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# Functional properties of pulse flours and their opportunities in spreadable food products

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RESEARCH ARTICLE

## Abstract

The pulse flours obtained from broad bean, red and green lentil were characterised in order to identify potential applications for obtaining food products with short ingredient list. The investigated pulse flours had similar protein contents (~22%), with the isoelectric point close to pH~4.4. Functional and rheological properties of the pulse flours were compared to the soy protein concentrate, which is widely used as main ingredient for obtaining spreadable food products. Among tested pulse flours, the highest values for water and oil binding capacities, and emulsifying properties were obtained in case of the broad beans. The soy protein concentrate had higher water binding capacity with respect to the broad bean flour, but lower functional properties involving the interaction with oils. The rheological shear tests performed under increasing, followed by decreasing shear rate, indicated a better stability of the suspensions based on pulse flours compared to the soy protein concentrate. The influence of native starch, modified starch and pectin on the rheological behaviour of the pulse flours and soy protein concentrate was also tested. As a general trend, the rheological behaviour was influenced by hydrocolloid nature and flour composition. The yield stress values indicated the easy spreadability of the pulse based samples, comparable to the soy products. The temperature ramp test highlighted high gelling temperatures for pulse and soy suspensions, hydrocolloid addition leading to earlier gelling process.

**Keywords:** broad bean, lentil, functional properties, rheology

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## 1. Introduction

Taking into account the climate change and growing world populations over the last century, together with biodiversity threats by monoculture farming and genetically modified organisms, an increasing concern for food security has been registered, and finding new and reliable proteins sources became one important research priority. Vegetables are valuable sources of food grade proteins, which are important macronutrients for human organism. Among vegetables, legumes represent a rich source of proteins (Makri *et al.*, 2005). The importance of legumes is expected to grow in the near future because of the additionally provided health benefits and soil fertility. In particular, pulses, which are dry seeds of legumes with low fat contents, are recognised as a food choice with significant potential for human health (Bassett *et al.*, 2010; Crépon *et al.*, 2010). They are known as good sources of carbohydrates with low glycemic load, proteins, dietary fibres, vitamins and minerals (Bassett *et al.*, 2010).

The present study is focused on the functionality of three different pulses: fava beans, red lentils and green lentils. Fava bean (*Vicia faba*), also known as broad bean, is one of the oldest crops used as protein source in human diet (Rubiales, 2010). Lentils are considered important pulse crop for the high protein content, and is also rich in other essential dietary components and trace elements. Moreover, Amarowicz *et al.* (2010) indicate lentils as potential source of prebiotic carbohydrates and phenolic compounds with high antioxidant capacity. There are some nutritious disfavours for which legumes are blamed. It includes trypsin inhibitors, together with vicilin and covicilin, two glucosidic aminopyrimidine derivatives from broad bean. However, the risk of favism is mentioned only in case of G6PD-deficient human individuals (Crépon *et al.*, 2010). Moreover, trypsin inhibitor proteins contain high levels of essential amino acids, which after denaturation during cooking process enrich the nutritional quality of the products (Kansal *et al.*, 2008).

Taking into account the increasing interest on affordable minimally processed and preserved products noticed in the last years, as well as the good perspectives of the food products with short ingredient list (no more than five ingredients), the present paper deals with preliminary investigations on the development of new short ingredient list spreadable food products based on pulse flours. The protein products currently used for this kind of food applications are mainly derived from soy. Considering the high price, allergenicity or difficulties in controlling the genetically modified organisms, more convenient protein sources with functional and nutritional benefits are to be investigated. Moreover the prevalence of soy crops over other protein rich legumes became a threat in terms of biodiversity, while the areas cultivated with pulses are in decline since the mid of the 20th century (Bassett *et al.,* 2010; Rubiales, 2010). The development of new food formulations based on pulses and the existence of sustainable technology to produce it, might allow increasing the demand for pulses as raw materials, therefore helping the revival of these crops.

The main objective of the study was to assess the suitability of using pulse flours, as main ingredients, for getting new spreadable food products. Functional and rheological properties of flours from broad beans, red and green lentils have been tested in model systems for helping a better understanding of their behaviour in complex food matrices. Despite higher protein content, the soy protein concentrate was considered as control, because is commonly used for obtaining commercially available spreadable food products. The addition of different carbohydrate polymers (native starch, modified starch and pectin) to the broad bean and lentil flours was also considered to get specific textural properties. The results of our investigation are promising, indicating the possibility of successfully using the minimally refined pulse flours for substituting the highly processed forms of the soy bean for obtaining spreadable food products.

## 2. Materials and methods

### Materials

The seeds of three different species of pulses from *Fabaceae* family, namely green and red lentils (*Lens culinaris*) and broad beans (*V. faba*) were purchased from a local store (Galati, Romania) specialised in selling vegetable products. Three types of hydrocolloids were used in the experiment: native potato starch (STÄRKINA 20.000, AGRANA Group, Vienna, Austria), modified pregelatinised starch (acetylated distarch adipate; AGENAJEL 20.309, AGRANA Group) and low methylated pectin (Brown Ribbon type). The soy protein concentrate was purchased from Ubimedia S.R.L. (Galati, Romania). All chemicals used in the experiments were of analytical grade.

### Flour obtaining

The pulse flours were obtained by grinding the seeds through a laboratory mill (WZ-2, Sadkiewicz Instruments, Bydgoszcz, Poland). In case of broad beans, a preliminary dehulling was performed by splitting the seeds followed by air separation to remove the coatings coarse particles. Further sieving of the pulse flours was carried out to remove the large size particles. In case of all studied pulse flours the maximum particle size was <500 μm and the yield after sieving was as follows: 65.98% in case of red lentil (particle size distribution of 52.74% ranging from 315 to 500 μm, 6.82% ranging from 180 to 315 μm, and 40.44% less than 180 μm), 75.91% in case of green lentil (particle size distribution of 55.33% ranging from 315 to 500 μm, 19.15% ranging from 180 to 315 μm, and 25.52% less than 180 μm), 93.94% in case of broad bean (particle size distribution of 16.82% ranging from 315 to 500 μm, 20.29% ranging from 180 to 315 μm, and 62.89% less than 180 μm). Obtained flours were sealed in glass containers and stored at room temperature (~22 °C) for further analyses.

### Proximate composition

The proximate composition of the pulse flours was determined as follows: the moisture content using the AACC 44-51 method (AACC International, 2000); the protein content through the semimicro-Kjeldahl method (Raypa Trade, Barcelona, Spain), using a nitrogen conversion factor of 6.25; fat content by extraction with ether using a Soxhlet extractor (SER-148, VELP Scientifica, Usmate Velate, Italy); total dietary fibres by an enzymatic-gravimetric method (Asp *et al.*, 1983) with Fibertec FOSS® Tecator (Merck, Darmstadt, Germany).

### Antioxidant activity

In order to determine the antioxidant activity of the pulses flours, the extraction was performed with 80% aqueous methanol solution at room temperature for 2 hours at 37 °C, using a magnetic stirrer. The mixture was afterwards centrifuged at 9,690×*g* for 10 minutes (Martinez-Villaluenga *et al.*, 2009). The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH RSA) was determined using the DPPH discolouring assay. A volume of 3.9 ml of DPPH solution ($6\times10^{-3}$ mol/l) was added to 0.1 ml extract, and the absorbance reduction was measured at 515 nm after 30 minutes of resting in dark at room temperature. For control sample the extract solution was replaced by methanol. The % DPPH RSA was calculated as (1–[$A_{sample}$ – $A_{control}$]) × 100.

### Functional properties

Protein solubility was determined using the method of Boye *et al.* (2010a) with slight modifications. A known quantity of flour sample was dispersed in distilled water and the pH was adjusted at various values ranging from 2 to 1 1, using HCl and NaOH solutions of appropriate concentrations. The samples were allowed to equilibrate for 30 minutes while continuously stirring, and afterwards were centrifuged at 4,900×*g* for 15 minutes. The protein content of the supernatant was determined using the Lowry method (Lowry *et al.*, 1951).

Water binding capacity (WBC, %) is the result of protein hydration. WBC is a measure of the maximum amount of water that can be absorbed by 1 g of tested material under centrifugation at reduced speed. Enough water quantity was added to the flour samples in order to saturate it, but avoiding the formation of a liquid phase (Boye *et al.,* 2010a). The hydrated samples were centrifuged at 880×*g* for 10 minutes.

Oil binding capacity was determined using the method proposed by Ogunwolu *et al.* (2009) with slight modifications. A mixture of 0.5 g pulse flour and 6 ml of sunflower oil was prepared in centrifuge tubes. After 30 minutes of resting period, samples were centrifuged at 755×*g* for 25 minutes. The supernatant was discarded and tubes with precipitate were let to drain for another 25 minutes. Oil binding capacity was expressed as ml oil/g of flour.

Foaming properties of studied pulse flours were determined using the methods described by Ogunwolu *et al.* (2009) with modifications. Six grams of flour were dispersed in 100 ml of phosphate buffer of pH 6.9 and stirred using a Philips blender for five minutes. The volume of the dispersion was measured before and after foaming. The foaming capacity was expressed as the volume increase after stirring, and foam stability was recorded after 10 minutes of storage at room temperature.

Emulsifying capacity was determined using de method described by Ionescu *et al.* (2009). Suspensions of 6% (w/v) concentration were obtained with a Philips blender. Sunflower oil was then progressively added using a laboratory burette under continuous stirring until the inversion point of the emulsion was observed (separation into two phases). The emulsifying capacity was reported as ml of oil/100 ml suspension.

### Rheological behaviour

Rheological tests were performed using a control-stress rheometer (AR2000ex, TA Instruments, Ltd., Leatherhead, UK) equipped with a Peltier temperature control system. A cone (plate geometry 40 mm in diameter, 2° cone angle) was used with a closing gap of 1000 μm. Rheological measurements were performed both on pulses flour suspensions and gels.

#### *Tests performed on pulses flour suspensions*

Pulse flour suspensions of 15% (w/v) were prepared in phosphate buffer 0.2M of pH 6.9. The concentration of the suspensions was established based on the least gelling concentration of the pulse flours, determined using the method described by Ogunwolu *et al.* (2009). When testing the influence of the hydrocolloids on the rheological properties of pulses flours, a hydrocolloid:flour ratio of 1:4 was used so that the dry base would remain the same (15% w/v).

Suspensions were prepared 20 minutes prior to every rheological test and let to rest at room temperature. An exception has been made in case of the time sweep tests, when samples were poured on the rheometer plate immediately after the appropriate mixing.

Flow behaviour of flour suspensions was studied with the stepped flow test, where the shear rate was increased from 0.1 to 100 $s^{-1}$, and then decreased back to 0.1 $s^{-1}$. Data were analysed using TA Rheology Advantage Data Analysis Software V 4.8.3 (TA Instruments, Ltd.) and the mathematical model of Herschel-Bulkley was applied to both upward curve and downward curve values (shear stress vs shear rate) in order to determine yield stress, viscosity and rate index parameters:

$$\sigma=\sigma_y+\eta\times\acute{Y}^n$$

$\sigma$-shear stress (Pa); $\sigma_y$-yield stress (Pa); $\eta$-viscosity (Pa·s); $\acute{Y}$-shear rate (1/s); $n$-rate index, also known as flow behaviour index (dimensionless).

The hysteresis areas, defined by the upward and downward curves, were used to estimate the thixotropic behaviour of the pulse flours suspensions.

In order to observe the time stability of the freshly obtained suspensions, a time sweep test was conducted for one hour at frequency of 1 Hz and strain of 0.1%.

The temperature ramp test was performed at frequency of 1 Hz and strain of 0.1% for observing the rheological behaviour during thermal treatment of targeted suspensions. The temperature was increased by 1.5 °C/min from 20 °C to 90 °C. The gelation temperature of pulse proteins was established using the method described by Ding *et al.* (2012), by fitting a third order polynomial equation to the storage modulus (G`) vs temperature data. Gelling temperature domain was then calculated by setting the first derivative of the equation.

### *Tests performed on pulses flour gels*

Gels were obtained by thermally treating the suspensions from 20 °C to 90 °C (~1.5 °C/min), followed by maintaining at 90 °C for five minutes. Obtained gels were cooled in running water and let to rest for ten minutes prior to rheological tests.

The linear viscoelastic region was first identified for each sample by running dynamic strain sweeps over the strain interval 0.01 to 100%. Afterwards a frequency sweep test was performed by increasing oscillation frequency from 0.1 to 10 Hz, at an imposed strain of 0.1%, in the linear viscoelastic region.

In order to identify the yield stress, a flow test was applied on each gel by increasing the shear rate from 0.1 to 5 $s^{-1}$. The final value of shear rate applied was established just before sliding phenomenon was observed.

All rheological tests were replicated three times.

### Statistical analysis

Statistical analyses were performed using Microsoft Excel Software (Redmond, WA, USA). The data were subjected to ANOVA Single Factor, considering a significance level of 95%. Each experiment was carried out in duplicate and the results were reported as mean values. Where appropriate, the Fisher`s least significant difference test ($P<0.05$) was used to determine differences between samples` means.

## 3. Results and discussion

### Proximal composition

Proximal composition of broad bean, red lentil and green lentil flours is represented in Table 1. In case of the broad beans the dehulling process followed by removal of the resulting large size coating allowed reducing the fibre content from 25% (USDA, 2014) to 11.55±0.32% which is closer to lentil flours (Table 1). All pulse flour samples had similar protein contents, of approximately 21%. The fat content of the studied pulse flours was rather low, but slightly higher when compared to the values reported by Boye *et al.* (2010a). These differences might be due to genomic, environmental or agronomic factors. In addition, sieving could concentrate the fat content of the flour fraction. This observation is in agreement with Toews and Wang (2013), who reported fat contents over 2% for the pulse protein concentrates. Anyway, the nutritional profile of the lipids extracted from lentil and fava beans indicated high amounts of unsaturated fatty acids (Vioque *et al.*, 2012).

**Table 1. Proximate composition and functional properties of studied pulse flours and soy protein concentrate.**

| | Broad bean flour | Red lentil flour | Green lentil flour | Soy protein concentrate |
|---|---|---|---|---|
| Proximate composition | | | | |
| Dry matter (%) | 90.24±0.05 | 91.51±0.12 | 88.49±0.04 | 90.48±0.09 |
| Protein (%) | 21.61±0.00 | 21.81±0.09 | 21.59±0.21 | 74.28±0.47 |
| Fat (%) | 1.27±0.12 | 1.77±0.09 | 1.18±0.03 | 0.57±0.01 |
| Ash (%) | 3.71±0.00 | 2.49±0.06 | 2.68±0.05 | 4.16±0.09 |
| Fibres (%) | 11.55±0.32 | 12.03±0.30 | 12.62±0.31 | 1.83±0.60 |
| % DPPH[1] | 23.24±0.07 | 31.36±0.01 | 48.86±0.00 | 9.27±0.01 |
| Functional properties | | | | |
| WBC[2] (g $H_2O$/g) | 0.96±0.002 | 0.73±0.01 | 0.69±0.05 | 5.59±0.13 |
| Oil binding capacity (g oil/g) | 1.92±0.016 | 0.81±0.13 | 0.96±0.08 | 1.22±0.03 |
| Emulsifying capacity (ml oil/100 ml dispersion) | 580±0.01 | 336±0.01 | 316±0.01 | 466±5.29 |
| Foaming capacity (%) | 22±0.00 | 68±0.00 | 50±0.00 | 50±0.00 |
| Foam stability (%) | 34.09±0.00 | 100±0.00 | 100±0.00 | 88.44±0.77 |

[1] DPPH = 2,2-diphenyl-1-picrylhydrazyl.

[2] WBC = water binding capacity.

Regarding the antiradical activity, the highest DPPH RSA value was found for the green lentil flour, double with respect to the broad bean flour. On the other hand, Halvorsen *et al.* (2002) reported that broad bean seeds have the highest antioxidants concentration (1.86 mmol/100 g) among ten studied pulses, while lentils have 0.49 mmol/100 g. The different trend in the antiradical activity of the pulse flours in case of our experiment is most probably due to the preliminary processing of the broad beans through dehulling before grinding, which, according to Boye *et al.* (2010a), significantly decreases the antiradical activity of the products. Many synergic bioactive compounds are found in vegetables: a-tocopherol, a-tocotrienol, vitamin C, lipoic acid, thiols, together with carotenoids, polyphenolic acids, sulfides, flavonoids, lignans, etc. (Halvorsen *et al.,* 2002). In case of the soy protein concentrate, the DPPH RSA value is significantly lower with respect to the pulse flours due to the advanced processing of the product which causes the removal of the fractions rich in antioxidant compounds.

## Functional properties

Food grade proteins, in addition to providing nutritional benefits, should also possess specific functional properties that facilitate processing and serve as basis for foodstuff performance. These properties highly influence the exploitation of new sources of food proteins.

Protein solubility is a useful indicator of protein performance in food systems, and influences other functional properties, such as emulsifying, foaming and gel forming abilities (Sikorski, 2001). Protein solubility varied significantly with the pulse flour ($P<0.05$), especially at alkaline pH (results not shown); the broad bean had the highest proteins solubility (31.49 g/100 g flour at pH 11.1), whereas the green lentil had the lowest solubility (16.68 g/100 g flour at pH 11.1). This fact might be due to the variability in the composition of pulses flour and to the different interactions between phenolic compounds and proteins, which were reported to induce changes in protein solubility (Labuckas *et al.*, 2008). In this respect Ozdal *et al.* (2013) stated that the interactions of proanthocyanidins with proteins can lead to decrease of protein solubility. The phenomenon is explained through the fact that the reaction of phenolic compounds with proteins facilitates proteins cross-linking, therefore changing the net charge of the protein molecules, which in turn affects the solubility.

The highest solubility was observed at low acidic and high alkaline pH values. The predominant proteins from pulses, albumins and globulins, are known to have the isoelectric point in the pH range of 4-5. In agreement with Torki *et al.* (1987), cited by Boye *et al.* (2010b), the isoelectric pH of albumin and globulin fractions is 3.9 and 4.2 in case of chickpea, whereas for lentil is 3.7 and 4.3, respectively. On the other hand Alamanou and Doxastakis (1997) reported a higher isoelectric pH of ~6.5 for albumins, and ~4.6 for globulins, when characterising lupin protein isolates. The isoelectric point of crystal albumin from *Lathyrus sativus* identified by Gaur *et al.* (2010) is reported to be at pH of 5.81. For all studied pulses our results comply with the literature; the minimum proteins solubility was identified in the pH range of 4.40 - 4.55. The isoelectric pH of the studied proteins from pulse flours is similar to the soy protein isolates (Hu *et al.*, 2010). In case of red lentil a rather high quantity of proteins remained dispersed in solution even at pHi (8.46 g/100 g flour). In this respect, Braudo *et al.* (2001), when studying legume protein functionality, suggested that interactions between legumin or hydrolysed legumin and polysaccharides in broad bean could increase their solubility at the isoelectric point and at higher pH values. On the other hand, Kerr *et al.* (2000) mentioned the high impact of the particle size on the functional properties of cowpea flours. Milling and sieving procedures affected, in particular, the water absorption, solid lost and protein solubility, through the ability of water to penetrate flour particles and to either absorb or carry away soluble components. The influence of particle size on protein functionality was also reported by other researchers (Ghavidel and Prakash, 2006).

Technological functionality of the broad bean, red and green lentils flours was further checked in terms of water binding capacity, oil binding capacity, emulsifying capacity and foaming properties, and was compared to the soy protein concentrate (Table 1).

The water binding properties of the broad bean flour was superior to the lentil flours, but was significantly lower with respect to the soy protein concentrate, mostly because of the high difference in terms of the protein content between tested products. The water binding properties are mainly determined by the interaction between water and macromolecular components of the pulse flours, such as proteins and polysaccharides. Concerning the protein fraction, some of the factors influencing the water retention capacity are the hydrophilic/hydrophobic balance of aminoacids, protein conformation, surface hydrophobicity, pH, ionic strength, temperature and concentration (Hettiarachchy and Kalapathy, 1998). The presence of lipids, sugars and tannins associated with proteins, affect the water absorption properties of the flours (Vioque *et al.*, 2012). On the other hand the high fibre contents might favour water absorption (Vioque *et al.*, 2012). Although the lentil flour had higher fibre contents compared to the broad bean, the water binding capacity was lower. These results might be explained by the higher contents of soluble proteins in the broad beans with respect to the lentil flours at native pH value. Our observations comply with Giménez *et al.* (2012), who reported high water absorption capacity for *V. faba* flour at thermal treatment. This phenomenon can be attributed to the high soluble protein content in broad beans which compete for water with other component such as starch (Ryan and Brewer, 2007).

The oil binding capacity of broad bean flour was superior to the soy protein concentrate, being almost double with respect to the lentil flour. Similar values for oil binding capacity (1.82 g oil/g flour) were obtained by Vioque *et al.* (2012) when studying the defatted broad bean flour. Concerning the red and green lentil, Boye *et al.* (2010a) reported higher values of the oil absorption capacity for the protein concentrates compared to our results. The protein concentration technique appeared to be a decisive factor influencing the oil binding properties. The ultrafiltration yielded protein concentrates with superior ability to bind and retain oil under centrifugation conditions. In particular, the oil binding capacity of the protein concentrate obtained through ultrafiltration (226 g oil/100 g) was almost double with respect to the one obtained through isoelectric precipitation (Boye *et al.*, 2010a).

The emulsifying capacity of the investigated pulse flours ranged between 316 and 580 ml oil/100 ml dispersion (Table 1). The emulsifying properties of the proteins highly depend on their intrinsic properties such as size, flexibility and surface properties (charge, the extent of hydrophobic exposed regions), and on the environmental factors such as ionic strength and pH (Turgeon *et al.*, 1992). The better emulsifying properties of broad bean flour might be explained by the higher protein solubility with respect to the lentil flour. On the other hand the protein-polysaccharide complexes contribute to the emulsifying properties of pulses flour by enhancing both the volume of emulsified oil and emulsion stability due to the steric repulsion effects (Alamanou and Doxastakis, 1997), therefore explaining better emulsifying behaviour of the broad bean flour with respect to the soy protein concentrate. Anyway, when estimating the emulsifying capacity per g protein of broad bean, one can see that our results are comparable with those reported by Karki *et al.* (2009) who indicated an emulsifying capacity for soy protein isolates of 445 g oil /g protein.

Regarding foaming properties, broad bean flour resulted to have limited capacity and stability compared to red and green lentil flour and soy protein concentrate. Kaur and Singh (2007) suggested that globular proteins, which in pulses prevail over the fibrous ones, have poor surface active properties, thus resulting in low foaming capacity. Makri *et al.* (2005) found better foaming capacity for broad bean proteins compared to lentil proteins. The differences between our results and those reported by Makri *et al.* (2005) for the protein isolates might be due to the non-protein content of the investigated pulse flours, such as fats or small-molecules with surfactant properties. Sikorski (2001) stated that fat content affected the foaming properties of lentil flours. Even if the foaming capacity was limited, the stability of the foams based on red and green lentil flours was very good (Table 1).

### Rheological behaviour

The rheological properties of the pulse flours suspensions at the lowest concentration (15%) which allows gel formation at thermal treatment, and of the thermally induced gels were further determined and compared to the soy protein concentrate based samples. Moreover, the effect of three different hydrocolloids (native starch, modified starch and pectin) frequently found in the spreadable food products composition on the rheological behaviour of the suspensions and gels was assessed.

Rheological behaviour of flour suspensions under flow conditions is presented in Figure 1 as shear stress vs shear rate rheograms. Both shear stress and viscosity values of the pulse flours based suspensions varied with the addition of different types of hydrocolloids. Regardless of shear rate values the soy protein concentrate alone and in admixture with native or modified starch exhibited higher shear stress values compared to the corresponding pulse flours suspensions (Figure 1A-C). In case of the mixtures with pectin, at low shear rates the broad bean based sample had the highest shear stress and viscosities values, being overtaken by the soy protein based sample at shear rate values over 20 $s^{-1}$ (Figure 1D).

The Herschel-Bulkley rheological model was used to evaluate the rheological behaviour of the samples, and the results are presented in Table 2. Taking into account the rate index values ($n<1$), one can appreciate the shear-thinning behaviour in case of most studied samples. The occurred exceptions concerned the suspension made of broad bean flour, soy protein concentrate, broad bean flour-native starch, and red lentil flour-native starch mixtures, where rate index values higher than 1 suggested a dilatant fluid. In all cases a dilatant region for viscosity (increasing viscosity with increasing shear rate) was observed in the shear rates domain of 0.1-1 $s^{-1}$ (data not shown). Ravi and Bhattacharya (2006) stated that agglomerated particles within dispersions can change their macromolecular structure during shear, showing thinning behaviour. Anyway, chances of particles re-association should be however considered, as it depends on the level of shear rate and temperature used.

The thixotropy of the studied flour suspension was estimated based on the hysteresis areas resulted when performing flow tests under increasing followed by decreasing shear rate. In the second shear step, when the shear rate is progressively decreased, the fluid reorganisation occurs. When the restructuring process takes place at a lower velocity in comparison to destruction, hysteresis loops are formed. All suspensions based on mixtures involving green lentil flour presented higher hysteresis values compared to the red lentil (Table 2). The highest values of the hysteresis area could be observed for broad bean flour suspensions in combination with modified starch and pectin. When combined with

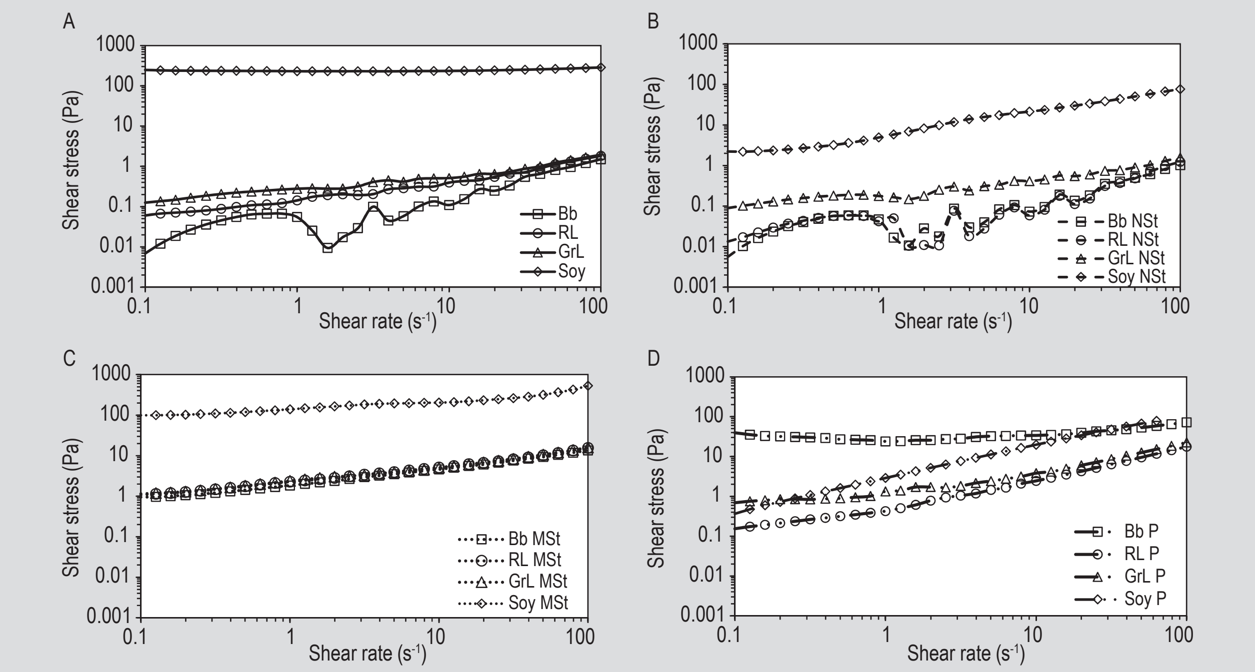

**Figure 1. Rheological behaviour of suspensions made of pulse flours and soy protein concentrate alone (A) or in admixture with native starch (B), modified starch (C), and pectin (D) during increasing shear rate flow test. Suspensions codes are to be interpreted as: Bb = broad bean; RL = red lentil; GrL = green lentil; Soy = soy protein concentrate; NSt = native starch; MSt = modified starch; P = pectin.**

**Table 2. Rheological parameters of the studied suspensions and gels derived from flow tests.**[1]

| Sample[2] | Suspensions | | | | Gels |
|---|---|---|---|---|---|
| | Yield stress (Pa) | Viscosity (Pa·s) | Rate index | Hysteresis area (Pa/s) | Yield stress (Pa) |
| Bb | 0.018±0.007$^{a}$ | 0.014±0.008$^{a}$ | 1.041±0.110$^{a}$ | 0.398±0.411$^{a}$ | 96.65±2.52 |
| RL | 0.144±0.129$^{ab}$ | 0.052±0.009$^{ab}$ | 0.748±0.066$^{b}$ | 3.340±0.226$^{ab}$ | 149.10±1.27 |
| GrL | 0.487±0.368$^{abc}$ | 0.038±0.011$^{abc}$ | 0.788±0.114$^{bc}$ | 11.800±0.424$^{c}$ | 126.20±1.27 |
| Soy | 232.660±0.761 | 0.390±0.094$^{a\text{-}d}$ | 1.146±0.030$^{ad}$ | 786.410±0.412 | 138.85±0.63 |
| Bb NSt | 0.022±0.005$^{a\text{-}d}$ | 0.006±0.001$^{a\text{-}e}$ | 1.129±0.019$^{ade}$ | 2.275±0.629$^{abd}$ | 44.36±1.07$^{a}$ |
| RL NSt | 0.025±0.003$^{a\text{-}e}$ | 0.002±0.000$^{a\text{-}f}$ | 1.383±0.029$^{d}$ | 6.815±0.374$^{a\text{-}e}$ | 153.50±0.97 |
| GrL NSt | 0.201±0.115$^{a\text{-}f}$ | 0.108±0.081$^{a\text{-}g}$ | 0.629±0.153$^{bcf}$ | 14.385±0.912$^{ce}$ | 185.10±1.13 |
| Soy NSt | 6.580±0.352 | 6.534±0.090 | 0.509±0.023$^{bfg}$ | 18.113±0.352$^{cef}$ | 133.86±0.36 |
| Bb MSt | 0.516±0.021$^{a\text{-}g}$ | 1.310±1.274$^{dh}$ | 0.542±0.126$^{bfgh}$ | 118.750±0.353 | 51.84±0.79$^{b}$ |
| RL MSt | 0.902±0.031$^{cg}$ | 1.238±0.899$^{dhi}$ | 0.557±0.121$^{bcfghi}$ | 87.300±0.424$^{g}$ | 48.80±2.33$^{ac}$ |
| GrL MSt | 1.087±0.679$^{cgh}$ | 1.079±0.692$^{a\text{-}i}$ | 0.573±0.043$^{bcfghij}$ | 89.600±0.565$^{g}$ | 42.21±0.49 |
| Soy MSt | 48.785±0.322 | 36.820±0.280 | 0.565±0.023$^{bcf\text{-}k}$ | 448.770±0.893 | 117.15±0.92 |
| Bb P | 23.210±0.042 | 1.094±1.094$^{a\text{-}j}$ | 0.879±0.342$^{abcl}$ | 772.500±1.480 | 130.60±0.76 |
| RL P | 0.179±0.086$^{a\text{-}gi}$ | 0.283±0.055$^{a\text{-}k}$ | 0.895±0.000$^{abcelm}$ | 8.363±0.191$^{a\text{-}ei}$ | 65.22±0.92 |
| GrL P | 0.785±0.063$^{cfghi}$ | 0.384±0.057$^{a\text{-}k}$ | 0.875±0.002$^{abclmn}$ | 12.720±1.350$^{cefi}$ | 50.56±0.86$^{bc}$ |
| Soy P | 0.025±0.002$^{a\text{-}gi}$ | 3.371±0.351 | 0.721±0.031$^{bcf\text{-}n}$ | 3.105±0.020$^{abdei}$ | 0.14±0.07 |

[1] Means with same superscript within same column do not differ significantly ($P$>0.05).

[2] Suspensions codes are to be interpreted as: Bb = broad bean; RL = red lentil; GrL = green lentil; Soy = soy protein concentrate; NSt = native starch; MSt: modified starch; P: pectin.

pectin, the broad bean flour formed a dense material with high viscosity (Table 2) (i.e. which would not trickle on glass side when it was reversed). Our results comply with the observations of Donato *et al.* (2005) who stated that globular protein-low methylated pectin mixtures formed at 20 °C stay homogenous and translucent even after centrifugation. The yield stress value determined by means of Herschel-Bulkley model for the broad bean-pectin suspension was significantly higher compared to all other combinations with native starch, modified starch and pectin. However, the yield stress values obtained for broad bean-pectin gels were significantly lower compared to other combinations like red lentil-native starch, green lentil-native starch or red lentil alone ($P<0.05$). In most cases, hydrocolloid addition favourably influenced the flow characteristics of studied gels by reducing the yield stress values. The importance of yield stress parameter is due to the fact that it reflects the spreadability of soft foods (Sun and Gunasekaran, 2009).

In order to assess the microstructural rearrangements occurring over time in the pulse flours and soy protein concentrate based suspensions, oscillatory time sweep tests were performed. The general trend of G` highly depended on added hydrocolloid (Figure 2).

The time dependent rheological behaviour of the suspensions varied with the type of pulse flour (Figure 2A). The red lentil flour suspension presented a typical hydration process, with increasing G` tendency. In case of green lentil flour the decrease of G` values was observed in the time range 2-30 minutes, probably due to the amylase catalysed starch hydrolysis. Further increase of G` values over 30 minutes could be mostly due to fibre hydration. Instead, in case of the broad bean flour and soy protein concentrate suspensions, no significant evolution of G` ($P>0.05$) were observed during the time sweep test. Distinct rheological behaviours were reported by Batista *et al.* (2005) for different vegetable protein isolates (pea, lupin and soy).

A different tendency was observed for the pulse flour suspensions supplemented with modified starch, which presented a significant decrease of G` values ($P<0.05$) during time sweep step (Figure 2C). All samples presented a viscoelastic behaviour, with storage modulus (G`) values prevailing over loss modulus (G``), and the shift angle (δ) values under 45° (data not shown), characteristic for soft solids (e.g. test average recorded: maximum: broad bean -23°; minimum: broad bean/pectin -7°). A perfect elastic material is defined by shift angle values close to 0°, whereas a completely viscous material has the shift angle near 90° (Tabilo-Munizaga and Barbosa-Cánovas, 2005).

Gelling properties of studied suspensions were determined through temperature ramp test. Over the considered temperature range for gelling process, the G` values were significantly higher than G`` ($P>0.05$), indicating a predominant elastic behaviour, with a solid like structure (Ahmed and Auras, 2011). After calculating the first derivative of the third order polynomial equation fitted on G` data, two temperature values were obtained, defining

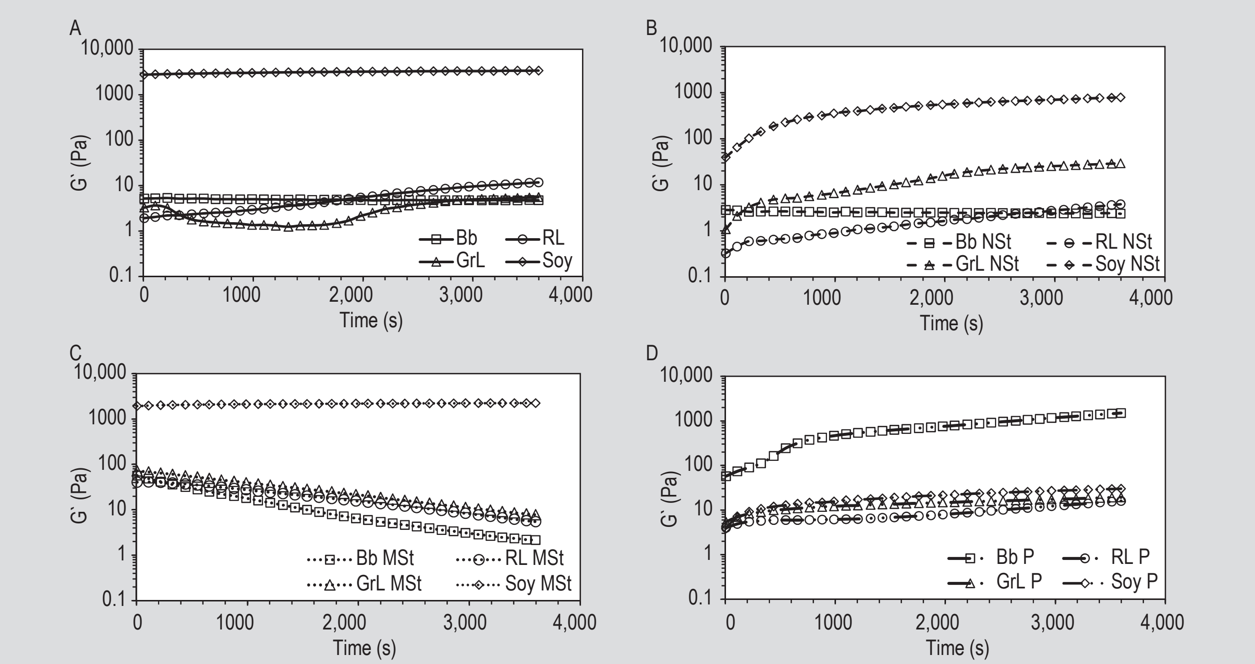

**Figure 2. Rheological behaviour of suspensions made of pulse flours and soy protein concentrate alone (A) or in admixture with native starch (B), modified starch (C) and pectin (D) during time sweep test. Suspensions codes are to be interpreted as: Bb = broad bean; RL = red lentil; GrL = green lentil; Soy = soy protein concentrate; NSt = native starch; MSt = modified starch; P = pectin.**

the domain where the gelling process occurred. In case of pulse flour suspensions the gelling process started at temperatures between 65 and 70 °C (Figure 3). In agreement with the observations of Renkema *et al.* (2000), in case of the soy protein concentrate the gelling process started at temperatures over 90 °C. The high differences between pulse flours and soy protein concentrate in terms of gelling temperatures might be explained by the presence of high amounts of carbohydrates in the flour samples. It is known that different starch varieties have gelling temperature near 60 °C (Fredriksson *et al.*, 1998). Regardless of the protein source, the hydrocolloid addition led to the decrease of the gelling temperature and the process accomplishment required a wider temperature range (Figure 3).

A particular case was registered for broad bean-modified starch suspension, where a realistic domain for gelling temperature could not be found (nine temperature ranges tested from 50 till 70 °C). An extensive review by Singh *et al.* (2007) on rheological properties of some chemical modified starches presents the factors that influence the gelling temperature as well as pic viscosity of starch suspensions. The modification method, reaction conditions and starch source appear being the most important factors influencing the rheological behaviour (Singh *et al.*, 2007). The combination between high swelling capacity of starch (up to several times with respect to the initial weight) and high water absorption capacity of broad bean flour at thermal treatment (Giménez *et al.*, 2012) could explain the smooth passage from dispersion to gel in this case.

Regarding broad beanpectin suspension, in order to identify a reliable gelling domain a wider temperature range, from 50 to 75 °C, had to be tested. As in previous case, it was not possible to identify an inflection point in the G`-temperature curve. Studies performed on heat-induced gelation of globular protein/gelling polysaccharide systems reported that internal structure and rheological properties are dependent on biopolymer nature, ions nature in the system as well as solvent conditions (Donato *et al.*, 2005). Fraeye *et al.* (2009) suggested that pectin gel strength is better explained by the pattern of methoxylation than by the degree of methoxylation, Moreover, the increasing calcium concentration favours gel strength through electrostatic pectin-calcium-interactions. Broad bean calcium concentration is reported by USDA (2014) to be double in comparison to lentil (all varieties), namely 103 vs 35-48 mg/100 g (depending on variety), thus explaining the strength of obtained suspensions and gels based on broad bean-pectin interactions.

The viscoelastic characteristics of the gels based on pulse flours and soy protein concentrate were monitored while performing frequency sweep tests (Figure 4). The frequency sweep tests are powerful tools which can be successfully used for getting information on mechanical spectra of materials, providing data about internal structure of gels (Batista *et al.*, 2005). When studying gelling ability of different vegetable proteins by means of rheological tools, Batista *et al.* (2005) mentioned the possibility of estimating the physical reinforcement of the primary gel

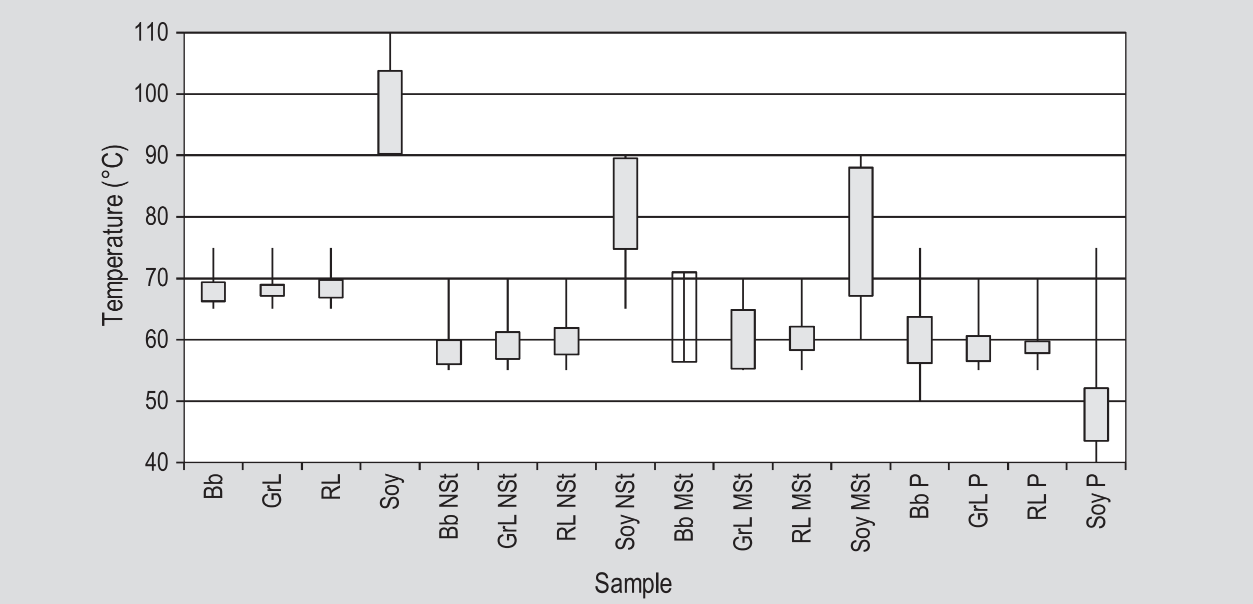

**Figure 3. Temperature domains where gelling process was observed for studied samples during temperature ramp test. Lines represent analysed temperature range, whereas columns represent obtained regions of gelling temperatures. Suspensions codes are to be interpreted as: Bb = broad bean; RL = red lentil; GrL = green lentil; Soy = soy protein concentrate; NSt = native starch; MSt = modified starch; P = pectin.**

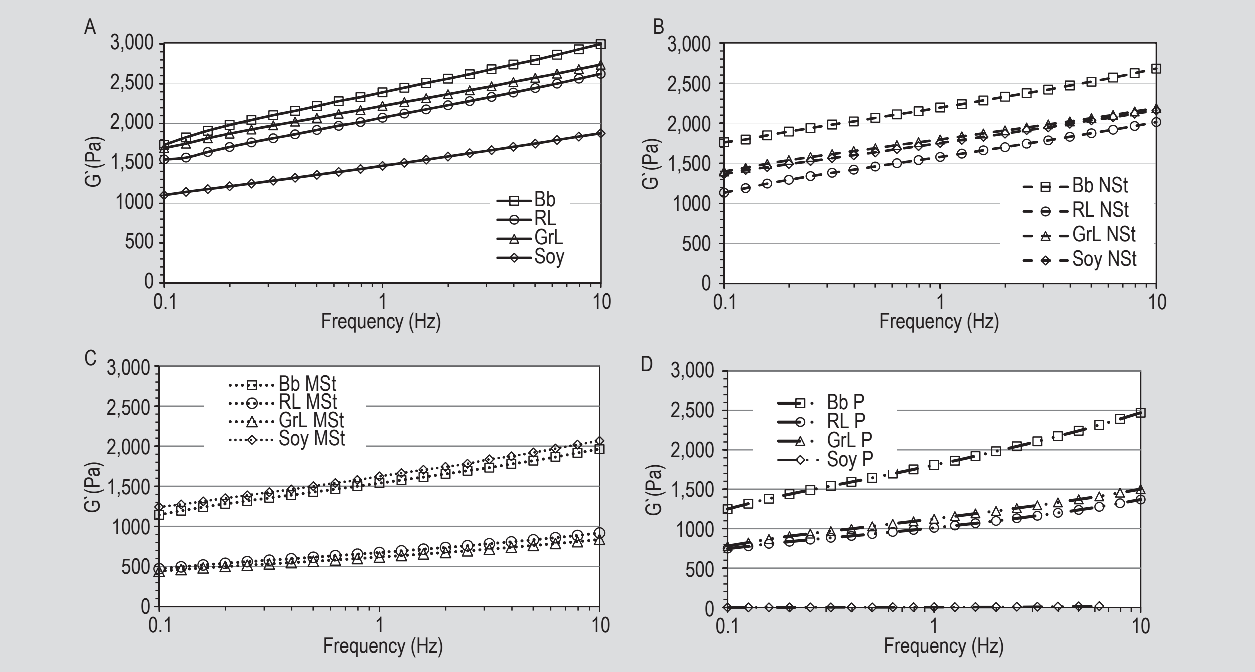

**Figure 4. Frequency dependent viscoelastic characteristics of gels made of pulse flours and soy protein concentrate alone (A) or in admixture with native starch (B), modified starch (C) and pectin (D). Samples codes are to be interpreted as: Bb = broad bean; RL = red lentil; GrL = green lentil; Soy = soy protein concentrate; NSt = native starch; MSt = modified starch; P = pectin.**

network leading to entangled network among the protein molecules through the frequency sweeps.

Analysing the results presented in Figure 4a one can see that soy protein concentrate exhibited the lowest G` values among the entire tested frequency range. Pulse flour gels (with no added hydrocolloid) were the most rigid ones, with a solid like behaviour ($\delta$=6.4-6.6, data not shown) with no significant differences between G` values (*P*>0.01). Regardless of the protein source the gels obtained with modified starch presented a softer structure, being followed by gel samples with pectin. For gels based on broad bean flour with addition of modified starch and pectin, higher G` values were obtained in comparison to lentil flour based gels (*P*>0.05). No statistically significant differences were registered among G` data of green lentil and red lentil flours in combination with previously mentioned carbohydrates (*P*>0.05).

## 4. Conclusions

The flours obtained from broad beans, green and red lentils have good technological and nutritional functionality, being suitable to replace the soy protein in different food applications. The broad bean flour had superior oil binding capacity and emulsifying properties to the lentil flours and soy protein concentrate. The rheological tests performed on the suspensions of pulse flour and soy protein concentrate alone or in admixture with different hydrocolloids, such as native starch, modified starch and pectin, indicated the shear-thinning behaviour and thixotropic properties for most of the samples. In order to estimate the suitability of the pulse flour to be used for obtaining spreadable food products, the spreadability of the heat induced gels was estimated based on the yield stress values. The results indicate that, similarly to the soy products, all tested pulse based samples are easy to spread. Regardless of protein source, the addition of hydrocolloids had a general softening effect, and caused the decrease of the gelling temperature of the mixtures. Pectin addition favoured gel strength of broad bean suspensions, appreciated to be the result of electrostatic pectin-calcium-interactions. These findings allow developing new food products based on pulses flour, such as soft gels or pâté like products. Future investigations are needed to check the properties of the cold-set gels produced from heated emulsions stabilised by pulse flours.

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## References

AACC International, 2000. Approved Methods of the American Association of Cereal Chemists, 10th edition. Methods 44-51, 56-81B, 54-30. The Association, St. Paul, MN, USA.

Ahmed, J. and Auras, R., 2011. Effect of acid hydrolysis on rheological and thermal characteristics of lentil starch slurry. LWT-Food Science and Technology 44: 976-983.

Alamanou, S. and Doxastakis, G., 1997. Effect of wet extraction methods on the emulsifying and foaming properties of lupin seed protein isolates (*Lupinus albus* ssp. *Graecus*). Food Hydrocolloids 11: 409-413.

Amarowicz, R., Estrella, I., Hernández, T., Robredo, S., Troszyńska, A., Kosińska, A. and Pegg, R.B., 2010. Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). Food Chemistry 121: 705-711.

Asp, N.G., Johansson, C.G., Hallmer, H. and Siljeström, M., 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. Journal of Agricultural and Food Chemistry 31: 476-482.

Bassett, C., Boye, J., Tyler, R. and Oomah, B.D., 2010. Molecular, functional and processing characteristics of whole pulses and pulse fractions and their emerging food and nutraceutical applications. Food Research International 43: 397-398.

Batista, A.P., Portugal, C.A., Sousa, I., Crespo, J.G. and Raymundo, A., 2005. Accessing gelling ability of vegetable proteins using rheological and fluorescence techniques. International Journal of Biological Macromolecules 36: 135-143.

Boye, J.I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E. and Rajamohamed, S.H., 2010a. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Research International 43: 537-546.

Boye, J.I., Zare, F. and Pletch, A., 2010b. Pulse proteins: processing, characterization, functional properties and applications in food and feed. Food Research International 43: 414-431.

Braudo, E.E., Plashchina, I.G. and Schwenke, K.D., 2001. Plant protein interactions with polysaccharides and their influence on legume protein functionality – a review. Food/Nahrung 45: 382-384.

Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P. and Duc, G., 2010. Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. Field Crops Research 115: 329-339.

Ding, Y., Liu, R., Rong, J., Liu, Y., Zhao, S. and Xiong, S., 2012. Rheological behavior of heat-induced actomyosin gels from yellowcheek carp and grass carp. European Food Research and Technology 235: 245-251.

Donato, L., Garnier, C., Novales, B. and Doublier, J.L., 2005. Gelation of globular protein in presence of low methoxyl pectin: effect of $Na^+$ and/or $Ca^{2+}$ ions on rheology and microstructure of the systems. Food Hydrocolloids 19: 549-556.

Fraeye, I., Doungla, E., Duvetter, T., Moldenaers, P., Van Loey, A. and Hendrickx, M., 2009. Influence of intrinsic and extrinsic factors on rheology of pectin-calcium gels. Food Hydrocolloids 23: 2069-2077.

Fredriksson, H., Silverio, J., Andersson, R., Eliasson, A.C. and Åman, P., 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. Carbohydrate Polymers 35: 119-134.

Gaur, V., Qureshi, I.A., Singh, A., Chanana, V. and Salunke, D.M., 2010. Crystal structure and functional insights of hemopexin fold protein from grass pea. Plant Physiology 152: 1842-1850.

Ghavidel, R.A. and Prakash, J., 2006. Effect of germination and dehulling on functional properties of legume flours. Journal of the Science of Food and Agriculture 86: 1189-1195.

Giménez, M.A., Drago, S.R., De Greef, D., Gonzalez, R.J., Lobo, M.O. and Samman, N.C., 2012. Rheological, functional and nutritional properties of wheat/broad bean (*Vicia faba*) flour blends for pasta formulation. Food Chemistry 134: 200-206.

Halvorsen, B.L., Holte, K., Myhrstad, M.C., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.B., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs Jr., D.R. and Blomhoff, R., 2002. A systematic screening of total antioxidants in dietary plants. Journal of Nutrition 132: 461-471.

Hettiarachchy, N.S. and Kalapathy, U., 1998. Functional properties of soy proteins. In: Functional properties of proteins and lipids. American Chemical Society, Washington, DC, USA.

Hu, X.Z., Cheng, Y.Q., Fan, J.F., Lu, Z.H., Yamaki, K. and Li, L.T., 2010. Effects of drying method on physicochemical and functional properties of soy protein isolates. Journal of Food Processing and Preservation 34: 520-540.

Ionescu, A., Aprodu, I., Zara, M. and Gurau, G., 2009. Functional characterization of lupin protein concentrate treated with bacterial transglutaminase. AUDJG – Food Technology 32: 9-19.

Kansal, R., Kumar, M., Kuhar, K., Gupta, R.N., Subrahmanyam, B., Koundal, K.R. and Gupta, V.K., 2008. Purification and characterization of trypsin inhibitor from *Cicer arietinum* L. and its efficacy against *Helicoverpa armigera*. Brazilian Journal of Plant Physiology 20: 313-322.

Karki, B., Lamsal, B.P., Grewell, D., Pometto III, A.L., Van Leeuwen, J., Khanal, S.K. and Jung, S., 2009. Functional properties of soy protein isolates produced from ultrasonicated defatted soy flakes. Journal of the American Oil Chemists' Society 86: 1021-1028.

Kaur, M. and Singh, N., 2007. Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. Food Chemistry 102: 366-374.

Kerr, W.L., Ward, C.D.W., McWatters, K.H. and Resurreccion, A.V.A., 2000. Effect of milling and particle size on functionality and physicochemical properties of cowpea flour. Cereal Chemistry 77: 213-219.

Labuckas, D.O., Maestri, D.M., Perelló, M., Martínez, M.L. and Lamarque, A.L., 2008. Phenolics from walnut (*Juglans regia* L.) kernels: antioxidant activity and interactions with proteins. Food Chemistry 107: 607-612.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin – phenol reagents. Journal of Biological Chemistry 193: 265-275.

Makri, E., Papalamprou, E. and Doxastakis, G., 2005. Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, broad bean) in admixture with polysaccharides. Food Hydrocolloids 19: 583-594.

Martinez-Villaluenga, C., Michalska, A., Frías, J., Piskula, M.K., Vidal-Valverde, C. and Zieliński, H., 2009. Effect of flour extraction rate and baking on thiamine and riboflavin content and antioxidant capacity of traditional rye bread. Journal of Food Science 74: 49-55.

Ogunwolu, S.O., Henshaw, F.O., Mock, H.P., Santros, A. and Awonorin, S.O., 2009. Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale L.*) nut. Food Chemistry 115: 852-858.

Ozdal, T., Capanoglu, E. and Altay, F., 2013. A review on protein – phenolic interactions and associated changes. Food Research International 51: 954-970.

Ravi, R. and Bhattacharya, S., 2006. The time-dependent rheological characteristics of a chickpea flour dispersion as a function of temperature and shear rate. International Journal of Food Science and Technology 41: 751-756.

Renkema, J.M., Lakemond, C.M., De Jongh, H.H., Gruppen, H. and Van Vliet, T., 2000. The effect of pH on heat denaturation and gel forming properties of soy proteins. Journal of Biotechnology 79: 223-230.

Rubiales, D., 2010. Faba beans in sustainable agriculture. Field Crops Research 115: 201-202.

Ryan, K.J. and Brewer, M.S., 2007. In situ examination of starch granule-soy protein and wheat protein interactions. Food Chemistry 104: 619-629.

Sikorski, Z.E., 2001. Functional properties of proteins in food systems. In: Sikorski, Z.E. (ed.) Chemical and functional properties of food proteins. CRC Press, Boca-Raton, FL, USA, 504 pp.

Singh, J., Kaur, L. and McCarthy, O.J., 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. Food Hydrocolloids 21: 1-22.

Sun, A. and Gunasekaran, S., 2009. Measuring rheological characteristics and spreadability of soft foods using a modified squeeze-flow apparatus. Journal of Texture Studies 40: 275-287.

Tabilo-Munizaga, G., Barbosa-Cánovas, G.V., 2005. Rheology for the food industry. Journal of Food Engineering 67: 147-156.

Toews, R. and Wang, N., 2013. Physicochemical and functional properties of protein concentrates from pulses. Food Research International 52: 445-451.

Torki, M.A., Shabana, M.K.S., Attia, N. and El-Alim, I.M.A., 1987. Protein fractionation and characterization of some leguminous seeds. Annals of Agricultural Science, Moshtohor 25: 277-291.

Turgeon, S.L., Gauthier, S.F., Mollé, D. and Léonil, J., 1992. Interfacial properties of tryptic peptides of β lactoglobulin. Journal of Agricultural and Food Chemistry 40: 669-675.

USDA, 2014. National nutrient database for standard reference, release 27. Nutrient Data Laboratory Home Page. Available at: http://www.ars.usda.gov/ba/bhnrc/ndl.

Vioque, J., Alaiz, M. and Girón-Calle, J., 2012. Nutritional and functional properties of *Vicia faba* protein isolates and related fractions. Food Chemistry 132: 67-72.