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INVESTIGATION OF RHEOLOGICAL CHARACTERISTICS AND STABILITY OF EMULSIONS BASED ON PEA, SOY, AND WHEY PROTEINS®

Badanie właściwości reologicznych i stabilności emulsji na bazie białek grochu, soi i serwatki®

Research equipment was purchased as part of the „Food and Nutrition Centre – modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (CŻiŻ)" co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014–2020 (Project No. RPMA.01.01.00-14-8276/17).
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Key words: emulsion stability, rheological properties, pea protein, soy protein, whey protein.

The article presents the results of research on the effect of the type and concentration of protein on the rheological properties and stability of O/W emulsions. The following proteins were used in the research: pea, soy and whey with a concentration of 0.5–10% in the water phase. The fat content of the emulsion was 20%. Studies have shown that among the tested proteins, pea protein provides the best emulsifying properties and emulsion stability even at low concentrations >0.5%. Moreover, it was found that the density, consistency coefficient and emulsion stability increased with increasing protein concentration.

Słowa kluczowe: stabilność emulsji, właściwości reologiczne, białko grochu, biało soi, białko serwatkowe.

W artykule przedstawiono wyniki badań dotyczące wpływu rodzaju i stężenia białka na właściwości reologiczne i stabilność emulsji typu O/W. Do badań użyto białka: grochu, soi i serwatki o stężeniu 0,5-10% w fazie wodnej. Zawartość tłuszczu w emulsji stanowiła 20%. Badania wykazały, że spośród badanych białek białko grochu zapewnia najkorzystniejsze właściwości emulgujące oraz stabilność emulsji już w niskich stężeniach >0,5%. Ponadto stwierdzono, że wraz ze wzrostem stężenia białka następował wzrost gęstości, wskaźnika konsystencji oraz stabilności emulsji.

INTRODUCTION

In a wide range of applications, emulsification is one of the most important functional properties of food proteins [5]. The proteins currently used by the food industry for their emulsifying abilities are mostly derived from milk (or caseins and whey), egg, soy, rice, and pea [12, 14, 16]. Applications for plant protein include vegan style yogurt, mayonnaise and dairy-free sports products, but it also used as partial dairy products protein substitutes for drinks.

Many proteins are surface-active molecules that can be used as emulsifiers due to their ability to facilitate formation, improve stability, and produce in oil-in-water emulsions with desired physicochemical properties [22]. The stabilization of emulsions by proteins results from their surfactant properties – they have the ability to adsorb at the interface, reduce the surface tension and retard the coalescence of the droplets by forming protective membranes around the droplets [19]. The stability of the food emulsion is the most important factor with respect to their industrial application rate and depends on the droplet size distribution of the dispersed phase, the rheology of the continuous phase, and the interaction between the particles of the dispersed phase [3].

The objective of this study was to evaluate the influence of protein type and concentration on the stability, flow characteristics, and oil droplet size of the stabilized emulsion by pea, soy and whey protein concentrates and to elucidate dependences between these variables.

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MATERIALS AND METHODS

Whey protein concentrate (80% protein) was obtained from Mlekovita (Poland). Pea protein and soy protein were purchased from Agnex (Poland). The pea and soy protein, according to the label, were 80% protein. Rapeseed oil was obtained from a local supermarket.

Emulsion preparation

Pea, soy and whey proteins were dispersed in distilled water and stirred for 2 h. Rapeseed oil (20%) was added to an aqueous phase containing 0.5, 1, 1.5, 2, 2.5, 5, 7.5 or 10% protein (pea, soy, or whey protein). A coarse emulsion was prepared using a rotor-stator homogenizer (Ultra-Turrax IKA T25 digital, Germany) at 10,000 rpm for 4 min, then the pre-emulsification of the system was subjected to pressure homogenization (Panda 2K; Niro Soavi, Italy) at a pressure of 60 and 20 MPa in the first and second stage, respectively.

Oil droplet size

The oil droplet size of protein-based emulsions was measured using a Cilas 1190 laser diffraction particle analyser (Cilas, France). Drops of samples were added to the sample dispersion unit (containing distilled water) until the obscuration index reached approximately 8% and the average droplet size was reported in terms of the mean volume diameter d50.

Density and pH

The emulsion density was determined using a Densito 30PX densimeter (Mettler Toledo, USA). The pH of the emulsion was determined using pH-meter CPO-505 with ERH-111 electrode (Electron, Poland).

Rheological characteristics

Emulsion flow curves were determined at 20 °C using a Haake Mars 40 rheometer (Thermo Scientific, Germany) in steady shear mode with a linearly increasing shear rate from 0.1 to 300 s^{-1} in a coaxial cylinder measuring system. The course of the tests was controlled using the Haake RheoWin 4.7 software. Flow curves were fitted by the Ostwald de Waele model [1] using the HAAKE software RheoWinDataManager 4.75 (HAAKE Co., Germany):

$$\tau = K \cdot \gamma^{n}$$

where: τ – shear stress [Pa],
K – consistency coefficient [Pa· s^n],
γ – shear rate [s^{-1}],
n – flow behaviour index.

Emulsion stability

Protein-based emulsion stability measurements were performed during storage for 0, 1, 3 and 7 days at 4 °C, using Turbiscan Lab® Expert (Formulaction SA, France), which collected data from the entire height of the vial every 40 µm. Round flat-bottomed vials were filled with the test emulsion (20 ml) to of ¾ their height. The Turbiscan Stability Index (TSI) was determined based on the Turbiscan Soft Lab software.

Statistical analysis

All measurements presented are the means of data from triplicate analysis. The statistical analysis was performed by the statistical software, Statistica version 13.1 (StatSoft, Poland). Analysis of variance (ANOVA) and Tukey's post hoc statistical tests were used to evaluate significant differences. The level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

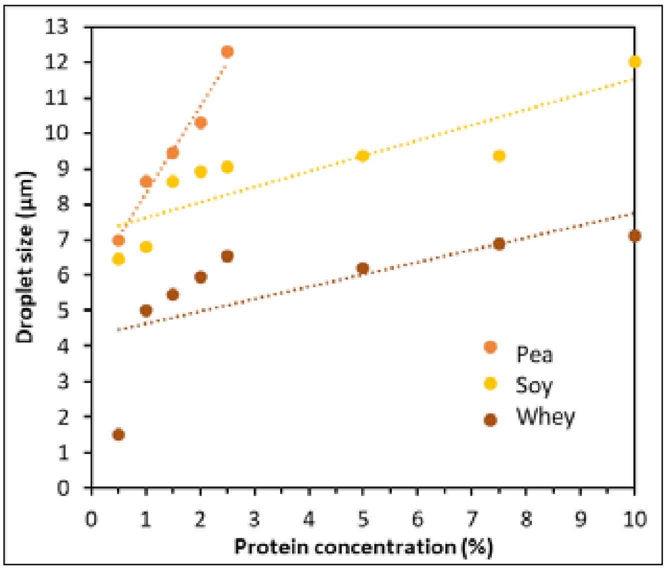

Fig. 1. Droplet size of emulsions with different protein type and concentration.
Rys. 1. Rozkład wielkości kropel w emulsjach o różnym rodzaju i stężeniu białka.
Source: Own study
Źródło: Opracowanie własne

The size of oil droplets in the emulsion ranged from 1.5 to 12.3 µm (Fig. 1). The whey protein emulsions were characterized by the smallest size of fat globules (<7.2 µm). Replacing animal-derived protein with plant resulted in an increase in the particle diameter of the dispersed phase. A higher oil droplet size was observed for the pea protein emulsion. Fernandez-Avila et al. [8] found that stabilized emulsions of soy protein isolate containing 20% oil phase showed a smaller size than emulsions of pea protein isolate emulsions, which was also confirmed in this study. Kopytowska and Domian [4] in research on spray drying of stabilized with pea protein also investigated the size of fat globules in emulsions obtained by pressure homogenization. The size of the dispersed phase of emulsions ranged from 6.4 to 9.8 µm. In the current study, the oil droplet size had values similar to those showed by Kopytowska and Domian [4]. Increasing protein concentration in the system from 0.5 to 2.5% increased the particle size of the dispersed phase which may indicate that oil droplet size is determined by the number of proteins and viscosity [6, 12]. As the viscosity of the system increases, the solubility of proteins (especially pea) is weaker, which may result in an increase in the droplet size of the emulsion. A further increase in protein concentration (5-10%) did not contribute to a considerable increase in the oil droplets. Mostly, higher protein levels favour higher surface loading, resulting in increased emulsifying potential and reduced oil droplet size [20]. However, the particle size in the emulsion system is determined not only by the size of the oil droplets covered by proteins, but also by the presence of protein aggregates [13, 18]. Furthermore, a reduction of shear forces, inertial forces and cavitation during the pressure homogenization process as a result of the increasing viscosity may limit the rearrangement of proteins at the oil-water interface and subsequently inhibit their ability to stabilise oil droplets [21]. Higher pea protein concentrations (5-10%) were not tested, because the emulsions were too thick. Pressure homogenization of emulsions with increasing addition of pea protein caused transforming from viscous-like to gel-like systems.

An important factor that determines the density of a substance is its chemical composition, as well as the internal structure of the product. The emulsion density ranged from 0.971 to 0.986 g cm^{-3} and increased with increasing protein concentration (Table 1). The replacement of whey protein with soy protein contributed to an increase in the density of the emulsion, while the opposite trend was observed in the case of pea protein. On the other hand, the greatest increase in density along with the increasing contribution of protein was observed for the emulsion with the addition of pea protein.

The pH values for the emulsion variants ranged from 5.82 to 6.88, which proves the slightly acidic nature (Table 1). A decrease in pH was observed with increasing protein concentration in the emulsion. The lowest pH values were obtained for the soy protein emulsions. The poorest protein emulsifying properties usually exist around its isoelectric point (pH~4.5) [10]. Emulsification improves as the pH moves away from the isoelectric point of the protein [5].

The physical stability of the systems during storage was described by the TSI (Turbiscan Stability Index). The TSI varies from 0 to 100 and this is a parameter to monitor the destabilization kinetics of the emulsions. The higher the TSI value, the greater the change in the sample and therefore the greater the instability of the system [7]. The type and concentration of protein as well as the storage time determine the stability of the emulsion (Fig. 2). Whey and soy emulsions with a protein concentration <5% showed TSI values above 4.0 after 7 days of storage (Fig. 2a-b). For these emulsions, an accumulation of the dispersed phase particles was observed

Table 1. Density, pH and rheological parameters of the Ostwald de Waele model (K, n) of emulsions with different protein type and concentration

Tabela 1. Gęstość, pH oraz parametry reologiczne modelu Ostwalda de Waele (K, n) emulsji o różnym rodzaju i stężeniu białka

Type of protein	Protein concertation (%)	Density (kg·m^{-3})	pH	*K* (mPa·s^n)	*n* (-)
pea	0.5	0.971^{a}	6.86^{i}	398.4^{a}	0.429^{def}
	1	0.972^{a}	6.85^{i}	2456.0^{b}	0.301^{abc}
	1.5	0.972^{a}	6.83^{i}	4271.3^{c}	0.271^{ab}
	2	0.976^{b}	6.81^{hi}	6495.7^{d}	0.241^{ab}
	2.5	0.980^{bc}	6.73^{g}	10105.3^{e}	0.203^{a}
soy	0.5	0.979^{b}	6.19^{d}	20.0^{a}	0.717^{ijk}
	1	0.980^{bc}	6.15^{d}	24.8^{a}	0.770^{k}
	1.5	0.980^{bc}	6.10^{cd}	38.4^{a}	0.738^{jk}
	2	0.981^{bc}	6.00^{bc}	169.2^{a}	0.619^{hi}
	2.5	0.982^{cd}	5.92^{b}	231.0^{a}	0.569^{gh}
	5	0.983^{cd}	5.82^{a}	571.5^{a}	0.493^{efg}
	7.5	0.985^{de}	5.81^{a}	2858.3^{b}	0.333^{bcd}
	10	0.986^{e}	5.80^{a}	3285.7^{b}	0.330^{bcd}
whey	0.5	0.976^{b}	6.81^{hi}	1.5^{a}	1.154^{lm}
	1	0.978^{b}	6.79^{h}	1.9^{a}	1.099^{m}
	1.5	0.978^{b}	6.78^{h}	3.4^{a}	1.066^{lm}
	2	0.979^{b}	6.72^{g}	3.5^{a}	1.060^{lm}
	2.5	0.980^{bc}	6.70^{g}	9.4^{a}	0.968^{l}
	5	0.981^{bc}	6.63^{f}	68.6^{a}	0.763^{k}
	7.5	0.981^{bc}	6.56^{e}	137.8^{a}	0.700^{ijk}
	10	0.983^{cd}	6.52^{e}	296.1^{a}	0.636^{hij}

Source: Own study

Źródło: Opracowanie własne

a)
b)
c)

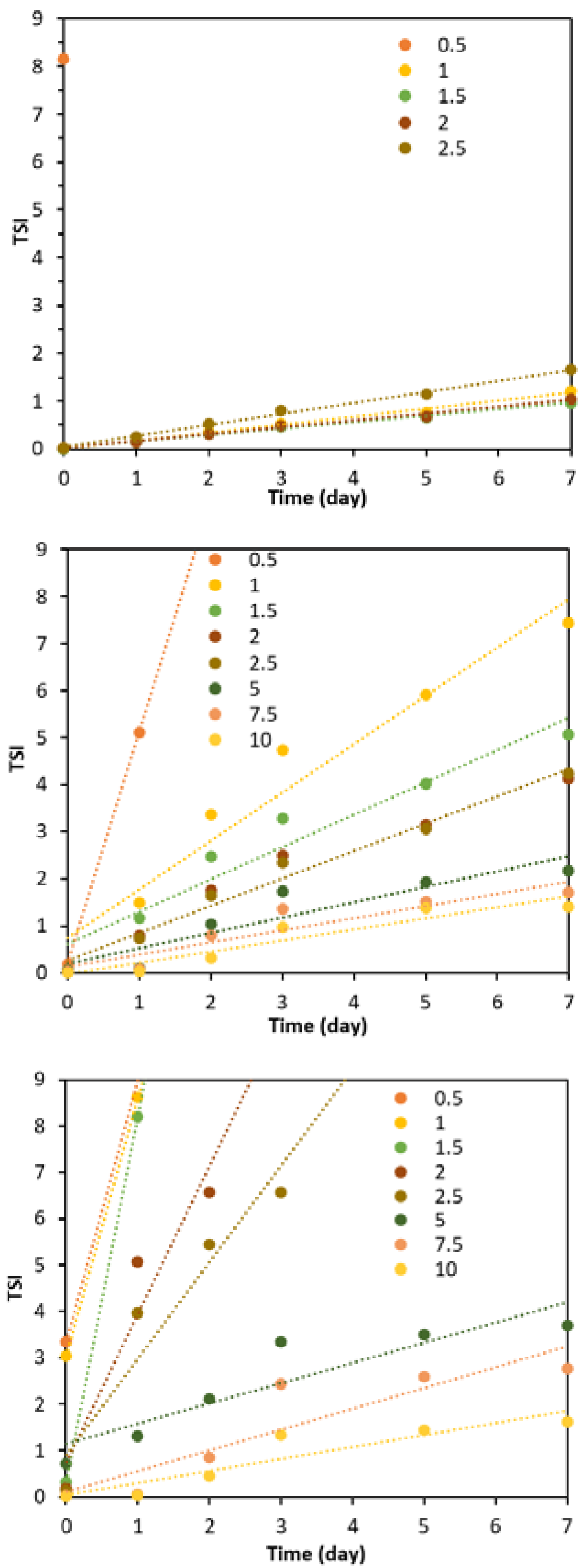

Fig. 2. Turbiscan Stability Index TSI of emulsions stabilized by pea (a), soy (b), and whey (c) protein at different protein concentration (0.5–10%).

Rys. 2. Wskaźnik stabilności TSI emulsji stabilizowanych białkiem grochu, soi oraz serwatki o różnym stężeniu białka (0,5–10%).

Source: Own study

Źródło: Opracowanie własne

in the upper part of the vial, and thus creaming was observed, indicating instability of the emulsion. It has previously been shown that, as the protein to fat ratio decreases in oil-in-water systems, the amount of protein available for emulsification decreases, leading to droplet coalescence [9]. The increase in protein concentration in the system contributed to the reduction of the TSI value, and thus to the improvement of the emulsion stability, which was also confirmed by Aziz el al. [2]. Protein concentration is an important parameter of emulsion stabilization due to a threshold level of protein required to create a protective barrier around the oil droplets [15]. The pea protein emulsions showed the greatest storage stability (Fig. 2c). Exceeding the protein content > 0.5% guarantees the achievement of stable emulsions for which the TSI value did not exceed 1.66. The smaller oil droplets diameters of whey protein - stabilized emulsions, compared to the emulsions with pea protein, did not respond to the higher whey protein emulsion stability. Pea protein may be a better stabilizer and WPC a better emulsifier. For emulsion with a pea protein content of 2.5%, a slight increase in the TSI value was observed, which may be the result of obtaining a larger size of oil droplets (Fig. 1). Larger droplet sizes lead to a lower interfacial layer, which consequently reduces stability of emulsion [17]. Results of TSI confirmed that pea protein in low concentrations may be a very good stabilizer and emulsifier of oil-in-water emulsions, as indicated in studies conducted by Sridharan et al. [16].

The flow behaviours of the emulsion were described by fitting the experimentally measured shear stress-shear rate data to the model. The shear-thinning behaviour of protein emulsions could be described by the Ostwald de Waele model [13]. Protein types and protein concentration significantly affected the consistency index (K) and flow behaviour index (n). Increased protein concentration in emulsions intensifies the shear-thinning effect, as evidenced by a decrease in the value of n and an increase in the value of the K which was also confirmed by Anema et al. [1], Lupi et al. [11]. Consistency index indicates the fluid viscosity. The K values in the emulsions were diversified, it is especially visible when the range of values for animal proteins (1.5–296.1 mPa·s^n) is compared to the values obtained for plant proteins, where this coefficient reached the value of 10105.3 mPa·s^n (2.5% pea protein) (Table 1). The pea protein variant showed significantly higher K values than soy and whey protein. The higher its value, the more viscous the emulsion is.

The flow behaviour index was close to 1 at 0.5–2.5% for the whey protein emulsion, further increasing the protein concentration led to a decrease of n values to 0.64 at 10% protein concentration, indicating that the samples at 0.5–2.5% whey protein concentration are Newtonian but as the protein concentration increased, the emulsions developed progressive more shear thinning (n < 1). The plant protein-based emulsions showed lower n values than the whey protein; lower n correspond to the more pronounced shear thinning. This indicates that the structure in emulsions at higher protein concentrations is rearranged to a less viscous state under the shear conditions [1].

SUMMARY

The mean droplet size in the emulsion ranged from 1.5 to 12.3 µm, which indicates the appropriate distribution of the fat globules and thus the possibility of obtaining more stable colloidal systems. An increase in the shear thinning effect was observed with increasing protein concentration in the emulsions; as evidenced by a decrease in the flow behaviour index and an increase the consistency index.

Pea protein emulsions showed the best storage stability. TSI stability index below 1.7 after 7 days of storage showed emulsions with pea protein concentration above 0.5%. Replacing animal-derived protein (whey) with pea protein allows for a stable emulsion during storage, despite the larger particle size of the dispersed phase.

The pea protein provides good emulsifying properties that allow it to meet the current consumer demands for alternative sources of plant protein. It is possible to further enhance the functionality of the pea protein and promote a wider use in the food industry.

PODSUMOWANIE

Średnia wielkość kropel w emulsji wahała się od 1,5 do 12,3 µm, co wskazuje na odpowiednie rozproszenie kuleczek tłuszczu, a tym samym na możliwość uzyskania bardziej stabilnych układów koloidalnych. Zaobserwowano wzrost efektu rozrzedzania ścinaniem wraz ze wzrostem stężenia białka w emulsjach; o czym świadczy obniżenie wskaźnika płynięcia i wzrost wskaźnika konsystencji.

Najlepszą stabilność podczas przechowywania wykazały emulsje na bazie białka grochu. Wskaźnik stabilności TSI poniżej 1,7 po 7 dniach przechowywania wykazały emulsje o stężeniu białka grochu powyżej 0,5%. Zastąpienie białka pochodzenia zwierzęcego (serwatki) białkiem grochu pozwala na uzyskanie stabilnej emulsji podczas przechowywania, pomimo większej wielkości cząstek fazy rozproszonej.

Białko grochu zapewnia dobre właściwości emulgujące, które pozwalają spełnić obecne zapotrzebowanie konsumentów na alternatywne źródła białka roślinnego. Możliwe jest dalsze zwiększenie funkcjonalności białka grochu oraz promowanie jego szerszego zastosowania w przemyśle spożywczym.

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