Physical properties of yogurt fortified with various commercial whey protein concentrates

Isabelle Sodini,[*] John Montella and Phillip S Tong

Dairy Product Technology Center, California Polytechnic State University, San Luis Obispo, California 93407, USA

Abstract: The effects of whey protein concentrates on physical and rheological properties of yogurt were studied. Five commercial whey protein concentrates (340 g kg^{-1} protein nominal) were used to fortify milk to 45 g protein kg^{-1}. Fermentation was performed with two different starters (ropy and non-ropy). Resulting yogurts were compared with a control yogurt enriched with skim milk powder. The water-holding capacity of the yogurt fortified with skim milk powder was $500 g kg^{-1}$ and ranged from 600 to 638g kg^{-1} when fortified with whey protein concentrates. Significant rheological differences have been noticed between the yogurts fortified with different whey protein concentrates, independent of the starter used. Three whey protein concentrates generated yogurts with a behavior similar to the control. The two others produced yogurt with lower firmness (15g compared with 17g), lower Brookfield viscosity (6Pa s compared with 9Pa s), lower yield stress (2Pa compared with 4Pa), lower complex viscosity (13 Pa s compared with 26 Pa s), and lower apparent viscosity (0.4 Pa s compared with 1 Pa s) than the control, respectively. The yogurts with the lowest firmness and viscosity were produced with concentrates which contained the highest amount of non-protein nitrogen fraction (160 g kg^{-1}

versus 126 g kg^{-1} of the total nitrogen), and the highest amount of denaturation of

the whey protein (262 versus 200 g kg^{-1} of the total nitrogen).

INTRODUCTION

Yogurt is a fermented milk product widely consumed around the world. In 2001, more than

9 million tons of yogurt were produced, mostly in Europe (6.6 millions tons).[1,2] However, it

is becoming more popular in other areas. For instance, the yogurt market in the USA has

grown over the past few years at an annual growth rate of between 3% and 10%.[3]

Manufacture of yogurt usually involves milk fortification with dairy ingredients to

increase the concentration of protein to 40–50 gprotein kg^{-1}. Polysaccharides and stabilizers

are sometimes added to improve texture. The fortified milk is homogenized, highly heated

(90^{0}C for 10 min), cooled to the fermentation temperature (42^{0}C) and inoculated with

starter. After inoculation, the process differs depending on the kind of yogurt produced. In

the case of set yogurt, the inoculated milk is filled into consumer cups and incubated to the

desired pH (4.5), then cooled to 4^{0}C without disturbing the curd. In the case of stirred

yogurt, the inoculated milk is filled into a tank where the fermentation occurs. After

fermentation, the gel is broken, then the yogurt is pumped through a fine mesh, cooled and

finally packaged into cups.[4]

Traditionally, skim milk powder (SMP) is used to enrich the milk before fermentation.

However, increased quality and availability of other dairy ingredients, such as whey protein

concentrates (WPCs), may provide a cost-effective alternative to skim milk powder. In

addition, whey proteins may offer functional properties different from the whole milk

proteins of skim milk powder. Whey protein concentrates are produced by ultrafiltration of

whey to enrich the protein fraction by removal of lactose, minerals and other low molecular

weight components. For manufacture of whey protein concentrates with a high protein content $(>600$–650 g kg$^{-1})$, an additional diafiltration step is applied. The protein contents of the WPCs typically range from 340 to 800 g kg^{-1}.[5]

  The effect of the replacement of SMP by WPC on textural and physical properties of yogurts has been studied by various authors. However, results are inconsistent. Some have reported a positive effect of WPC on yogurt firmness and/or viscosity.[6–8] In contrast, the studies of Guin´ee *et al*,[9] and GuzmanGonzalez *et al*[10] reported a viscosity similar or weaker when SMP was replaced by WPC. The effect of WPCs on the water-holding capacity of yogurt is also inconsistent. GuzmanGonzalez *et al*[10] and Cheng *et al*[6] showed a better ability to retain water in yogurts containing WPC than in those enriched with SMP. However, Guin´ee *et al*,[9] Modler *et al*[11] and Remeuf *et al*[7] noticed the opposite effect of WPC addition on water retention in yogurt.

  These apparent contradictions could be due to many reasons. First, there were different methods used to determine the physical and rheological properties of yogurt in these studies. Some methods focus on the viscoelastic behavior (when the yogurt structure is not affected), while other methods evaluated the flow behavior (when the initial yogurt structure is altered). Differences in starters used to ferment the milk may also influence yogurt properties.[12,13]

  Finally, variations in the functional properties of commercial WPCs[14,15] may explain some of the inconsistencies between studies. Modler *et al*[11] compared three WPCs with 330 to 530 g kg^{-1} protein. They reported variation in syneresis (210 to 280 g kg^{-1} of whey expelled during a drainage test) and firmness (49.5 to 78.9 g) of the three yogurts. Guin´ee *et al*[9] compared four whey protein concentrates (350 to 750 g kg^{-1} protein). They noticed variation in water-holding capacity (370 to 550 g kg^{-1} of whey expelled during a centrifugation test) and apparent viscosity (0.16 to 1.12 Pa s). GuzmanGonzalez *et al*[10] demonstrated variation in

water-holding capacity (100 to 300 g kg^{-1} of whey expelled during a centrifugation test) and Brookfield viscosity (10 to 30 Pa s) with six yogurts produced with six different whey protein concentrates (340 to 800 g kg^{-1} protein).

In recent years, changes in processing whey have improved the consistency and functionality of WPCs. Nevertheless, variation in properties of yogurts fortified with WPCs may still exist. Our objectives were to: (1) characterize the properties of yogurts fortified with commercial WPCs (340 g kg^{-1} protein nominal), and (2) determine whether differences in yogurt properties can be related to differences in physicochemical properties of the WPCs.

MATERIALS AND METHODS

Starters and ingredients

The starter cultures were blends of strains of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp *bulgaricus, Lactobacillus acidophilus*, Bifidobacterium and *Lactobacillus paracasei* subsp *casei*. Two different starters were used: Yog-Fast 11 and Yog-Fast 17 (Chr Hansen, Milwaukee, WI, USA). They were characterized as ropy and non-ropy starters, respectively. They were stored at −70 $^{\circ}$C in a concentrated form prior to use.

Six commercial dairy ingredients were used: a low-heat skim milk powder and five commercial whey protein concentrates 340 g kg^{-1} (WPC1 to WPC5). These concentrates had been provided by different facilities which manufacture WPC from whey resulting from manufacture of cheddar (WPC2, WPC4, WPC5), Mozzarella (WPC1) or various other cheeses (Monterey Jack, Mozzarella, Muenster, and Manchego) (WPC3).

Yogurt preparation

Milk was standardized to a fat level of 10 g kg^{-1} by blending pasteurized non-fat milk and pasteurized homogenized whole milk (35 gkg^{-1} fat). The protein content was enriched to 45 g protein kg^{-1} by the addition of skim milk powder (SMP) or whey protein concentrate (WPC1 to WPC5). One-liter flasks were filled with the standardized and fortified milk, heated without agitation for 55 min in a water bath at 90 $^{\circ}$C, then cooled for 1 h in an ice bath, and stored overnight at 4 $^{\circ}$C.

 The following day, the milk was preheated to 42°C, inoculated with 0.2 g l^{-1} commercial culture, then incubated at the same temperature until a pH of 4.50 was reached. Fermentation was stopped by rapidly cooling the fermented milk to 25°C. This was accomplishing by placing the bottles in an ice bath for 15 min while manually stirring the yogurt. The cooled fermented milk was then poured into 100-ml cups at a flow rate of approximately 6lh^{-1} by means of a 50-ml syringe (orifice 1 mm diameter) and was stored overnight at 4 $^{\circ}$C. The shear created by pumping the yogurt from a syringe was to simulate the texturization of stirred yogurt which occurs commercially by pumping set yogurt through perforated screens, then filling heads for packaging.

Physicochemical analyses

The pH values of 100 g kg^{-1} reconstituted whey protein concentrates in deionized water were determined at 20 $^{\circ}$C with a pH meter model 34 (Beckman, Fullerton, CA, USA). Measurements were done in duplicate.

 The levels of total nitrogen (TN), nitrogen soluble at pH 4.6 (SN), and non-protein nitrogen (NPN) were determined in the whey protein concentrates, as well as in the mixes, before and after heating, by the Kjeldahl method.[16] All measurements were carried out in duplicate.

A multiplication factor of 6.38 was used to convert nitrogen to protein. The non-protein fraction (NP), expressed as protein equivalent, was calculated as NPN × 6.38. The true protein fraction (TP) was calculated as $(TN − NPN) × 6.38$. The fraction of protein soluble at pH 4.6 (SP) was calculated as $(SN − NPN) × 6.38$. The fraction of protein insoluble at pH 4.6 (IP) was calculated as $(TN − SN) × 6.38$.

The fraction of protein soluble at pH 4.6 was determined in the mixes before and after heating (SP1 and SP2, respectively). The extent of protein denaturation (D) occurring in the mixes during the heating was calculated as described by Mottar et al.[17]

$$D = \frac{SP_1 - SP_2}{SP_1} \times 100\%$$

Moisture, fat content and ash were determined in whey protein concentrate. Moisture was determined Yogurt fortification with whey protein concentrates by drying for 5 h in a vacuum oven at 100 °C.[18] Fat content was determined by the Babcock method.[19] Ash content was determined by ignition at 550 °C in an electric muffle furnace.[20] All the measurements were carried out in triplicate. Lactose content was calculated by difference {total solid − (total protein + fat + ash)} as described by GuzmanGonzalez et al.[10]

Physical evaluations

Three different analyses were carried out on the yogurts after 16h of storage at 4 °C to determine their physical properties. Before each analysis, the yogurt was gently stirred by making five up and down movements of a spoon in the yogurt cup to ensure homogeneity, as reported by Remeuf et al.[7]

The water-holding capacity was determined by a procedure adapted from GuzmanGonzalez et al.[10] A sample of about 20g of yogurt (Y) was centrifuged for 10 min at

1250 x g at 4 $^{\circ}$C. The whey expelled (W) was removed and weighed. The water-holding

capacity (WHC, gkg^{-1}) was calculated as: WHC = $(Y - W)/Y$ x 1000

The measurement was carried out in triplicate.

  A puncture test was carried out with a TAXT2 texture analyzer (Stable Micro Systems

Ltd, Haslemere, UK) using a 2.5-cm acrylic cylinder probe. The test speed was fixed at 2

mm s^{-1} and the penetration depth was 10 mm. The sample temperature was 4°C. The test

was replicated three times. Firmness was defined as the force necessary to reach the

maximum depth.

  Viscosity was measured using a Brookfield DVII viscosimeter (Brookfield,

Middleboro, MA, USA) with a Helipath (T Spindle, type D) rotated at 2.5 rev min^{-1}

during 1 min. Sample temperature was

4 $^{\circ}$C. The test was replicated three times. The viscosity determined with the Brookfield

viscometer is known as the Brookfield viscosity in the following text of this paper to

distinguish it from the apparent viscosity measured by the rheometer.

Rheological evaluations

Three tests were conducted at 10 $^{\circ}$C with a controlled stress rheometer (model SR5000;

Rheometric Scientific Inc, Piscataway, NJ, USA), used in harmonic and stationary modes. The

rheometer was equipped with a concentric cylinder device consisting of a cup (32 mm diameter)

and a bob (29.5 mm diameter, 44.25 mm length). About 17 ml of yogurt sample was transferred

into the cup of the rheometer and the bob was lowered until its whole surface was covered. Five

minutes were allotted for the sample temperature to equilibrate to 10 $^{\circ}$C prior each analysis.

  Measurements in harmonic mode were used to characterize the viscoelastic behavior (where

original sample structure is not affected), and carried out using a stress sweep (1–100 Pa,

frequency 1 Hz, 20 cycles per decade of stress) to determine the range of linear viscoelasticity. The complex viscosity, in Pa s, was calculated at 1 Pa, which was in the range of linear viscoelasticity.

Measurements in stationary mode (where the original sample structure is disrupted) were used to characterize the flow behavior. The determination of yield stress was carried out following the procedure of Benezech *et al*.[21] The sample of yogurt was subjected to a small shear stress (1 Pa) and the shear stress was increased in increments of 0.5 Pa until the recorded strain increased continuously. The duration of each step was 30 s. The yield stress in Pa was defined as the stress required to increase the strain continuously. Apparent viscosity in Pa s was determined after 3 min of shearing by applying a stress of 20 Pa.

For all tests, three replicates were performed and a fresh sample was used for each replicate.

Statistical analyses

Results were evaluated statistically using Minitab[TM] 13.1 Software (Minitab Inc, State College, PA, USA). A one-factor analysis of variance was performed to determine the effect of the dairy ingredient on the protein composition of the mixes (true protein content and degree of denaturation for the whey protein). A two-factor analysis of variance with interaction was performed to determine the effects of both starter and dairy ingredient on the physical and rheological properties of the resulting yogurts. For each main effect, a multiple comparison of treatment means was performed using Tukey's pair-wise comparison at a *a*-level of 5%. The relationships between physical and rheological properties of the yogurt were investigated by evaluating the correlation matrix.

RESULTS AND DISCUSSION

Physicochemical characteristics of the dairy powders

Proximate composition of the dairy powders

The proximate compositions on a dry matter basis of the five commercial WPC are given in

table 1. The pH was between 6.30 and 6.5, except for WPC5, for which pH was 6.84.

Probably a neutralization with NaOH was done for this product before the drying, as

previously reported.10,15 Fat content was in the range 21–37 g kg−1 and ash content was

approximately 70 g kg−1. These values are comparable with those obtained by Guzman

Gonzalez *et al*.10 For total protein, the content was higher than 340 g kg−1. One whey protein

concentrate (WPC2) had a significantly higher total protein content *(*376.5 g kg−1*)* than the

others *(*350–360 g kg−1*)*. [Table 1]

Nitrogen fractions of the dairy powders

The contents of the nitrogen fractions of the whey protein concentrates (non-protein nitrogen,

soluble and insoluble protein at pH 4.6) are detailed in Table 2. The whey protein concentrate

WPC5 had a high non-protein nitrogen content (160 g kg−1 of the nitrogen fraction) compared

with approximately 126 g kg−1 for the other whey protein concentrates; the difference was

significant *(p ≤ 0.05)*. This could be due to a higher level of microbial proteolysis during cheese-

making and the storage of whey before concentration and drying, or to a higher retention of the

NPN fraction during ultrafiltration. The fraction of insoluble protein at pH 4.6 estimates the

denaturation of the whey protein occurring during manufacture.15 The fractions of insoluble

protein at pH 4.6 determined in our study (190 to 260 g kg−1 of the nitrogen fraction) were

higher than those obtained by GuzmanGonzalez *et al*10 (50 to 210 g kg−1of the nitrogen

fraction). There were significant differences *(p ≤ 0.05)* between the five whey protein

concentrates. The fraction of insoluble protein at pH 4.6 was 262 g kg−1 of the nitrogen fraction for WPC4, compared with 200 g kg−1 of the nitrogen fraction for the other WPC. Therefore, the heat treatment was probably more severe for this product. [Table 2] [Table 3] [Figure 1] [Figure 2]

Physicochemical characteristics of the mixes

The true protein content and the degree of denaturation of the whey protein in the mixes, which are key factors for the physical and rheological properties of the yogurts,22 were determined for each replicate. The average values are reported in Table 3. The content of true protein was not significantly different between the six mixes (approximately 41 g protein kg−1). The denaturation of the whey protein fraction during the heating was high (72.9–77.5%), due to the high heat treatment applied to the fortified milk. This high heat treatment is commonly used in yogurt manufacture to obtain the desired texture. By applying different heat treatments to mixes, it was demonstrated that the highest viscosity was obtained with the highest heat treatment, because it led to the highest level of whey protein denaturation.23 The denatured whey protein resulting from the heating of the milk interacts with κ-casein, coats the micelles, and enhances the cross-linking of the gel.17 The degree of denaturation was not significantly different between the five mixes enriched with WPC (*ca* 76.6%).

Physical and rheological properties of the yogurts

Effect of starter on physical and rheological properties of the yogurts

The effect of starter on the properties of the yogurt was significant $(p \leq 0.01)$ for two rheological parameters: the yield stress and the complex viscosity. These results are presented in Figs 1 and 2, respectively. Independent of the ingredient used to fortify the milk, the yogurt obtained with the ropy starter gave lower values for complex viscosity and yield stress compared with those

obtained with the non-ropy starter. On average, yield stress and complex viscosity were equal to 2.85 Pa and 18.5 Pa s, respectively, for yogurt fermented with ropy starter, compared with 3.83 Pa and 22.8 Pa s for yogurt fermented with nonropy starter. These results are consistent with those previously reported by Rohm *et al*,[13] Hess *et al*[12] and Hassan *et al*.[24] The lower value observed in yogurt produced with the ropy starter can be attributed to the production of exopolysaccharide by the starter. The filaments of exopolysaccharides interfere with the casein network. It can be assumed that protein strand formation and protein–protein interaction is partly inhibited by the exopolysaccharides, thus reducing the rigidity of the resulting yogurt gel.

Effect of dairy powders on physical and rheological properties of the yogurts

The effect of the dairy ingredient source used for fortification was significant *(p ≤ 0.01)* for all the physical and rheological parameters of yogurt evaluated. The results are summarized in Table 3. The water-holding capacity of yogurts enriched with whey protein concentrates was higher than that of control yogurt. The water-holding capacity of WPC enriched yogurts was 601 to 636 g kg^{-1}, compared with 501 g kg^{-1} for control yogurt, which represented a 25% difference. These results are comparable with previous works,[6,10] and may be due to a higher cross-linkage of the network noticed in yogurts fortified with whey protein concentrates.[7,8]

  For the other descriptors (firmness, Brookfield viscosity, rheological parameters), the highest values were obtained with the yogurts enriched with skim milk powder (control) or with whey protein concentrates WPC1, WPC2, and WPC3, while the lowest values were recorded with the yogurts fortified with whey protein concentrates WPC4 and WPC5. The data demonstrated a strong variation in functionality of the commercial

preparations of 340 g kg^{-1} WPC for yogurt application, and agreed with the results of previous studies using WPC.[9–11]

Relationships between the physical and rheological properties of the yogurts and the physicochemical characteristics of the dairy powders

Some differences in physicochemical characteristics of the dairy powders were found in WPC4 and WPC5, and these resulted in yogurts with the lowest firmness, Brookfield viscosity, yield stress, and complex and apparent viscosity, compared with the other WPCs. There was a higher content of NPN in WPC5, and a higher level of denatured whey protein in WPC4, than in the other whey protein concentrates. A high level of NPN suggests a high level of proteolysis, and could influence the functionality of the whey protein concentrate. A high level of denaturation of whey protein has been shown to decrease the firmness of a heat-induced whey protein gel.[15] For lactic gels, no studies have been carried out to relate the degree of denaturation of the whey protein concentrates to their functionality. However, the acid gelation behavior of co-heated casein micelle/whey mixtures has been recently compared with systems where whey proteins were heated separately before mixing with the casein micelles.[25] The results demonstrated that the presence of denatured whey protein before mixing with casein led to a less homogeneous gel with a more open structure than in a system where whey proteins are denatured in the presence of casein. These authors suggest that a pre-denaturation of the whey protein is detrimental to the structure of the gel because it generates some large aggregates of denatured whey proteins, which would not coat the casein micelles. This could partially explain the lower texturing ability of WPC4 compared to the other WPC's.

Other physicochemical characteristics are probably involved in the definition of the texturing ability of the whey protein concentrates. The balance between the minerals, which has not been determined in this study, has been shown to affect the physical properties of the yogurt.[10] [Table 4] [Figure 3]

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Ingredient	pH[b]	Fat	Ash	Crude protein	Lactose[c]
WPC1	6.31^c	35.6^{ab}	64.0^e	354.1^b	546.3
WPC2	6.47^b	24.9^{cd}	69.4^b	376.5^a	529.2
WPC3	6.49^b	29.7^{bc}	76.3^a	348.8^b	545.2
WPC4	6.30^c	36.8^a	65.9^d	359.9^b	537.4
WPC5	6.84^a	21.0^d	67.1^c	353.9^b	558.0

[a] Means within a column not sharing a common superscript differ significantly ($p \leq 0.05$).
[b] Measured in a $100\,\text{g kg}^{-1}$ reconstitution.
[c] Calculated by difference.

Table 1. Compositions *(*gkg−1*)* on a dry matter basis of the five commercial whey protein concentrates (WPC1 to WPC5). averages of two determinationsa.

Ingredient	Non-protein nitrogen	Insoluble protein at pH 4.6	Soluble protein at pH 4.6
WPC1	123^b	209^b	668^{ab}
WPC2	107^b	193^b	700^a
WPC3	114^b	207^b	679^{ab}
WPC4	118^b	262^a	620^b
WPC5	160^a	189^b	651^{ab}

[a] Means within a column not sharing a common superscript differ significantly ($p \leq 0.05$).

Table 2. Contents *(*gkg−1*)* of the nitrogen fractions of the five commercial whey protein concentrates (WPC1 to WPC5), (averages of two determinations)a.

	SMP	WPC1	WPC2	WPC3	WPC4	WPC5
Protein composition of the mixes						
True protein (g kg^{-1})	41.8^a	40.5^a	40.9^a	40.3^a	40.6^a	40.0^a
Whey protein denaturation (%)	72.9^b	77.5^a	77.2^a	76.1^{ab}	76.7^a	75.5^{ab}
Physical and rheological properties of the yogurts						
Water-holding capacity (g kg^{-1})	501^c	636^a	638^a	631^a	625^{ab}	601^b
Firmness (g)	17.7^a	17.3^a	16.2^b	16.3^b	15.1^c	15.2^c
Brookfield viscosity (Pa s)	9.33^a	10.28^a	9.46^a	8.54^{ab}	7.40^{bc}	6.32^c
Yield stress (Pa)	3.62^a	3.93^a	4.25^a	3.57^a	2.55^b	2.27^b
Complex viscosity (Pa s)	25.8^a	24.3^a	22.9^{ab}	20.4^b	16.0^c	13.4^c
Apparent viscosity (Pa s)	1.10^a	0.78^b	0.84^b	0.58^c	0.40^{cd}	0.38^d

One mix was enriched with skim milk powder (SMP). The five others were enriched with various commercial whey protein concentrates (WPC1 to WPC5). The presented values are the means obtained with two different starters (ropy and non ropy). Each assay has been performed in duplicate. Means within a row not sharing a common superscript differ significantly ($p \leq 0.05$).

Table 3. Physicochemical characteristics (true protein content and whey protein denaturation), of the mixes, and physical and rheological properties (water-holding capacity, firmness, Brookfield viscosity, yield stress, apparent viscosity, and complex viscosity) of the resulting yogurts.

	Water-holding capacity (g kg^{-1})	Firmness (g)	Brookfield viscosity (Pa s)	Yield stress (Pa)	Complex viscosity (Pa s)
Firmness (g)	−0.466	—			
Brookfield viscosity (Pa s)	0.025	0.709^c			
Yield stress (Pa)	−0.085	0.622^c	0.739^b		
Complex viscosity (Pa s)	−0.316	0.865^a	0.796^b	0.887^a	
Apparent viscosity (Pa s)	−0.445	0.609^c	0.703^c	0.462	0.656^c

^{a,b,c} Significant coefficient, $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$, respectively.

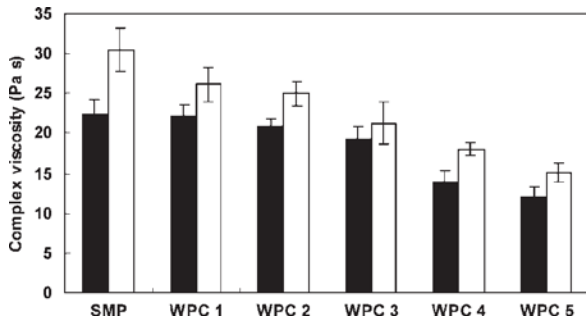**Table 4.** Correlation between physical properties of yogurts.

Figure 1. Effect of milk fortification and starter on complex viscosity of yogurt. Milk was enriched with skim milk powder (SMP) or with various commercial whey protein concentrates (WPC1 to WPC5). The milk was fermented by a ropy starter (_) or a non-ropy starter (_). The presented data correspond to two replications of each combination of milk fortification and starter
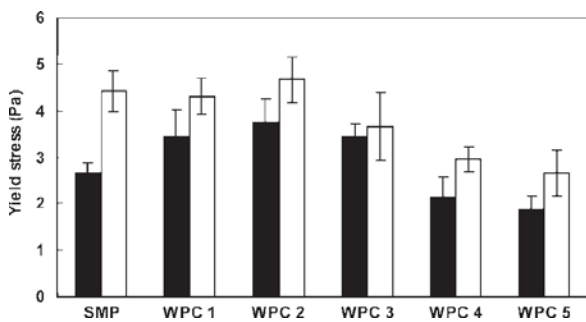

Figure 2. Effect of milk fortification and starter on yield stress of yogurt. Milk was enriched with skim milk powder (SMP) or with various commercial whey protein concentrates (WPC1 to WPC5). The milk was fermented by a ropy starter (_) or a non-ropy-starter (_). The presented data correspond to two replications of each combination of milk fortification and starter.
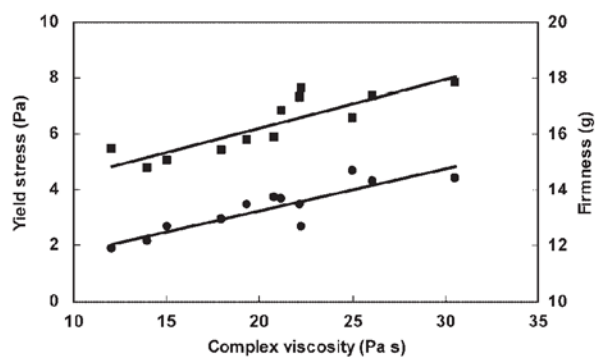
Figure 3. Relationship between complex viscosity (Pa s) and yield stress (Pa) (ž) and between complex viscosity (Pa s) and firmness (g) (_) for 12 yogurts obtained with different combinations of milk fortification and starter. The presented data correspond to two replications of each combination.