

RESEARCH ARTICLE

Effect of added bovine casein and whey protein on the quality of camel and bovine milk yoghurts

Bhawna Sobti, Hajer A. A. Al Teneiji, Afaf Kamal-Eldin*

Department of Food, Nutrition and Health, United Arab Emirates university, P.O. Box 15551, Al-Ain, UAE

ABSTRACT

The aim of this study is to evaluate the effect of fortification of camel milk (CM) and bovine milk (BM) with 1-4% of bovine casein (CN) and/or whey protein (WP) on the quality of yoghurts after 12 hours of fermentation. Response surface methodology was used to design the experiment. Yoghurts were evaluated for pH, acidity, hardness and rheology. The results revealed that addition of WP and CN significantly improved the hardness ($p < 0.000$), and the rheological properties; storage modulus (G' , $p < 0.005$), and loss modulus (G'' , $p < 0.005$), viscosity ($p < 0.005$) and $\tan \delta$ (G''/G' , $p < 0.05$) of CM yoghurts. Both types of yoghurts attained highest viscosity, G' and G'' values at highest level of WP (4%) and intermediate level of CN (2.5%) while increased CN ratio showed negative effects on yoghurts rheology. It can be concluded that the amount of total proteins as well as altering CN-to-WP ratio are important for the formation of strong gel network and the production of good quality set yoghurts.

Keywords: Yoghurt; Camel milk; Bovine milk; Texture; Rheology

INTRODUCTION

Fresh and fermented camel milks (CM) is gaining increasing importance worldwide as a healthy alternative to bovine milk (BM) (Technavo, 2018). CM is claimed to possess a number of therapeutic properties including anti-diabetic (Agrawal et al., 2007, Mirmiran et al., 2017, Ayoub et al., 2018), anti-carcinogenic (Magjeed, 2005), and anti-hypertensive effects (Quan et al., 2008) and has been recommended to be consumed by children who are allergic to bovine milk (El-Agamy et al., 2009). However, the exploitation of CM faces challenges because of difficulties in processing by conventional technologies used in the processing of bovine milk as well as some sensory limitations (Ghnnimi et al., 2015; Haliu et al., 2016; Berhe et al., 2017).

Yoghurt is a popular fermented dairy product widely consumed by people all over the world. It is prepared by adding starter cultures (mainly *Streptococcus spp.* and *Lactobacilli spp.*) to heat-treated milk to allow fermentation of caseins (CN) to become unstable at their isoelectric point (pH 4.6 in bovine milk) and to coagulate forming different networks (Damin et al., 2009). Heat treatment of milk above 70 °C prior fermentation allows the denaturation of the

global whey proteins (WP) and the formation of disulfide bonds between β -lactoglobulin (β -LG) and κ -casein (κ -CN) leading to firm gels (Lucey et al., 1998; Guyomarc'h et al., 2003; Anema, 2006). This bond formation depends upon number of factors including protein concentrations, pH, time, and temperature (Oldfield et al., 1998).

For a fermented product like yoghurt, texture is a very important attribute that affects its quality, consumer acceptability and commercial production. In comparison to BM, it is difficult to produce fermented products with good texture and rheology from CM (Attia et al., 2001), which only forms weak gels more preferable for drinking (Al haj & Al Kanhal, 2010). Mohamed et al. (1990) also reported that CM failed to reach a gel-like structure even after 18 h incubation with lactic acid cultures. The inability of camel milk to form hard viscous gels is mainly attributed to its lack of β -lactoglobulin and lower amounts of κ -casein (Kappeler et al., 1998); and high whey protein to casein ratio (Shamsia, 2009). β -Lactoglobulin and κ -casein are essential for gel formation due to interactions among them and between them and other milk proteins (Donato & Guyomarc'H, 2009). It was found that the texture and rheology of bovine yoghurts can be improved by dry matter fortification,

*Corresponding author:

Prof. Afaf Kamal-Eldin, Department of Food, Nutrition and Health, United Arab Emirates university, P.O. Box 15551, Al-Ain, UAE.
Tel.: 00971501390248. E-mail: Afaf.kamal@uaeu.ac.ae

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e.g. by the addition of dairy ingredients such as skim milk powder, whey proteins, caseins, and caseinates (Sodini and Béal, 2003; Damin et al., 2009; Peng et al., 2009). Herrero and Requena (2006) also reported that the addition of whey protein concentrate enhanced the textural properties of goat milk yoghurts. Increasing the levels of denatured whey proteins showed significant effects on reducing gelation time, increasing gelation pH after acidification, and improving the hardness of yoghurt gels (Lucey et al., 1999). Puvanenthiran, (2002) also demonstrate the positive effect of altering CN: WP ratio on the yield stress of set yoghurts by fortification either with whey protein isolate or sodium caseinates. The present study was undertaken to explore the effect of fortification of camel milk with bovine caseins and whey proteins on pH, acidity, texture, and rheology of CM yoghurts in comparison with BM yoghurts.

MATERIAL AND METHODS

Materials

Camel milk powder (Al Ain Dairy, Al Ain, UAE) and cow milk powder (Nido, Nestle, Dubai) were purchased from the local supermarkets in Al Ain, UAE. Yoghurts were prepared using lyophilized starter culture YoFlex®Express 1.0, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in the ratio of 1:1 (w/w) (Chris-Hansen, Denmark). Bovine casein (CN) (Gold Standard 100% Casein, Downers Grove, IL, USA) and whey protein isolate (WP) (About Time, Pittsburgh, PA, USA) were purchased from local market of Alain, UAE.

Yoghurt preparation

Yoghurt samples were prepared from bovine and camel milk powders by dissolving 25 g of powder in 200 ml of water according to manufacturer's instructions. Indigenous level of WP present in reconstituted BM and CM was 1.8% and 1.375% while indigenous level of CN was 1.437% and 1.27%, respectively. Commercial whey protein isolate (WP) at 1-4% and bovine casein (CN) at 1-4% was added to both milks before pasteurization at 85 °C for 15 min. Total WP and CN at five different levels in both yoghurts, CN (1.63-6.06%) and whey proteins (1.75-6.42%). The footnote of (Table 1) has the details for five levels of WP and CN in yoghurt samples. Milk samples were fermented with the bacterial starter culture viz. *S. thermophilus* and *L. delbrueckii* ssp. *Bulgaricus*. The level of the starter culture was 0.05% for camel milk and 0.025% for bovine milk and the fermentation was performed at 43 °C for 12 hrs.

Titrateable acidity and pH

The titratable acidity of the samples was estimated using standard method ISO/TS 11869:2012 (IDF/RM 150:2012) and the pH was determined using a digital pH meter (OHAUS Europe GmbH, Greifensee, Switzerland).

Texture profile analysis (TPA)

The hardness of the yoghurt samples were determined using Brookfield texture analyzer-CT III (Middleboro, MA, USA) equipped with a 4.5 kg load cell. TPA was carried out by a compression test that generated a plot of force (g) versus time (s) in one complete run. A 25-mm-diameter perplex cylindrical probe (TA11/1000) with a test speed of 1 mm/s and 3mm of target distance was used to measure the textural profile of the yogurt samples and their hardness was calculated by the software program of the equipment.

Dynamic rheology

Rheological measurements were carried out using Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA) with a cone-plate geometry, in which the rotating cone was 60 mm in diameter and the cone angle was 4° with a gap of 225 µm. Oscillatory rheology (frequency sweep) tests were carried out at 30 °C at the same deformation (1%) and frequencies ranging from 0.01 to 10.0 Hz. The storage modulus (elastic modulus G'), the loss modulus (viscous modulus, G'') and the apparent viscosity η^* (Pa.s) were determined by the equipment software in all cases. The loss tangent, or $\tan \delta$, was calculated as G''/G' .

Statistical design and analysis

All statistical analyses were completed using a computer software package (Minitab 18, Minitab Inc., State College, PA, USA). Thirteen experiments were performed as per Central Composite rotatable design (CCRD) with two independent variables (WP and CN) and five levels of each variable. The CCRD design was used to estimate the effect of the addition of WP and CN on pH, acidity, texture and rheology. All 13 experiments were repeated three times and each analysis was performed in triplicate. Each response was fitted to a quadratic polynomial model as per the following equation

$$y = \beta_0 + \sum_{i=1}^4 \beta_i x_i + \sum_{i=1}^4 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} x_i x_j$$

Where y is the response, β_0 is constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the interaction coefficient. X_i and X_j are independent variables. The level of significance used in this study was $p < 0.05$ or lower.

RESULTS AND DISCUSSION

The characteristics of pure camel and bovine milk yoghurts

Fig. 1 presents photos of the yoghurts obtained from pure bovine and camel milks prepared as discussed above. The pure camel milk (CM) yoghurt has a soft liquidly texture compared to the set bovine milk (BM) yoghurt. The acidity,

Table 1: Experimental design with the total levels of casein (CN) and whey protein (WP) in camel and bovine milk yoghurts

Expt no.	Independent Variables*		Yoghurt characteristics (Dependent Variables)													
			Bovine Milk							Camel Milk						
	WP	CN	Acidity (%)	pH	Hardness (g)	Viscosity (Pa.s)	G' (Pa)	G'' (Pa)	Tan δ (G''/G')	Acidity (%)	pH	Hardness (g)	Viscosity (Pa.s)	G' (Pa)	G'' (Pa)	Tan δ (G''/G')
1	0	-2	0.509	4.21	48	262	2555	566	0.221	1.378	3.83	15	17	172	37	0.215
2	0	0	0.543	4.26	80	614	5960	1367	0.229	1.439	3.90	20	124	1202	278	0.231
3	-1	-1	0.487	4.17	48	446	4335	981	0.226	1.293	3.81	13	4	42	9	0.214
4	0	0	0.534	4.28	70	599	5829	1314	0.225	1.452	3.87	21	129	1249	316	0.253
5	-2	0	0.598	4.28	72	588	5761	1325	0.229	1.431	3.81	15	12	129	29	0.225
6	1	1	1.159	4.04	141	906	8646	2110	0.244	1.602	4.04	32	260	2522	605	0.240
7	0	0	0.825	4.01	90	615	5973	1330	0.222	1.627	3.92	23	118	1139	277	0.243
8	0	0	0.881	4.03	72	601	5834	1314	0.225	1.623	3.92	22	120	1165	281	0.241
9	1	-1	0.842	4.04	56	425	4139	923	0.223	1.492	3.91	22	95	932	212	0.227
10	-1	1	0.649	4.36	124	548	5294	1307	0.246	1.654	3.92	23	46	444	101	0.227
11	2	0	1.123	4.03	52	987	9570	2273	0.231	1.250	4.02	31	450	4534	1033	0.228
12	0	2	1.028	4.36	85	942	9096	2317	0.254	1.662	4.02	28	179	1733	427	0.246
13	0	0	0.643	4.30	63	595	5819	1347	0.231	1.365	4.02	23	127	1228	297	0.242

*The total level of the independent variables casein (CN) and whey proteins (WP)
 For camel milk: CN: -2 (1.63%), -1 (2.25%), 0 (3.75%), 1 (5.25%), 2 (5.87%); WP: -2 (1.75%), -1 (2.37%), 0 (3.87%), 1 (5.37%), 2 (6.00%)
 For bovine milk: CN: -2 (2.18%), -1 (2.44%), 0 (3.94%), 1 (5.44%), 2 (6.06%); WP: -2 (1.82%), -1 (2.80%), 0 (4.30%), 1 (5.80%), 2 (6.42%)



Fig 1. Photographs showing the appearance of yoghurts from pure bovine milk, pure camel milk, and camel milk fortified with whey protein (4%) and casein (2.5%).

pH, textural and rheological properties of the two type yoghurts are presented in (Table 2). Pure CM yoghurt was found to be more acidic and lower in pH than the pure BM yoghurt. The decrease in pH follows the increase in titratable acidity caused by the microbial activity converting lactose to lactic acid (Attia et al., 2001; Prasanna et al., 2013, Costa and Conte-Junior, 2015; Costa et al., 2016). The difference in pH between the CM and BM yoghurts was attributed to differences in buffering capacities and proteolytic activity (Attia et al., 2001; Al-Sheraji et al., 2013). Pure CM yoghurt showed extremely lower hardness, viscosity, storage modulus (G') and loss modulus (G'') values compared to pure BM yoghurt supporting the liquid consistency and weak gel structure (Table 2). Since the storage modulus is responsible for strength and number of bonds in a network (Roefs et al., 1990; van Vliet et al., 1991), the results obtained here indicate lower number of bonds in the CM yoghurt gel network compared to those in BM yoghurt gels.

The weak body and poor texture of fermented CM yoghurts may be attributed to several factors; primarily to the lack of β -LG (El-Agamy et al., 2009) and the low concentration

Table 2: The characteristics of yoghurts made of pure camel milk (CM) versus pure bovine milk (BM)*

Parameters	Pure BM yoghurt	Pure CM yoghurt
Acidity (%)	0.08±0.07	1.2±0.03**
pH	4.3±0.05	3.7±0.04**
Hardness (g)	52.5±4.33	11.8±0.29***
Viscosity (Pa.s)	661±22	0.57±0.08***
G' (Pa)	6425±215	5.5±0.78***
G'' (Pa)	1461±74	1.3±0.17***
Tan δ (G''/G')	0.227±0.004	0.243±0.004*

*Means and standard deviations of three preparations.

Yoghurt from CM is significantly different from BM: *p<0.01, **p<0.001, and ***p<0.0001

of κ -CN (Kappeler, 1998) and secondarily to the different molecular structures of the proteins (Shamsia, 2009), and the large size of casein micelles (Kamal et al., 2017). It is believed that disulfide bonding between β -LG and κ -CN during the heat treatment preceding fermentation is essential for firm yoghurt structure. Thus, fortification of milk with CN and WP was foreseen to be important for enhancing the functional and nutritional properties and preventing textural defects such as poor gel firmness and syneresis (Sodini and Béal, 2003; Séverin and Wenshui, 2005; Marafon et al., 2011).

Effect of addition of WP and CN on characteristics of fortified CM and BM yoghurts

(Table 1) presents the effects of fortification of CM *versus* BM with different levels of WP and CN (range 1-4%) on yoghurt characteristics, namely acidity (%), pH, hardness (g), viscosity (Pa.s), G' (Pa) and G'' (Pa). The endogenous levels of CN and WP were: 1.8% and 1.4 % in BM; and 1.2 % and 1.4% in CM, respectively. The regression coefficients obtained by multiple regression fitting of the response variables (titrable acidity, pH, hardness, viscosity, G', G'', and tan δ) to the levels of the independent variables (total CN and total WP) by the quadratic (or cubic) models are presented in (Table 3). This analysis shows that CM yoghurts start with higher acidity and low pH compared to BM yoghurts and that the fortification with WP and CN has opposing effects on the acidity of CM yoghurt and the pH of BM yoghurts leading to non-significant overall models. Peng et al. (2009) published similar findings on the CN-fortification of BM yoghurts showing increased buffering capacity, i.e. resistance to pH change, at around pH 5.

Hardness and rheology are important textural characteristics for set-type yoghurts as they are important quality parameters in consumer acceptability. (Table 3) shows a large difference between CM and BM yoghurts in their initial hardness, viscosity, G', G'', and tan δ and how these are affected by the addition of WP and CN. The fortification with WP significantly increased the hardness in CM yoghurts as well as the viscosity, G', and G'' in both CM and BM yoghurts (p<0.01) without effects on

tan δ . This can be explained by formation of high level of crosslinking within the gel network on fortifying the yogurt mix with WP, thereby increasing the viscosity of samples (Remeuf et al., 2003). The interaction effects of WP, i.e. WP*WP, were positive in both CM and BM yoghurts but not significant. The addition of CN had positive effects on the hardness, viscosity, G', G'', and tan δ in both CM and BM yoghurts (p<0.05) but the interaction effect, CN*CN, was negative but insignificant in the case of CM yoghurts' hardness, viscosity, G', and G'' and BM yoghurts' viscosity, G', and G''. Although non-significant, the interactions within WP and between WP and CN lead to increased viscosity, G', and G'' in CM and BM yoghurts. On the other hand, interactions within CN contributed negatively, but insignificantly, to the viscosity, G', and G'' in CM and BM yoghurts (Table 3). Because of these negative interactions, the optimum concentration of CN in all yoghurts was about 2.5% (Fig. 2). Interactions between the additives and the indigenous proteins and other components of the milks are included in the constant of the models, which were found highly significant suggesting that these "unknown" interactions play very important roles. Despite large effects of added WP and CN on viscosity, G', and G'' of fortified compared to pure CM yoghurts, they did not alter the tan δ values (i.e. G''/G') indicating no change in the gel-to-liquid behavior despite the change in hardness and viscosity. This is desired for the consistency of yoghurt.

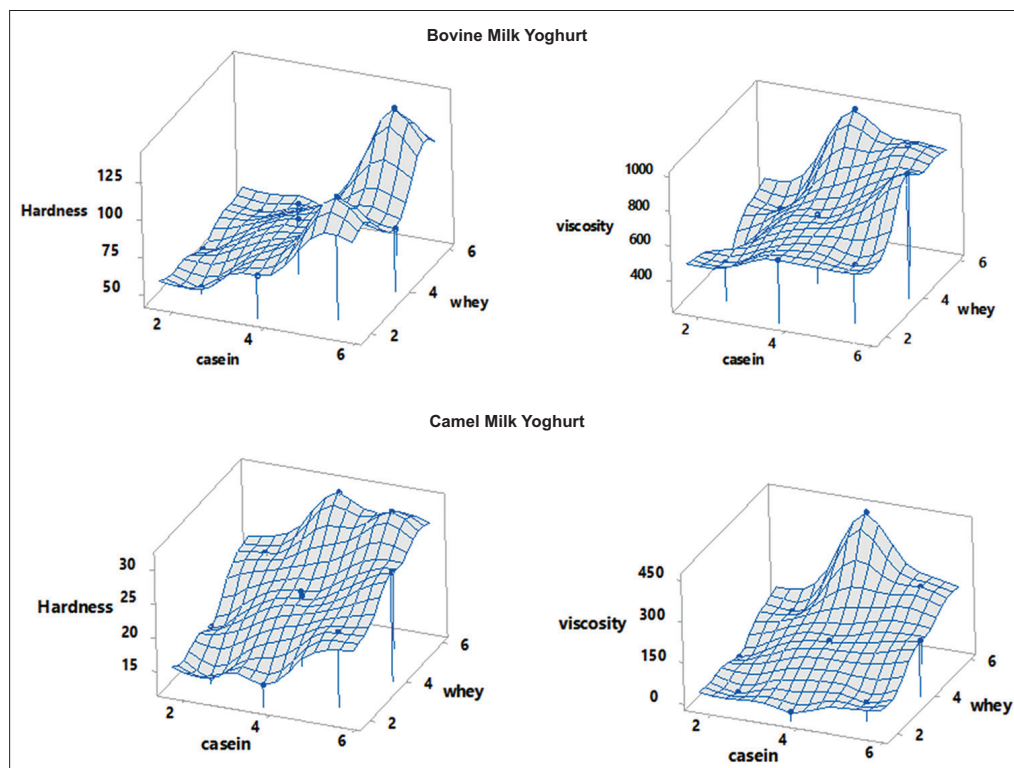
The effects of WP and CN addition is complicated by their contribution to the content of total solids (TS) and the effect of TS on the hardness of two yoghurts showing a strong and significant correlation between TS and the hardness of CM yoghurts and on the rheology of CM and BM yoghurts (Fig. 3). Ibrahim and Zubeir (2016) also documented similar findings on yoghurt prepared from camel-sheeps' milk mixtures with improved texture owing to its have higher total solids, fat and protein content than in comparison to those obtained from CM alone. These results are in agreement with Anema et al. (2006) who also explained the influence of protein concentration and total solid content of heated milk on the formation of whey protein-casein complexes. The interactive effect of WP and CN in CM and BM yoghurts is conflicting showing a trend towards increased pH and decreased hardness in CM but opposite effects in BM (Table 3). It was discussed that some interactions between WP and κ -casein will make micelles less sensitive to pH decrease and enhance their solvation rather than their aggregation leading to weaker gels (Oldfield et al., 2000; González-Martínez et al., 2002).

This result suggest that decreasing the CN: WP ratio may significantly affect yoghurt rheology, which is in agreement with others who revealed that the ratio of proteins in the yoghurt formulations, not just increasing total solids

Table 3: Model and estimated regression coefficients for the independent variables (casein and whey protein) and their significance in affecting yoghurt quality according to the modelDependent variable=constant + C₁ (WP) + C₂ (CN) + C₃ (WP*WP) + C₄ (CN*CN) + C₅ (WP*CN)

Dependent variables	Coefficients for the independent variables						Model Significance
	Constant	C ₁	C ₂	C ₃	C ₄	C ₅	
Bovine milk yoghurts							
Titration acidity	+0.685***	+0.2**	+0.151**	+0.08	+0.034	+0.039	Significant (p<0.05)
pH	+4.174***	-0.102*	+0.048	-0.026	+0.038	-0.048	Non-significant
Hardness	+74.8***	-0.43	+26.5*	+0.43	+2.72	+2.3	Non-significant
viscosity	+604.8***	+112.8**	+193.2**	+62.9	-29.7	+94.8	Highly significant (p<0.005)
G'	+5885***	+1068**	+1840**	+606	-314	+887	Highly significant (p<0.005)
G''	+1334.5***	+260.8**	+498.5**	+159.7	-19	+215	Highly significant (p<0.005)
Tan δ (G''/G')	+0.226	0	+0.011**	+0.002	+0.006**	0	Highly significant (p<0.005)
Camel milk yoghurts							
Titration acidity	+1.501***	-0.014	+0.109*	-0.061	+0.029	-0.063	Non-significant
pH	+3.925***	+0.065**	+0.063**	-0.005	-0.001	+0.002	Significant (p<0.05)
Hardness	+21.433***	+5.262**	+4.685**	+0.815	-0.06	-0.167	Highly significant (p<0.000)
Viscosity	+123.6***	+115.7**	+54.3*	+37.9	-28.5	+30.8	Highly significant (p<0.005)
G'	+1197***	+1150**	+525*	+403	-286	+297	Highly significant (p<0.005)
G''	+290***	+265.8**	+129.7*	+83	-66.3	+75.2	Highly significant (p<0.005)
Tan δ (G''/G')	+0.242***	+0.004	+0.009**	-0.008*	-0.006	0	Significant (p<0.05)

Significance of the coefficients: *p<0.05, **p<0.01, ***p<0.005

**Fig 2.** Surface plots showing interactive effects of WP and CN interactions on hardness and viscosity of bovine and camel milk yoghurt.

content, has a strong effect on yoghurt gel rheology and texture (Lobato-Calleros et al., 2004; Lucey et al., 1999; Sandoval-Castilla et al., 2004). The WPs have consistently shown improving effects on the rheological parameters of fortified CM and BM yoghurts (Table 3 and Fig. 3) supporting their participation in gel formation. This observation is well supported by the drastic increase in G'

and G'' values of CM yoghurts gels which was comparable to that of pure BM yoghurt gels, and shows an improvement in gel structure from liquid to solid like structure. The positive effect of addition of WP on yoghurt viscosity, firmness and gel strength (G') and reduced syneresis have also been reported by many authors (e.g. Lucey et al., 1999; Bhullar et al., 2002; Haque and Ji, 2003; Remeuf et al., 2003;

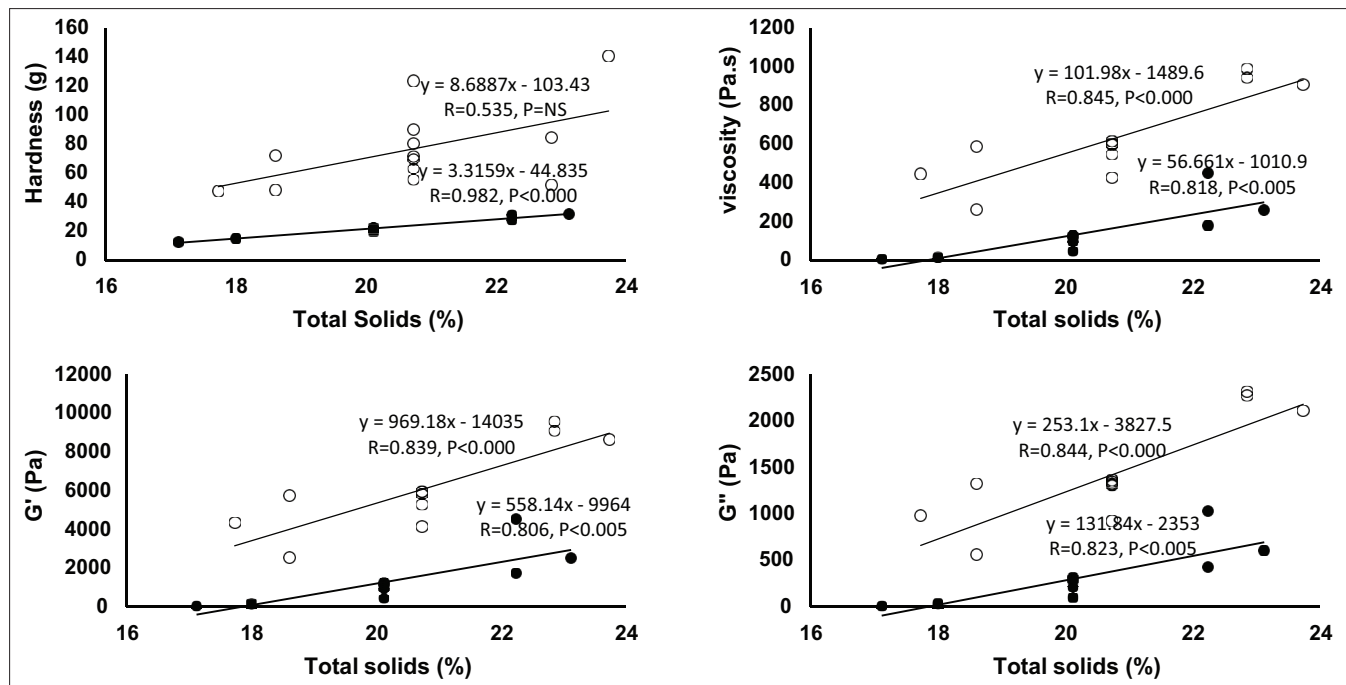


Fig 3. The effects of total solids (%) on bovine milk and camel milk yoghurt hardness, viscosity, G' and G''. Bovine milk yoghurts (○) and camel milk yoghurts (●).

Isleten and Karagul-Yuceer, 2006; Tamime and Robinson, 2007; Lee and Lucey, 2010). In addition, increasing the WP: CN reduces the pores size and strengthens the gel network (Puvanenthiran et al., 2002). The negative effects of CN: CN interactions on viscosity, G' and G'' of both the yoghurts (Table 3) are supported by previous reports (Smits and Van Brouwershaven, 1980; Noh et al., 1989) but cannot be precisely explained at this point. However, certain polymers may lead to self-association and suppression of cross complexes when present at high concentrations (Bungenberg, 1952, Burgess, 1994). In addition, increasing the ratio of other CNs (α s- and β -caseins) in milk may hinder the aggregation of κ -CN with the denatured WP. Moreover, Zhang et al. (2005) demonstrated that disordered milk CNs may have a “chaperone-like” effect that inhibits the unfolding of globular WPs and the development of heat induced CN: WP complexes *via* phosphoserine residues as well as hydrophobic surface interactions.

CONCLUSIONS

Yoghurt from pure camel milk has liquid consistency and weak gel structure compared with pure bovine milk yoghurt. Fortification with WP and CN was found to be a viable way to improve the texture and rheology of CM yoghurts. Stronger gel networks were produced in both CM and BM as a result of increased total solid and whey protein contents. The addition of bovine casein showed an optimal effect at the level of 2.5% suggesting negative interactions at higher concentrations. The model study

presented in this papers allowed a logical description of the individual and interactive effects of WP and CN on the texture and rheology of fortified CM and BM yoghurts. More studies are needed to further understand the nature of the different complexes and how the gel structure of yoghurts can be optimized, e.g. by the addition of different hydrocolloids.

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Author's contribution

Kamal-Eldin, A. and Sobti, B. planned and supervised the research. Teneiji, H.A.A and Sobti, B. performed the experiments. Sobti, B. processed the data and wrote the manuscript along with Kamal-Eldin, A.

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