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THE INFLUENCE OF WPC 80 ADDITIVE ON THE STABILITY OF WATER-IN-OIL EMULSIONS*

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Abstract
The effect of 0, 1.5, 5, 10 and 15 (g 100 g^{-1} of emulsion) WPC 80 additive (80% whey protein concentrate) on the pH, physical, oxidative and microbiological stability of the water-in-oil emulsion was studied during 16-week storage at ~20°C at 4-week intervals. All determined features were significantly affected by the supplementation. The most beneficial as regards storage stability was the emulsion with 5% of WPC 80. This treatment was the most resistant to oxidative changes showing low increase of the concentration of conjugated diene hydroperoxides (from 0.92 to 1.04 mg g^{-1}) and of the thiobarbituric acid reactive substances (from 0.83 to 1.37 mg malondialdehyde g^{-1}) as well as only slight increment (by 0.47 log CFU g^{-1}) of the microorganisms number during storage. Thus, the results of the present study revealed that whey proteins can be applied in the proper amount to produce cosmetic emulsions composed of natural ingredients and with reasonable storage stability.

Key words: whey protein concentrate, water-in-oil emulsion, stability, oxidation

Whey products, including whey protein concentrates (WPC) with protein content ranging from 35 to 80%, are widely used as food and non-food ingredients (e.g., in cosmetic and pharmaceutical formulations) because of their nutritional and functional properties. Whey proteins are characterized by the wide range of functional properties like water binding capacity, thickening (Herceg et al., 2007) and gelling ability (Lucey, 2008), they also facilitate the formation and stabilization of foams and emulsions (Sliwinski et al., 2003). Due to their molecular properties proteins are able to lower interfacial tension and during emulsifications they form a protective layer on the droplets of the dispersed phase which stabilizes emulsions against flocculation and coalescence. Whey proteins are capable of stabilizing emulsions in the

following order: β-lactoglobulin > α-lactalbumin > bovine serum albumin (Darewicz and Dziuba, 2005).

Whey proteins are globular proteins, consisting of the following main fractions: α-lactalbumin (20%), β-lactoglobulin (60%), bovine serum albumin (3%), immunoglobulin, the structure of which is stabilized by disulfide bonds (S-S) (Zhu et al., 2010). Whey proteins, particularly bovine serum albumin and lactoferrin, are characterized by a high content of disulfide bonds and thiol groups (-SH). Reactivity of SH groups (e.g., in cystein) retards lipid oxidation and as a result increases oxidative stability of emulsions (Worobiej et al., 2008). SH groups in proteins could reduce hydroperoxides (primary lipid oxidation products) as well as aldehydes and ketones (secondary lipid oxidation products) through the donation of the most labile hydrogen atom (Viljanen, 2005). In addition, whey proteins such as bovine serum albumin and lactoferrin act as antioxidants through chelation of the transition metals. Antioxidant ingredients are really important both in food products and in the human organism because they reduce negative changes induced by the reactive oxygen species (Ries et al., 2010).

Whey proteins and peptides show also antimicrobial properties. Lactoferrin and its peptide derivative – lactoferricin were shown to exhibit antifungal, antibacterial, antiviral, antiparasite, antitumor activities and stimulate immune system (Zimecki and Artym, 2005). Caseinomacropeptide stimulates the immunomodulatory functions through productions of human macrophage such as U937 cells and inhibition of the production of IgG (immunoglobulin G) antibodies (Korhonen and Pihlanto, 2006). α-lactalbumin has been reported to show antiviral properties, whereas milk enzymes, i.e., lysozyme and lactoperoxidase work together with lactoferrin in combating bacteria (Zimecki and Artym, 2005).

In the past, whey was perceived as a waste by-product in the dairy industry. Recently there has been a great concern regarding the whey components which exert biological activity, especially whey proteins. In the present work the attempt was made to use whey protein concentrate as a texturizing and bioactive component of the cosmetic emulsion. The impact of WPC 80 additive in different concentrations on the stability, microbiological quality and antioxidant capacity of water-in-oil emulsions during storage was also evaluated. The present study was established to find the new application of whey products and to develop the novel emulsion formulation for possible application in cosmetic preparations which is based exclusively on the natural ingredients.

Material and methods

Materials

Whey protein concentrate – WPC 80 (80% protein, max. 5.5% water, 8% fat, 3.5% ash, max. 8% lactose) was purchased from Bartex (Pasłęk, Poland). Grape (*Vitis vinifera*) seed oil and olive (*Olea europaea*) fruit oil were obtained from Olitalia (Index Food, Luboń, Poland), lanolin (*Adeps lanae* anhydrous), cocoa (*Theobroma*

cacao) butter, beeswax (*Cera alba*) from Pharma Cosmetics, (Kraków, Poland), vitamin A palmitate 1.0 MIU g^{-1} and dl-α-tocopheryl acetate were 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). The reagents and media used for the microbiological study were derived from Biocorp (Warszawa, Poland), the reagents for the antioxidant stability determination were purchased from Chempur (Piekary Śląskie, Poland) and TBA reagent from Sigma Aldrich (St. Louis, USA). 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 (g 100 g^{-1} of emulsion) of WPC 80 were produced by the recipe and method developed at the University of Agriculture in Krakow (Tabaszewska et al., 2012, 2014). The basic composition of the w/o emulsions was as follows: oil phase – 60%, water phase with WPC 80 – 39% and additives – 1% (w/w). The oil phase comprised: *Vitis vinifera* seed oil, *Olea europaea* fruit oil, *Adeps lanae* anhydrous, *Theobroma cacao* butter, *Cera alba* and the water phase constituted: distilled water and WPC 80. The following additives were also applied: vitamin A palmitate 1.0 MIU g^{-1}, dl-α-tocopheryl acetate, sodium benzoate, citric acid, ethyl alcohol, *Oleum lavandulae*. Briefly, WPC 80 was mixed with distilled water at ambient temperature for 2 hours using a magnetic stirrer. At the first stage emulsions were produced by adding pre-heated mixture of the solutions of water phase and sodium benzoate into the oil phase with simultaneous mixing with increasing speed with a laboratory blender at 68±2°C in the water bath. Subsequently, pre-emulsions were cooled during continuous mixing to ambient temperature. During the first stage of cooling (~50°C) they were mixed with citric acid, which was prior to mixing dissolved in the small amount of water and homogenized with other additives (Unidrive 1000 homogenizer, Germany) at the rate of 10000 rpm for 5 min. During homogenisation emulsions were cooled in an ice-water bath to avoid temperature increase up to ~20°C.

The produced emulsions were stored at room temperature (20±1ºC) and subjected to analyses directly after production and after 4, 8, 12 and 16 weeks of storage.

pH measurement of the water phase

Briefly, 4 g of the emulsion sample was boiled with 30 mL of distilled water and filtered through medium-hard paper filter. After cooling to ambient temperature, pH of filtrate was measured using an Elmetron (Zabrze, Poland) CP-411 pH-meter (Polish Standard PN-93/C-04842).

Measurement of the physical stability

The centrifugal method, adapted from the method of Saffla and Actona was employed for the determination of the stability of emulsions according to the procedure described by Jędrzejkiewicz and Florowska (2007). The samples of emulsions were put into the 10 mL scaled centrifuge tubes. The tubes were incubated at 37ºC for 24 h and centrifuged (3500 × g) for 10 min using the MPW-331 centrifuge (Mecha-

nika Precyzyjna, Warszawa, Poland). Stability of emulsion (SE) was calculated using the following formula, where V_1 denotes volume (mL) of emulsion in a tube after centrifugation and V_0 denotes volume of free oil phase:

$$SE(\%) = \frac{V_1 - V_0}{V_1} * 100$$

Measurement of the concentration of conjugated diene hydroperoxides (CDHP)

The CDHP were isolated and analysed by the spectrophotometric method described by Kiokias et al. (2007) and by the modification of the method described by Mestdagh et al. (2011). The sample (approximately 0.03 g) was mixed with 10 mL of isooctane/2-propanol mixture (2:1 v/v) and vortexed (three times for 10 s). This mixture was filtered through Merc Millipore, GNWPO2500 (25 mm filter diameter, 0.2 µm pore size). The absorbance was measured at $\lambda=232$ nm using the UV-Vis Helios Gamma spectrophotometer (Thermo Electron Corporation, Cambridge, UK).

The concentration of CDHP was calculated using the molar absorptivity ($e=26{,}000$) and molecular mass (280 g mol^{-1}) of linoleic acid.

Measurement of thiobarbituric acid reactive substances (TBARS)

The content of TBARS was evaluated by the spectrophotometric method described by Kiokias et al. (2007). Briefly, the sample of emulsion (approximately 0.06 g) was mixed with 0.7 mL of distilled water and 2.0 mL of TBA solution (prepared by mixing 15 g of trichloroacetic acid, 0.375 g of thiobarbituric acid, 1.76 mL of 12 N HCl and 82.9 mL of distilled water) in a test tube. Tubes were boiled in a water bath for 15 min, then cooled to ambient temperature (10 min) and centrifuged for 15 min (2000xg). The absorbance of the water phase was measured at $\lambda=532$ nm using the UV-Vis Helios Gamma spectrophotometer (Thermo Electron Corporation, Cambridge, UK).

The amounts of TBARS were calculated using the molar extinction coefficient (1.56×10^5 mol^{-1} mL^{-1}) of the red TBA reaction product and expressed as mg MDA (malondialdehyde) per g of emulsion.

Evaluation of the microbiological quality

The total viable count (TVC) of microorganisms was tested using the plate microbiological method by the procedure described in Polish Standard (PN-80/C-77022). PS37 agar was used as a media and suitable dilutions were made using sterile peptone water (1 g – peptone, 8.5 g – NaCl, per 1 L) supplemented with 20% (w/w) aqueous solution of Tween 80 (25% (v/v)).

Statistical analysis

All types of emulsions were prepared in three replicates and all analyses were carried out in duplicate ($n=6$). The results are expressed as mean ± standard error. For the estimation of the effect of WPC 80 addition and the storage time, the obtained results were subjected to two-way analysis of variance 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 9.0 (StatSoft, Inc., Tulsa, OK, USA).

Results

Emulsion stability

In the examined emulsions no visible destabilization, such as coalescence, "breaking of emulsion" was observed irrespective of the amount of added whey protein concentrate (WPC 80) during 12 weeks of storage at ambient conditions. At the 16th week of the storage the first symptoms of the emulsion destabilization occurred, the stability of emulsion (SE) measured by the centrifugal method was still high (99.2%) in the emulsions with 0, 1.5 and 5% of WPC 80 additive, whereas for emulsions with 10 and 15% concentrations of WPC 80 it was slightly lower and amounted to 98.4%. Physical stability of an emulsion is a very important factor not only from an aesthetical point of view but it also greatly influences oxidative stability of the oil phase and microbiological quality (Kryża and Stodolnik, 2007).

The pH of the dispersed phase as affected by the different concentrations of WPC 80

The acidity of the water phase greatly affects the stability of the whole system and the most optimal conditions for the stability of emulsions containing proteins are achieved near their isoelectric point (Taherian et al., 2011).

Table 1. Least square means for the content of CDHP (conjugated diene hydroperoxides), TBARS (thiobarbituric acid-reactive substances) expressed as MDA (malondialdehyde) concentration, pH and TVC (total viable count) as affected by the emulsion type (TE) and storage time (ST)[1]

	CDHP (mg g^{-1})	TBARS (mg MDA g^{-1})	pH	TVC (log cfu g^{-1})
Type of emulsion (percentage of WPC 80 additive; n=30)				
0	0.97 a	1.29 b	5.62 a	0.04 b
1.5	0.97 a	0.94 a	5.79 b	0.31 c
5	0.97 a	0.90 a	6.06 c	0.71 a
10	0.98 a	1.23 b	6.11 d	0.83 a
15	0.89 b	1.73 c	6.21 e	1.25 d
Storage time (weeks; n=30)				
0	0.86 a	0.89 a	6.00 b	0.66 bc
4	0.86 a	0.82 a	5.93 a	0.52 b
8	0.96 b	1.20 b	5.93 a	0.59 b
12	1.00 c	1.21 b	5.97 ab	0.75 c
16	1.06 d	1.81 c	5.99 b	0.88 a
Source of variance				
TE	*	*	*	*
ST	*	*	*	*
TE × ST	*	ns	*	ns

[1]Values are means for all emulsion types and storage times.
a, b, c – values in columns with different letters differ significantly (P≤0.05).
* statistically significant (P≤0.05), ns – statistically not significant (P>0.05).

In the present study, pH level of the emulsions was affected by both the emulsion type and storage time and also a statistically significant interaction between these two factors was stated (Table 1). Mean pH values of the dispersed phase in the analysed emulsions significantly increased with increasing concentration of added WPC 80, and the smallest differences were observed for 5–15% WPC 80 concentrations (Table 1, Figure 1). These differences could result from the fact that pH of the WPC 80 alone was in the range of 6.0–6.25. Although some fluctuations in the pH values were observed throughout the whole study the mean initial and final pH levels were almost the same. The direction of these changes highly depended on the emulsion type (Figure 1). For the emulsion without WPC 80 additive and for that with the smallest protein fortification (1.5%) a significant decrease of pH values was observed after 4 or 8–12 weeks (respectively for the former and latter type) which was followed by further pH increase to the value close to the initial one. On the contrary, pH of the emulsions with medium protein contents (5, 10%) was stable during the whole experiment, whereas for the emulsion with 15% WPC 80 concentration the observed significant decline in the pH level was maintained from the 8th week to the end of the study.
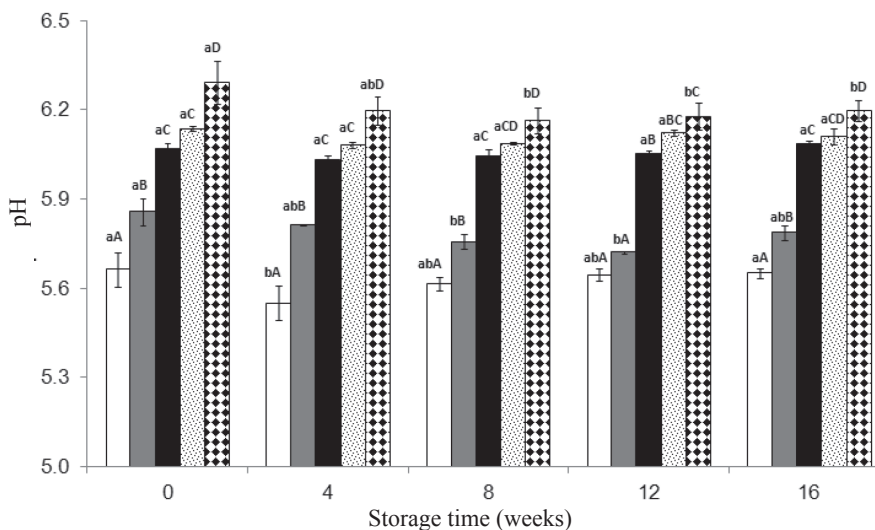

Figure 1. The pH values of emulsions with different concentrations of WPC 80 during room temperature storage (□ 0.0 (%); ▨ 1.5 (%); ■ 5.0 (%); ▨ 10.0 (%); ▨ 15.0 (%), values are means ±SE; n=6). Different capital letters denote statistically significant differences (P≤0.05) between results obtained for different emulsion types at the given storage time. Different lower case letters denote statistically significant differences (P≤0.05) between results obtained for a certain emulsion type during storage period

The effect of WPC 80 additive on the oxidative stability of the oil phase

Both studied oxidative stability characteristics, i.e. the concentration of conjugated diene hydroperoxides (CDHP) and thiobarbituric acid reactive substances (TBARs) were highly affected by the WPC 80 additive and storage time. Moreover,

in the case of CDHP the significant interaction between both factors of the two-way analysis of variance was observed (Table 1).
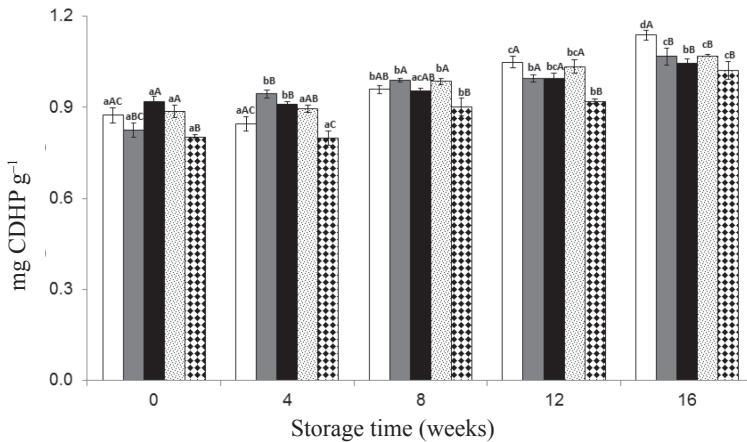

Figure 2. The conjugated diene hydroperoxides (CDHP) values of emulsions with different concentration of WPC 80 during room temperature storage (☐ 0.0 (%); ▨ 1.5 (%); ■ 5.0 (%); ▨ 10.0 (%); ▨ 15.0 (%), values are means ±SE; n=6). Different capital letters denote statistically significant differences (P≤0.05) between results obtained for different emulsion types at the given storage time. Different lower case letters denote statistically significant differences (P≤0.05) between results obtained for a certain emulsion type during storage period
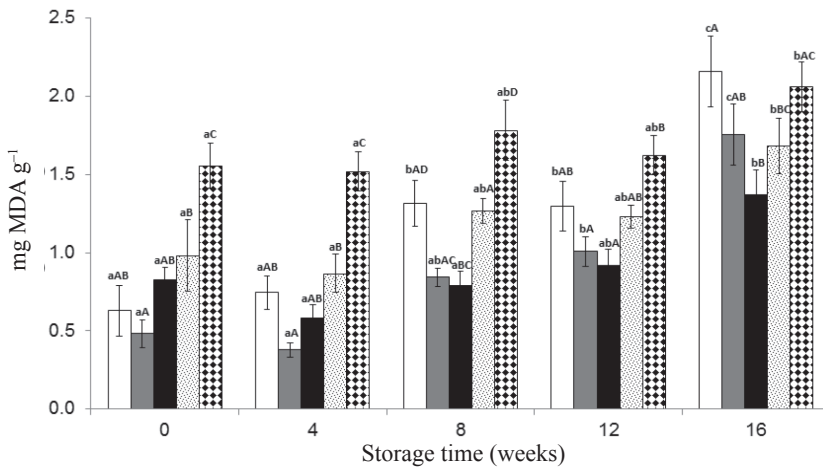

Figure 3. The TBARS (thiobarbituric acid reactive substances; secondary lipid oxidation products) values of emulsions with different quantity of WPC 80 during room temperature storage (☐0.0 (%); ▨ 1.5 (%); ■ 5.0 (%); ▨ 10.0 (%); ▨15.0 (%), values are means ±SE; n=6). Different capital letters denote statistically significant differences (P≤0.05) between results obtained for different emulsion types at the given storage time. Different lower case letters denote statistically significant differences (P≤0.05) between results obtained for a certain emulsion type during storage period

In the present study, the concentration of the CDHP in the lipid phase reached the lowest level in the emulsion with 15% of WPC 80, whereas other treatments were characterized by the similar level of this parameter which gradually increased throughout the storage period (Table 1). The higher differences could be observed regarding the level of thiobarbituric acid reactive substances (TBARS). The lowest concentrations of TBARS were stated in the emulsions with 5 and 1.5% of WPC 80 additive and the highest in the emulsion supplemented with 15% of WPC 80. During the storage period the content of TBARS initially decreased (after 4 weeks of storage) which was followed by further increment of their concentration (Table 1).

Generally, the lowest level of CDHP during the entire storage time was found for the emulsion with the highest content of WPC 80 (Figure 2). However, the difference between the final and initial level of CDHP in this treatment amounted to 0.22 mg g^{-1}. In the emulsion without WPC 80 additive slight (P>0.05) initial decrease of the CDHP concentration was found during the first 4 weeks of storage followed by fast and significant increase during the next weeks of the study (Figure 2). The overall increase of the CDHP concentration observed for this emulsion was 0.27 mg g^{-1}. The emulsions supplemented with 1.5, 5 and 10% of WPC were characterized by the comparable average levels of CDHP for the whole study (Table 1), but the rates of the oxidative changes during the experiment were different depending on the emulsion type (Figure 2). The emulsion with 5% WPC 80 was the most resistant to oxidative deterioration as significant increase of the CDHP content was observed after 12 weeks of storage and the overall growth of this factor amounted to 0.12 mg CDHP g^{-1}. For other emulsions with WPC incorporation, the increase of the concentration of these primary lipid oxidation products ranged from 0.18 to 0.24 mg g^{-1} respectively for the emulsion with 10% and 1.5% of WPC.

The further stage of the lipid oxidation process is connected with formation of the lipid degradation products like malondialdehyde (MDA). Figure 3 presents the changes of the level of secondary lipid oxidation products, i.e. TBARS (thiobarbituric acid reactive substances) in the examined emulsions during their storage. At the beginning of the experiment the emulsion with the highest WPC 80 additive contained the highest amount of TBARS but during the study only slight increase of their level was noticed (ΔTBARS = 0.50 mg MDA g^{-1}). The emulsions with 1.5 and 5% WPC 80 additive had the lowest concentration of TBARS during the entire storage period and the increase of TBARS value during the study amounted to 1.28 and 0.54 mg MDA g^{-1}, respectively. The most intensive oxidative changes were stated for the emulsion non-supplemented with whey proteins (ΔTBARS = 1.53 mg MDA g^{-1}).

The effect of WPC 80 additive on the microbiological quality of the emulsion

The microbiological quality determines the safety and the shelf life of the emulsion preparations. This feature was significantly affected by the type of emulsion and storage duration (Table 1). The total count of microorganisms increased with increasing concentration of WPC 80 in the emulsion (Table 1, Figure 4), which could be the result of the number of microorganisms provided with the emulsion ingredients. However, for the whole storage period the count of determined microbiota did not exceed the permissible level (maximum 1000 cfu g^{-1} of cosmetic preparation)

(Dz. U. Nr 9, poz. 107, 2003). The level of microflora was determined at the similar level for the first 8 weeks of the experiment followed by the increase of the emulsion microbiological contamination during prolonged storage. The highest amount of microorganisms was present in the emulsion with 15% of WPC 80 (up to 45 cfu g^{-1}).
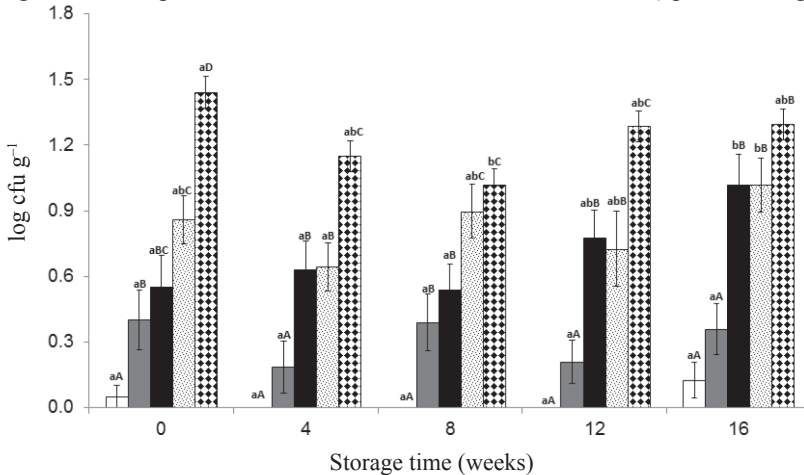

Figure 4. The total viable count (TVC) of microorganisms in the emulsions with different concentration of WPC 80 during room temperature storage (□ 0.0 (%); ▨ 1.5 (%); ■ 5.0 (%); ▨ 10.0 (%); ▨ 15.0 (%), values are means ±SE; n=6). Different capital letters denote statistically significant differences (P≤0.05) between results obtained for different emulsion types at the given storage time. Different lower case letters denote statistically significant differences (P≤0.05) between results obtained for a certain emulsion type during storage period

Discussion

An emulsion is a mixture of two or more immiscible liquids, from which one should be dispersed in another in the form of small droplets. It should be characterized by the proper physical stability during storage as this feature significantly affects the qualitative, utilization and aesthetic parameters of an emulsion (Kryża and Stodolnik, 2007). Ye (2008) in his research reported that addition of proteins positively affected the emulsion stability. In the cited study, emulsion stability increased with increasing concentration of WPC up to 1%, whereas the subsequent addition of WPC to the amount of 6% did not change this feature significantly. Results of our study are not fully consistent with this observation as the emulsions without supplementation were characterized by the same stability as those fortified with 1.5 and 5% of WPC 80. On the contrary, the results of the study conducted by Dybowska (2011) indicate that with increasing WPC 80 addition from 3 to 10% the stability of emulsions decreases as the result of the excessive amount of emulsifier. This phenomenon also cannot be confirmed by the results obtained in the present study as the decline of the emulsion stability was observed only when the WPC 80 additive was 15%, which can be caused by the different composition of the oil phase.

The pH value has a great impact on the stability of emulsions with whey protein additive. The study of Taherian et al. (2011) revealed that emulsions characterized by lower pH (3.4) were more stable during storage than those with higher pH (6.4). This phenomenon is also confirmed in the present study (section 3.1). Also the results of the study conducted by Śliwa et al. (2010) indicated that emulsions are more stable under the pH conditions close to the isoelectric point of the protein.

The emulsion quality and safety is affected by many factors, such as resistance of oil phase against oxidative changes. The rate of oxidative changes is influenced by the emulsion type, size of the droplets of the dispersed phase, composition, storage conditions and duration etc. (Osborn and Akoh, 2004; Ries et al., 2010). Also emulsions rich in unsaturated fatty acids are more susceptible to oxidation (Mestdagh et al., 2011). To retard these changes natural or synthetic antioxidant substances may be applied, which include also the proteins and peptides derived from whey (Elias et al., 2008).

Antioxidative functions of whey proteins have been reported by many authors (Kiokias et al., 2007; Elias et al., 2008). They are known to reduce the amount of formed hydroperoxides, to inhibit formation and to scavenge free radicals from lipids. These features are widely exploited, e.g. in the meat industry to prolong product shelf-life (Elias et al., 2008). In the examined emulsions antioxidative action was observed, especially as regards CDHP increase during storage in the emulsion enriched with 5% of WPC 80. This treatment was also effective in the reduction of the MDA level increment during the experiment. Kiokias et al. (2007) reported that higher WPC additive contributes to the better separation of the emulsion phases and as a consequence prevents penetration of the substances stimulating oxidative changes into the oil phase. β-lactoglobulin which is the main protein in the whey fraction (35%) exerts the strong reduction ability against hydroperoxides and thiobarbituric acid reactive substances (TBARS), both in its native form and after heat treatment at 50–90ºC (Elias et al., 2007). Also the study of Tong et al. (2000) revealed the effect of whey proteins on the inhibition of lipid oxidation through the inactivation of lipid hydroperoxides. In the present research the changes were not so noticeable between the emulsions containing up to 10% of WPC 80, which is also confirmed by the results obtained by Osborn and Akoh (2004). The antioxidant capacity of whey proteins (in the form of isolate, WPI) was also subjected to investigation by Ries et al. (2010), who found that with the increasing concentration of whey proteins up to 10% the total concentration of formed lipid hydroperoxides decreased but simultaneously during the storage their level significantly increased. The inhibiting effect of WPI towards lipid oxidation was also reported by Peña-Ramos and Xiong (2003). The reducing effect of whey proteins against CDHP formation in our study was achieved only when 15% of WPC 80 was applied, which may result from the higher concentration, purity and biological activity of proteins in isolate than in the WPC 80.

Regardless of the fact that increasing WPC 80 additive contributed to higher microorganisms content (Pawlik, 1999) at the same time their growth during storage period was slowed down. It could result from the antibacterial and bacteriostatic action of proteins, e.g., lactoperoxidase and lysozyme, present in WPC 80, which exert their antimicrobial action particularly against *E. coli* and *Listeria innocula*

(Recio and Visser, 2000). Even stronger bactericidal and bacteriostatic properties have also been reported for peptides derived from whey proteins, e.g., products of pepsin, trypsin and alcalase hydrolysis of α-lactalbumin and β-lactoglobulin have been shown to exert bacteriostatic activity against pathogenic microorganisms, such as: *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* (Dereck et al., 2006). On the other hand, these whey protein hydrolysates were not effective against *Salmonella enterica Cip5858*, *Listeria innocua R1007* and *Streptococcus mutans Cip103220T* (El-Zahar et al., 2004). The microbiological quality could also be influenced by the physical emulsion stability, e.g. resistance to flocculation and coalescence.

In conclusion, the additive of whey protein concentrate (WPC 80) significantly affected the estimated parameters. It was advantageous in terms of lowering oxidative changes of the dispersing phase, particularly it influenced retardation of the formation of conjugated diene hydroperoxides during prolonged storage. On the other hand, the lowest amount of the TBARs was observed for the emulsions with 1.5 and 5% WPC 80, whereas the lowest increase of the TBARs content during storage was noticed for 15% WPC 80 treatment. The supplementation with WPC 80 provided also proper microbiological quality to the obtained emulsions as the number of viable microorganisms was within the permissible range during 16 weeks of storage at room temperature and enhanced physical stability of emulsions containing up to 5% of WPC 80 additive. Thus, the results of the present study revealed that 5% concentration of WPC 80 is optimal to ensure the best emulsion quality.

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