STUDIA UBB CHEMIA, LXIII, 1, 2018 (p. 199-214)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2018.1.15

PULSE FLOUR BASED EMULSIONS – THE EFFECT OF OIL TYPE ON TECHNOLOGICAL AND FUNCTIONAL CHARACTERISTICS

INA VASILEAN[a], IULIANA APRODU[a], ION VASILEAN[a], LIVIA PATRAȘCU[a,*]

ABSTRACT. Three different types of whole pulse flours (broad bean, green and red lentils) were used to obtain emulsions and thereafter heat-set gels, and their properties were compared to the soy protein concentrate. The influence of different vegetable oils (sunflower, canola and palm) on emulsions and heat-set gels properties was investigated by considering the antioxidant activity and rheological characteristics. The emulsifying capacity resulted fairly similar in case of all protein sources in combination with sunflower and canola. Slightly higher emulsifying capacity was observed in case of soy-canola oil emulsion compared to soy-sunflower oil emulsion. The palm oil presented limited capacity to form emulsions. Among studied proteins sources, the highest antioxidant capacity was recorded for broad bean flour. DPPH radical scavenging activity of obtained emulsions was influenced by oil type. It was also observed that antioxidant activity was affected by the thermal treatment. Rheological measurements showed no stability for pulse flour based emulsions, samples entering directly into the transition phase towards flowing. The heat-set gels presented linear viscoelastic regions with higher yield point values in comparison to emulsions. The oil used for preparing the emulsions had a significant influence on yield point value both for emulsions and gels.

Keywords: *Emulsions, Fatty acids, Natural antioxidants, Rheology, Legumes*

INTRODUCTION

The growing interest on vegetable proteins as alternatives to animal ones is related to the nutritional benefits, agricultural aspects and biodiversity preservation. Among vegetables, the legume seeds and in particular pulses represent a rich source of proteins with significant potential to provide benefits for human health [1, 2, 3].

[a] *Universitatea Dunarea de Jos, Facultatea de Stiinta si Ingineria Alimentelor, Str. Domneasca, Nr. 111, RO-800201 Galati, Romania.*
[*] *Corresponding author: livia.patrascu@ugal.ro*

Technological processing of vegetable foods products might involve passing through an emulsified state before becoming final ready to eat products, such as pate or cake batters. Knowledge of the rheological characteristics of the emulsion state, is crucial for efficient handling the quality of the final product, because many of the sensory attributes of food emulsions like creaminess, thickness, smoothness, spreadability, pourabilty, flowability, brittleness, and hardness are directly related to their rheological properties [4].

Many studies report on the properties of vegetable emulsions containing leguminous proteins from lupine, soy, broad bean or pea as emulsifiers and stabilizers [5, 6, 7, 8, 9, 10]. When referring to emulsion based foodstuff, lipid peroxidation is one of the major concerns, and emulsifying the protein sources in their natural matrices containing bioactive compounds is an appealing alternative to synthetic antioxidants in fighting free radicals. In this respect, leguminous seeds represent important sources of natural antioxidants such as phenolic compounds like flavanols and condensed tannins, tocopherols, and vitamin C [11]. However, thermal treatment at high temperatures was reported to decrease the antioxidant properties of pulses, while increasing the amount of total available phenols [12]. On the other hand, Xu and Chang [13] stated that processing the pulse legumes through cooking decreased both antioxidant capacities and total phenolic, while Chakraborty and Bhattacharyya [14] reported increasing the antioxidant capacities for some pulses. These differences are due to different cooking methods applied in different studies for pulses treatment.

The physicochemical properties of emulsions are highly influenced by all major constituents. In particular, proteins influence both emulsion formation and stability. The ratio between polar and non-polar groups within protein structure is considered one of the major factors that modulate proteins functionality as emulsifiers. Also, the properties of the oil phase influence the texture and spreadability of the final products [15]. The ratio between linear saturated fatty acids and branched unsaturated fatty acids in oils was reported to mainly determine the performances of the oils in stabilizing the emulsion structure [4]. However there is limited knowledge regarding the effect of oil nature on the physicochemical characteristics of the emulsion based gels. To the best of our knowledge there is no scientific data on proteins emulsifying capacity as a function of oil type.

On the other hand, there is the increasing interest for minimally processed products with short ingredient list and high nutritional functionality [16, 17]. In line with the FAO mission of increasing the awareness on the importance of developing sustainable food production based on pulses, and also considering the new trends in human nutrition the present study was focused on minimally refined flours from red and green lentils and broad

beans for substituting the highly processed forms of the soy bean. The aim of the study was to emphasize the effect of different oil types on antioxidant activity and rheological characteristics of pulses flour based emulsions and thermally induced gels. Three frequently used food grade oils were tested, namely sunflower, canola and palm oils.

RESULTS AND DISCUSSION

Emulsifying capacity

The emulsifying capacity of the pulse flours was estimated as volume of oil required for emulsion break down due to phase inversion, and was compared to the soy protein concentrate (Figure 1) which is commonly used in a large variety of food products due to the good emulsifying properties.

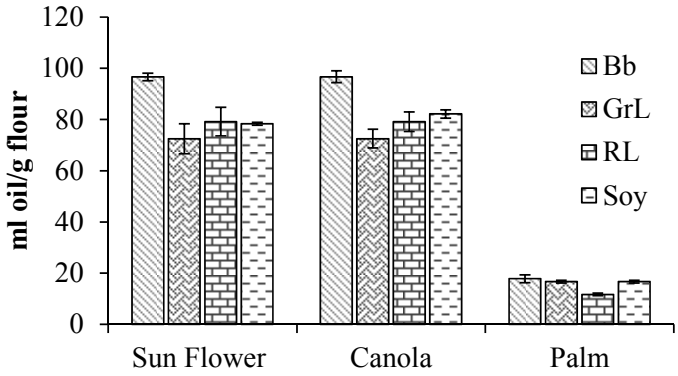

Figure 1. The influence of selected oils on emulsifying capacity of proteins

The emulsifying capacity of proteins is influenced by the molecular structure of the compounds prevailing in the composition of the oils, and in particular by the ratio between saturated and unsaturated fatty acids [4]. The fatty acids composition of the oils considered in the study is given in Table 1. As one can see, canola oil presented the least percentage of saturated fatty acids, with the ratio of saturated and unsaturated acids of 1:13.38, followed by sunflower oil with the ratio of 1:9. Palm oil had the highest quantity of saturated fatty acids with a ratio of saturated and unsaturated acids of 1:1.3.

Regardless of the protein source, the emulsifying capacity resulted fairly similar in case of emulsions prepared with sunflower and canola oils ($p>0.05$), with slightly higher values in case of soy-canola oil emulsion compared to

soy-sunflower oil emulsion. All investigated protein sources displayed limited capacity to form emulsions in combination with palm oil (Figure 1). The limited capacity of the protein derivatives to form emulsions with palm oil might be due to fat destabilization as a consequence of crystallization phenomena. Moreover, in case of the palm oil based emulsions the consistency of the system during the entire period of emulsification was observed to be significantly lower compared to sunflower or canola oil where thick emulsions were obtained before the breakdown.

Table 1. Oils composition in saturated acids according to label

Oil type	**Declared composition**
Sunflower	Lipids – 97 % from which saturated – 10.1% monounsaturated – 83.6% polyunsaturated – 3.9%
Canola	Lipids – 92 % from which saturated – 6.4% monounsaturated – 58.7% polyunsaturated – 26.9%
Palm	Lipids – 100 % from which saturated – 43% monounsaturated – 45% polyunsaturated – 12%

Available scientific reports indicate that protein emulsions with 10 to 20 wt% palm oil concentrations were passed through a high-pressure valve homogenizer in order to reduce droplet size and to assure thus emulsion stability [22, 20].

Emulsions stability

Emulsions instability and break down is known to be dictated by many physicochemical processes. Several studies indicate different mechanisms leading to emulsion destabilization, such as Ostwald ripening, creaming, gravitational sedimentation and flocculation, spontaneous coalescence and coalescence under stress [26, 22].

The stability of the investigated emulsions over 24 hours of storage at 10°C is presented in Figure 2. The destabilization of the emulsions with droplet sizes larger than a few microns mainly occurs due to creaming and sedimentation phenomena. According to our results the stability of the emulsions was mainly influenced by the protein sources rather than the nature of oil. As one can see the most stable formulations were those based on soy protein concentrate with no separated phases. In case of broad bean based emulsions, only the sample prepared with palm oil separated a liquid

phase. Regardless of the oil used for preparing the emulsions, all lentils flour based samples separated liquid and/or foam phases. However, the lowest stability with 2 - 2.5 cm^3 of total separated phase was observed in case of the red lentils based emulsions (Figure 2).

Radical scavenging activity

Because different classes of antioxidants respond to different analytical methods, three types of assays were used in order to cover both hydrogen atom transfer mechanism (ABTS radical scavenging method) and electron transfer mechanism (DPPH radical scavenging assay and total phenols) [27].

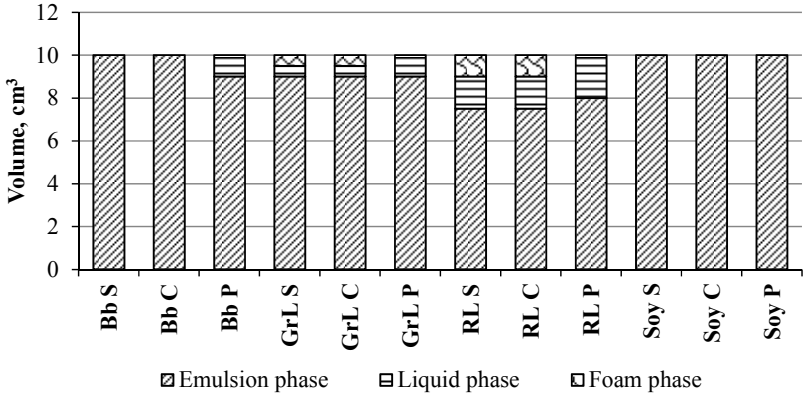

Figure 2. Stability of the emulsions prepared with broad bean (*Bb*), red lentils (*RL*) or green lentils (*GrL*) flours and sun flower (*S*), canola (*C*) or palm (*P*) oil after 24 h of storage at 10°C

The antioxidant properties were determined for pulses flours, derived emulsions and heat-set gels, and were compared to the soy protein concentrate (Table 2).

Table 2. Proximal composition and antioxidant capacity of protein derivatives used in the present study

	Broad bean flour	Green Lentil flour	Red Lentil flour	Soy Protein Concentrate
Moisture, %	9.76±0.05	11.51±0.04	8.49±0.12	9.52±0.09
Total protein, %	21.61±0.00	21.59±0.21	21.81±0.09	74.28±0.47
Total phenolics, FA mg/g	8.31±0.15	5.15±0.02	3.44±0.01	7.18±0.01
EC50 of DPPH, mg	10.26±0.03	12.55±0.00	28.92±0.01	25.72±0.00
TEAC, µmol Trolox/g	11.01±0.11	9.07±0.01	3.93±0.01	4.88±0.01

Total phenolic content is highly important, as the phenolic compounds were reported to be related with many health benefits and also were found responsible for most of the antioxidant properties of the methanolic extracts of pulse seeds [28]. In addition, Elias et al. [29] reported that proteins can act as antioxidants in food products, through multiple mechanisms such as inactivation of reactive oxygen species, scavenging free radicals, chelation of prooxidative transition metals, reduction of hydroperoxides, and alteration of the physical properties of food systems.

The highest total phenolic content was obtained for the broad bean flour, followed by soy protein concentrate, green lentil and red lentil (Table 2). Higher phenolic content for green lentil in comparison to red lentil was also reported by Amarowicz et al. [30, 31]. The high content of polyphenols in the broad bean flour can be explained by the presence of insoluble fiber content (hulls) that are known to concentrate most polyphenols of the bean seeds. Antioxidant activity in terms of EC50 of DPPH and TEAC presented a similar trend to total phenolic content in case of pulse flours. The highest DPPH radical scavenging activity was recorded for broad bean flour while for red lentil flour was registered the lowest activity. Despite the high content of total phenols, the DPPH radical scavenging activity and TEAC of the soy protein concentrate was fairly low. The antioxidant activity of the soy protein derivatives is mainly due to polyphenolic compounds like isoflavones (genistein and daidzein) [32], chlorogenic acid isomers, caffeic acid and ferulic acid [33]. However, soybean isoflavones and their glycosides were found to be no effective in scavenging the DPPH free radicals [34, 32].

The antioxidant properties of emulsions and gels were correlated with data obtained for the corresponding protein sources (Table 3). Thus trend of the antioxidant potentials of the emulsions and gels resulted as follows: red lentil < soy < green lentil < broad bean. As expected, the type of oil used to prepare the emulsions had no significant effect on the total phenolic content ($p>0.05$). In case of the DPPH antioxidant activity significant differences were obtained between groups defined based on the oil nature ($p<0.05$), both for emulsions and gels. The highest DPPH radical scavenging activity was obtained in case of samples prepared with sun flower oil, followed by those with palm and canola oils. Thermal treatment determined a significant decrease of DPPH radical scavenging activity in case of all gel samples ($p<0.05$). As a general trend TEAC antioxidant activity presented no significant differences after thermal treatment ($p>0.05$), with slight differences only in case on broad bean based emulsions and gels.

Rheological behavior

Rheological behaviour of the tested food grade oils is presented in Figure 3 as viscosity and shear stress responses to the applied shear rate.

Canola and sunflower oils presented Newtonian behavior, while palm oil showed a shear thinning behavior with a yield stress of 25.64 Pa and a slightly visible thixotropy area of 1040 Pa/s (determined with Hershel-Bulkley equation).

Table 3. Antioxidant capacity of obtained emulsions and heat-set gels

Samples		Total phenolics, FA mg/g	EC50 of DPPH, mg	TEAC, µmol Trolox/g
Emulsions	Soy+Sunflower	2.16±0.01	227.68±0.01	0.67±0.01
	Soy+Canola	2.14±0.01	205.28±0.01	1.16±0.01
	Soy+Palm	2.14±0.01	212.30±0.01	1.08±0.01
	Broad bean+ Sunflower	2.33±0.01	43.64±0.01	2.68±0.01
	Broad bean+Canola	2.30±0.01	47.10±0.01	2.62±0.01
	Broad bean+Palm	2.31±0.01	46.58±0.01	2.51±0.01
	Green Lentil+ Sunflower	0.83±0.01	78.85±0.01	1.79±0.01
	Green Lentil+Canola	0.81±0.01	82.64±0.01	1.53±0.01
	Green Lentil+Palm	0.81±0.01	79.22±0.01	2.13±0.01
	Red Lentil+ Sunflower	0.55±0.01	396.55±0.01	1.20±0.01
	Red Lentil+Canola	0.53±0.01	516.62±0.01	0.78±0.01
	Red Lentil+Palm	0.55±0.01	451.90±0.01	0.97±0.01
Gels	Soy+ Sunflower	2.72±0.01	127.67±0.01	1.14±0.01
	Soy+Canola	2.69±0.01	147.52±0.01	1.73±0.01
	Soy+Palm	2.71±0.01	135.47±0.01	1.62±0.01
	Broad bean+ Sunflower	2.99±0.01	40.93±0.01	3.11±0.01
	Broad bean+Canola	2.97±0.01	42.83±0.01	3.71±0.01
	Broad bean+Palm	2.98±0.01	41.72±0.01	3.41±0.01
	Green Lentil+ Sunflower	0.99±0.01	61.07±0.01	1.62±0.01
	Green Lentil+Canola	0.96±0.01	64.20±0.01	2.38±0.01
	Green Lentil+Palm	0.98±0.01	63.78±0.01	2.13±0.01
	Red Lentil+ Sunflower	0.78±0.01	213.27±0.01	0.81±0.01
	Red Lentil+Canola	0.78±0.01	247.55±0.01	0.85±0.01
	Red Lentil+Palm	0.76±0.01	231.23±0.01	0.96±0.01

When studying palm oil rheological behavior as influenced by crystallization, Tarabukina et al. [35] identified the 17-22°C range as the onset of crystallization process. At room temperature, palm oil is a semi-solid material

with rheological properties depending on the amount, size and tri-dimensional organization of its fat crystal network [35]. Viscosity values resulted fairly similar for sunflower and canola oils ($p>0.05$), and significantly different compared to the palm oil. Our results comply with the observation of Santos et al. [36], who reported a Newtonian behavior for different edible oils, through which sunflower and rape seed, at temperatures ranging between 10 and 80°C.

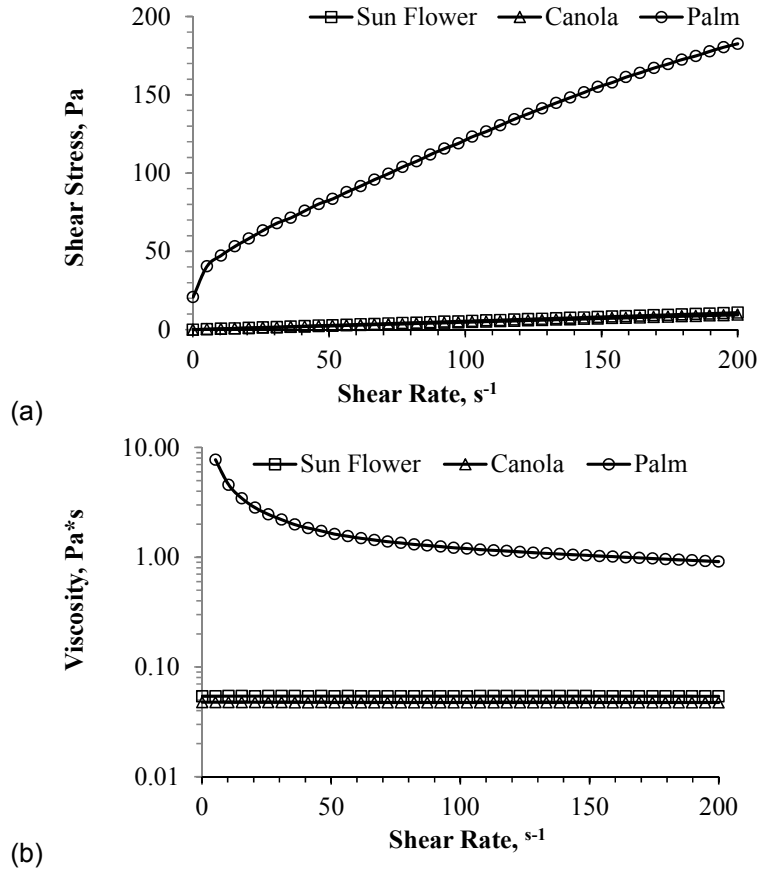

Figure 3. Evolution of shear stress (a) and viscosity (b) of selected vegetable oils under forced flow conditions

Rheological behavior of the obtained emulsions was studied at 20°C in low amplitude oscillatory conditions, by increasing the strain values from 0.01 to 100% at a constant frequency of 1 Hz. Emulsions obtained from broad bean (Figure 4a) and lentil flour (Figure 4 b and c) presented no stability no

LVR was registered), displaying even from the beginning of the tested strain interval a behavior specific to the transition phase, when particles just start moving. Soy protein emulsions prepared with sunflower and canola oils presented limited structure stability, with LVR up to a critical strain of 1%, while emulsion obtained with palm oil resisted only up to ~ 0.5% strain. In all studied protein sources the highest G` values were obtained for the emulsions prepared with palm oil. The G` and G`` values were significantly influenced by the flour type. The lowest G` and G`` values, ranging from 1 to 3 Pa, were obtained for red lentils based emulsions, followed by green lentils and broad bean. The emulsions based on soy protein concentrate presented the highest G` and G`` values, revealing a strong solid like behavior with G` values up to 5000 Pa, and the lowest delta values of ~ 9° in the linear viscoelastic region (data not presented).

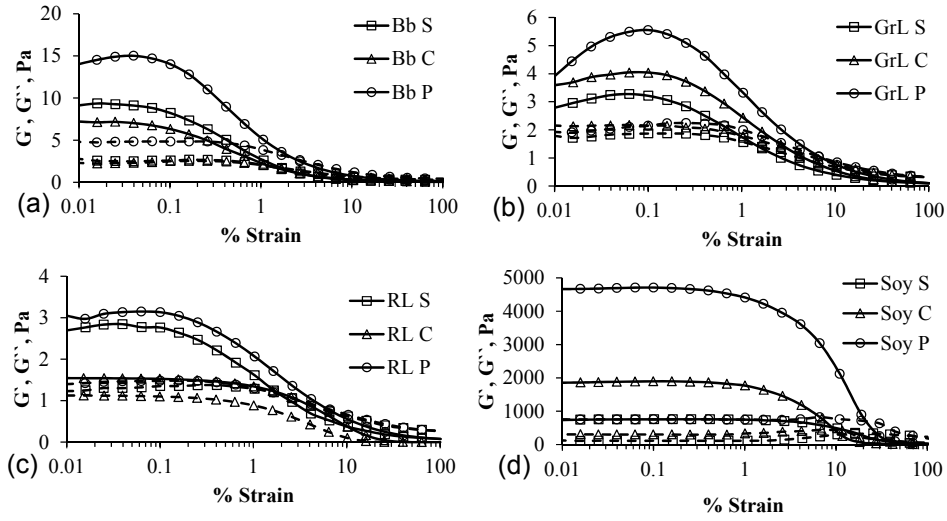

Figure 4. Rheological behavior, under strain sweep, of the emulsions based on broad bean flour (a), red lentils flour (b), green lentils flour (c) and soy protein concentrate (d). Emulsions were prepared with sunflower (*S*), canola (*C*) or palm (*P*) oils. Full lines stand for G`, dashed lines stand for G``; *Bb* - broad bean flour, *RL* - red lentils flour, *GrL* - green lentils flour

The proper beginning of flow, also known as the yield point, was determined as the strain value at G`/G`` crossover as represented in Table 4. In case of most proteins sources the canola oil based samples had the earliest beginning of flow, followed by sunflower and palm oils. When discussing the influence of the protein source on the yield point, generally the lowest values were obtained in case of broad bean based emulsions, followed by lentils and soy protein derivative.

The heat-set gels presented similar viscoelastic characteristics for the three investigated pulse flours, with strong solid like behaviors as can be seen in Figure 5.

Table 4. Yield point of pulse flour based emulsions and gels determined as the strain value (%) at G`/G`` crossover

	Sample	Sunflower Oil	Canola Oil	Palm Oil
Emulsions	Soy	15.52±0.98%	9.28±0.05	25.31±1.12
	Broadbean	2.13±0.15%	1.33±0.78	2.68±0.10
	Green Lentil	1.65±1.02%	3.15±0.22	8.38±0.06
	Red Lentil	4.13±1.17%	4.05±0.84	6.61±0.43
Gels	Soy	22.21±0.66%	22.51±1.54	22.49±1.34
	Broadbean	33.50±1.83%	35.95±2.02	17.38±0.80
	Green Lentil	40.00±1.68%	18.44±0.70	10.61±0.04
	Red Lentil	31.99±0.31%	34.72±0.64	33.17±0.51

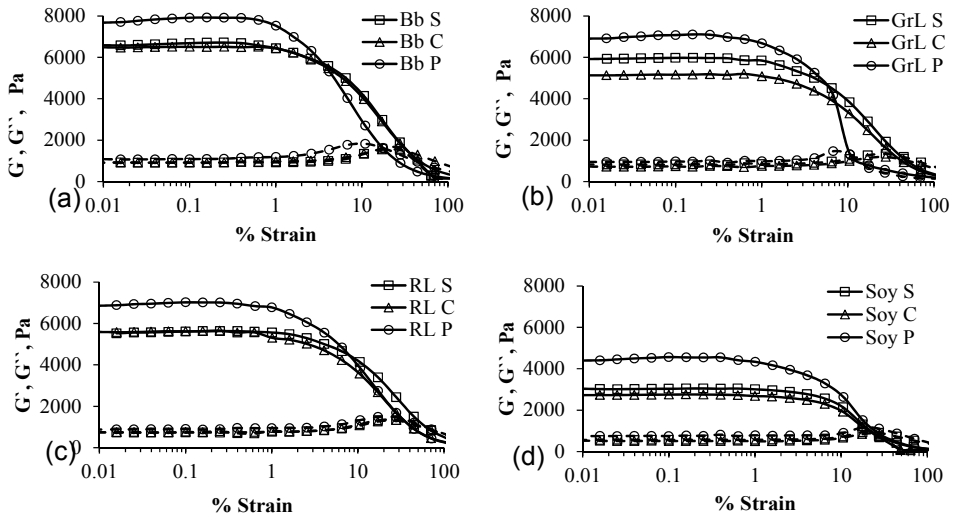

Figure 5. Rheological behavior of obtained heat –set gels under strain sweep (broad bean (*Bb*), red lentils (*RL*), green lentils (*GrL*) flours, sun flower (*S*), canola (*C*), palm (*P*)). Full lines stand for G` and dashed lines stand for G``

The high G` values in case of pulse flour based gels are explained by the presence of polysaccharides and fibers [18], but also by the protein unfolding during thermal treatment that favored the development of a stable entangled network [37]. Lower G` values were obtained in case of soy protein based gels in comparison to emulsions. It seems that at pH values higher than 5 the thermal treatment softens the structure of the soy proteins gels, due to the decreased number of protein molecules involved in establishing the network [38]. In case of all studied gels the structure remained unchanged up to strain values of 1%, marking the end of the linear viscoelastic region. The yield point values of the gels appeared at significantly higher strain values compared to the emulsions. A particular behavior was observed for gels obtained with palm oil. In case of emulsions the samples with palm oil presented the highest G`/G`` crossover strain value, whereas after gel formation higher yield point values were determined for samples prepared with sunflower and canola (Table 4). No influence of oils on yield point of soy protein based gels was noticed.

CONCLUSIONS

The emulsifying capacity of the flours obtained from broad beans, green and red lentils is comparable to the soy protein concentrate. Regardless of the protein source, no significant difference in terms of emulsifying capacity and emulsions properties were found between samples prepared with sunflower and canola oils. All studied protein sources presented limited capacity to form emulsions in combination with palm oil which could be given by fat destabilization due to crystallization phenomena. The stability of the emulsions varied significantly with protein source and the oil type. The antioxidant activity of emulsions and heat-set gels was significantly influenced by the protein source. Rheometric measurements showed that sunflower and canola oils are Newtonian fluids, while palm oil behaved as a thixotropic fluid. Moreover, a strong solid like behavior was observed for the palm oil containing emulsions. Even if the rheological tests performed on emulsions indicated the significant influence of both protein sources and oil type, after the thermal treatment no important differences were found between samples prepared with different oils. Finally it can be stated that, given the high content of saturated fatty acids, the palm oil alone is not suitable for obtaining stable emulsions and further heat-set gels, while oils with high ratios of saturated *vs*. unsaturated acids ratio presented similar technological and functional characteristics.

EXPERIMENTAL SECTION

Materials

Three different pulses, namely broad beans (*Vicia faba*), red and green lentils (*Lens culinaris*) purchased from a local store (Galati, Romania), were considered in the study. The pulse flours were obtained at laboratory scale as specified by Patrascu et al. [18]. Proximal composition of obtained samples is presented in Table 1. Because the majority of foodstuff that implies the use of vegetable proteins is based on soy protein derivatives (concentrates and isolates), a soy protein concentrate (Ubimedia S.R.L., Galati, Romania) was used as control.

Three different food grade refined vegetable oils were considered for the investigations: bottled sunflower (produced in Romania), canola (produced in Romania) and palm (produced in Malaysia) oils purchased from a local supermarket (Galati, Romania). All materials were stored in dark conditions at room temperature until use.

All chemicals used in the experiments were of analytical grade. The reagents 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and ferulic acid were purchased from Sigma Aldrich Chemie GmbH (Taufkirchen, Germany), while Folin Ciocalteu reagent was purchased from Merck and Co., Inc. (New York, USA).

Proximate composition

The physicochemical composition of the obtained pulse flours was determined using the following methods: the moisture content through the AACC 44-51 method (AACC International, 2000); the protein content using the semimicro-Kjeldahl method (Raypa Trade, R. Espinar, S.L., Barcelona, Spain).

Emulsifying capacity

In order to assess the emulsifying capacity of pulse flours and soy protein concentrate as a function of selected oils the method proposed by Ionescu et al. [19] was used. First suspensions of 6% (w/v) concentration were prepared in 0.2M phosphate buffer of pH 7. The oil was progressively added to the suspensions, using a laboratory burette, under continuous stirring with a 650W Philips blender, until the inversion point of the emulsion was observed. The emulsifying capacity was reported as ml of oil/g flour. In the case of palm oil, a preliminary melting was performed through storage for 24 h at 40°C. In order to prevent crystallization a similar palm oil preparation for emulsion formation was applied by Pongsawatmanit et al. [20].

Emulsion preparation

Suspensions were first prepared by adding 65 ml of tap water to 15 g of pulse flour under continuous manual stirring. The concentration of the pulse flour suspensions was established by first determining the least gelling concentration using the method described by Ogunwolu et al. [21]. A volume of 20 ml of oil was then added to the homogenous mixture while mixing the ingredients at 1500 rpm with a Philips RH13 vertical mixer for 5 minutes.

It is commonly accepted that a true emulsion consists of two phases, oil and water, with the dispersed droplet size between 0.1 and 100 μm. Taking into account that the protein derivatives used in the present study had particles size lower than 500 μm, we consider that the obtained system is an O/W emulsified protein dispersion. Anyway, for convenience the term *emulsion* will be further used in the paper.

Emulsion stability

The stability of the emulsions over 24 h of storage at 10°C was determined by filling a known quantity of emulsion (10 ml) into laboratory tubes and measuring phase separation. In agreement with the method of Thanasukarn et al. [22] with modifications, the separated layers were visually estimated at the end of the resting period. In our study the following phases were considered: “emulsion phase” which is a layer similar to the initial emulsion, “liquid phase” an intermediate white watery layer with little traces of free oil, and “foam phase” a dense and more opaque phase separated on the top of the emulsion.

Heat-set emulsion gels

Fresh emulsions were poured into glass containers, hermetically sealed with food grade metal caps and thermally treated in a water bath (JSWB-06T, JSR) by increasing the temperature with ~1.5°C/min until 90°C, where was maintained for 30 minutes. The obtained products had a paté like structure, and were further termed gels. All samples were stored at 10±1°C for further analysis.

Radical scavenging activity

In order to investigate the antioxidant activity of obtained emulsions, gels and flours, first a methanol extraction of active compounds was carried. The extraction of the active compounds from pulse flours, emulsions and gels responsible for the antioxidant activity was performed by mixing 1 g of sample with 10 mL of 80% aqueous methanol solution, followed by stirring for 2 h at room temperature using a magnetic stirrer at 300 rpm and centrifugation at 9,690×g for 10 minutes.

Determination of total phenolic contents

The concentration of total phenolic compounds was determined using the Folin-Ciocalteu method proposed by Deng et al. [23] with slight modification. A volume of 0.2 ml of extract solution was mixed with 1.5 mL Folin-Ciocalteu reagent, previously diluted with water 1:10, v/v. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 60 g/L sodium carbonate was added. After 90 min of reaction at room temperature, absorbance was read at 725 nm. Acidified methanol was used as blank. Results were expressed as mg ferulic acid equivalents (FAE)/g sample.

DPPH-radical scavenging assay

Volumes of 0.1 mL extracts were added to 3.9 mL DPPH in methanol solution of 6×10^{-5} mol. A control sample containing the same volume of solvent in place of extract was used to measure the maximum DPPH absorbance. After a resting period of 30 min in dark conditions the absorbance was recorded at 515 nm. The DPPH radical scavenging activity was expressed as EC50 values after the method of Anagnostopoulou *et al*. [24].

Antioxidant activity by ABTS radical cation decolorization assay

ABTS assay was based on the slightly modified method of Re *et al.* [25]. ABTS radical cation ($ABTS^{\cdot+}$) was obtained by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{\cdot+}$ stock solution was then diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 20 µL of sample to 1.48 mL of diluted $ABTS^{\cdot+}$ solution, absorbance was measured after exactly six min. The antioxidant activity was expressed as micromoles of Trolox equivalent antioxidant capacity (TEAC) per gram of sample.

Rheological tests

Rheological characterization of oils, emulsions and gels was performed by using a control-stress Rheometer (AR2000ex, TA Instruments), equipped with a Peltier jacket temperature control system. A cup and conical cylinder geometry assembly was used, with the bob diameter of 28 mm and 42 mm in length.

Rheological characteristics of considered oils were studied at 20°C under forced flow conditions, by submitting samples to a stepped flow test. Shear rate was increased from 0.1 to 200 s^{-1} and then decreased back to 0.1 s^{-1}. Results were plotted as the shear stress response/viscosity *vs.* shear rate, in order to determine the hysteresis formed by the destructured - restructured material. The thixotropic behavior was estimated as the decreasing viscosity under increasing shear rate. When appropriate (namely in the case of palm oil),

data were analyzed with Herschel-Bulkley equation: $\sigma = \sigma_y + \eta \times \dot{Y}^n$, where σ = shear stress (Pa); σ_y = yield stress (Pa); η = viscosity (Pa·s); $\dot{Y}$ = shear rate (s^{-1}); n = rate index, also known as flow behavior index (dimensionless).

Rheological properties of emulsions and gels were analyzed under oscillatory flow in small amplitude conditions, by performing a strain sweep test under increasing strain from 0 to 100%, with an oscillation frequency of 1 Hz. The linear elastic region and the beginning of flow were determined as described by Patrascu et al. [18].

Statistical analysis

Statistical analysis was performed using Microsoft Excel Software. 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.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS – UEFISCDI, project number PN-II-RU-TE-2014-4-0618.

REFERENCES

1. E. Makri, E. Papalamprou, G. Doxastakis, *Food Hydrocolloids,* **2005**, *19*, 583.
2. C. Bassett, J. Boye, R. Tyler, B.D. Omah, *Food Research International*, **2010**, *43*, 397.
3. K. Crépon, P. Marget, C. Peyronnet, B. Carrouée, P. Arese, G. Duc, *Field Crops Research*, **2010**, *115*, 329.
4. D.J. McClements, “Food emulsions: principles, practices, and techniques”, CRC Press, **2004**, chapter 1.
5. A. Raymundo, J.M. Franco, J. Empis, I. Sousa, *Journal of the American Oil Chemists' Society*, **2002**, *79*, 783.
6. W. Jang, A. Nikolov, D.T. Wasan, K. Chen, B. Campbell, *Industrial and Engineering Chemistry Research*, **2005**, *44*, 4855.
7. R.R. Roesch, M. Corredig, *Journal of Food Science-Chicago*, **2002**, *67*, 2837.
8. F. Donsì, B. Senatore, Q. Huang, G. Ferrari, *Journal of Agricultural and Food Chemistry*, **2010**, *58*, 10653.
9. A.C. Karaca, N. Low, M. Nickerson, *Food Research International*, **2011**, *44*, 2742.
10. Y. Ladjal-Ettoumi, H. Boudries, M. Chibane, A. Romero, *Food Biophysics*, **2016**, *11*, 43.
11. R. Amarowicz, R. B. Pegg, *European Journal of Lipid Science and Technology*, **2008**, *110*, 865.
12. H. Han, B.K. Baik, *International Journal of Food Science and Technology*, **2008**, *43*, 1971.
13. B.J. Xu, S.K.C. Chang, *Journal of Food Science*, **2008**, *73*, H19.

14. A. Chakraborty, S. Bhattacharya, *Journal of Applied Pharmaceutical Science*, **2014**, *4*, 65.
15. D.P. Moran, "Fats in Food Products", Springer US, **1994**, chapter 5.
16. R.K. Keservani, R.K. Kesharwani, N. Vyas, S. Jain, R. Raghuvanshi, A.K. Sharma, *Der Pharmacia Lettre,* **2010**, *2*,106.
17. I. Goldberg, "Functional Foods: Designer foods, Pharmafoods, Nutraceuticals" Springer Science and Business Media, **2012**.
18. L. Patrascu, I. Banu, I. Vasilean, I. Aprodu, *Food Science and Technology International,* **2017**, *9(1)*,67.
19. A. Ionescu, I. Aprodu, M. Zara, G. Gurau, *The Annals of the University Dunarea de Jos of Galati–Food Technology*, 2009, *32(1)*, 9.
20. R. Pongsawatmanit, T. Harnsilawat, D. J. McClements, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **2006**, *287*, 59.
21. S.O. Ogunwolu, F.O. Henshaw, H.P. Mock, A. Santros, S.O. Awonorin, *Food Chemistry*, **2009**, *115*, 852.
22. P. Thanasukarn, R. Pongsawatmanit, D.J. McClements, *Food Hydrocolloids*, **2004**, *18*, 1033.
23. G.F. Deng, X. Lin, X.R. Xu, L.L. Gao, J.F. Xie, H.B. Li, *Journal of Functional Foods*, **2013**, *5*, 260.
24. M.A. Anagnostopoulou, P. Kefalas, V.P. Papageorgiou, A.N. Assimopoulou, D. Boskou, *Food Chemistry*, **2006**, *94*,19.
25. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free radical biology and medicine*, **1999**, *26*, 1231.
26. D. Langevin, S. Poteau, I. Hénaut, J.F. Argillier, *Oil and Gas Science and Technology*, **2004**, *59*, 511.
27. E. Shalaby, S. Shanab, *African Journal of Pharmacy and Pharmacology,* **2013**, *7*, 528.
28. B.A. Cevallos-Casals, L. Cisneros-Zevallos, *Food Chemistry*, **2010**, *119*, 1485.
29. R.J. Elias, S.S. Kellerby, E.A. Decker, *Critical reviews in food science and nutrition,* **2008**, 48, 430.
30. R. Amarowicz, I. Estrella, T. Hernández, M. Dueñas, A. Troszynska, A. Kosinska, *International Journal of Molecular Sciences*, **2009**, *10*, 5513.
31. R. Amarowicz, I. Estrella, T. Hernández, S. Robredo, A. Troszyńska, A. Kosińska, R. B. Pegg, *Food Chemistry*, **2010**, *121*, 705.
32. C.H. Lee, L. Yang, J.Z. Xu, S.Y.V. Yeung, Y. Huang, Z.Y. Chen, *Food Chemistry*, **2005**, *90*, 735.
33. D.E. Pratt, P.M. Birac, *Journal of Food Science,* **1979**, 44, 1720.
34. J.H. Mitchell, P.T. Gardner, D.B. McPhail, P.C. Morrice, A.R. Collins, G.G. Duthie, *Archives of Biochemistry and Biophysics*, **1998**, *360*, 142.
35. E. Tarabukina, F. Jego, J.M. Haudin, P. Navard, E. Peuvrel-Disdier, *Journal of Food Science*, **2009**, *74*, E405.
36. J. Santos, I. Santos, M. Conceição, S. Porto, M.F.S.A. Trindade, A. Souza, A. Araújo, *Journal of thermal analysis and calorimetry*, **2004**, *75*, 419.
37. A. Raymundo, J. Franco, C. Gallegos, J. Empis, L. Sousa, *Nahrung-Food*, **1998**, *42*, 220.
38. J.M. Renkema, H. Gruppen, T. Van Vliet, *Journal of Agricultural and Food Chemistry*, **2002**, *50*, 6064.