

INFLUENCE OF EMULSIFYING MIXTURES ON THE STABILITY AND ODOUR INTENSITY OF OIL-IN-WATER EMULSIONS

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Abstract. The studies showed that stability of the oil-in-water (o/w) emulsions depended statistically significantly on concentration of natural emulsifier (sodium caseinate) as well as concentration of synthetic emulsifiers (sodium dodecyl sulfate – SDS or Tween 60) added to the emulsifying mixtures. The highest stability of the emulsions evaluated towards creaming was observed in samples containing 2 wt% natural emulsifier and 1 wt% Tween 60 or 0.4 wt% SDS. Increase of concentration of the synthetic emulsifiers decreased surface protein concentration and average droplet diameter $D[4,3]$ what contributed to the higher thermodynamic stability of the emulsions. It was also noticed that those process caused higher retention of lemon aroma (negative values of Pearson's correlation coefficients calculated between concentrations of emulsifiers and aroma intensities). The lowest release of lemon odour from o/w emulsions was observed in sample stabilized by mixture composed of 2 wt% sodium caseinate with 1 wt% Tween 60. The achieved results suggest, that mixtures composed of sodium caseinate with synthetic emulsifiers, particularly with Tween 60 may be applied to manufacture food emulsions containing no more than 30 wt% oil which belong to the low fat mayonnaises.

Key words: oil-in-water emulsion, sodium caseinate, Tween 60, SDS, emulsion stability, odour intensity, surface protein concentration

INTRODUCTION

Oil-in-water (o/w) emulsions are important elements in the formulation of foods, and therefore have to be prepared in such way to be stable [Dalglish 1997]. To achieve this result they are produced by application exhibiting appropriate emulsifying capacity and activity emulsifiers [Pearce and Kinsella 1978]. The two most important types of surface-active material in food are proteins and small-molecule surfactants or “emulsifiers” [Dickinson et al. 1999, Pallandre et al. 2007] which compete for the emulsion interface during and after formation of the emulsion [Dalglish 2006]. The coexistence

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of both proteins and emulsifiers may bring about emulsion destabilization [Dickinson and Ritzoulis 2000, Bortnowska 2008] and affect odour perception recognized as the major quality determinant in food systems [Taylor et al. 2001]. In o/w emulsions, flavour release is mainly dependent on the affinity of volatile compounds for the liquid phases, but may be also affected by microstructure [Druaux and Voilley 1997, Roos 1997, Seuvre et al. 2000]. The microstructure is characterized by the nature of the dispersed phase, the surface area of the lipid-water interface and the nature and amount of the surface-active agent adsorbed at the interface [Charles et al. 2000]. Milk proteins are good emulsifiers and therefore are used as important ingredients in food emulsions [Srinivasan et al. 1996]. However, other emulsifier such as non-ionic Tween 60 (polyoxyethylene sorbitan monostearate) is also widely used [Stauffer 2001]. Euston et al. [1995] reported that Tween 60 partially displaced proteins of sodium caseinate from the oil-water interface. Other study showed that presence of Tween 20 (polyoxyethylene sorbitan monolaurate) and sodium caseinate in o/w emulsions led to the depletion flocculation what enhanced emulsion creaming [Dickinson et al. 1999]. The anionic surfactant SDS (sodium dodecyl sulfate) has been studied because of its ability to denature, and associate with, a wide variety of proteins [Fairley et al. 1996]. Demetriades and McClements [2000] demonstrated that the physicochemical properties of whey protein-stabilized emulsions can be modified by utilizing SDS-protein interactions also Dickinson and Ritzoulis [2000] reported that presence both SDS and sodium caseinate in an emulsion system increased the overall stability with respect to creaming. However, there are not reports regarding in which way protein-surfactant interactions influence aroma compounds release and if this process is dependent on the stability of emulsion.

The objective of this research was to study influence of sodium caseinate applied as emulsifying mixtures with SDS or Tween 60 on the stability of o/w emulsions and odour intensity of lemon sensed orthonasally.

MATERIALS AND METHODS

Materials

Sodium caseinate produced by Minne S.A. de CV Mexico, purchased from Duncan Sp. z o.o. (Kamień Pomorski). Tween 60 (polyoxyethylene sorbitan monostearate) and SDS (sodium dodecyl sulfate) with the estimated hydrophile-lipophile balance (HLB) of 15 and 40, respectively, purchased from Sigma-Aldrich (Poznań). Vegetable rapeseed oil (Kruszwica), purchased in retail outlet. Natural lemon flavouring (6.0% lemon oil dissolved in rapeseed oil) manufactured by the Pollena-Aroma Aromatic Substances Factory (Warsaw), purchased directly from the manufacturer. All of the chemicals used were of analytical grade, purchased from Hartim Sp. z o.o. (Szczecin).

Emulsion preparation

The emulsion aqueous phase was prepared by dissolving an appropriate amount of surface-active material (sodium caseinate or its mixtures with Tween 60 or SDS) in disodium hydrogen phosphate-citric acid aqueous solution at pH 7.0 with 0.04 wt% sodium azide added to protect against microbial contamination. The mixture of oil and

aqueous solution was homogenised using an MPW 302 laboratory homogeniser (Mechanika Precyzyjna, Warszawa) for 30 s at 3500 rpm and flavoured by addition of 0.1 wt% natural lemon flavouring. All samples contained the same amount of rapeseed oil (30 wt%) and varied with the composition and concentration of emulsifying mixtures composed of sodium caseinate: 0.2, 0.6, 1.0 or 2.0 wt%) with addition of SDS or Tween 60: 0.2, 0.4, 0.6 or 1.0 wt%. The control samples were emulsions containing: 0.2, 0.6, 1.0 or 2.0 wt% sodium caseinate.

Emulsion stability

Emulsion stability was characterized in terms towards creaming [Le Denmat et al. 2000, Huang et al. 2001] and changes of the average droplet diameters $D[4,3]$, interpreted as the thermodynamic stability [Dickinson and James 1999]. The stability of the emulsions (ES) towards creaming was assessed after accelerated ageing. The emulsions (8 ± 0.1 ml) were placed into 10 ml centrifugation tubes and centrifuged at 1983.6 g for 10 min at room temperature ($22 \pm 0.5^\circ\text{C}$). The emulsion stability (ES) was calculated as follows:

$$\text{ES (\%)} = (\text{remaining emulsion height} / \text{initial emulsion height}) \cdot 100$$

Average droplet diameter $D[4,3]$

Sample droplet sizes were measured by the method described by Quintana et al. [2002], using microscope (Matic[®] B1 Series, Carlzeiss Jena equipped with a built in camera and software *Multiscan v. 11.06*, Computer Scanning Systems). Droplet size measurements were made using 0.1 and 0.01 scales. Average droplet diameter $D[4,3]$ was calculated for each sample according to the method described by Dickinson and James [1999].

Surface protein concentration

The surface protein concentration ($\text{mg} \cdot \text{m}^{-2}$) was determined by the method described by Haque et al. [1988]. The protein content was assayed by the Kjeldahl method (1026 Distilling Unit and 1007 Digester, Tecator AB, Höganäs, Sweden). A factor 6.38 was used to convert nitrogen to protein content [AOAC 1995].

Viscosity measurement

Viscosity of emulsions was investigated using a Rheotest 2 – 50 Hz – type RV2 viscometer equipped with S/S1 cylinder. The measurements were carried out at $22 \pm 0.5^\circ\text{C}$ and shear rate at 437.4 (s^{-1}).

Odour intensity

Odour intensity was evaluated by an internal panel consisting of eight assessors. The panel was selected according to PN-ISO 8586-1 [1996]. During training sessions the assessors were trained how to evaluate orthonasally odour intensity using a scale from 0 to 9 [PN-ISO 4121 1998]. Samples (20 ml) were served in plastic cups with

metal lids at ambient temperature, approx. 22°C. Assessors were told to open the cup take a deep sniff and after that determine intensity of lemon odour [PN-ISO 5496 1997]. Six samples were evaluated at individual speed in 20 min sessions. The emulsions were served in individual randomised order. All samples were evaluated during 4 h on four sequential days.

Statistical analysis

Three replicates were conducted for all measured parameters and data were statistically treated using Statistica[®] 6.0 program. Two-way ANOVAs were made with design parameters (sodium caseinate and SDS or Tween 60 concentration) as fixed effect on both stability and odour intensity data. Tukey's multiple comparison test was performed to identify significant differences between means ($p \leq 0.05$). The extent of correlations between SDS or Tween 60 concentrations and: stability towards creaming, D[4,3], odour intensity, surface protein concentration and viscosity values was determined by Pearson's correlation coefficients.

RESULTS AND DISCUSSION

The studies showed that with increasing concentration of sodium caseinate with SDS or Tween 60 in emulsifying mixtures, improved the stability of majority of the emulsions evaluated towards creaming and these changes in range of investigated series of samples were statistically significant (Fig. 1). The average stability of the emulsions with addition of 0.2 wt% sodium caseinate was indicated at the level of 34.5% and in samples containing 2 wt% sodium caseinate increased by 5.9% (Fig. 1). However, addition of synthetic emulsifiers to the emulsifying mixtures caused changes of the stability of investigated emulsions in the range from 35.4 to 44.0%. A very high stability of the emulsions was observed in samples with addition of 2 wt% sodium caseinate and 0.8-1.0 wt% Tween 60 whereas in emulsions stabilized by emulsifying mixtures containing SDS, the optimal concentration of synthetic emulsifier varied from 0.4-0.6 wt%, particularly in those stabilized by sodium caseinate with concentration higher than 1 wt% (Fig. 1). Correlations calculated between increasing concentration of synthetic emulsifiers and stability of the emulsions demonstrated that values of coefficient (r) were much higher and in majority statistically significant in samples containing Tween 60 than SDS, regardless of the quantity of sodium caseinate in emulsifying mixtures (Table 1 and 2). Studies of the emulsions in relation to their thermodynamic stability (D[4,3] parameter) indicated that addition of sodium caseinate as well as synthetic emulsifiers decreased average droplet diameters D[4,3] (Fig. 2). Increase of concentration of natural emulsifier from 0.2 to 2.0 wt% induced decrease of average droplet diameter by 36% whereas addition of synthetic emulsifiers to the emulsifying mixtures was correlated negatively at the higher level with stability in samples containing SDS than Tween 60 (Table 1 and 2), and the highest differences of average droplet diameter values D[4,3] between control sample formed with 0.2 wt% sodium caseinate and those stabilized by mixtures containing 0.2 wt% sodium caseinate with 1 wt% SDS or Tween 60 were indicated as 0.46 and 0.50 μm , respectively. Two-way ANOVA evidenced highly significant effects between stability of emulsions and concentrations of natural

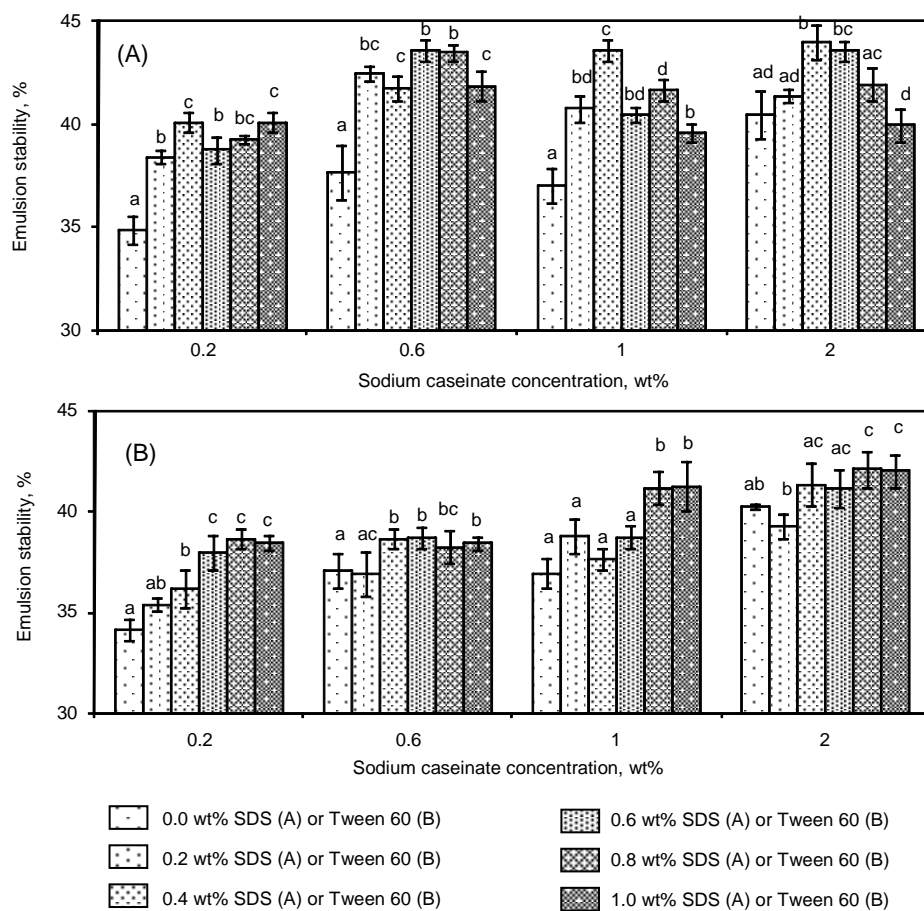


Fig. 1. Effect of SDS (A) or Tween 60 (B) addition to emulsifying mixtures with sodium caseinate on the stability towards creaming of o/w emulsions. Means marked with different letters within samples containing: 0.2, 0.6, 1.0 or 2.0 wt% sodium caseinate are significantly different at $p \leq 0.05$

Table 1. Correlation coefficients (r) and significance level (p) between measured parameters and concentration of SDS in emulsifying mixtures containing sodium caseinate

Sodium caseinate concentration wt%	Stability		D[4,3]		Odour intensity		Surface protein concentration		Apparent viscosity	
	r	p	r	p	r	p	r	p	r	p
0.2	0.752	ns	-0.847	0.05	-0.615	ns	-0.879	0.05	0.935	0.01
0.6	0.639	ns	-0.792	ns	-0.400	ns	-0.699	ns	0.300	ns
1.0	0.301	ns	-0.943	0.01	-0.937	0.01	-0.851	0.05	0.785	ns
2.0	-0.038	ns	-0.885	0.02	0.489	ns	-0.782	ns	0.529	ns

ns – non significant.

Table 2. Correlation coefficients (r) and significance level (p) between measured parameters and concentration of Tween 60 in emulsifying mixtures containing sodium caseinate

Sodium caseinate concentration wt%	Stability		D[4,3]		Odour intensity		Surface protein concentration		Apparent viscosity	
	r	p	r	p	r	p	r	p	r	p
0.2	0.962	0.01	-0.890	0.02	-0.885	0.02	-0.869	0.05	-0.693	ns
0.6	0.724	ns	-0.859	0.05	-0.892	0.02	-0.531	ns	0.851	0.05
1.0	0.893	0.02	-0.699	ns	-0.865	0.05	-0.564	ns	0.909	0.02
2.0	0.838	0.05	-0.789	ns	-0.855	0.05	-0.838	0.05	0.429	ns

ns – non significant.

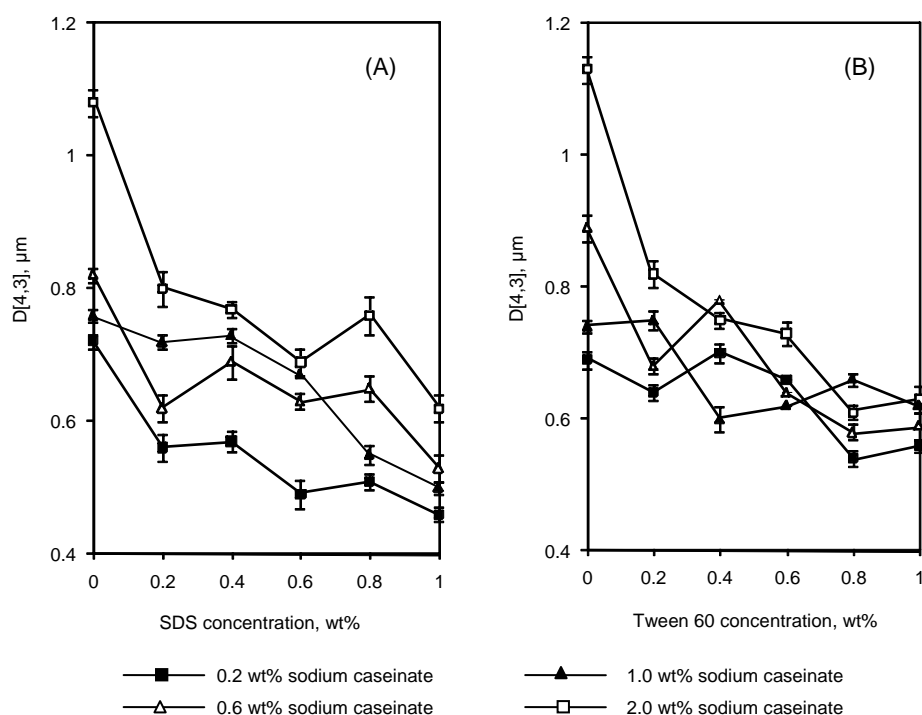


Fig. 2. Effect of SDS (A) or Tween 60 (B) addition to emulsifying mixtures with sodium caseinate on the average droplet diameter $D[4,3]$ of o/w emulsions

emulsifier ($F = 133.71$, $F = 133.48$) as well as synthetic emulsifiers ($F = 44.57$, $F = 110.03$) in samples stabilized by mixtures containing sodium caseinate with SDS or sodium caseinate with Tween 60, respectively (Table 3 and 4). Sodium caseinate is a mixture of caseins which are flexible, amphipathic and rapidly adsorb at the oil-water interface [Haque et al. 1988, Euston et al. 1995], these proteins can also absorb high amount

Table 3. Two-way analysis of variance of influence of SDS addition to emulsifying mixtures with sodium caseinate on the stability and odour intensity of o/w emulsions

Attribute	Sodium caseinate effect			SDS effect			Sodium caseinate × SDS interaction		
	F	p	df	F	p	df	F	p	df
Stability	133.48	0.001	3	110.03	0.001	5	13.16	0.001	15
Odour intensity	86.55	0.001	3	6.87	0.001	5	4.04	0.001	15

Table 4. Two-way analysis of variance of influence of Tween 60 addition to emulsifying mixtures with sodium caseinate on the stability and odour intensity of o/w emulsions

Attribute	Sodium caseinate effect			Tween 60 effect			Sodium caseinate × Tween 60 interaction		
	F	p	df	F	p	df	F	p	df
Stability	137.71	0.001	3	44.57	0.001	5	5.52	0.001	15
Odour intensity	95.85	0.001	3	46.41	0.001	5	3.55	0.001	15

of water increasing stability of the emulsion [Lubbers et al. 1998], therefore it may be supposed that sodium caseinate added with higher concentration increased volume of emulsified phase and improved stability of the emulsions measured towards creaming. These results are in accordance with those reported by Lemam and Kinsella [1989] who found that with higher concentration of micellar casein the percentage of emulsion separation decreased. On the other hand, higher stability of the emulsions with increasing concentration of synthetic emulsifiers may be explained as influence of their very good interfacial activity and possibility to immobilize molecules of water between hydrophilic portions of surfactants [Stauffer 2001, Kamande et al. 2000]. The other explanation is that sodium caseinate may form a complex with SDS at the interface and this complex may provide greater steric stabilization although such interaction has not been reported regarding Tween 60. Further studies are probably required to elucidate why some of the samples containing: 0.6, 1.0 or 2.0 wt% sodium caseinate and SDS with concentration higher than 0.6 wt% demonstrated decrease of the stability. Probably, it cannot be explained as the result of competitive adsorption between proteins of sodium caseinate and SDS because at lower surface protein concentration (0.2 wt% sodium caseinate in bulk phase) addition of SDS improved stability of the emulsions. Also these results are not in agreement with those reported by Dickinson and Ritzoulis [2000] who demonstrated that the 30 vol% n-tetradecane-in-water emulsions made with 2 wt% sodium caseinate were unstable after addition of 10 wt% SDS, but they used other methods to evaluate stability of the emulsions and therefore it is difficult to compare directly these results. To summarise, there were not significant interactions between surface protein concentration and stability towards creaming of the emulsions regardless of synthetic emulsifiers applied. In contrast, the lower surface protein concentration probably had the main effect on decrease of average droplet diameters $D[4,3]$ what was observed in all studied emulsions with increasing concentration of SDS or Tween 60. Similar results also were achieved regarding emulsions stabilized by mixtures com-

posed of dried egg yolk with Tween 65 [Bortnowska 2008]. Dickinson et al. [1999] suggested that surfactants produce lower interface tension than proteins and it might be the reason that with displacement of proteins from interface they were able to increase dispersion of oil phase whereas slight changes of D[4,3] diameter in emulsions containing more than 0.4 wt% SDS or Tween 60 probably depended on critical micelle concentration (CMC) of synthetic emulsifiers, because with concentration higher than CMC the thermodynamic activity of surfactant is not increasing and the interfacial tension is nearly at the same level [Stauffer 2001]. Increase of the stability of emulsions affected intensity perception of lemon odour from studied emulsions. It was observed that intensity of odour decreased in majority of the samples with increasing concentration of emulsifiers. Statistical analyses evidenced relatively very highly correlated coefficients

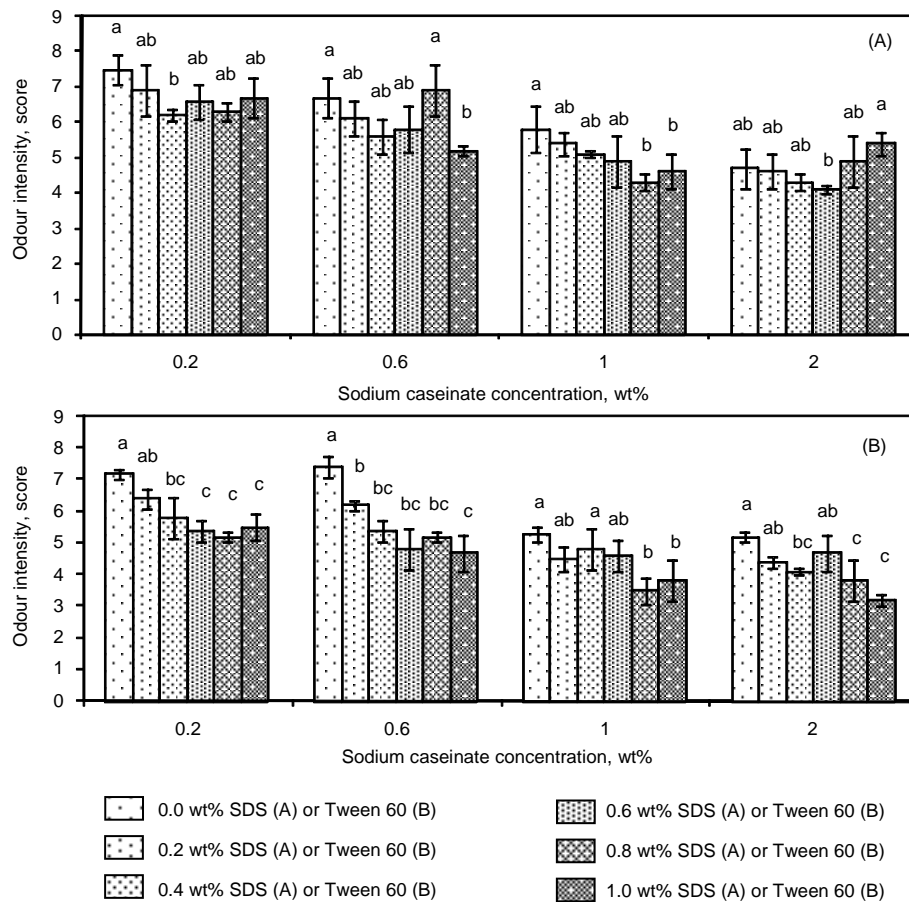


Fig. 3. Effect of SDS (A) or Tween 60 (B) addition to emulsifying mixtures with sodium caseinate on the odour intensity of o/w emulsions flavoured with lemon flavouring. Means marked with different letters within samples containing: 0.2, 0.6, 1.0 or 2.0 wt% sodium caseinate are significantly different at $p \leq 0.05$

(r) at the significance level: $p = 0.05$; 0.02 or 0.01 (Table 1 and 2). The most intensive odour with an average of 6.7 scores was sensed from samples stabilized by 0.2 wt% sodium caseinate with SDS regardless of its concentration in emulsifying mixtures but observed changes were not statistically significant in majority of the samples (Fig. 3). Whereas, the lowest release of lemon odour evidenced at the level of 3.2 scores was reported from emulsion stabilized by mixture containing 2 wt% sodium caseinate and 1 wt% Tween 60 (Fig. 3). Two-way ANOVA indicated that perception of lemon odour released from emulsions was higher affected by concentration of sodium caseinate than synthetic emulsifiers but also was noticed that addition of Tween 60 influenced more perception of odour intensity than the same concentration of SDS ($F = 46.41$ and $F = 6.87$, respectively; Table 3 and 4). Intensity of odour sensed orthonasally is dependent on the rate of aroma compounds released from emulsion to the vapour phase and this process in the considerable extent is affected by the viscosity of emulsion, therefore the observed suppression of aroma release may be attributed to the changes of rheological properties with increasing concentration of emulsifiers (Fig. 3 and 5). Furthermore, the research demonstrated that intensity of lemon aroma applied with oil as the carrier rather did not depend on surface protein concentration in food emulsions stabilized with emulsifying mixtures containing different natural to synthetic emulsifiers ratio.

In summary, it can be said that with increasing concentration of SDS or Tween 60, in emulsifying mixtures with sodium caseinate, increased the stability of o/w food emulsions what also improved retention of lemon aroma in studied samples particularly in those composed of synthetic emulsifier with HLB = 15. Therefore, it seems that for preparation of food emulsions containing no more than 30 wt% oil to which, among other things, belong low fat mayonnaises, it is purposeful to use mixtures composed of

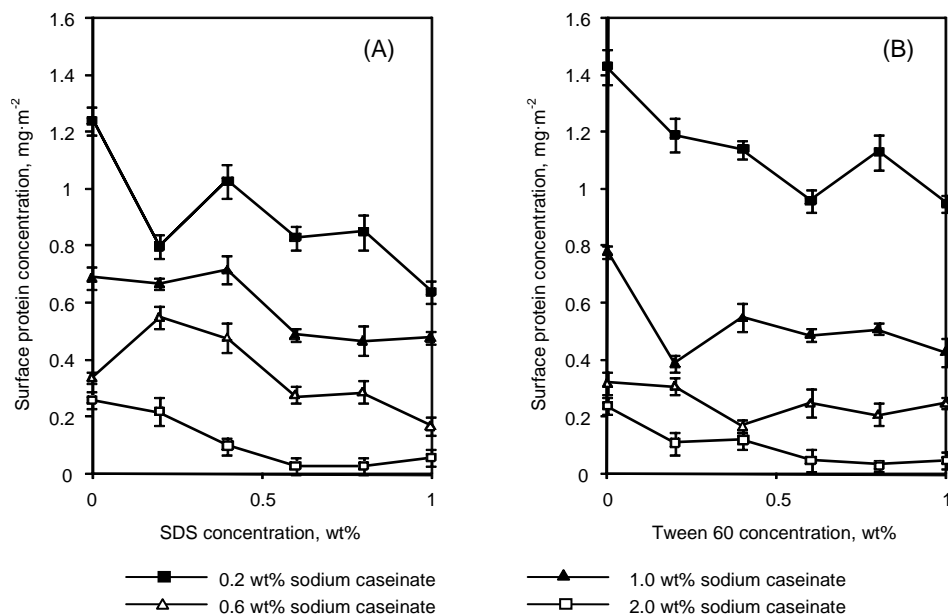


Fig. 4. Effect of SDS (A) or Tween 60 (B) addition to emulsifying mixtures with sodium caseinate on the surface protein concentration of sodium caseinate in o/w emulsions

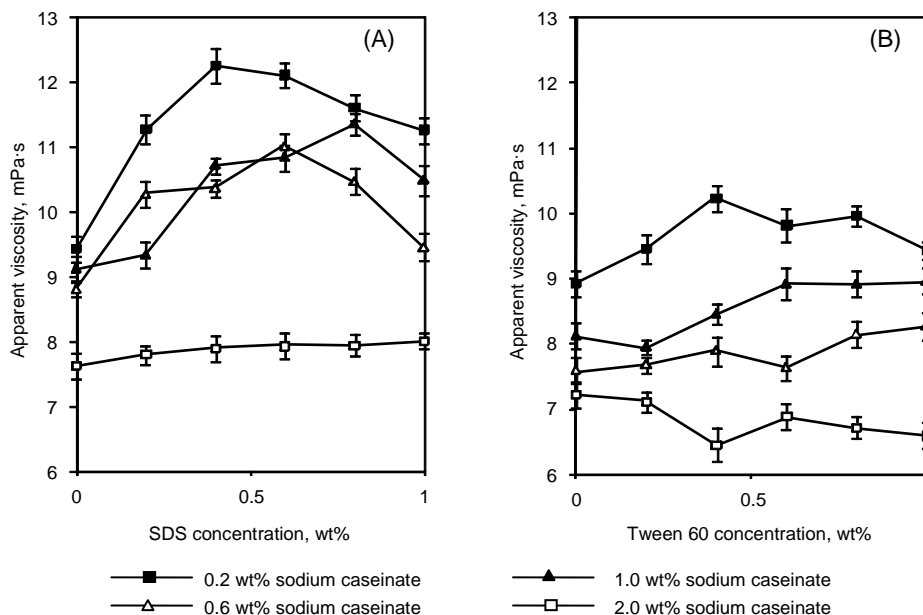


Fig. 5. Effect of SDS (A) or Tween 60 (B) addition to emulsifying mixtures with sodium caseinate on the apparent viscosity of o/w emulsions

natural and synthetic emulsifiers which forming specific microstructure may decrease very rapid release of lipophilic aroma compounds. Moreover, it may be supposed that the consumer's odour intensity perception of the other lipophilic aroma compounds added to the investigated emulsions can be the same as odour of lemon. However, it can not be excluded, that the same lipophilic aroma compounds applied with other carriers may change odour intensity of aromatized samples, therefore it would be advisable to perform additional investigations with the aim to study influence of new environment on release of non-polar aroma compounds.

CONCLUSIONS

1. Increase of the quantity of synthetic emulsifiers in emulsifying mixtures with sodium caseinate improves stability of the emulsions measured towards creaming with optimal concentration from 0.4 to 0.6 wt% SDS or 0.8 to 1.0 wt% Tween 60.
2. The competitive adsorption at the oil-water interface between proteins of sodium caseinate and molecules of SDS or Tween 60 affects thermodynamic stability of o/w emulsions and rather not change stability with respect to the creaming.
3. Intensity of lemon odour decreases with higher stability of emulsions measured towards creaming and demonstrates the lowest value in sample stabilized by emulsifying mixture containing 2 wt% sodium caseinate and 1 wt% Tween 60.
4. Changes of emulsions' viscosity as the result of addition of synthetic emulsifiers may induce differences in perception of odour intensity in flavoured emulsions.

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WPLYW MIESZANIN EMULGUJĄCYCH NA STABILNOŚĆ I INTENSYWNOŚĆ ZAPACHU EMULSJI TYPU O/W

Streszczenie. Badania wykazały, że stabilność emulsji typu o/w zależała w sposób statystycznie istotny od stężenia emulgatorów zarówno naturalnego (kazeinianu sodu), jak i syntetycznych (dodecylosiarczan sodu – SDS lub Tween 60) w mieszaninie emulgującej. Największą stabilność na podstawanie (śmietankowanie) obserwowano w próbach zawierających 2% (w/w) emulgatora naturalnego i 1% (w/w) Tweenu 60 lub 0,4% (w/w) SDS. Wzrost stężenia emulgatorów syntetycznych wpływał na zmniejszenie stężenia białek zaadsorbowanych na powierzchni międzyfazowej olej-woda oraz średniej średnicy kuleczek oleju D[4,3], co zwiększało termodynamiczną stabilność emulsji, a także zatrzymywało coraz większą ilość aromatu cytrynowego (ujemne wartości współczynników korelacji Pearsona liczone pomiędzy stężeniem emulgatorów a intensywnością zapachu). Intensywność zapachu cytrynowego najbardziej zmniejszył dodatek do emulsji 2% (w/w) kazeinianu sodu i 1% (w/w) Tweenu 60. Wydaje się, że mieszaniny kazeinianu sodu z emulgatorami syntetycznymi, a szczególnie Tweenem 60 mogą być wykorzystane do produkcji emulsji spożywczych, zawierającej nie więcej niż 30% (w/w) oleju, do których należą między innymi majonezy niskotłuszczowe.

Słowa kluczowe: emulsja typu o/w, kazeinian sodu, Tween 60, SDS, stabilność emulsji, intensywność zapachu, powierzchniowe stężenie białek

Accepted for print – Zaakceptowano do druku: 5.12.2008

For citation – Do cytowania: Bortnowska G., 2009. Influence of emulsifying mixtures on the stability and odour intensity of oil-in-water emulsions. Acta Sci. Pol., Technol. Aliment. 8(1), 35-46.