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Milk Fat Substitution by Microparticulated Protein in Reduced-fat Cheese Emulsion: The Effects on Stability, Microstructure, Rheological and Sensory Properties

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Abstract Fat reduction in the formulation of cheese emulsion causes problems in its flowability and functional characteristics during spray-dried cheese powder production. In order to eliminate these problems, the potential of using microparticulated whey protein (MWP) in cheese emulsions was examined in this study. Reduced-fat white-brined cheese emulsions (RF) with different dry-matters (DM) (15%, 20%, and 25% excluding emulsifying salt) were produced using various MWP concentrations (0%–20% based on cheese DM of emulsion). Their key characteristics were compared to full-fat cheese emulsion (FF). MWP addition had no influence on prevention of the phase separation observed in the instable group (RF 15). The most notable effect of using MWP was a reduction in apparent viscosity of RF which significantly increased by fat reduction. Moreover, increasing the amount of MWP led to a decrease in the values of consistency index and an increase in the values of flow behavior index. On the other hand, using high amounts of MWP made the emulsion more liquid-like compared to full-fat counterpart. MWP utilization also resulted in similar lightness and yellowness parameters in RF as their full-fat counterparts. MWP in RF increased glossiness and flowability scores, while decreased mouth coating scores in sensory analyses. Fat reduction caused a more compact network, while a porous structure similar to FF was observed with MWP addition to RF. In conclusion, MWP showed a good potential for formulation of reduced-fat cheese emulsions with rheological and sensorial characteristics suitable to be used as the feeding liquid in the spray drying process.

Keywords reduced-fat, cheese powder, rheology, white-brined cheese, microparticulated protein

Introduction

Cheese powder can be used as a flavoring agent and/or nutritional ingredient for the manufacture of many food products (Erbay et al., 2015; Pisecky, 2005). For the

production of cheese powder by spray drying process, it is necessary to prepare the liquid form of cheese, described as cheese emulsion. This is mainly prepared by melting the cheese with water and emulsifying salts using heat (Koca et al., 2015; Varming et al., 2014). The production of cheese emulsion resembles the production of spreadable processed cheese, but the expectations from emulsion differ due to the requirements of the spraying process. The main target is to prepare homogeneous, uniform, stable and pumpable emulsion for a fine atomization (Kelimu et al., 2017; Uргу et al., 2018; Varming et al., 2014).

Milk fat has the ability to improve texture, acts as a flavour carrier and modifies the visual characteristics of dairy products (Koca and Metin, 2004). The fat reduction cause some undesirable changes in textural, functional and sensorial quality of dairy products such as excessively firm, rubbery, poor meltability, undesirable color and flavour. The removal of fat from casein matrix causes a much tighter para-casein network, resulting in an increase in viscosity of cheese emulsion system. This can cause problems during atomization, but can be tackled by changing the formulation of cheese emulsion e.g., addition of fat replacers. Fat replacers are used to substitute or mimic the functions of fats in dairy products. Microparticulated whey protein is a water-dispersible fat replacer; manufactured from whey protein concentrate in a process that primarily involves thermal aggregation at intensive shearing and low pH (Singer et al., 1990). The small size and round shape of MWP provide fat mimicking properties and improve creaminess of the products (Liu et al., 2016). Addition of MWP to the cheese can help to reduce the hardness due to the weakening of the casein structure and improve meltability and flowability characteristics (Aryana and Haque 2001; Koca and Metin, 2004; McMahon et al., 1996). When used in emulsion systems, it can be helpful to enhance emulsification and stabilization, as well as to contribute creaminess, mouthfeel and opacity. Although studies on cheese emulsion have been increased in recent years (Hougaard et al., 2015; Kelimu et al., 2017; Ray et al., 2016; Varming et al., 2014), fat reduction and also addition of MWP in cheese emulsion system has not been studied yet. The current study investigated the effects of using MWP for imitation of fat in reduced-fat formulation. Therefore, variations in the stability, rheology, microstructure and sensory of reduced-fat cheese emulsions were compared to full-fat cheese emulsion.

Materials and Methods

Materials

Reduced-fat and full-fat white-brined cheeses were supplied by Pinar Dairy Company (Izmir, Turkey). Cheese samples were packaged in rectangular aluminium packages with brine and used to prepare emulsions after one month storage at 4°C. The average moisture, protein, fat contents and pH values of full-fat and reduced-fat cheeses were 53.51%, 13.87%, 26.50%, 4.72 and 64.98%, 19.46%, 11.13%, 4.88, respectively. Simplese[®], the microparticulated whey protein (0.01–3 microns particle size, 3.9% fat, 53% protein, 5% ash, 6.49 pH and 1.31% acidity) as fat replacer (CP Kelco Co., Lille Skensved, Denmark), and JOHA[®] emulsifying salt were provided by Azelis Company (Istanbul, Turkey) and Kipa Chemical Company (Istanbul, Turkey), respectively.

Cheese emulsion preparation

Cheese emulsions were prepared with ground white cheese, water and emulsifying salt of 3% (based on cheese weight) by using the same procedure according to Koca et al. (2015). According to this procedure, the ingredients were heated by adding hot water at 80°C and sheared in a blender (model LB10S, Waring, Torrington, CT, USA) at 6,000 rpm for 1 min. Subsequently, the slurry was heated in a water bath to 80°C, then sheared again at 6,000 rpm for 10 min. Authors found the suitable DM to be 25% for full-fat white-brined cheese emulsion for the pilot plant spray drier. So, in this research, the

highest dry matter content of cheese emulsion was 25% considering the increasement in viscosity of RF. Dry matter contents of RF were 15%, 20%, and 25% (excluding emulsifying salt). In the preparation of RF with fat replacer, MWP was added directly to replace 5%, 10%, 15%, and 20% of cheese dry matter. The quantities of MWP were chosen based on manufacturers recommendation and our preliminary experiments. FF was also prepared with 25% DM for comparison. Cheese emulsions were prepared in triplicate.

Compositional analysis

Moisture, fat and protein contents of cheese emulsions were determined by gravimetric (IDF, 1982), Van-Gulik (IDF, 1981) and Kjeldahl (AOAC, 2007) methods, respectively. Ash content was carried out using gravimetric method (AOAC, 2007). pH value was measured by using a digital pH meter (pH 320, WTW, 82362 Weilheim, Germany), and titratable acidity was expressed as percent lactic acid (AOAC, 2007). All the measurements were performed in triplicate.

Emulsion stability

Emulsion stability was determined by the centrifugation method according to Hougaard et al. (2015) with slight modifications. 30 mL of emulsion was centrifuged at 1,600×g for 5 min. Emulsions were described as stable if no phase separation was observed after the test. Instability was observed as separation into three phases. In instable groups, each separated phase was measured and compared to their weight. The effects of the holding time on stability were all evaluated using the same procedure in which the samples were placed in a water bath at 45°C for 60 min. All measurements were done in triplicate.

Rheological properties

Rheological properties were measured using a rotational cylinder viscometer (Haake 550 Viscotester, Thermo Scientific, Waltham, MA, USA) fitted with a SV-DIN sensor. The thermostatic bath (Haake DL 30 Thermo, Electron Corporation, Waltham, MA, USA) was used to regulate the temperature at 45°C during the measurements. The viscosity and shear stress of samples were recorded by increasing shear rates from 1 to 1,000/s in 360 s. The shear stress values were plotted against the shear rates from the average measurements of 3 trials. The average apparent viscosity results were reported at 250/s. In addition, average the shear stress versus shear rate for stable emulsions were used to analyze flow properties by fitting to the Power Law model (Eq. 1). The rheological model equation was:

$$\tau = K\gamma^n \quad (1)$$

where τ is the shear stress (Pa), γ is the shear rate (1/s), K is the consistency index (Pa.sⁿ) and n is the flow behavior index.

The root square mean error (RSME) and chi-square (χ^2) values were also determined by using Equations 2 and 3, respectively.

$$RMSE = \sqrt{\Sigma(x - y)^2 / N} \quad (2)$$

$$\chi^2 = \frac{\Sigma(x - y)^2}{N - n} \quad (3)$$

where x is the measured value, y is the predicted value, N is the number of data points, and n is number of parameters in model.

Color evaluation

The color of the samples was measured using a Hunter Color Flex colorimeter (Hunter Associates Laboratory, Reston, VA, USA) and expressed as L^* (lightness), a^* (greenness and redness) and b^* (yellowness and blueness).

Microscopic evaluation

Microscopic evaluation was carried out using a motorized light microscope (PSARON Floptik HPTS 150, AIV Labs, Ankara, Turkey) which has $\times 4$, $\times 10$, $\times 40$, and $\times 100$ objective lenses for imaging. Emulsions were carefully dropped in the centre of a glass microscope slide and examined at room temperature.

Sensory evaluation

The sensory evaluation was performed in agreement between three trained assessors that have been working on cheese emulsion for years. Grittiness, mealiness, graininess, flowability, mouth coating, glossiness and color were determined using scales from 1 (representing none for grittiness, mealiness, graininess, glossiness, flowability / watery for mouth coating / extremely white for color) to 7 (representing very high degree of grittiness, mealiness, graininess, glossiness, flowability / high consistency for mouth coating / dark yellow for color).

Statistical analysis

Statistical data analyses were performed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant differences between the means were evaluated by analysis of variance (ANOVA) and Duncan post hoc test. Statistical significance between mean values was set at $p < 0.05$.

Results and Discussion

Chemical composition

The fat reduction in cheese is compensated by the increase in both protein and water contents (Koca and Metin, 2004). Therefore, the protein content of reduced-fat white cheese emulsion was higher compared to that of full-fat counterpart (Table 1). Although the addition of MWP at different levels slightly decreased the protein content of reduced-fat emulsion in a specific dry matter, it was not significant ($p > 0.05$). The added fat replacer was whey protein-based. Therefore, in the emulsion the amount of whey protein will increase whereas the casein amount will decrease. On the other hand, fat levels of emulsion decreased as the addition of MWP increased due to the lower cheese amount added to emulsion formulation. Due to the similar reason, pH values of the reduced-fat cheese emulsions (5.16–5.42) showed a slight increase with an increase in fat replacer, whereas the acidity levels decreased because of the high pH value (6.49) of MWP.

Emulsion stability

Stability refers to the ability of cheese emulsion to resist phase separation over centrifugation and holding time. The phase separation after centrifugation was not observed for RF 25 and RF 20 as well as FF 25 and they remained stable during 60

Table 1. Chemical composition of white-brined cheese emulsions

Samples	MWP based on DM (%)	DM (%)	Fat (%)	Ash (%)	Protein (%)	Acidity (% lactic acid)	pH
FF 25	-	27.16±0.09 ^B	14.2±0.28 ^A	3.42±0.12 ^B	8.67±0.30 ^D	1.51±0.02 ^C	5.04±0.01 ^C
RF 25	-	27.90±0.06 ^{aA}	6.80±0.14 ^{ab}	3.87±0.03 ^{bA}	16.25±0.36 ^{aA}	1.80±0.01 ^{aA}	5.16±0.03 ^{aB}
	5	27.79±0.03 ^b	6.60±0.13 ^{ab}	3.90±0.02 ^{ab}	16.07±0.48 ^a	1.71±0.05 ^b	5.17±0.02 ^a
	10	27.72±0.03 ^b	6.30±0.14 ^b	3.93±0.05 ^{ab}	15.96±0.35 ^a	1.57±0.03 ^c	5.18±0.04 ^a
	15	27.60±0.04 ^c	5.95±0.07 ^c	3.94±0.05 ^{ab}	15.73±0.28 ^a	1.56±0.01 ^c	5.20±0.03 ^a
	20	27.53±0.03 ^c	5.25±0.07 ^d	3.98±0.02 ^a	15.51±0.26 ^a	1.52±0.02 ^c	5.22±0.04 ^a
RF 20	-	22.50±0.05 ^{aC}	5.40±0.14 ^{aC}	3.22±0.06 ^{bB}	12.95±0.31 ^{aB}	1.62±0.02 ^{aB}	5.17±0.02 ^{bB}
	5	22.07±0.04 ^b	4.95±0.21 ^b	3.26±0.03 ^b	12.88±0.27 ^a	1.57±0.02 ^b	5.19±0.01 ^{ab}
	10	21.86±0.04 ^c	4.85±0.07 ^{bc}	3.29±0.02 ^{ab}	12.70±0.23 ^a	1.56±0.01 ^b	5.20±0.02 ^{ab}
	15	21.72±0.03 ^d	4.50±0.14 ^c	3.32±0.03 ^{ab}	12.62±0.33 ^a	1.53±0.02 ^{bc}	5.21±0.02 ^{ab}
	20	21.61±0.05 ^e	4.05±0.07 ^d	3.38±0.03 ^a	12.48±0.18 ^a	1.50±0.01 ^c	5.24±0.03 ^a
RF 15	-	16.30±0.01 ^{aD}	3.95±0.07 ^{aD}	2.55±0.08 ^{aC}	9.68±0.36 ^{aC}	1.23±0.05 ^{aD}	5.27±0.02 ^{cA}
	5	16.24±0.03 ^b	3.60±0.14 ^b	2.57±0.05 ^a	9.57±0.21 ^a	1.20±0.02 ^a	5.30±0.03 ^c
	10	16.17±0.02 ^c	3.45±0.07 ^{bc}	2.59±0.02 ^a	9.48±0.34 ^a	1.19±0.02 ^a	5.34±0.04 ^{bc}
	15	16.12±0.02 ^d	3.25±0.07 ^{cd}	2.60±0.02 ^a	9.38±0.25 ^a	1.15±0.03 ^{ab}	5.38±0.03 ^{ab}
	20	16.03±0.02 ^e	3.00±0.14 ^d	2.62±0.03 ^a	9.32±0.27 ^a	1.08±0.01 ^b	5.42±0.01 ^a

The results are presented as mean±SD.

^{a-c} Means with the different letter in the column for each reduced-fat cheese emulsion sample group having different MWP are significantly different ($p < 0.05$).

^{A-D} Means with the different letter in the column for full-fat and reduced-fat emulsions without MWP are significantly different ($p < 0.05$).

MWP, microparticulated whey protein; FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM; RF 15, reduced-fat cheese emulsion with 15% DM.

min holding time, whereas RF 15 was not stable at any stages (Table 2). In other words, to have a stable emulsion of reduced-fat white cheese, it was necessary to prepare an emulsion with at least 20% DM. These data confirm the results in previous study on cheese emulsion reported by Uргу et al. (2018). In fact, proteins are the primary emulsifiers in the cheese emulsion system and responsible for the resistance to the changes during centrifugation (Cernikova et al., 2017; Lobato-Calleros et al., 2006). The casein-dominated matrix of reduced-fat cheese improved the degree of emulsification and caused high degree of cross-linking in the network exhibiting high resistance to the changes during centrifugation. On the other hand, a decrease in DM resulted in a decrease in protein level in the system which lowered the emulsifying ability. The addition of MWP did not have any considerable effects on improving the stability in RF 15. Moreover, increasing the amount of MWP had also no effect on stability. In contrast to our result, it is reported that whey proteins are generally used to stabilize emulsions due to their capability to adsorb at the oil and water interface (Sun et al., 2015). It is difficult to compare our results with emulsion or dispersion systems studied, because the emulsion system in cheese emulsion is more complex. The stability of the cheese emulsions depends on the casein content, pH, ionic strength and temperature and their effects on the molecular interactions of the adsorbed protein. In present study, MWP was added to replace of cheese dry matter, so the addition of MWP caused a reduction in the casein content of emulsion, which is the primary emulsifiers in the cheese matrix. The cheese emulsion is as a stable oil-in-water emulsion supported by a gel network of emulsified casein proteins. So, the casein amount is also important for the reactions between emulsifying salt and protein during melting process.

Table 2. Phase separation of white-brined cheese emulsions after 0 and 60 min

Samples	MWP based on DM (%)	Precipitated phase (%)		Liquid phase (%)		Fat phase (%)	
		0	60	0	60	0	60
FF 25	-	No phase separation observed					
RF 25	-	No phase separation observed					
	5						
	10						
	15						
	20						
RF 20	-	No phase separation observed					
	5						
	10						
	15						
	20						
RF 15	-	36.66±0.01 ^a	35.67±0.23 ^a	60.02±0.83 ^a	60.59±0.58 ^a	3.08±0.04 ^b	3.23±0.52 ^b
	5	36.67±0.06 ^a	35.83±2.10 ^a	58.89±2.12 ^a	60.43±2.25 ^a	3.46±0.59 ^{ab}	3.65±0.18 ^b
	10	36.66±0.70 ^a	37.85±2.78 ^a	57.84±0.06 ^a	57.55±2.47 ^a	4.10±0.07 ^a	4.40±0.32 ^a
	15	37.47±0.42 ^a	36.45±0.09 ^a	57.95±0.24 ^a	58.78±0.15 ^a	3.92±0.20 ^{ab}	4.20±0.10 ^{ab}
	20	37.57±0.34 ^a	36.75±1.44 ^a	58.22±1.44 ^a	59.26±1.37 ^a	3.93±0.54 ^{ab}	3.82±0.11 ^{ab}

The results are presented as mean±SD.

^{a,b} Means with the different letter in the column for reduced-fat cheese emulsion sample group with 15% DM having different MWP are significantly different ($p < 0.05$).

MWP, microparticulated whey protein; FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM; RF 15, reduced-fat cheese emulsion with 15% DM.

Rheological measurements

Rheological properties in cheese emulsion is a key factor for a fine atomization in the spray drying process. Rheological measurements were applied to stable emulsions (FF 25, RF 25, and RF 20 with different MWP concentration), because the instable emulsion (RF 15) should not be used as a feed during atomization. In addition, our pre-experiments showed that instable emulsions had very low viscosity because of insufficient emulsification, which causes misconception for comparison. The fat reduction caused significant increases in the shear stress and average apparent viscosity values of the samples (Fig. 1 and Table 3). Reduction in DM from 25% to 20% decreased apparent viscosity, but RF 20 had still higher apparent viscosity (0.405 Pa·s) when compared to FF 25 (0.138 Pa·s). In reduced-fat cheese, a high level of protein that tends to make the cheese firm and difficult to melt caused an increase in shear rate and apparent viscosity. The protein content of RF 20 had still higher than that of FF 25 (Table 1). Besides, the dry matter of a reduced-fat emulsion should not be decreased to 15% due to its instability problem. The reduction of fat content in cheese emulsion had an adverse effect on flowability and resulted in problems in pumping and droplet formation during atomization due to their high viscosity. Both RF samples without MWP having higher shear stress values were observed as too viscous for atomization. Our hypothesis was that addition of MWP can decrease stress values and increase flowability of reduced-fat emulsions. MWP particles have good lubrication properties due to small particle size and spherical shape which called as a ball-bearing mechanism (Liu et al., 2016). Aryana and Haque (2001) reported that microparticulated protein imparts discontinuity to the protein matrix, just as milk fat globules do in full-

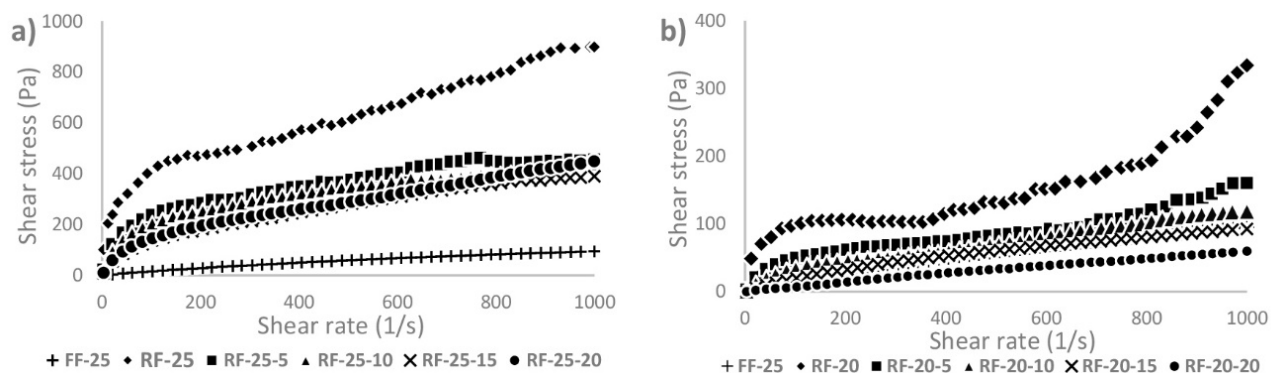


Fig. 1. The comparison of shear stress values of full-fat (25% dry matter) and reduced-fat (a, 25% dry matter; b, 20% dry matter) cheese emulsions with different microparticulated whey protein concentration. FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM.

Table 3. Average apparent viscosities and power law model parameters of stable cheese emulsions

Samples	MWP based on DM (%)	Apparent Viscosity (Pa·s)	K (Pa·s ⁿ)	n	r ²	RMSE	χ ²
FF 25	-	0.138	0.55	0.75	0.999	0.38	0.16
RF 25	-	1.928	31.02	0.48	0.998	4.17	20.58
	5	1.178	31.62	0.40	0.992	2.55	7.82
	10	1.086	27.98	0.41	0.999	1.76	3.15
	15	0.788	12.71	0.50	0.999	1.67	3.30
	20	0.842	13.67	0.50	0.999	2.19	5.45
RF 20	-	0.405	1.25	0.75	0.996	2.09	5.03
	5	0.257	0.64	0.78	0.996	0.40	0.18
	10	0.187	0.50	0.80	0.997	0.94	0.95
	15	0.134	0.46	0.82	0.999	0.05	0.02
	20	0.086	0.12	0.91	0.998	0.49	0.26

K, consistency index; n, flow behavior index; r², coefficient of determination; χ², chi-square.

MWP, microparticulated whey protein; FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM.

fat cheese, which results in softer low-fat cheese. Fortunately, the use of MWP had positive effects on the viscosity and flowability of samples. It was able to interrupt the continuity of the firm para-casein network, to improve creaminess and to reduce the viscosity. Shear stress values of RF dramatically decreased even with the addition of the lowest level of MWP (5% based on DM). In our study, one of the aims was to find the optimum amount of MWP which imitates the flow behavior of FF 25. In earlier studies, FF 25 was found suitable for feeding to pilot plant spray drier (Erbay et al., 2015; Koca et al., 2015). The reduced-fat samples with 25% DM in all concentrations of MWP had higher apparent viscosity values than that of FF 25. With respect to 20% DM, the use of more than 10% MWP based on DM showed similar values to FF 25. In addition, RF 20 containing more than 15% MWP had relatively low viscosity compared to FF 25. These results showed that the amount of MWP used was very important for the feeding properties of emulsion used for cheese powder production.

The shear stress versus shear rate profiles clearly indicated that samples had non-Newtonian rheological properties. All the stable emulsions exhibited pseudoplastic (shear thinning) behavior ($n < 1$) (Table 3), meaning that resistance to flow decreases

with the increase in shear rate and the change in viscosity was independent of time. Rheological parameters were changed with the fat reduction and the MWP addition amount. RF 25 showed higher K and lower n values, but increasing amount of MWP added to the sample helped to decrease in K values, indicating lower consistency of the reduced-fat cheese emulsions. Thus, the addition of MWP to reduced-fat cheese emulsion system helps to reduce the consistency and increase the flow behavior. The rheological parameters of RF 20 with MWP more than 10% were found similar to full-fat emulsion. Kelimu et al. (2017) reported that the addition of some dairy ingredients (sodium caseinate, butter milk powder and their combinations) had a significant change in the rheological parameters of Cheddar-soft cheese emulsion system, higher K and lower n values were obtained in samples with the emulsifying salt, whereas the opposite tendency was observed with the addition of sodium caseinate.

Microscopic evaluation of white cheese emulsions

The micrographs of FF 25, RF 25, RF 20, and RF 20 with 15% MWP were shown in Fig. 2. The microscop image of FF 25 was chosen for the indication of the stable and pumpable white cheese emulsion and RF 20 with 15% MWP which gives the similar rheological properties with FF 25 was represented as an indication of a fat replacer effect. RF 20 was also given as a control sample for evaluating the effects of fat replacer. Images were recorded from $\times 10$ objective lenses of the microscope. Higher magnification lenses could not be used for imaging due to the compact structure of stable emulsions. Fat content had a major influence on the visual properties of emulsion. RF 20 had a more compact and uniform network that consisted of less fat globules (Fig. 2c) compared to FF 25 (Fig. 2a). Fat globules in FF 25 showed a more open network with more space between protein aggregates. The higher protein amount in RF 20 covered the fat globules and resulted in an increase in the interactions between the protein chains. This was more obvious for the samples RF25 having very compact and uniform structure (Fig. 2b). The addition of MWP in RF 20 resulted in the formation of porous structure similar to FF 25, having fat globules entrapped in the protein structure (Fig. 2c). Therefore, MWP addition to reduced-fat formulation helps to imitate microstructure to full-fat counterparts.

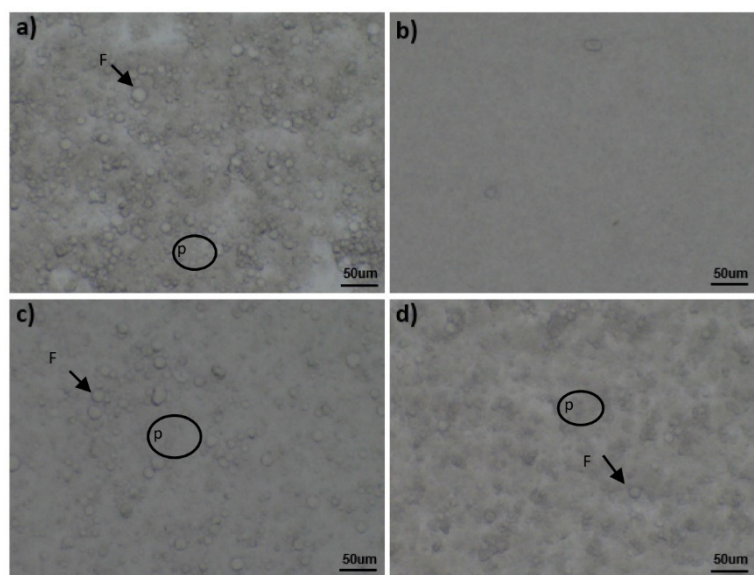


Fig. 2. Microscopic images of FF 25 (a), RF 25 (b), RF 20 (c), and RF 20 with 15% MWP based on DM of emulsion (d). F, fat globules; P, protein network; MWP, microparticulated whey protein; FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM.

Color evaluation of white cheese emulsions

Color characteristics were significantly affected by dry matter, fat content and the addition of fat replacer (Table 4). Lightness was found higher in FF when compared to RF due to the high light scattering ability of fat globules, whereas high amount of caseins in RF cheese caused a negative effect on light scattering attributes. Addition of MWP significantly improved light scattering ability of RF ($p<0.05$), because of breaking the casein network in the same manner as fat globules. Similar to our results, Chung et al. (2014) stated that the addition of MWP to suspensions increased the lightness of the samples which was attributed to the influence of the protein particles on light scattering. Yellow color of dairy products generally increases as the fat content increases. This was noted in the white-brined cheese emulsion system. The increasing in MWP concentration also increased b^* values. This could be attributed to the small size of MWP particles providing fat mimicking properties. All the cheese emulsion samples had a negative a^* (greenness) values. Lower fat and dry matter contents, as well as higher amount of MWP contributed the green color of the samples.

Sensory evaluation of cheese emulsions

Fat content and addition of fat replacer had significant effects on glossiness, flowability and mouth coating (Table 5). RF 25 and 20 had lower flowability and glossiness scores, and higher mouth coating scores compared to those of FF 25. The addition of MWP to reduced-fat formulation significantly increased the sensory scores of glossiness and flowability and

Table 4. Color parameters of white-brined cheese emulsions

Samples	MWP based on DM (%)	L*	a*	b*
FF 25	-	93.68±0.06 ^A	-0.63±0.01 ^A	14.44±0.17 ^A
RF 25	-	91.30±0.13 ^{dB}	-1.36±0.01 ^{aB}	13.51±0.01 ^{dB}
	5	91.60±0.07 ^c	-1.68±0.05 ^b	14.55±0.15 ^c
	10	91.79±0.08 ^{bc}	-1.78±0.16 ^b	15.07±0.05 ^b
	15	91.95±0.01 ^{ab}	-1.82±0.10 ^b	15.20±0.01 ^b
	20	92.11±0.02 ^a	-1.88±0.02 ^b	15.48±0.02 ^a
RF 20	-	91.01±0.01 ^{dB}	-1.62±0.01 ^{aC}	13.09±0.02 ^{dB}
	5	91.15±0.04 ^d	-1.65±0.02 ^a	13.41±0.02 ^c
	10	91.46±0.01 ^c	-1.75±0.01 ^b	13.91±0.06 ^b
	15	91.71±0.08 ^b	-1.84±0.02 ^c	14.35±0.11 ^a
	20	91.91±0.09 ^a	-1.97±0.02 ^d	14.47±0.03 ^a
RF 15	-	86.86±0.95 ^{cC}	-1.84±0.01 ^{aD}	11.62±0.33 ^{dC}
	5	89.69±0.19 ^b	-2.00±0.12 ^b	12.20±0.03 ^c
	10	90.61±0.12 ^{ab}	-2.14±0.01 ^{bc}	12.52±0.12 ^{bc}
	15	90.89±0.12 ^a	-2.24±0.04 ^{cd}	12.87±0.06 ^{ab}
	20	91.61±0.03 ^a	-2.37±0.3 ^d	13.22±0.04 ^a

The results are presented as mean±SD.

^{a-d} Means with the different letter in the column for each reduced-fat cheese emulsion sample group having different MWP are significantly different ($p<0.05$).

^{A-D} Means with the different letter in the column for full-fat and reduced-fat emulsions without MWP are significantly different ($p<0.05$).

MWP, microparticulated whey protein; FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM; RF 15, reduced-fat cheese emulsion with 15% DM.

Table 5. The sensory scores of white-brined cheese emulsions

Samples	MWP based on DM (%)	Flowability	Glossiness	Mouth coating	Color
FF 25	-	5	7	4	3
RF 25	-	1	5	7	4
	5	3	5	6	4
	10	3	6	5	4
	15	4	6	4	4
	20	4	7	4	4
RF 20	-	3	5	5	4
	5	3	6	5	4
	10	4	6	4	4
	15	5	7	3	4
	20	5	7	3	4

MWP, microparticulated whey protein; FF 25, full-fat cheese emulsion with 25% DM; RF 25, reduced-fat cheese emulsion with 25% DM; RF 20, reduced-fat cheese emulsion with 20% DM.

decreased the scores of mouth coating. However, addition of MWP more than 15% concentration to RF 20 could result in higher flowability than FF 25, but the same smooth mouthfeel. MWP can soften the cheese texture (Koca and Metin, 2004) and gives a creamy and smooth mouthfeel (Liu et al., 2016) that may help to soften the reduced-fat cheese emulsion structure. Sensory results of the emulsions were in line with the rheological results.

The grittiness, mealiness and graininess were also evaluated by assessors (data not shown). These characteristics, which are not desired in cheese emulsion due to the clogging of the atomizer, were not detected in all samples. It was also noted that foreign flavor was not detected even in the samples with maximum amount of MWP. The instable emulsions were also evaluated, and these samples were perceived as too grainy. These particles can cause atomization problems and to form scorched particles in the drying chamber.

Conclusion

The stability, rheological properties and microstructure of cheese emulsion are key characteristics for the efficiency of spray drying and final powder quality. Fat reduction in white-brined cheese emulsion significantly increased the apparent viscosity that caused atomization problems during the spray drying process. The ability of MWP on improving the rheological properties and stability of reduced-fat white-brined cheese emulsions were investigated. The use of MWP significantly reduced the apparent viscosity, and reduced-fat cheese emulsions with 20% dry matter and more than 10% of MWP based on cheese dry matter had closer apparent viscosities to full-fat cheese emulsion with 25% dry matter. Our results showed that using MWP in reduced-fat formulation may be a good alternative to obtain pumpable emulsion by softening the structure and providing low apparent viscosity. MWP could not improve the stability of the instable emulsion whereas the stable emulsions kept their stability. This investigation provided valuable information about the emulsions made from reduced-fat brined-cheese by filling the research gap in this field. On the other hand, the amount of MWP used for cheeses having different composition and properties could change. Therefore, the optimum amounts need to be determined for different kinds of cheeses. In addition, further researches are necessary to investigate the effects of reduced-fat cheese emulsions with MWP on cheese powder

characteristics and to compare them with the characteristics of full-fat cheese powders for the benefit of end-users.

Conflicts of Interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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Author's Contributions

Conceptualization: Urgu M, Koca N. Data curation: Urgu M, Ünlütürk S, Kaymak-Ertekin F, Koca N. Formal analysis: Urgu M, Türk A, Koca N. Methodology: Urgu M, Ünlütürk S, Kaymak-Ertekin F, Koca N. Software: Urgu M. Validation: Ünlütürk S, Kaymak-Ertekin F, Koca N. Investigation: Koca N. Writing - original draft: Urgu M, Koca N. Writing - review & editing: Urgu M, Türk A, Ünlütürk S, Kaymak-Ertekin F, Koca N.

Ethics Approval (IRB/IACUC)

This article does not require IRB/IACUC approval because there are no human and animal participants.

References

- AOAC. 2007. Official methods of analysis of AOAC International. 18th ed. AOAC International. Gaithersburg, MD, USA.
- Aryana KJ, Haque ZU. 2001. Effect of commercial fat replacers on the microstructure of low-fat Cheddar cheese. *Int J Food Sci Tech* 36:169-177.
- Cernikova M, Nebesarova J, Salek RN, Rihackova L, Bunka F. 2017. Microstructure and textural and viscoelastic properties of model processed cheese with different dry matter and fat in dry matter content. *J Dairy Sci* 100:4300-4307.
- Chung C, Degner B, McClements DJ. 2014. Development of reduced-calorie foods: Microparticulated whey proteins as fat mimetics in semi-solid food emulsions. *Food Res Int* 56:136-145.
- Erbay Z, Koca N, Kaymak-Ertekin F, Ucuncu M. 2015. Optimization of spray drying process in cheese powder production. *Food Bioprod Process* 93:156-165.
- Hougaard AB, Sijbrandij AG, Varming C, Ardo Y, Ipsen R. 2015. Emulsifying salt increase stability of cheese emulsions during holding. *LWT- Food Sci Technol* 62:362-365.
- IDF [International Dairy Federation]. 1981. Milk–determination of fat content–Gerber butyrometers. IDF standard 105.
- IDF [International Dairy Federation]. 1982. Cheese and processed cheese — Determination of the total solids content. IDF Standard 4a.

- Kelimu A, Felix da Silva D, Geng X, Ipsen R, Hougaard AB. 2017. Effects of different dairy ingredients on the rheological behaviour and stability of hot cheese emulsions. *Int Dairy J* 71:35-42.
- Koca N, Erbay Z, Kaymak-Ertekin F. 2015. Effects of spray-drying conditions on the chemical, physical, and sensory properties of cheese powder. *J Dairy Sci* 98:2934-2943.
- Koca N, Metin M. 2004. Textural, melting and sensory properties of low-fat fresh kashar cheeses produced by using fat replacers. *Int Dairy J* 14:365-373.
- Liu K, Stiegeri M, van der Linden E, van de Velde F. 2016. Effect of microparticulated whey protein on sensory properties of liquid and semi-solid model foods. *Food Hydrocoll* 60:186-198.
- Lobato-Calleros C, Rodriguez E, Sandoval-Castilla O, Vernon-Carter EJ, Alvarez-Ramirez J. 2006. Reduced-fat white fresh cheese-like products obtained from W1/O/W2 multiple emulsions: Viscoelastic and high-resolution image analyses. *Food Res Int* 39:678-685.
- McMahon DJ, Alleyne MC, Fife RL, Oberg CJ. 1996. Use of fat replacers in low fat Mozzarella cheese. *J Dairy Sci* 79:1911-1921.
- Pisecky J. 2005. Spray drying in the cheese industry. *Int Dairy J* 15:531-536.
- Ray CA, Gholamhosseinpour A, Ipsen R, Hougaard AB. 2016. The effect of age on Cheddar cheese melting, rheology and structure, and on the stability of feed for cheese powder manufacture. *Int Dairy J* 55:38-43.
- Singer NS, Yamamoto S, Latella J. 1990. Protein product base. European Patent Specification EP 0 250 623 B1.
- Sun C, Wu T, Liu R, Liang B, Tian Z, Zhang E, Zhang M. 2015. Effects of superfine grinding and microparticulation on the surface hydrophobicity of whey protein concentrate and its relation to emulsion stability. *Food Hydrocoll* 51:512-518.
- Urgu M, Unluturk S, Koca, N. 2018. Effects of fat reduction on the stability, microstructure, rheological and color characteristics of white-brined cheese emulsion with different emulsifying salt amounts. *Korean J Food Sci Anim Resour* 38:866-877.
- Varming C, Hougaard AB, Ardo Y, Ipsen R. 2014. Stability of cheese emulsions for spraydrying. *Int Dairy J* 39:60-63.