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ORIGINAL ARTICLES

Effect of Protein Type on the Physical Properties of Milk Fat Emulsions

M. Abd El-Aziz; Faten L. Seleet; A.F. Farrag; M.M. El-Shiekh and A.F. Sayed

Dairy Department, National Research Centre, Dokki, Cairo, Egypt.

ABSTRACT

The physical properties of milk fat emulsions formulated with 20% butter oil and ~ 80% protein solutions (3.2% protein) of sodium caseinate (SC), mixed sodium caseinate with whey proteins (SCW, 3:2), mixed sodium caseinate with soy proteins (SPS, 3:2) or mixed sodium caseinate with both of whey proteins and soy proteins (SCWS, 3:1:1) were evaluated. The results showed that SC emulsion had the best whipping ability and fat destabilization ($P < 0.05$), while SCS emulsion had the best creaming rate, proteins load and fat destabilization among all protein emulsions. SCW and SCWS emulsions had medium proteins load, fat destabilization, and whipping ability between SC and SCS emulsions. The shear stress (apparent viscosity) of SCS emulsion was the highest while that of SCWS was the lowest; although the differences among all emulsions were not significant ($P > 0.05$). These results can be useful in selecting suitable protein type and percentage for the preparation of milk fat emulsion.

Key words: Sodium caseinate, whey protein, soy protein, physical properties, milk fat emulsion.

Inroduction

Emulsions are complex and unstable thermodynamically systems, composed of two immiscible liquids phase. In oil/water emulsion, oil is dispersed in a continuous aqueous phase in a form of very small fat droplets. A thermodynamic balance between the two emulsion phases is assured by the use an emulsifier, which decreases the interfacial tension and keeps the emulsion stability. Proteins and emulsifiers are widely used as functional ingredients for the formation and stabilization of emulsions. These molecules contain simultaneously polar and non-polar regions, which give them surface-active properties (Rouimi *et al.,* 2005).

The surface activity of milk proteins results in to their rapid adsorption at the oil-water interface during emulsification, producing a stabilizing layer that protects oil droplets against subsequent flocculation or coalescence (Vega & Roos, 2006; Singh & Ye, 2009; Ye, 2008; McClements, 2010 and Singh, 2011). Hu *et al.* (2003) reported that whey proteins can be used as emulsifiers due to their excellent surface activities, which can avoid the coalescence or creaming process of the dispersed phase, giving stability to the oil/water interface. The native forms of whey proteins are characterized by rigid and compact structures stabilized by covalent and non-covalent intermolecular interactions. Under heat treatment, whey proteins undergo unfolding, interaction of reactive groups such as thiol groups (-SH), formation of intermolecular disulphide bonds (S-S) and protein aggregates. In food emulsions, partial denaturation of whey proteins can result in the formation of a thicker coating than the native form and increased emulsion stability (Kiokias *et al.,* 2007).

Currently there is considerable and increasing interest in the health benefits of soy-containing foods, in particular their role in lowering the potential incidence of certain cancers (Messina et al., 2009). Due to its nutritional value and low cost, currently, soy protein is the largest commercially available vegetable protein in the world (Messina & Barnes, 1991). Globular proteins such as soy and whey protein are used as emulsifiers in number of commercial food (Al-Bakkush, 2008). Sipos (1994) reported that the multiplicity of groups attached to the peptide chain of the protein, such as lipophilic, polar, nonpolar, negatively and positively charged groups, enables soy proteins to associate with many different types of compounds.

The thermal stability of food protein ingredients is dependent on a variety of conditions including their interaction with water, pH and ionic strength (Mohamed & Xu, 2003 and Ryan *et al.,* 2008). Recently, Ryan *et al*. (2008) reported that inclusion of calcium salts reduced the thermal stability at 140°C of aqueous dispersions of soy protein isolate and soy protein hydrolysate ingredients. Extensive research has been carried out to understand the adsorption process, the composition and the structure of the adsorbed protein layers and how they influence the physical and chemical properties of emulsions (Singh, 2011). Therefore, the aim of this work was to study the effect of protein type; casein, whey protein and soy protein, on the physical properties of reformulated milk fat emulsion.

Corresponding Author: M. Abd El-Aziz, Dairy Department, National Research Centre, Dokki, Cairo, Egypt.
E-mail: mabdelaziz69@yahoo.com

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Materials And Methods

1. Materials:

Fresh butter was obtained from dairy processing unit, Faculty of Agriculture, Cairo University, Giza, Egypt. Butter oil was prepared by the method of Amer *et al* (1985). Sodium caseinate (LACTONAT EN) was obtained from Lactoprot Deutschland GmbH, Germany. Whey protein concentrate (WPC) was imported from Agri. Mark, USA. Soy protein isolate was purchased from Lustrel Laboratoires SAS (Saint Jean de Vedas, France). The chemical composition of used proteins is shown in Table 1 as data of the supplier.

Table 1: Chemical composition of used protein as the data of the supplier.

Items	Protein type		
	Sodium caseinate	WPC	SPI
Total Protein (%)	> 88.5	> 81	> 89
Moisture (%)	< 6.0	< 5.3	< 5.0
Fat (%)	< 2.0	< 5.5	< 0.5
Carbohydrate (%)	< 0.3	< 10	< 0.3
Ash (%)	< 4.5	< 3.0	< 4.5

WPC, Whey protein concentrate; SPI, soy protein isolate

2. Methods:

Emulsions formulation:

Sodium caseinate, whey protein and soy protein solutions (3.2% protein) were prepared by dispersing the required amount of protein in distilled water under continuous stirring at room temperature for 4 h. Emulsions were formulated from 20.0% butter oil and 80.0% SC, SCW, SPS or SCWS blends to create four treatments as described in Table 2. Formulated emulsions were preheated to 60ºC, homogenized using laboratory double stage homogenizer (Rannie, Copenhagen, Denmark), 13.6 MPa first stage and 3.5 MPa second stage, then heated to 78ºC for seconds and cooled to 4±2ºC. Sodium azide was added at the rate of 0.02% as preservative. All formulated emulsions were prepared in triplicates.

Table 2: Abbreviations and description of formulated milk fat emulsions.

Abbreviations	Formulated milk fat emulsions
SC	20.0% butter oil and 80.0% sodium caseinate solution.
SCW	20.0% butter oil and 80.0% mixed sodium caseinate and whey protein (3:2) solution.
SCS	20.0% butter oil and 80.0% mixed sodium caseinate and soy protein (3:2) solution.
SCWS	20.0% butter oil and 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution.

Shear stress:

Shear stress of milk fat emulsions was measured according to Farrag *et al*. (2006) using a coxial cylinder viscometer (Bohlen V88, Sweden) attached to a workstation loaded with V88 viscosity program. The system C_{30} was filled with emulsion at the measurement temperature of 20°C. The rheological parameter (shear stress) was carried out in the up mode at shear rate ranging from 88 to 924 S^{-1}.

Creaming rate:

Emulsion instability was measured as the rate of creaming over 1 wk of storage as described by Scott *et al.* (2003). After their preparation, (d 0), emulsions were placed in 100 ml graduated cylinders up to mark , capped, and stored at 4 ±2°C. Initial fat content was determined and fat content of the lower phase of each emulsion was analyzed in duplicate after 1, 3 and 7 days of storage using the Gerber method (AOAC, 2007). The creaming rate was calculated as follows:

$$Creaming\ rate\,(\%) = \frac{\%\ fat\ in\ original\ emulsion - \%\ fat\ in\ lower\ phase}{\%\ fat\ in\ original\ emulsion}\ x\ 100$$

Fat destabilization index:

Fat destabilization index was determined according to Goff and Jordan (1989) by dilution of formulated emulsion ((1:500 distilled water) before and after whipping for 20 min in a mixer ((Heidolph No. 50 111, Type RZRI, Germany) at speed setting 10 and measurement of the turbidity (absorbance) at 540 nm.

Protein load:

The adsorbed protein on the surface of fat globules was determined by measuring the protein content **(**AOAC, 2007**)** of the aqueous phase of formulated emulsion before and after centrifugation (Sigma Laborzentri Fugen, 2 K15, Germany) at 10,350 xg for 30 min at 20ºC (Cano-Ruiz and Richter, 1997) and freezing at -30°C for 45 min. The adsorbed protein was calculated from the difference between the initial and final proteins of the aqueous phase and expressed as protein load.

Whipping properties:

Whipping ability measurement was made at 5 min intervals for a total of 20 min. A 200 ml formulated emulsion was whipped in a mixer ((Heidolph No. 50 111, Type RZRI, Germany) at speed setting 10 under freezing conditions according to procedure described by Smith *et al.* (2000). The stability of the whipped emulsion was measured as described by Mangino *et al.* (1987). A 100 ml whipped emulsion (after 20 min) was left in graduated cylinders at 25±2ºC and the decrease in the volume was measured at 30 min intervals for a total of 120 min.

Statistical analysis:

Analysis of variance (ANOVA) and LSD test were conducted using a Statistical Analyses System (SAS, 2004). A probability to $p \leq 0.05$ was used to establish the statistical significance.

Results And Discussion

Creaming rate:

The effect of protein type on the creaming rate of milk fat emulsion during storage at 4 ±2°C for 7 days is shown in Table 3. The Duncan test showed that the protein types had a substantial effect on the instability of milk fat emulsion ($P < 0.05$). SC emulsion showed significantly higher cream rate ($P < 0.05$) compared to other emulsions throughout the storage period. Also, the creaming rate of SCWS emulsion was higher than that of SCW or SCS emulsion, the difference being significant only at day 7. However, there was no significant variation ($P > 0.05$) in creaming rate of SCW and SCS emulsions. Over the storage period, all emulsions displayed an increased rate being significant ($P < 0.05$) at day 1, 3 and 7 for SC emulsion, whereas for SCWS emulsion the changes were significant at day 3 and 7. Earlier studies have reported that the creaming rate could be related to protein load, droplet size distribution and/or emulsion viscosity (Wiking, 2005; Abd EL-Aziz, 2008 and Raikos, 2010). A strong negative relationship ($r = -0.52^{**}$) was formed between the proteins load (Fig. 2) and creaming rate (Fig 1). A similar observation was found by Abd EL–Aziz (2008). Van Lent *et al.* (2008) showed that high homogenization pressures led to small fat globules and consequently less free proteins in the serum because of the higher surface area that needs to be covered. The protein molecule acts at the water/fat interface to produce stable emulsion, preventing the oil droplets from coalescing. Also, in this respect, the creaming rate of emulsion may be inversely correlated with droplet size distribution (Elizalde *et al.*, 1988). (Sliwinski *et al.,* 2003) documented that the amount of protein adsorbed at the emulsion droplet increased with increasing temperature for whey protein stabilized emulsions. However, in this study, the creaming rate was not related to the emulsion viscosity.

Table 3: The creaming rate of formulated milk fat emulsions as affected by protein types during storage at 4 ±2°C for 7 days.

Emulsions	Creaming instability (%)		
	1 day	3 days	7 days
SC	21.0^{c} ±0.30	27.5^{b} ±2.50	42.0^{a} ±4.00
SCW	1.87^{f} ±0.05	2.05^{ef}±0.13	2.44^{ef}±0.52
SCS	2.00^{ef}±0.08	2.43^{ef}±0.15	3.97^{ef}±0.19
SCWS	4.05^{ef}±0.07	6.90^{de}±1.00	10.74^{d} ±1.16

Means with the same letters are not significantly different ($P < 0.05$); SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution.

Proteins load:

Lipid emulsion is stabilized by a layer of protein adsorbed at the surface of fat globules (Krog, 1991 and Millqvist-Fureby *et al.,* 2001). Therefore, the protein load may be influenced by the type of protein used in milk fat emulsion. As shown in Fig 1, the presence of whey protein or soy protein with sodium casienate in emulsion increased the protein load on the oil–water interface compared with sodium casienate. SC emulsion had significantly the lowest protein load ($P < 0.05$) than SCW and SCS emulsions. However, the protein load of SCS emulsion was higher than that of SCW emulsion ($P > 0.05$). The increasing in protein load could be attributed to the decrease in fat globule size (Fatouh *et al.,* 2006) and the increase in the thickness of the protein layer covering the oil droplet, which increases the density of the droplet (Monahan *et al.,* 1996). Also, native proteins move more quickly to the oil-water interface facilitating the covering, while aggregated denatured proteins results in the formation of thick membranes on this interface (Kiokiasl *et al.,* 2007). Ye (2008) indicated that caseins adsorb preferentially at the oil–water interface at high protein concentrations, whereas at low protein concentrations (< 3.0%), whey proteins were preferentially adsorbed in comparison to caseins. However, the presence of both soy and whey proteins with sodium casienate in emulsion (SCWS) decreased the protein load compared to SCW or SCS containing emulsion ($P < 0.05$).

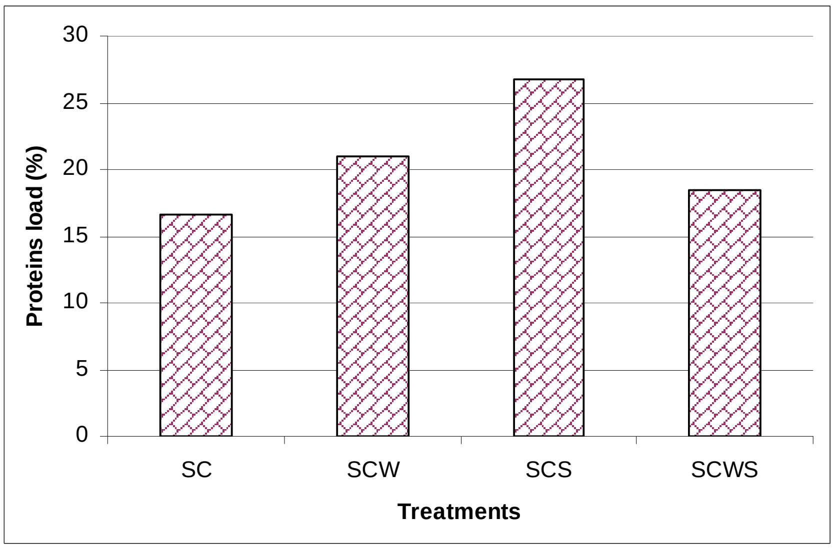

Fig. 1: The proteins load of formulated milk fat emulsions as affected by protein types [SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution].

Fat destabilization:

Fat globules are mechanically damaged by the shear forces and the ice crystallization process, which leads to agglomeration and partial coalescence of the fat globules (Koxhalt *et al*., 2001). Fig 2 illustrates the fat destabilization of SC, SCW, SCS and SCWS emulsions after whipping for 20 min under freezing conditions. SCS emulsion had the highest fat destabilization ($P < 0.05$), while SC emulsion had the lowest fat destabilization ($P < 0.05$). Fat destabilization of SCWS and SCW emulsions was higher than that of SC emulsion, the difference being significant only between SCWS and SC emulsions ($P < 0.05$). Also, differences in fat destabilization between SCWS and SCW emulsions were found no significant. This means that, soy protein and whey protein had adverse effects on the fat destabilization, however, fat destabilization was more pronounced in soy protein containing emulsion (SCS). Also, fat destabilization may be correlated with the type of protein covering the oil droplet. Positive correlation was found between fat destabilization and protein load ($r = 0.67^{**}$).

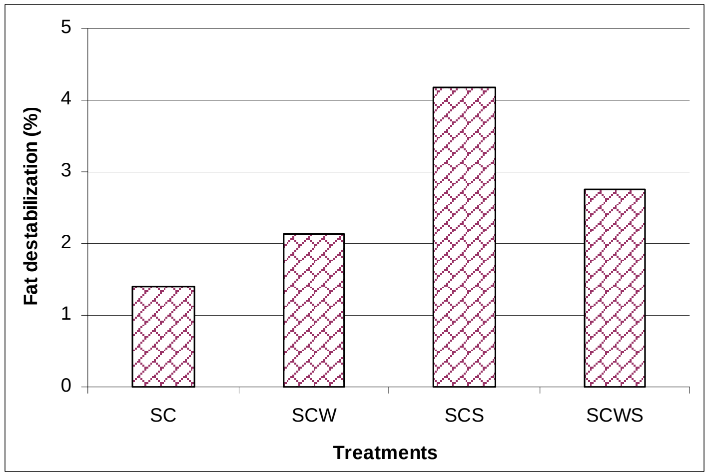

Fig. 2: Fat destabilization of formulated milk fat emulsions as affected by protein types under freezing condition for 20 min **[**SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution].

Whipping ability:

Whipping ability determines the ease with which air can be incorporated into the emulsion. Whipping ability of SC, SCW, SCS and SCWS emulsions under freezing condition for 5, 10, 15 and 20 min are shown in Table 4. The Duncan test showed that the protein type had significant effect on the whipping ability ($P < 0.05$). The whipping ability values of SC emulsion were significantly higher ($P < 0.05$) than those of other emulsions during whipping time (20 min). A similar observation was found by Indrawati *et al*. (2008). They observed higher whipping ability in the case of sodium caseinate stabilized foams. The whipping ability is markedly affected by molecular size and structure of protein. Disordered, small and flexible proteins reduce the surface tension earlier and faster than ordered, rigid and larger proteins (Martin *et al.,* 2002). Also, the whipping ability values of SCW emulsion were higher than those of SCS and SCWS emulsions from min 10 onwards, the difference being significant only at 15 and 20 min. The whipping ability values were similar in both SCS and SCWS emulsion throughout the whipping time ($P > 0.05$). These results indicate that, soy protein showed higher negative effect in whipping ability of emulsion than whey proteins. The decrease in whipping ability may be related to the high fat destabilization ($r = -0.72^{**}$), which may retard air incorporation.

Table 4: The whipping ability of formulated milk fat emulsions as affected by protein types under freezing condition for 20 min.

Emulsions	Whipping ability (%)			
	5 min	10 min	15 min	20 min
SC	86.3^{b} ±0.72	91.3^{ab} ±0.72	96.2^{a} ±1.20	98.8^{a} ±0.92
SCW	37.5^{g} ±1.44	48.8^{def} ±3.60	65.0^{c} ±4.33	71.8^{c} ±4.05
SCS	40.0^{fg} ±2.88	43.8^{efg} ±2.16	50.0^{def} ±1.44	52.5^{d} ±1.24
SCWS	33.8^{g} ±5.05	41.3^{efg} ±3.06	51.4^{de} ±5.05	53.8^{d} ±4.94

Means with the same letters are not significantly different ($P < 0.05$); SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution

Foaming instability:

As shown in Table 5, statistical analysis showed that the protein type had significant effect on the foaming instability of milk fat emulsions ($P < 0.05$). In particular, the SC emulsion had the highest foaming instability, while the SCWS emulsion had the lowest foaming instability. This indicates that, emulsion of higher whipping ability had also higher foaming instability and vice versa. The SCW and SCS emulsions had medium foaming instability ($P < 0.05$), although the foaming instability of SCW emulsion was higher ($P < 0.05$) than that of SCS emulsion. Globular protein results in higher foam stability due to its ability to form cohesive, elastic and viscous film (Phillips, 1981). Also, Zhoa *et al.* (2008), found the partial coalescence (which reduce the foaming stability) of whipped cream with sodium caseinate was higher than those with whey proteins. By increasing the time, the percentage of foaming instability increased in all emulsion except SCW emulsion; that was more stable compared with other emulsions.

Based on foaming instability relative to the percentage of air incorporated (Table 6), the foaming instability of SCW emulsion was higher than that of SC emulsion at min 30 and 60 but remained stable and similar in both emulsions. In this respect, Marinova *et al.* (2009) reported that adsorbed casein layers were denser and thicker thus ensuring better stabilization than the globular whey proteins. The foaming instability of SCS emulsion was lower than that of SC and SCW emulsions at min 30, 60 and 90, after which the foaming instability of SCS emulsion was the highest ($P < 0.05$). A similar to Table 5, SCWS emulsion had the lowest foaming instability compared to other emulsions ($P > 0.05$).

Table 5: The foaming instability of formulated milk fat emulsions as affected by protein types at 25 ±2°C for 120 min.

Emulsions	Faoming instability (%)			
	30 min	60 min	90 min	120 min
SC	33.0^{b} ±0.58	42.0^{a} ±0.58	47.4^{a} ±1.44	47.5^{a} ±1.44
SCW	31.0^{b} ±2.02	32.0^{b} ±2.02	32.5^{b} ±3.90	32.5^{b} ±3.90
SCS	15.5^{c} ±0.29	15.5^{c} ±0.87	21.0^{c} ±0.58	29.5^{b} ±0.87
SCWS	0.00^{e} ±0.00	2.50^{de} ±0.29	4.00^{de}±0.36	8.50^{d} ±0.29

Means with the same letters are not significantly different ($P < 0.05$); SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution

Table 6: The foaming instability of formulated milk fat emulsions relative to foaming capacity as affected by protein types at 25 ±2°C for 120 min.

Emulsions	Foaming instability (%)			
	30 min	60 min	90 min	120 min
SC	33.4^{bc}±0.34	42.5^{abc}±0.28	48.1^{ab} ±1.11	48.1^{ab}±1.11
SCW	45.1^{ab}±6.07	46.5^{ab} ±6.17	47.1^{ab} ±7.32	47.1^{ab}±7.32
SCS	29.6^{c} ±1.36	33.5^{bc} ±2.57	40.2^{bc} ±2.21	56.4^{a} ±3.20
SCWS	0.00^{e} ±0.00	4.60^{de} ±0.10	7.60^{de} ±0.72	16.2^{d} ±2.09

Means with the same letters are not significantly different ($P < 0.05$); SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution

Rheological properties:

The results of shear stress of SC, SCW, SCS and SCWS emulsions are given in Fig. 3. The shear stress of SC and SCS emulsion (on increasing shear rate, corresponding to the upward curve) were higher values than that of SCW emulsion and SCWS, indicating that SC and SCS emulsion has higher apparent viscosity than SCW and SCWS emulsion. This was similar to the observation of Zhoa *et al*. (2008), who found higher improvement in texture characteristics of whipped cream made with sodium caseinate than that made with whey proteins. The shear stress of SCS emulsion was slightly higher ($P > 0.05$) than that of SC emulsion. This is confirmed by the higher ($P < 0.05$) protein load (Fig 1) of SCS as compared to other emulsions. Also, there was no difference in the values of shear stress of SCW and SCWS emulsion except at shear rate 446 and 639 S^{-1}, the shear stress of SCW emulsion was higher than that of SCWS emulsion ($P > 0.05$).

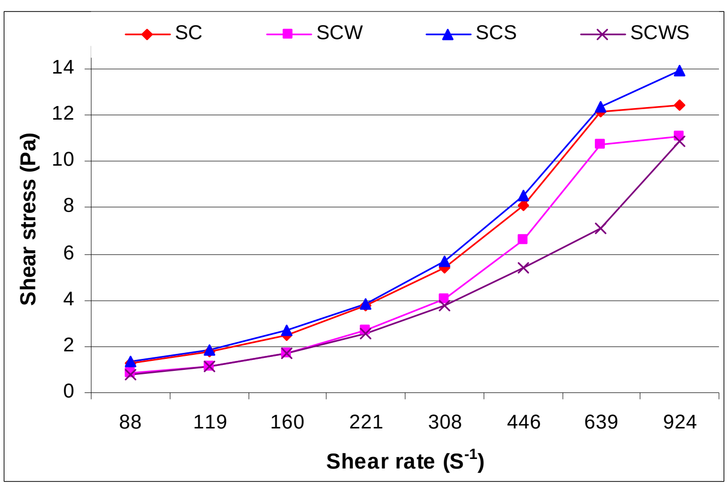

Fig. 3: The shear stress of formulated milk fat emulsions as affected by protein types at shear rate ranging from 88 to 924 S^{-1}. [SC, 80.0% sodium caseinate solution; SCW, 80.0% mixed sodium caseinate and whey protein (3:2) solution; SCS, 80.0% mixed sodium caseinate and soy protein (3:2) solution; SCWS, 80.0% mixed sodium caseinate, whey protein and soy protein (3:1:1) solution].

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