank. One TIU is defined as a decrease in A410 by 0.01 in 10 min using the large-scale assay (Kakade *et al.*, 1974). Chymotrypsin inhibitory activity (CIA) was measured by using N-benzoyl-L-tyrosine ethyl ester (BTEE) as specific substrate. A 35 µL sample of a solution of chymotrypsin (0.02 mg/mL in 1 mM HCl) was incubated with appropriate quantities of protease inhibitor (PI) for 2 min at 30°C, and 500 µL of 0.1 M Tris–HCl, pH 7.8, containing 0.1 M $CaCl_2$. BTEE (500 µL of 1 mM) was added to the mixture, and CIA was determined by following the change in absorbance at 256 nm over 5 min. One chymotrypsin inhibitor unit (CIU) was defined as that which would give a reduction in absorbance of 0.01 at 256 nm relative to chymotrypsin control reactions, in a defined assay volume (10 mL) (Clemente *et al.*, 2004). Phytic acid analysis was performed following the method of Latta and Eskin (1980), by using the Wade reagent (0.03% iron (III) chloride, 0.3% sulfosalicylic acid). Phytic acid (dodecasodium salt) from corn was supplied by Sigma (USA) and used as a standard. The condensed tannins were determined using Vanilin-HCl method as described by Osman (2004).

Statistical analysis

All analysis was conducted in triplicate, and the values reported as mean ± SD. Data were subjected to a statistical analysis (Statistica v.5.0 software). Significant differences between means were calculated by one-way analysis of variance (ANOVA) using planned comparisons (LSD) test at $p < 0.05$.

Results and discussion

The weight of 100 seeds of the legumes ranged between 4.46-70.08 g, with *L. culinaris* and *V. faba* being lighter and heavier, respectively (Table 1). The average length and width of the seeds also ranged between 4.85 mm (L) and 2.85 mm (W), and between 14.26 mm (L) and 9.90 mm (W) for *L. culinaris* and *P. vulgaris*, respectively. The density, hydration capacity, hydration index, swelling capacity and swelling index also showed a wide range of values depending on the legume type (Table 1). The density of five seeds of legumes varied from 0.79 to 1.31 g/mL, the highest being *C. arietinum* and the lowest being *P. sativum*. *C. arietinum* had a significantly ($p < 0.05$) higher density followed by *L. culinaris*, *V. faba*, and *P. vulgaris*. *L. culinaris* had the lowest hydration capacity, hydration index, and swelling capacity and index. This might be a reflection of the relative hardness and impermeability of the lentil seed´s coat as compared to the other seeds. *C. arietinum* had the highest hydration capacity and swelling index followed by *P vulgaris*. Hence, they would require less cooking and germination time, and these characteristics might also influence the preference of consumers and processors for these seeds (Akinyele *et al.*, 1986; Bishnoi and Khetarpaul, 1993).

The chemical composition of the legume seeds is shown in Table 2. The crude protein content of these legumes ranged from 20.10 to 26.37% (Table 2) and significant differences ($p < 0.05$) were observed among seed types. The highest crude protein content was found in faba beans (26.37%). The chick pea seeds were found to be rich in fat (7.75%) and total carbohydrate (64.65%). These values were similar to those reported by Mohan and Janardhanan

Table 1. Seed mass, length, width, bulk density, hydration capacity, hydration, swelling capacity and swelling index.

Parameter	Chick pea	Lentil	Faba bean	Common bean	Pea
Seed mass (g/100 seeds)	46.65 ± 0.81[c]	4.46 ± 0.05[e]	71.34 ± 3.48[a]	52.86 ± 1.97[b]	22.85 ± 0.31[d]
Length (mm)	10.37 ± 0.15[c]	4.86 ± 0.02[e]	13.72 ± 0.10[b]	14.27 ± 0.20[a]	6.46 ± 0.13[d]
Width (mm)	8.14 ± 0.06[d]	n.[d].	9.74 ± 0.15[a]	9.91 ± 0.19[a]	7.05 ± 0.09[c]
Bulk density (g/mL)	1.31 ± 0.03[a]	1.25 ± 0.00[b]	1.22 ± 0.00[c]	1.04 ± 0.00[d]	0.79 ± 0.00[e]
Hydration capacity (g/seed)	0.33 ± 0.00[b]	0.03 ± 0.00[e]	0.30 ± 0.00[c]	0.36 ± 0.00[a]	0.21 ± 0.01[d]
Hydration index	0.81 ± 0.02[b]	0.70 ± 0.01[c]	0.51 ± 0.03[d]	0.71 ± 0.00[c]	0.94 ± 0.00[a]
Swelling capacity (mL/seed)	0.35 ± 0.02[a]	0.03 ± 0.00[d]	0.31 ± 0.02[b]	0.35 ± 0.00[a]	0.22 ± 0.00[c]
Swelling index	1.09 ± 0.03[a]	0.93 ± 0.00[b]	0.64 ± 0.05[d]	0.78 ± 0.04[c]	0.49 ± 0.00[e]

Data are means ± SD of three determinations ($n = 3$). Data with different superscript letters within the same row differ significantly ($p < 0.05$). n.d.: not determined.

Table 2. Biochemical composition (g/100 g) and gross energy (kcal/kg) of legume seeds.

Parameter	Chick pea	Lentil	Faba bean	Common bean	Pea
Moisture	0.72 ± 0.58[c]	11.17 ± 0.39[b]	12.35 ± 0.30[a]	11.06 ± 0.46[b]	9.22 ± 0.44[d]
Ash	3.01 ± 0.26[b]	1.93 ± 0.35[c]	3.14 ± 0.16[b]	4.53 ± 0.05[a]	2.98 ± 0.14[b]
Fat	7.75 ± 0.13[a]	2.51 ± 0.1[d]	3.24 ± 0.02[c]	4.61 ± 0.00[b]	2.87 ± 0.29[d]
Carbohydrate	64.65 ± 1.91[a]	62.03 ± 0.30[b]	59.72 ± 2.84[c]	61.95 ± 1.11[c]	65.47 ± 0.68[a]
Protein	20.10 ± 0.20[c]	20.39 ± 0.19[c]	26.37 ± 0.29[a]	21.85 ± 0.20[b]	20.48 ± 0.51[c]
Crude fibre	2.48 ± 0.16[d]	2.89 ± 0.21[d]	7.71 ± 0.13[a]	3.51 ± 0.18[c]	5.46 ± 0.12[b]
Total starch	42.29 ± 0.29[c]	43.44 ± 0.31[b]	37.65 ± 0.22[e]	38.89 ± 0.39[d]	44.40 ± 0.41[a]
Gross energy	5,214.22 ± 21.99[a]	4,978.73 ± 11.38[b]	4,943.85 ± 8.05[cd]	4,954.85 ± 12.22[bc]	4,927.19 ± 9.51[d]

Data are means ± SD of three determinations (n = 3). Data with different superscript letters within the same row differ significantly ($p < 0.05$).

(1994). The amino acid profile of the legume flours is presented in Table 3. The results of amino acid composition indicated little variation in the contents of total essential and non-essential amino acids. However, variation existed in the individual amino acid contents, particularly for arginine, histidine and methionine. The arginine contents varied from 16.51 mg/g flour in common bean to 23.75 mg/g in faba bean. Among the five legumes, lysine, alanine, cysteine and proline were found to be rich in pea, while phenylalanine and serine were found in appreciable amounts in chick pea and common bean, respectively. The total essential and non-essential amino acids were maximum in pea. The most abundant components of essential and non-essential amino acids were leucine and glutamic acid, whereas the least abundant were methionine and cysteine. These legume proteins were found to be rich (approximately 20%) in aspartic acid and glutamic acid, which constitute important reservoirs of amino groups for the body. Glutamic acid has received attention as a primary fuel source for the intestinal tract, especially controlling glycogen synthesis and protein degradation (Mahan and Escott-Stump, 1996). The proportion of essential amino acids with respect to total amino acids was 38%, and the percentage of essential amino acids to non-essential amino acids was 0.6, which achieved the reference values recommended by FAO/WHO (40% and 0.6%) (FAO and WHO, 1973). These data are comparable to those reported for grain legumes by Degussa (2006). It is known that the nutritive value of proteins depends primarily on the capacity to satisfy the needs of nitrogen and essential amino acids (Pellet and Young, 1980). However, observations are accumulating to suggest that legume proteins are not only a valuable source of amino acids but might also induce several physiological effects in animals or humans consuming legume- or legume protein-based diets. The chemical structure of this particular group of proteins is probably the ultimate reason to explain their nutritional and/or physiological behaviour

(Rubio, 2000; Rubio and Clemente, 2009).

The crude fibre content was lower than 7.71% in all cases. Starch constituted the major storage carbohydrate (37.65 to 44.40%) in these legumes, with peas having the highest amount (44.40%). The highest energy value was observed for chick pea (5,214.22 kcal/kg) and the lowest was found in pea (4,927.19 kcal/kg). The values for lentils, beans, and faba beans were 4,978.73, 4,954.85, and 4,943.85 kcal/kg, respectively (Table 3). Dietary fibre includes NSP, oligosaccharides, lignin, and associated plant substances (Pawar and Thompkinson, 2014). NSP are referred to as the polysaccharides that cannot be degraded by endogenous enzymes and therefore reach the colon almost indigested. Individual NSP groups have different chemical and physical characteristics that result in a variety of physiological effects within the intestine (Căpriţă et al., 2010). The NSP measured as constituent sugars (Table 3) showed that glucose was the most abundant sugar in NSP of all legumes, followed by arabinose, galactose, xylose, mannose and rhamnose. The concentration of rhamnose was comparatively low. NSP of common bean was particularly high in arabinose (47.15 mg/g). Significantly higher amounts of total NSP were reported for pea (192.51 mg/g) than for other legumes in the present work. That difference was mainly due to higher amounts of glucose, galactose and uronic acids in pea seed NSP. Pea NSP contents were significantly higher than those reported for lentil and broad bean (Elhardallou and Walker, 1993). The hypolipidemic effects and cardioprotective benefits associated with dietary soluble fibre consumption are well documented in human clinical trials, animal feeding studies, epidemiological investigations and meta-analysis reports. However, the mean total daily fibre intake amongst adults in most industrialised countries is well below 25 g, the minimal amount recommended by various health organisations (Pawar and Thompkinson, 2014).

Table 3. Amino acid, carbohydrate and starch composition of legume seed meals.

Component	Chick pea	Lentil	Faba bean	Common bean	Pea
Amino acids (mg/g)					
Isoleucine (Ile)	9.09	8.64	8.70	9.16	9.83
Leucine (Leu)	15.95	15.48	15.85	15.72	16.75
Lysine (Lys)	14.77	14.56	14.90	15.51	18.11
Methionine (Met)	2.91	2.57	2.20	2.98	2.33
Phenylalanine (Phe)	12.99	11.45	9.77	11.36	12.04
Threonine (Thr)	7.52	7.07	7.65	9.06	8.25
Valine (Val)	9.30	9.50	9.54	10.47	10.75
Histidine (His)	5.83	5.80	6.21	7.72	6.71
Total essential AA	**78.36**	**75.07**	**74.82**	**81.98**	**84.77**
Alanine (Ala)	8.89	8.30	9.00	9.19	10.19
Arginine (Arg)	21.81	17.88	23.74	16.51	23.35
Asparticacid (Asp)	23.33	21.63	24.07	26.95	26.67
Glutamicacid (Glu)	31.94	30.11	34.89	35.49	36.48
Glycine (Gly)	8.10	9.71	9.03	8.46	9.67
Proline (Pro)	9.73	9.95	9.73	8.56	11.10
Serine (Ser)	10.41	9.89	10.55	13.42	10.83
Cysteine (Cys)	1.95	1.22	1.59	0.91	2.41
Tyrosine (Tyr)	6.58	6.65	7.71	10.43	7.86
Total non-essential AA	**122.74**	**115.34**	**130.31**	**129.92**	**138.56**
Total AA	**201.09**	**190.42**	**205.12**	**211.89**	**223.33**
Carbohydrates (mg/g)					
Rhamnose	1.11 ± 0.06^{ab}	0.23 ± 0.10^{d}	0.71 ± 0.07^{c}	0.97 ± 0.08^{b}	$1.18 \pm 0,09^{a}$
Arabinose	40.18 ± 4.57^{b}	22.95 ± 0.63^{d}	18.76 ± 0.96^{ad}	47.15 ± 4.99^{a}	30.87 ± 1.19^{c}
Xylose	3.42 ± 0.32^{d}	6.72 ± 0.55^{c}	8.50 ± 0.81^{b}	12.17 ± 0.91^{a}	8.89 ± 1.08^{b}
Mannose	8.90 ± 0.36^{b}	8.92 ± 0.19^{b}	8.94 ± 0.49^{b}	11.13 ± 0.59^{a}	8.69 ± 0.33^{b}
Galactose	15.97 ± 0.64^{c}	16.62 ± 2.51^{c}	18.79 ± 2.78^{dc}	26.47 ± 3.99^{a}	22.83 ± 1.37^{ab}
Glucose	43.20 ± 2.21^{c}	63.54 ± 6.77^{b}	96.46 ± 9.00^{a}	50.75 ± 3.73^{c}	99.81 ± 7.13^{a}
Uronic acids	13.91 ± 1.26^{bc}	12.44 ± 1.18^{c}	19.68 ± 2.08^{a}	16.55 ± 1.09^{b}	20.60 ± 2.04^{a}
Total NSP	126.70 ± 6.21^{e}	131.45 ± 10.25^{d}	172.26 ± 13.06^{b}	165.21 ± 10.17^{c}	192.51 ± 6.05^{a}
Starch, %					
RDS	8.64 ± 0.17^{b}	7.12 ± 0.57^{c}	4.11 ± 0.10^{d}	2.84 ± 0.12^{e}	10.70 ± 0.12^{a}
SDS	6.17 ± 0.79^{a}	22.85 ± 0.72^{bc}	27.95 ± 1.07^{a}	23.95 ± 0.26^{b}	21.65 ± 0.22^{c}
RS	6.75 ± 1.10^{c}	13.41 ± 1.07^{a}	5.25 ± 0.90^{c}	11.45 ± 0.69^{b}	11.17 ± 0.64^{b}

Data are means $\pm$ SD of three determinations ($n = 3$). Data with different superscript letters within the same row differ significantly ($p < 0.05$).

Table 4. Minerals composition of legume seed meals (mg/100 g seed flour).

Mineral	Chick pea	Lentil	Faba bean	Common bean	Pea
Ca	156.89 ± 0.94^{a}	50.15 ± 0.63^{cd}	117.29 ± 1.89^{b}	50.32 ± 0.45^{c}	48.43 ± 0.33^{d}
P	797.71 ± 1.37^{b}	299.45 ± 3.84^{e}	$1,187.97 \pm 7.53^{a}$	503.92 ± 0.20^{c}	352.79 ± 0.98^{d}
Mg	68.75 ± 0.81^{b}	46.20 ± 0.21^{d}	76.16 ± 0.68^{a}	77.33 ± 0.76^{a}	60.41 ± 0.98^{c}
Fe	6.80 ± 0.04^{a}	6.54 ± 0.12^{b}	5.88 ± 0.09^{d}	6.18 ± 0.02^{c}	4.32 ± 0.07^{e}
Cu	0.95 ± 0.01^{c}	0.87 ± 0.01^{d}	1.48 ± 0.01^{a}	1.31 ± 0.01^{b}	0.76 ± 0.01^{e}
Zn	5.07 ± 0.08^{b}	5.67 ± 0.11^{a}	5.67 ± 0.11^{a}	5.08 ± 0.09^{b}	2.19 ± 0.04^{d}
Na	12.82 ± 0.31^{b}	3.82 ± 0.13^{e}	25.03 ± 0.47^{a}	4.55 ± 0.09^{d}	6.20 ± 0.15^{c}
K	$1,135.96 \pm 3.85^{b}$	838.56 ± 7.64^{e}	$1,285.43 \pm 11.02^{a}$	$1,093.68 \pm 14.60^{c}$	883.89 ± 7.14^{d}

Data are means $\pm$ SD of three determinations ($n = 3$). Data with different superscript letters within the same row differ significantly ($p < 0.05$).

The mineral composition (Table 4) showed high contents (mg /100 g seed flour) of phosphorus (299.45-1187.97), magnesium (46.20-77.33), iron (4.32-6.80), sodium (3.82-25.03) and potassium (838.56-1285.43). Potassium was the most abundant mineral present in all legume seeds, and faba bean contained the highest amount (1285.43 mg/100 g). In addition, all samples examined contained a higher amount of phosphorus and potassium than the other macro–elements analysed. The seeds are also good sources of calcfium (48.43-156.89 mg/100 g). In contrast, low amounts of copper (0.76-1.48 mg/100 g) and zinc (1.85-5.67 mg/100 g) were found. Besides, the Na/K ratio was very low (0.006-0.01), which is important from a nutritional point of view because a high Na/K ratio is usually linked to higher hypertension incidence (Zhou and Han, 2006). The mineral profiles shown in the present work were similar, but slightly lower, than those in other legumes such as Brazilian legumes, and Mexican and North American beans (Meiners *et al.*, 1976; D'Mello *et al.*, 1985). Such variations in the content of minerals for legume samples might be due to their genetic origins, geographical sources and/or soil conditions.

Dietary starches are classified based on the bioavailability considering both the kinetic component and the completeness of digestibility; therefore classified as rapidly digestible, slowly digestible and indigestible (resistant) fractions (Englyst *et al.*, 1992a). The starch digestibility of pulses is affected by factors such as cell-wall structure (Hoover and Zhou, 2003), presence of anti-nutritional factors such as protease inhibitors, phytates and polyphenols (Yadav and Khetarpaul, 1994; Bravo *et al.*, 1998; Siddhuraju and Becker, 2005), high amylose content (Hoover and Zhou, 2003; Tharanathan and Mahadevamma, 2003), and high content of viscous soluble dietary fibre components (Tharanathan and Mahadevamma, 2003). The levels (%) of RDS, SDS and RS were in the ranges of 2.84-10.70, 21.65-27.95, and 5.25-11.45, respectively, and there were significant differences between seed types (Table 3). Common bean flour had the lowest RDS content (2.84%). The SDS content followed the following decreasing order: faba bean (27.95%), chick pea (26.17%), beans (23.95%), lentil (22.85%) and pea (21.65%). The RS content in chick pea (6.75%) and faba bean flour (5.25%) was much lower than that of lentil (13.41%), pea (11.17%) and bean (11.45%) flours. The values of RDS, SDS and RS in the present work were comparable to those previously reported for lentil, chick pea and pea (Chung *et al.*, 2008), while they were substantially lower than those for mung bean starches (Sandhu and Lim, 2008). The digestibility can be also affected by

the physicochemical properties of the starch, which are influenced by processing or storage conditions (Du *et al.*, 2014). Legumes have been shown to contain significant amounts of RS in comparison with other products such as cereals, tubers and unripe fruits (Jenkins *et al.*, 1982; Bravo *et al.*, 1998). For this reason, the starch digestion rate and therefore the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in reduced glycaemic and insulinemic post-prandial responses in comparison with cereal grains or potatoes (Jenkins *et al.*, 1982; Tovar *et al.*, 1992).

Legume seeds contain a variety of constituents which can interfere with appetite, absorption and metabolism. Table 5 summarises the protease inhibitors (TIU and CIU) and phytate values determined in the seeds. The levels of anti-nutritional factors may be due to the variety, climatic conditions, location, irrigation conditions, type of soil and year during which the plants grow (Urbano *et al.*, 2000). Protease inhibitors are widespread anti-nutrient substances which block either trypsin or chymotrypsin activity, thereby reducing digestibility. The trypsin inhibitor activity (TIA) and chymotrypsin inhibitor units (CIU) of different legume seeds varied from 2.27 to 16.22 mg/g and from 1.77 to 27.15 CIU/mg, respectively. Common bean showed significantly higher values of both TIA and CIU. However, pea and faba bean flours had very low TIA and CIU values. It is very difficult to compare the enzyme inhibitory activities of legumes, as reported by different investigators, primarily because of the differences in methods and units used. Kumaraguru Vasagam *et al.* (2007) reported trypsin inhibitor values (using the same method as used in the present work) of 13.7 and 14.9 mg of trypsin inhibited/g of cow pea and mung bean, respectively. Faba bean showed lower CIU values than the other investigated legumes. The average CIU of soybean was 9.8 (Abu-Tarboush, 1998), which is significantly lower than that observed in the present work for all legumes tested except common bean (27.15). These inhibitors are relatively thermolabile and their inhibitory activity can be reduced considerably by an appropriate thermal treatment (Alonso *et al.*, 2000; Shimelis and Rakshit, 2007). Apart from their traditionally investigated anti-nutritional effects, protease inhibitors are currently being investigated as colorectal cancer chemopreventive agents. Although the therapeutic targets and the action mechanism have not yet been elucidated, the emerging evidence suggests that they exert their preventive properties via serine protease inhibition (Clemente and Arques, 2014; Utrilla *et al.*, 2015).

Phytates were estimated as phytic acid in the

Table 5. Anti-nutrients content of legume seed meals.

Component	Chick pea	Lentil	Faba bean	Common bean	Pea
Trypsin inhibitors (TIU/g)	7.14 ± 0.17^b	$2.71 \pm 0,10^c$	2.84 ± 0.21^c	16.22 ± 0.51^a	2.27 ± 0.00^d
Chymotrypsin inhibitors CIU/mg	3.86 ± 0.09^b	3.09 ± 0.27^d	1.77 ± 0.08^e	27.15 ± 1.38^a	3.61 ± 0.33^c
Phytic acid (%)	1.54 ± 0.09^c	2.40 ± 0.09^a	2.40 ± 0.09^a	2.15 ± 0.05^b	1.21 ± 0.10^d
Tannins (mg/100 g)	165.68 ± 6.24^c	282.30 ± 23.73^b	151.13 ± 5.79^c	410.93 ± 5.82^a	161.26 ± 0.16^c

Data are means $\pm$ SD of three determinations (n = 3). Data with different superscript letters within the same row differ significantly ($p < 0.05$).

present work. Phytic acid concentration ranged from 0.93% to 2.40% (Table 5), which is in the range of published phytate amounts in legumes (Urbano *et al.*, 2000). The phytic acid can be reduced by processing methods such as soaking and cooking (Reddy *et al.*, 1982), or by the use of phytases (Urbano *et al.*, 2007). Phytates (hexaphosphates of myo-inositol) are common anti-nutrients in plant seeds. They chelate di- and trivalent mineral ions, such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}, resulting in reduced bioavailability of trace minerals to consumers (Duffus and Duffus, 1991; Welch, 2002). However, a number of animal and epidemiological studies demonstrate beneficial effects of dietary phytic acid including decreased risk of heart disease, renal stone formation and colon cancer (Urbano *et al.*, 2000; Fox and Eberl, 2002). Dietary phytate may also have health benefits for diabetes patients by lowering the blood glucose response through a reduction in the rate of starch digestion and by slowing gastric emptying (Thompson, 1993). Likewise, phytate has also been shown to regulate insulin secretion (Barker and Berggren, 1999).

Significant differences among legume seeds were found in the tannin content which ranged from 410.93 mg/100 g (common bean) to 151.12 mg/100 g (faba bean). The production of phenolics has been shown to be dependent on environmental conditions (Weidner *et al.*, 2000). Tannins have been reported to inhibit the digestive enzymes and thereby lower digestibility of some nutrients, particularly proteins and carbohydrates (Reddy *et al.*, 1985; Brand *et al.*, 1990). However, tannins may be involved in the prevention of some forms of cancer acting as agents inducing nucleosome-sized DNA fragmentation, which is a biochemical hallmark of apoptosis. There is a well-established evidence of anti-carcinogenic activity of tea tannins in animals. They also may protect against LDL-oxidation, inhibit platelet aggregation, reduce the systolic blood pressure and the level of plasma cholesterol, at least in animal and cohort studies, thus preventing cardiovascular diseases (Santos-Buelga and Scalbert, 2000; Multari *et al.*, 2015).

Conclusion

In conclusion, the five legume seeds studied were good sources of protein and carbohydrates. The mineral concentration values were high, especially those of K, P and Ca. These seed flours could be also considered as good sources of dietary fibre, NSP and RS, which qualifies them as healthy food ingredients. The presence of anti-nutritional factors such as protease inhibitors deserves closer attention as bioactive components potentially useful in the prevention of pathologies such as colorectal cancer. The nutritional qualities of these pulses make them quite suitable for a balanced human diet, and a more extended cultivation is recommended in Algeria.

Acknowledgement

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