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INFLUENCE OF WPC 80 ADDITION ON COLOUR, DROPLET SIZE AND STORAGE STABILITY OF W/O EMULSIONS

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Abstract

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Whey proteins are characterized with ability to form and stabilize foams and emulsions. The size and distribution of the dispersed droplets significantly affect the stability and colour reflectance ability (colour parameters) of the emulsion. The W/O (water-in-oil) emulsions with different concentrations of WPC 80 (whey protein concentrate with 80% protein), i.e., 0; 1.5; 5; 10; 15% were produced and analysed. The emulsions differed regarding colour parameters, the preparations with higher protein supplementations were darker, more red and yellow and their colour was more saturated. The size of the dispersed phase droplets was not affected up to 10% WPC 80 additive, whereas at higher protein concentrations they were significantly larger. No significant changes of the droplet size in the dispersed phase and of the quality of added whey proteins were observed throughout the experiment duration.

Key words: whey protein concentrate, emulsions, SDS-PAGE, droplet size

Introduction

Emulsion is a system consisting of at least two liquid immiscible phases. Emulsions can be classified into two groups: simple emulsions and multiple emulsions. In simple emulsions, droplets of one liquid phase (dispersed phase) are dispersed in another immiscible liquid phase (dispersing phase). Simple emulsions are of two types: W/O (dispersed phase – water; continuous phase – oil) and O/W (dispersed phase – oil; continuous phase – water). Multiple emulsions are emulsions of emulsions, it means that dispersed phase is at the same times continuous phase for smaller droplets of the liquid immiscible phase (Pal, 2007, 2011).

Emulsions are thermodynamically unstable systems. They tend to be destabilized with changes observed in their structure, size and distribution of the dispersed droplets (Tcholakova et al., 2006; Manoi and Rizvi, 2009). The huge impact on the emulsion stability has an addition of a surface active agent. Surfactants have two main functions, one is to allow the formation of emulsions through decreasing the interfacial tension between phases and the second is to stabilize droplets in an emulsion (Pal, 2011). Milk protein, e.g., caseins and whey proteins are natural surfactants. Whey proteins (WPs) facilitate the formation and stabilization of the emulsions. Protein adsorption at the water-oil interface (at the surface of the dispersed droplets) of an W/O emulsion create an interfacial layer which exerts stabilization effect through steric and electrostatic repulsion as WPs contain charged amino acids (Sliwiński et al., 2003; Manoi and Rizvi, 2009). The most important whey protein to stabilize an emulsion is β – lactoglobulin (60% of the whey protein fraction), followed by α – lactalbumin (20%) and bovine serum albumin (3%) (Farrell et al., 2004; Darewicz and Dziuba, 2005).

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Low-degree hydrolyzed WPs enhance the emulsion stability, whereas highly hydrolyzed to short-chain peptides and free amino acids may have a negative impact on the process of the emulsion formation and further may decrease the system stability (Ye and Singh, 2006). Emulsions prepared with the significant incorporation of the hydrolysed WPs usually require addition of emulsifiers to ensure the preparation stability (Ye et al., 2004).

Appearance, including colour, is determined by a combination of factors and is one of the most important parameter of the product quality. Colour of emulsions mainly depends on the absorption and scattering light ability. Light absorption in an emulsion depends on the concentration and type of chromophoric materials, e.g., dyes, whereas light scattering is influenced by the microstructure, refractive index, size and concentration of any particle of the emulsion. The lightness of emulsions is influenced by the concentration and size of the dispersed droplets (Chanamai and McClements, 2001, 2002a).

Due to excellent physicochemical features, including emulsifying, foaming and stabilizing properties, WPs are widely used as ingredients in food (Ye and Singh, 2006) and cosmetic (Kalicka et al., 2010) preparations. However, these applications as well as scientific reports are usually limited to the O/W emulsions. This work was established to estimate the effect of whey protein concentrate (WPC 80 v/v) addition on the droplet size of the dispersed phase, emulsion colour parameters and the behaviour of the added proteins during storage in the reverse, i.e. W/O cosmetic emulsion.

Materials and Methods

Materials

Whey protein concentrate (WPC 80) was purchased from Bartex (Pasłęk, Poland). The oil phase components used in the study included: grape (*Vitis vinifera*) seed oil and olive (*Olea europaea*) fruit oil obtained from Olitalia (Index Food, Luboń, Poland), lanolin (*Adeps lanae* anhydrous), cocoa (*Theobroma cacao*) butter, beeswax (*Cera alba*) purchased from Pharma Cosmetics, (Krakow, Poland). The following additives were also used: vitamin A palmitate 1.0 MIU g^{-1} and dl-α-tocopheryl acetate purchased from DSM (Mszczonów, Poland), sodium benzoate, citric acid from Chempur (Piekary Śląskie, Poland), ethyl alcohol from Polmos (Bielsko-Biała, Poland), lavender oil (*Oleum lavandulae*) from Sabana (Warszawa, Poland). All other reagents used were of analytical grade.

Preparation of water-in-oil (W/O) emulsions

Water-in-oil (W/O) emulsions with 0 (control); 1.5%; 5%; 10%; 15% (w/w) addition of WPC 80 were produced by the recipe and procedures developed at the University of Agriculture in Krakow (Tabaszewska et al., 2012, 2014). The W/O emulsions were composed of 60% of oil phase and 40% of water phase (WPC 80 aqueous solution – 39% and additives – 1%) (w/w). Full description of the preparation procedure was given in the previous work (Tabaszewska et al., 2015). Prepared emulsions were stored at ambient temperature (20 ± 1ºC) and analysed directly after production as well as after 5, 10 and 15 weeks of storage.

Colour analysis

Measurement of colour was performed by the transmitance method (measuring geometry d/0°, illuminant D65, range 400-700 nm at 20 nm intervals, optical path length 20 mm) in terms of the CIE*Lab* system (1976) using the CM-3500d spectrophotometer (Konica Minolta Sensing Inc., Japan) as described by Wołosiak (2009). The following CIE $L^*a^*b^*$ parameters were determined: L^* - lightness (from 0 (black) to 100 (white)), a^* coordinate ((-) green (+) red colour), b^* coordinate ((-) blue (+) yellow colour), C – chroma (measure of colour intensity).

Droplet size measurement

The droplet size (diameter) of the emulsions was measured using the MT5310L light microscope (Meiji, Japan) which was coupled with a digital camera (Moticam 2300 3.0 megapixels, Germany) and PC with Motic Image Plus 2.0 ML software (Germany). The method described by Dybowska (2006) with some modifications was applied. The droplet size was observed in six fields of view, using the 40 x magnifications.

SDS-PAGE analysis

Analyzed emulsion treatments were mixed with SDS-PAGE reducing sample buffer, triturated vigorously with a porcelain mortar and pestle until the water phase was expelled and left at ambient temperature for 20 minutes so as to separate phases. From the released water phase of the emulsion 20 μL of the sample was taken followed by the addition of denaturing buffer (40 μL). Electrophoretic separation was run using 5% thickening gel and 16% separating gel with the use of Mini-PROTEAN 3 cell apparatus (Bio-Rad Laboratories, Richmond, USA). Separated protein bands were stained with Coomassie brilliant blue, then discoloured, scanned and analyzed using GELSCAN v. 2.0 software (Kucharczyk, Poland).

Statistical analysis

All types of emulsions were prepared and analysed in three independent series. Analyses of colour were performed in duplicate whereas diameter of the dispersed droplets was determined in triplicate. Results are expressed as mean±standard error. For the estimation of the effect of WPC 80 addition and time of storage, the obtained results were subjected to two-way analysis of variance (ANOVA) and the significance of differences between the means was determined on the basis of Duncan test at the significance level of $P<0.05$. The statistical analysis was performed using Statistica 10.0 software (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

Appearance, including colour, is an impression perceived by the sense of sight, which usually determines the will to possess or reject a certain product (Chantrapornchai et al., 1999; McClements, 2002ab). The analyzed emulsions differed greatly as regards colour profile Figure 1. Lightness of the emulsion (*L**) noticeably decreased with the increase of the WPC 80 concentration ($P<0.05$) with the exception of the lack of a significant difference between the emulsion without whey proteins and that containing 1.5% of WPC 80. This parameter was not subjected to any changes during storage. The share of green colour coordinate (-*a**) with the increasing WPC 80 additive decreased significantly in favour of the red colour (+*a**). During storage of the emulsion without WPC 80 addition the significant increase of the green colour parameter was noticed. The *a** colour coordinate in the emulsion fortified with 1.5% of WPC 80 was not subjected to any changes throughout the storage period. In other whey protein treatments there was observed slow increase of the red colour, and these changes were statistically significant. The share of *b** colour attribute as well as chroma index (C) changed significantly ($p<0.05$) with the increase of WPC 80 concentration, the share of the yellow coordinate (+b*) as well as colour intensity (C) of the emulsion increased. On the other hand, there was no significant changes in these colour parameters during storage.

Evans et al. (2010) observed that the solutions containing WPC 80 are not transparent and the *L** value decreases with the reduction of transparency. The higher WPC 80 addition the lower is transparency and *L** value. This is consistent with the results of the present study as with the increasing WPC fortification the lightness of the emulsions decreased.

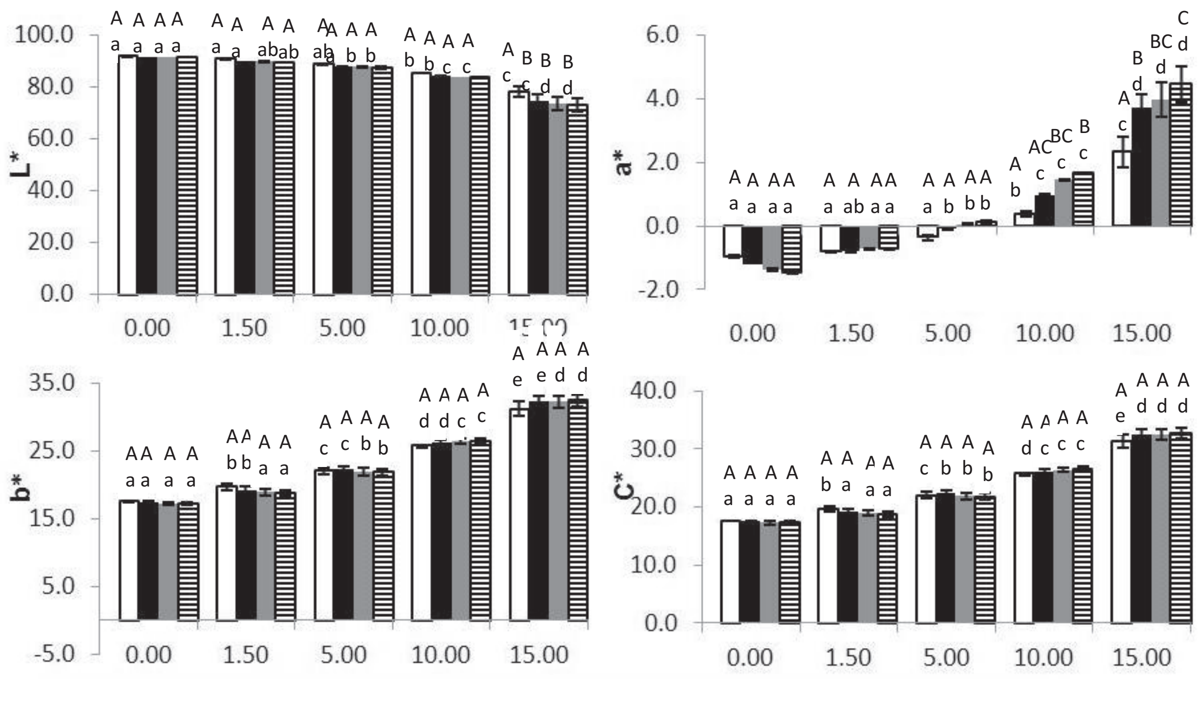

Fig. 1. The colour *L*a*bC parameters of the emulsions with different concentrations of WPC 80 (%) during storage. Storage at week: 0 (blank columns), 5th (black columns), 10th (grey columns), 15th (striped columns). Vertical bars correspond to standard error (n = 6), different upper case/lower case letters denote statistically significant differences (P ≤ 0.05) between the means of the same protein content during storage or at the same storage time for variable pro tein contents, respectively**

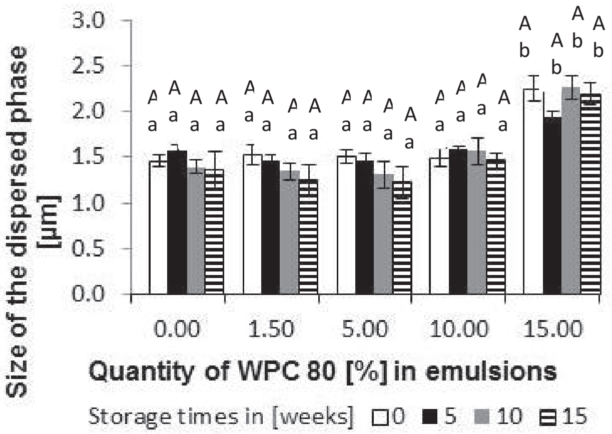

Fig. 2. The droplet sizes of emulsions with different WPC 80 concentrations during room temperature storage. Vertical bars correspond to standard error (n=9), different upper case/lower case letters denote statistically significant differences (P ≤ 0.05) between the means of the same protein content during storage or at the same storage time for variable protein contents, respectively.

The changes of the colour profile affected by the WPC 80 addition may result from the light reflectance by the droplets of the dispersed phase. McClements (2002a) reported, that with increasing radius of the dispersed phase droplets above 0.1μm the value of *L** colour parameter decreases and at the same time an increase of *a** and *b** values is observed. Also the research of Chantrapornchai and co-workers (1999) on the changes of the *L*, a*, b** colour parameters as affected by the droplet size of dispersed phase in emulsions of different colours indicate the similar results for the red-coloured emulsion. Increase of the droplet diameter of the internal phase up to 10 μm resulted in the very slight increase of *a** and *b** values. On the other hand, lightness (*L**) of the emulsion steadily decreases with increasing droplet size of the internal phase. On the contrary to emulsion lightness, the colour chroma (C) increases with the increase of the internal droplet radius above 0.1 μm, as was indicated by the McClements (2002a). The results of the present investigations are only partly consistent with the above statements. In the our study the decrease of the *L** parameter and concomitant increase of the *a**, *b** coordinates as well as colour saturation (C) were observed with increasing concentration of added WPC 80, however only the emulsion with 15% of WPC 80 was characterized by the significantly higher droplet diameter than other emulsions (~2 μm vs 1.5 μm). This indicates that the investigated W/O emulsions were much more sensitive to the WPC 80 concentration-induced modifications in colour parameters than to the changes of the sizes of internal water-phase droplets. However, it should be highlighted that the discrepancies between results may result from the emulsion type, as cited authors investigated the characteristics of reverse O/W emulsions.

The size of the dispersed phase droplets has a huge impact on the stability of preparations as well as their colour profiles (Kiokias et al., 2007; McClements, 2002a). The droplet diameter of the dispersed phase in the emulsions containing up to 10% of WPC 80 was equal to approximately 1.5 μm and was significantly different (P<0.05) from that measured for the emulsion with 15% WPC addition, which amounted to above 2μm (Figure 2 and 3). There were no statistically significant changes observed of the droplet size during storage, however slow tendency for lowering of the droplet size was noticed for the emulsions with 1.5 and 5% WPC 80 concentration.

Similar results were obtained by Dybowska (2003), who reported that emulsion with 12% WPC content was characterized by the higher droplet diameter of the dispersed phase (1 - 4 μm) than did the emulsion with 6% additive of WPC. Research of Ye (2008) indicates, that the 2-10% addition of protein concentrate did not influence significantly the droplet size of the dispersing phase. The same was obtained in the present work for the emulsions with 5 and 10% addition of WPC 80.

During processing or storage proteolytic microorganisms, which may penetrate into the products with raw ma-

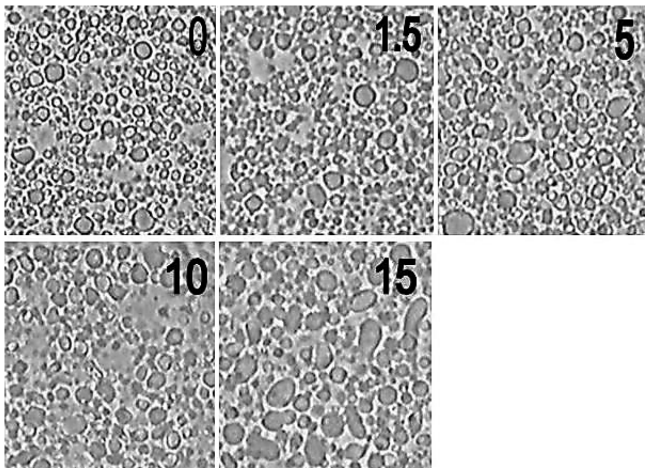

Fig. 3. Photographs of the droplets in emulsions with different concentrations of WPC 80 (The numbers in photos: 0, 1.5, 5, 10, 15 are the WPC 80 concentrations (%)).

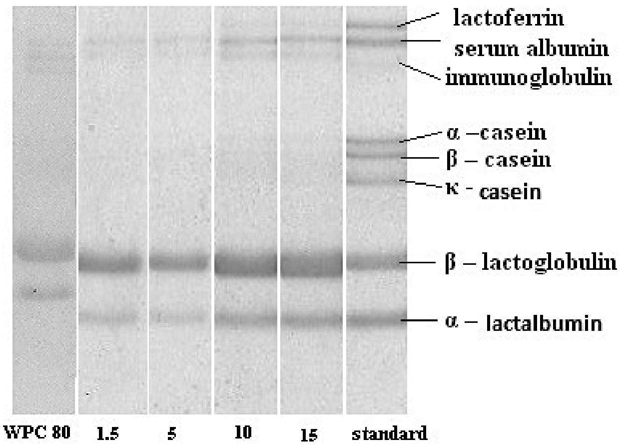

Fig. 4. The SDS-PAGE electrophoretogram of the whey proteins (WPC 80) added to the emulsions in the amount of (w/w) 1.5; 5; 10; 15% after 15 weeks of storage

terials, can be responsible for degradation of the intact proteins into smaller peptides (Scherze and Muschiolik, 2001) and thus they can lower the stability of the emulsion system. According to the results of Euston and co-workers (2001) emulsions prepared with the addition of whey protein hydrolysate were less stable than those prepared with whey protein concentrate. In the present study whey proteins added to the emulsion during production stage maintained their size within the 15-week storage period. No degradation of the polypeptide chain of the certain proteins to the smaller peptides was observed regardless of the applied WPC 80 concentration (Figure 4).

Conclusions

Addition of whey protein concentrate (WPC 80) into the water-in-oil emulsion formula significantly affected the colour parameters of the emulsion. As the WPC 80 addition increased the emulsions became darker and simultaneously the increase of the red and yellow coordinates and colour saturation was observed. The diameter of the droplets of the dispersed phase was similar in the emulsions containing WPC 80 in concentrations up to 10%, whereas at higher concentration it increased. No significant changes were observed regarding analysed parameters during storage period. Moreover, there was not observed any degradation of the whey proteins to the smaller peptide fraction within the 15 weeks of the experiment.

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