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ORIGINAL ARTICLE

# Optimization of low-fat set-type yoghurt: effect of altered whey protein to casein ratio, fat content and microbial transglutaminase on rheological and sensorial properties

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**Abstract** In the present study the amount of whey protein to casein ratio (W/C ratio) (0.22–0.7), fat content (FC) (0.5–1.5%) and microbial transglutaminase (mTGase) (0.0–1.0 U/g substrate protein) in the formulation of low fat set type yoghurt, prepared from reconstituted milk, were optimized by response surface method to achieve the best rheological properties and sensory characteristics. The selected parameters consisted of higher consistency coefficient, consistency, texture and mouth feeling and lower syneresis and pH. The optimum condition was found to be 0.97, 0.46 and 0.5% of the enzyme concentration, W/C ratio and FC respectively. Results showed that thermal denaturation increased up to 33% with increasing W/C ratio significantly. All samples showed non-Newtonian shear thinning behavior. Increasing in W/C ratio from 0.22 to 0.7 raised the consistency coefficient, yield stress and textural parameters such as hardness and consistency of yoghurt. Enzyme addition (together with starter culture addition) increased yoghurt viscosity and yield stress. In sensory evaluation W/C ratio affected the texture and mouth feeling of yoghurt samples and the influences of FC and mTGase were not significant. Yoghurt syneresis was highly affected by FC, W/C ratio and mTGase.

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**Keywords** Fat content · Microbial transglutaminase · Whey protein to casein ratio · Yoghurt

## Introduction

Yoghurt is made by milk fermentation using thermophilic homo-fermentative lactic acid bacteria (*Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus*). The rheological and sensory attributes of yoghurt are influenced with many factors such as milk base, starter culture and processing conditions (Tamime and Robinson 1999).

Milk total solids significantly control the physical properties of yoghurt (Jaros and Rohm 2003). Fortification of solid content can be done by using skim milk powder (SMP), whey protein concentrate (WPC), milk protein concentrate (MPC), butter milk powder (BMP), caseinates (Na or Ca-caseinate) or by concentration through evaporation (EV) or reverse osmosis (RO). The amount and kind of proteins, even protein genetic polymorphism, in dry matter, have a significant effect on yoghurt texture (Sodini et al. 2004; Soukoulis et al. 2007).

Addition of whey products, because of their excellent nutritional and functional properties in addition to low-cost, enriches the milk protein. Casein micelles affect the coagulation in unheated milk, while heat treatment has the significant effect on major whey proteins (WPs), i.e. $\beta$-lactoglobulin and $\alpha$-lactalbumin (Lucey 2009). Conformational changes of $\beta$-lactoglobulin, due to heating, cause to form disulfide bridges between WPs. Denatured-WPs also can form disulfide links with $\kappa$-casein at the surface of casein micelles (Donato et al. 2007; Vasbinder and de Kruif 2003). Denaturation of WPs is important for increasing of stiffness, firmness (elastic modulus), viscosity and water holding capacity (WHC) of yoghurt gels (Donato et al. 2007; krzeminski et al. 2011; Lucey et al. 1998; Remeuf et al. 2003).

During the last two decades, because of the growing attention for low calorie and healthy products, consumers have trend to use of low fat yoghurts (Ozer et al. 2007) but the functional role of fat in yoghurt makes this difficult. The fat content (FC) of yoghurt causes improvement of physical characteristics such as gel firmness and reduction of whey separation (Ramchandran and Shah 2008). Fat also improves the mouth feel and increases creaminess in yoghurt (Lucey et al. 1998). However, since using natural or synthetic gums as stabilizers in yoghurt production is not allowed in many countries, different methods should be employed in order to achieve favorable texture in non-fat or low fat yoghurts (Ozer et al. 2007).

Enzymatic protein crosslinking is used with the aim of changing functionality of food proteins. The high specificity of these reactions causes to minimize undesirable co-reactions that can produce toxic side products. Microbial transglutaminase (mTGase, EC 2.3.2.13) is the first commercial crosslinking enzyme in food processing. Transglutaminase (TGase) induces acyl-transfer reactions between glutamine and lysine residues in certain proteins (Sanli et al. 2011; Sodini et al. 2004). Among milk proteins, caseins, because of their open structure, are more suitable substrate for mTGase, but globular conformation of WPs prevents the formation of covalent links between them by enzymatic reactions. The cross-linking of whey protein (WP) can be induced when they are unfolded or denatured by heat treatment (Bonisch et al. 2007a; Sanli et al. 2011). Yoghurt samples treated with TGase, showed lower syneresis and higher gel strength, gel firmness, viscosity, consistency index and degree of protein polymerization (Bonisch et al. 2007a; Ercili-Cura et al. 2013; Gauche et al. 2009; Lorenzen et al. 2002; Sanli et al. 2011).

Considering higher nutritional value and lower price of WPC than SMP with salutary impact of low fat products, the main objective of this study was to optimize rheological and sensory properties of low fat set type yoghurt formulation with altered whey protein to casein ratio (W/C ratio) and using mTGase.

## Materials and methods

### Preparation of yoghurt culture

Thermophilic yoghurt culture (YoFlex® Express 1.0) was supplied from Chr. Hansen Company's representative (Tehran, Iran), added into 1 L sterilized reconstituted skim milk at about 25 °C. After mixing, 1 ml of this mixture was used for 1 L of yoghurt milk according to the manufacturer's instruction.

### Manufacture of yoghurt

Set type yoghurt samples were prepared according to Sanli et al. (2011) with modifications. SMP with 33% (w/w) total protein (TP), was reconstituted with distilled water to 3.3% TP. W/C ratios were adjusted to 0.22, 0.46 and 0.7, by adding blends of WPC (82% TP) and MPC (66.7% TP) (SMP and WPC were a gift from Mashhad Milk Powder Industries Co. and MPC was purchased from Pegah Dairy Industries Co., Mashhad, Iran). Homogenized/pasteurized cream with 25% FC (Kalleh Dairy Industries Co., Amol, Iran) was added to achieve 0.5, 1.0 and 1.5% (w/w) fat. Dairy powders and cream were dispersed in reconstituted milk using a lab mixer (Model SM-56, Sunny, Germany). Prepared yoghurt milk was kept in refrigerator (4 °C for at least 12 h to become fully hydrated. Then it was preheated to 60 °C and homogenized (15,000 rpm, 2 min) using an ultraturrax homogenizer (T25, IKA, Freiburg, Germany). Homogenized milk was heated at 85 °C for 15 min and subsequently cooled to 42 °C. Yoghurt starter culture (YoFlex® Express 1.0 Chr. Hansen) and mTGase (enzyme activity of 100 Units (U) per g powder, was a gift from BDF Co. representative, Tehran, Iran) at enzyme concentrations of 0.5 and 1 $Ug^{-1}$ substrate protein, were added to prepared milk simultaneously. After mixing and packaging into 100-ml coded plastic cups, the fermentation process continued at 42 °C in the laboratory incubator until the pH value reached 4.6. Yoghurt samples were cooled to 4 °C, stored at refrigeration temperature and analyzed for rheological and sensory characteristics (Sanli et al. 2011).

### Rheological analysis

#### *Instrumental texture analysis*

After 24 h of storage, mechanical properties of yoghurt were estimated at 4 °C using a Brook field texture analyzer (CT3 Texture Analyzer, Brook field, USA) equipped with 38.1-mm of diameter cylindrical probe (according to Gonzalez-Martinez et al. 2002, with modifications). Set yoghurt was prepared in containers with 54.7-mm internal diameter, compressed until 20% deformation achieved with 2 mm $s^{-1}$ penetration speed. Textural parameters, including consistency (total work required in order to achieve desired deformation) expressed as mJ, hardness (the peak of compression force required for desired deformation) expressed as g were studied for each sample.

### Steady shear test

After 24 h of storage, time-independency flow behavior of yoghurt samples was measured using a rotational viscometer (Bohlin Model Visco 88; Bohlin Instruments, Malvern, UK) equipped with a measuring spindle (C30) and a heat circulator (Model F12-MC; Julabo Labortechnik, GmbH D-77960, Seelbach, Germany). In order to reduce the time dependency effect, about 15 g of prepared sample was placed into the cup and permitted to equilibrate at 25 °C and 50 $s^{-1}$ shear rate for 180 s. The effect of shear stress on rheological behavior of yoghurt was studied in a logarithmic shear rate increase from 0 to 300 $s^{-1}$ at 25 °C. In order to describe flow behavior of yoghurt samples, results were fitted with Power Law (Eq. 1) and Casson (Eq. 2) models. Consistency coefficient and flow behavior index were described with Power Law model:

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

where $\tau$ is the shear stress (Pa), K is the consistency coefficient ($Pa.s^{n}$) which displays the shear stress at the shear rate of 1.0 $s^{-1}$, $\dot{\gamma}$ is the shear rate (1/s), and n is the flow behavior index (dimensionless) and reflects the closeness to Newtonian fluid. In Casson model:

$$\sqrt{\tau} = K_0 + K\sqrt{\dot{\gamma}} \quad (2)$$

$$\text{Syneresis } (\%) = \frac{\text{Mass of separated whey from the yoghurt gel during centrifugation}}{\text{Initial yoghurt mass}} * 100 \quad (5)$$

$K$ ($Pa^{0.5}$) and $K_0$ ($Pa.^{0.5}\ s^{0.5}$) are constant. Casson yield stress, $\tau_{0c}$ (Pa) and plastic viscosity, $\eta_c$ (Pa.s) can be written as:

$$\tau_{0c} = (K_o)^2 \quad (3)$$

$$\eta_c = (K)^2 \quad (4)$$

### Sensory analysis

The sensory characteristics of yoghurt samples, comprising of color, aroma, texture with the spoon, flavor and mouth feeling, were evaluated by students (4 male and 6 female) of MSc and PhD students of Food Science and Technology. The panelists didn't have any relationship with our study. They also were not expert but they were trained to evaluate the selected quality properties. Samples were prepared into 100 ml plastic containers, coded and stored at 10 °C, commonly used temperature for sensory evaluation of fermented dairy products (Krzeminski et al. 2011). The panelists evaluated sensory properties using a 5 point hedonic scale from 1 for undesirable sample to 5 for highly desirable sample. Each sample was replicated twice.

### Physicochemical analysis

The amount of milk solid non-fat (MSNF, g/100 g w/w), FC (g/100 g w/w), pH (for yoghurt samples at 21st days of storage) and acidity (% Lactic acid) were determined according to Institute of Standards and Industrial Research of Iran (ISIRI 1753 and 2852 respectively). The pH value was determined using a pH-meter (Metrohm Ltd., model 691, Herisau, Switzerland). All measurements were done in duplicate.

#### *Syneresis*

Whey separation tendency in yoghurt (syneresis) was determined at 21st days of storage according to Gauche et al. (2009). 15 g of yoghurt was weighed in glass tube (125 mm height and 15 mm internal diameter) and centrifuged (350 g, 10 min at about 6 °c (Andreas Hettich, GmbH & Co.KG, Tuttlingen, Germany). The separated whey was weighed and the syneresis was expressed in percentage according to Eq. 5. All measurements were done in duplicate.

#### *Whey protein denaturation (WPD)*

The effect of heat treatment on WPD of yoghurt milk was calculated as a percentage amount of denatured-WPs in heated milk divided by the amount of native WPs in unheated milk. Details of this method were described by Remeuf et al. (2003).

### Statistical analysis

The main effects of FC, W/C ratio and mTGase on rheological and sensory properties of yoghurt samples were evaluated using a response surface methodology (RSM). Statistical analysis was done by Design-Expert v.6.0.2 software. The MATLAB v.2014 software (Math Works INC., Natick, USA) was used to fit viscometer data with power law, Herschel–Bulkley and Casson models. Data obtained from denaturation was analyzed by Minitab v.16 statistical software (Minitab INC., PA, USA) to evaluate the effect of W/C ratio on protein denaturation. Means were compared by Tukey test ($P < 0.05$).

## Results and discussion

### Time-independent properties

Rheological properties of set-type yoghurt samples were studied via curve fitting of steady shear test results by the Herschel–Bulkley, power Law and Casson models. Negative yield stress, obtained using Herschel–Bulkley model, shows that it is not a suitable model to describe the yoghurt flow behavior. High values of $R^2$ and low values of RMSE in power Law and Casson models show that they can be used to describe the rheological behavior of set-type yoghurt samples.

#### *Consistency coefficient*

As shown in Table 1, yoghurt viscosity was highly affected by W/C ratio (B) ($P < 0.001$) and mTGase (C) ($P < 0.01$). No significant effect was observed for FC (A) ($P > 0.05$).The interactions of A and B (AB) or B and C (BC) on yoghurt viscosity were negative and highly significant ($P < 0.001$).

In our study the consistency coefficient of yoghurt increased, with increasing W/C ratio (Fig. 1a). We didn't take the amount of protein constant, in formulating of yoghurt with various W/C ratio, so total protein content (PC) of yoghurt milk enhanced from 3.27 to 5.06 (%) with increasing W/C ratio (Table 2). After heat processing of yoghurt milk, the amount of denatured-WP also increased from 43.48 to 76.46 (%) (Fig. 3).

In addition to milk-PC which is one of the most important factor on physical and rheological behavior of yoghurt (Sodini et al. 2004), if high heat treatment is given to denature the WP, an increase in WP and decrease in casein to whey protein ratio (C/W ratio) cause gel firmness to increase (Lucey 2004). Because of WPD, yoghurt milk heat treatment (above 70 °C can lead to change of the structural and functional properties of the casein micelles. The denatured-WPs interact with themselves and with κ-casein, coats the micelle surface (Donato et al. 2007). Increase in TPC and WPD with increasing W/C ratio in our study can be contributed to the increase in the yoghurt viscosity. Since in our study heating was applied at about natural pH (6.7–6.8), the sulphydryl interchange between κ-casein and WP could occur at the casein micelle surface and increase of WP caused to saturate all cross-linking capacity in heated milk (Puvanenthiran et al. 2002). Our results were in agreement with Remeuf et al. (2003), who observed that >50% denaturation level caused high bridging capacity of WP. These authors attributed the yoghurt viscosity to high level of cross-linking within the yoghurt gel enriched with WPC. However, Puvanenthiran et al. (2002) studied the effect of alteration C/W ratio on visco-elastic properties of set type yoghurt and showed a decrease in gel viscosity with decreasing in C/W ratio. The difference in yoghurt viscosity between the current study and their study may be due to the difference in the heating pH because interactions between WPs and κ-casein and size of aggregates are different in various pH of heating. Since heat treatment in their study was carried out at above natural pH, it caused increase of the dissociation of κ-casein-WP aggregates, formation of soluble aggregates and decreased of the gelation pH.

Because of enzymatic cross-linking of milk proteins covalently by mTGase, increasing the level of enzyme from 0 to 1 (U $g^{-1}$ protein) gave the higher viscose gel (Fig. 1b). Similar results are available from Bonisch et al. (2007b) and Ozer et al. (2007) on cow's milk yoghurt. Since TGase can turn protein monomers into high molecular weight polymers, enzyme treatment increases the consistency coefficient (Gauche et al. 2009).

**Table 1** Significance levels of the analysis of variance for the effects of A: fat content, B: whey protein to casein ratio and C: microbial transglutaminase on responses

| Response | A | B | C | $A^2$ | $B^2$ | $C^2$ | AB | AC | BC |
|---|---|---|---|---|---|---|---|---|---|
| Consistency coefficient (K) | n.s. | *** | ** | – | – | – | *** | n.s. | *** |
| Flow behavior index (n) | *** | *** | * | – | – | – | *** | n.s. | *** |
| Yield stress ($\tau_{0c}$) | n.s. | *** | *** | n.s. | *** | n.s. | n.s. | n.s. | n.s. |
| Consistency | n.s. | *** | n.s. | *** | n.s. | n.s. | n.s. | n.s. | n.s. |
| Hardness | n.s. | *** | n.s. | n.s. | *** | n.s. | n.s. | n.s. | n.s. |
| Texture | n.s. | * | n.s. | n.s. | *** | n.s. | n.s. | n.s. | n.s. |
| Mouth feeling | n.s. | *** | n.s. | n.s. | *** | n.s. | n.s. | n.s. | n.s. |
| pH | n.s. | *** | n.s. | n.s. | *** | n.s. | * | n.s. | n.s. |
| Syneresis | *** | *** | ** | n.s. | *** | ** | *** | n.s. | *** |

*n.s* not significant

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

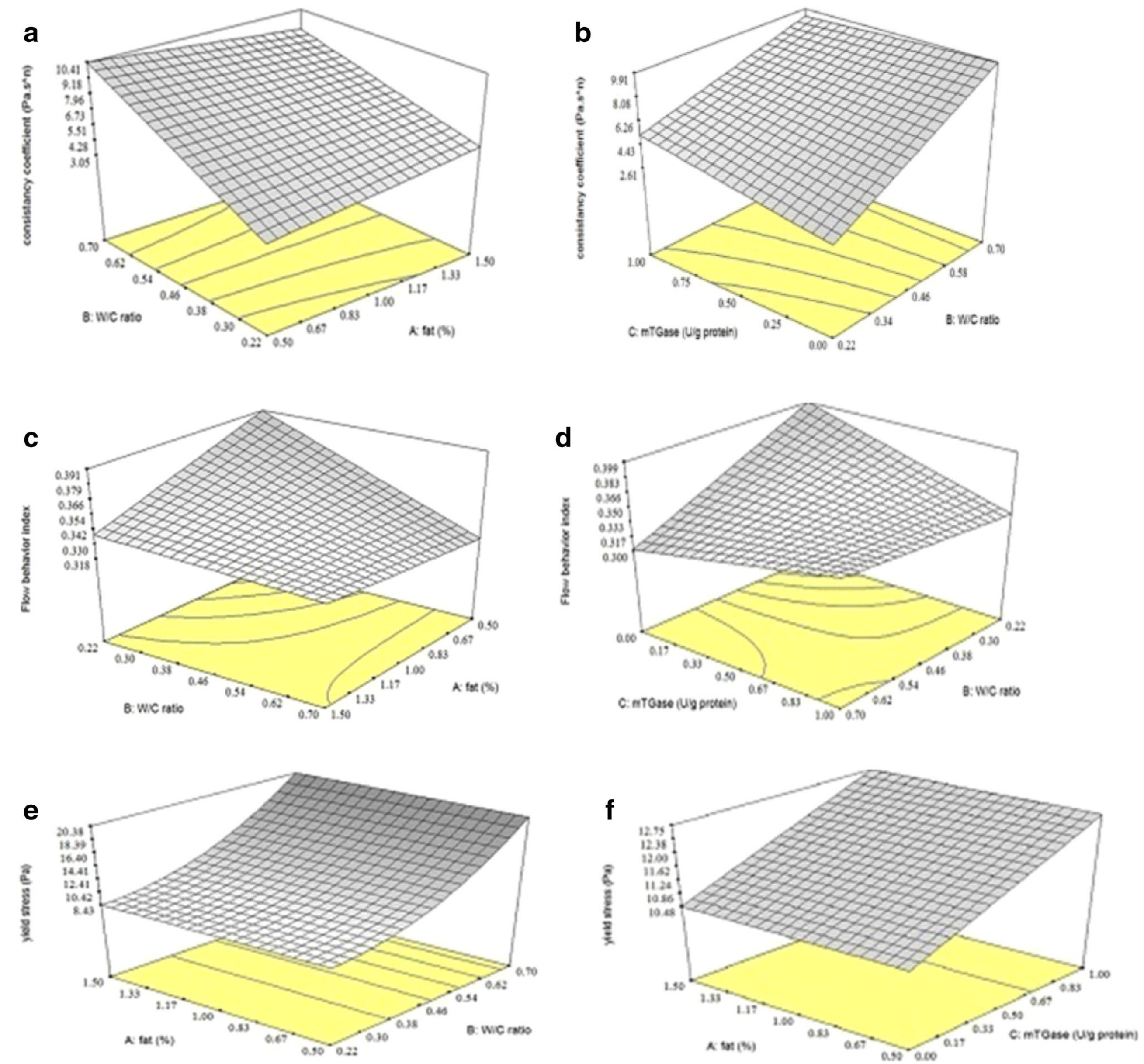


**Fig. 1** Response surface plot for the effect of variables on, **a** and **b** consistency coefficient, **c** and **d** flow behavior index, **e** and **f** yield stress of low-fat set type yoghurt

Although the enzyme effect on yoghurt viscosity was positive, the interaction between WP and mTGase was negative (Table 1). Actually native structure of WP has less ability for enzymatic cross-linking, however; using a severe heat treatment increases cross-linking ability of WPs by increasing their thermal denaturation (Sanli et al. 2011). In our study, mTGase was not used in high level, Because of using it simultaneously with the fermentation process (Lorenzen et al. 2002) and also reduction the negative effects of enzyme (Bonisch et al. 2007b; Ozer et al. 2007). Negative interaction between WPs and mTGase, despite of high level of protein denaturation at high W/C ratio, might be due to the low concentration of enzyme. As it was reported by Truong et al. (2004) increase in enzyme/substrate ratio in the WPI solution causes to widespread cross-linking of WPI and therefore increase in the apparent viscosity significantly. Bonisch et al. (2007b) also reported that TGase must be added at above 3 (U/g protein) in order to achieve a $\eta > 0.07$ Pa s.

*Flow behavior index*

FC (A), W/C ratio (B), the interactions of A and B (AB) or B and C (BC) had highly significant effect on flow

**Table 2** Physicochemical properties of yoghurt milk with different W/C ratio: whey protein to casein ratio

| W/C ratio | Acidity (% L.A) | L/WP | Lactose (%) | WP (%) | Protein (%) | pH | MSNF (%) |
|---|---|---|---|---|---|---|---|
| 0.22 | $0.17 \pm 0.00^{a}$ | 9.43 | $5.56 \pm 0.10^{a}$ | 0.59 | $3.27 \pm 0.01^{a}$ | $6.76 \pm 0.00^{a}$ | $10.19 \pm 0.04^{a}$ |
| 0.46 | $0.20 \pm 0.00^{b}$ | 5.98 | $7.74 \pm 0.22^{b}$ | 1.29 | $4.11 \pm 0.03^{b}$ | $6.71 \pm 0.00^{b}$ | $10.88 \pm 0.01^{b}$ |
| 0.7 | $0.26 \pm 0.00^{c}$ | 4.09 | $8.54 \pm 0.32^{c}$ | 2.08 | $5.06 \pm 0.06^{c}$ | $6.68 \pm 0.00^{c}$ | $11.71 \pm 0.05^{c}$ |

*L/WP* lactose to whey protein, *WP* whey protein, *MSNF* milk solid non fat

[a–c] Means in the same column not sharing a common superscript differ significantly ($P < 0.05$)

behavior index ($P < 0.001$). The effect of mTGase (C) was significant ($P < 0.05$) (Table 1). Owing to n < 1 the Power Law model displayed the non-Newtonian shear thinning (Pseudo plastic) behavior of yoghurt samples.

Physical properties of yoghurt are highly influenced by the degree of aggregated or fused micelle in acid gels. The reaction between $\kappa$-casein and denatured-WPs especially $\beta$-lactoglobulin, at the surface of casein micelles that causes micelle fusion, is influenced by W/C ratio. Since the micelle solvation increases and the micelle fusion reduces in WPC enriched yoghurt milk (Remeuf et al. 2003) this parameter probably gave the lower amount of flow behavior index in our study at high level of W/C ratio (Fig. 1c). A similar result obtained by Gauche et al. (2009) which gained lower amount of 'n' at higher level of added milk whey.

The addition of mTGase increased the shear thinning behavior of yoghurt samples, but its interaction with W/C ratio caused decrease of pseudo plastic properties (Fig. 1d). Enhancement of shear thinning behavior of yoghurt samples by the level of enzyme, might be due to simultaneously decrease in intensities of casein-WP bands and increase in quantity of high-molecular weight polymers that don't participate in the gel network (Ozer et al. 2007).

### *Yield stress*

Yield stress was highly affected by W/C ratio (B) and mTGase (C) ($P < 0.001$). FC and interaction of variables did not have significant effect on it ($P > 0.05$) (Table 1). Increase in total-PC (Table 2), WPD (Fig. 3) and hardness (Fig. 2b) of yoghurt samples with increasing W/C ratio, as well as enzymatic corss-linking of milk protein by mTGase, with increasing the level of enzyme, contributed to increase of yield stress (Fig. 1e, f). Actually the number and strength of protein–protein links, the relaxation times and the strands direction in the gel network affect in yield attributes of yoghurt gel (Lee and Lucey 2010). Our results were in good agreement with Kuecuekcetin (2008), who found a significant effect of C/W ratio on yield stress. He attributed the difference in $\tau_0$ of yoghurt samples to the difference between PC.

## Texture properties

### *Consistency*

Yoghurt consistency was highly affected by W/C ratio (B) and quadratic effect of FC ($A^2$) ($P < 0.001$).

Previous studies showed that protein is the most effective ingredient in increasing yoghurt consistency and the effect of fat is at the secondary importance (Keogh and O'kennedy 1998). Not only the ratio of protein to total solid but also the ratio of W/C influences the texture of yoghurt. Actually denatured-WPs play an important role in protein cross-linking (Sodini et al. 2004). In our study, increase in yoghurt consistency is a result of increase in total-PC and WPD with increase in W/C ratio (Fig. 2a). Puvanenthiran et al. (2002) also presented similar results. However the result of Soukoulis et al. (2007) is in contrast with our study. They reported that the addition of whey powder had negative influence on consistency of set type yoghurt.

As shown in Fig. 2a, FC exhibited two different effects. The consistency of gels reduced at 0.5–1% FC whereas it increased at 1–1.5% level. Fat globules act as structure promoters within the protein network, because they associate with themselves and also with denatured-WPs at the surface of casein micelles so increase of fat globules enhances the yoghurt firmness (Krzeminski et al. 2011; Lucey et al. 1998), while restricted fat in MSNF, because of the fat action as a lubricant, reduces the necessary force to fracture (Pereira et al. 2006).

### *Hardness*

The quantity of casein and FC, milk homogenization, thermal processing, starter culture, yoghurt pH, incubation temperature and hardness measuring temperature are effective variables on hardness (Walstra et al. 1999). Statistical analysis showed the influence of W/C ratio and its quadratic effect were highly significant ($P < 0.001$), the effect of fat and enzyme were covered by high level of W/C ratio (Fig. 2b and Table 1). Increase in hardness was a result of increase in TP and denatured-WP content in

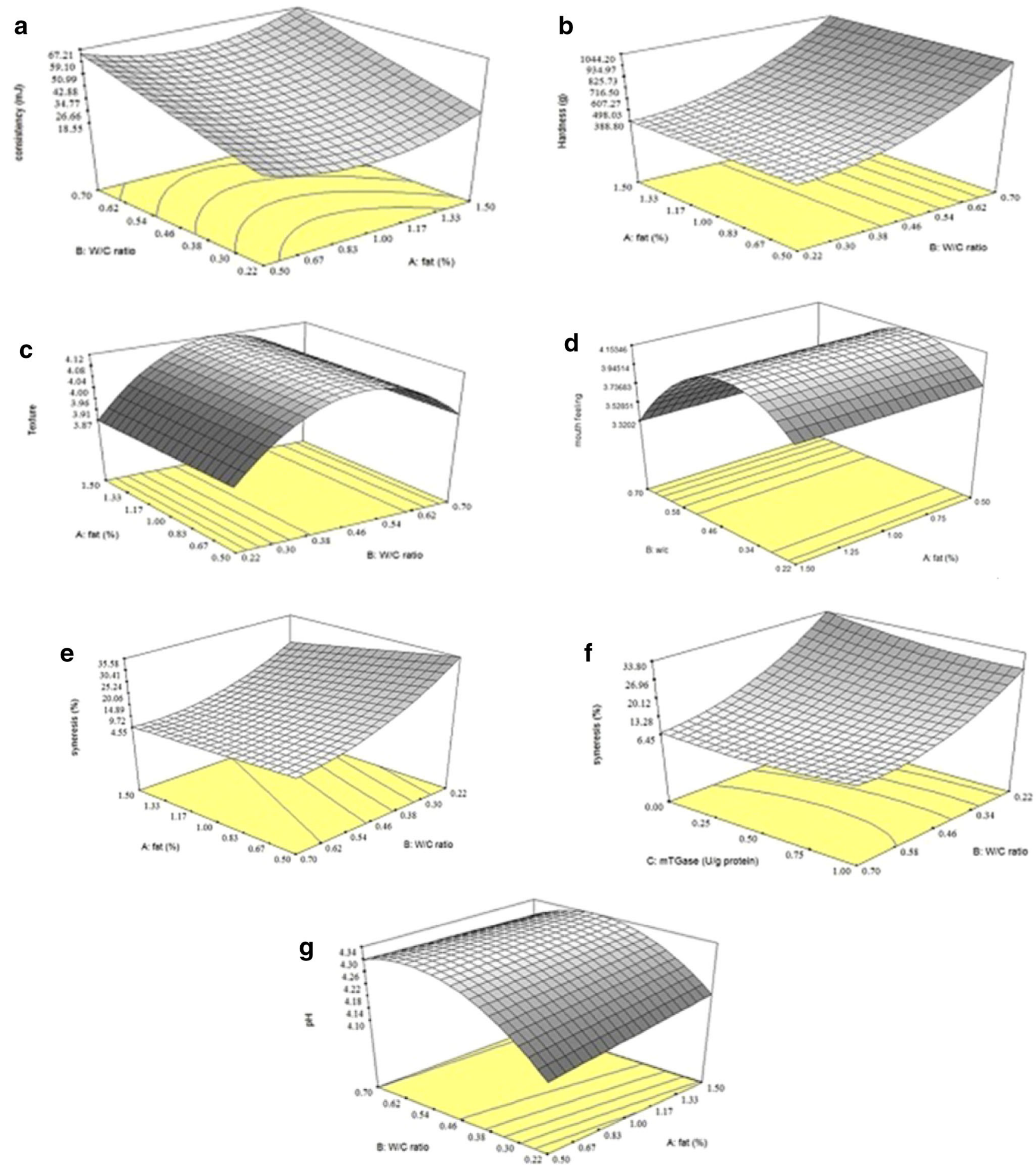


**Fig. 2** Response surface plot for the effect of variables on, **a** consistency, **b** hardness **c** texture, **d** mouth feeling **e** and **f** syneresis and **g** pH of low-fat set type yoghurt

yoghurt samples which was in agreement with Akalın et al. (2012). Association of denatured-WPs to the surface of casein micelles via covalent disulfide bonds, was recommended to be a main factor for the enhancement of yoghurt firmness (Krzeminski et al. 2011). However Amatayakul et al. (2006) and Soukoulis et al. (2007) reported that the addition of WP had a harmful influence on firmness. The contradiction in result between our study and Amatayakul et al. (2006) might be due to the difference in WPD of formulated milk.

## Sensory characteristics

### *Texture of yoghurt*

As shown in Table 1, W/C ratio had a significant effect on yoghurt texture ($P < 0.05$). FC and mTGase didn't show significant influence ($P > 0.05$). The highest scores were given to the yoghurt samples with 0.46 W/C ratio and samples with 0.22 W/C ratio received the lowest scores (Fig. 2c). Yoghurt texture should be smooth, uniform and spoonable (Lucey 2004). In the highest level of W/C ratio, samples were firm, more brittle and had gelatin like texture that was not accepted by panelists. Low solid content and WPD in the ratio of 0.22 induced weak body which obtained the low score by panelists.

### *Mouth feeling*

In our study Mouth feeling was highly affected by W/C ratio and its quadratic effect ($P < 0.001$). The influences of FC and mTGase were not significant ($P > 0.05$) (Table 1). In reality their effects were covered by WPs. Yoghurt samples with 0.46 W/C ratio received the highest scores while panelists gave the lowest scores of mouth feeling to the samples with 0.7 W/C ratio (Fig. 2d).

If W/C ratio was kept in definite extent, it would be possible to achieve a desirable product (Sodini et al. 2004). In this study, yoghurt gels with high ratio of W/C had undesirable aftertaste. Because of water absorption properties of WP, less freshness texture enhanced in high level of W/C ratio. Gelatin like texture and less freshness led to less palatable and the samples couldn't produce the favorable mouth feel. Therefore, despite week body of 0.22 ratios it received higher mouth feel scores than 0.70 ratios. Using of whey powder in yoghurt production at the level of 2% could only improve its flavor and has negative influence on the other evaluated sensory attributes such as firmness, texture, syneresis and palatable (Soukoulis et al. 2007).

Prepared yoghurt samples were also evaluated for flavor, odor and color but panelists couldn't recognize significant differences between them. According to the result of Ott et al. (2000) the least difference in sensory parameters could be attributed to use of similar strains of starter culture in all samples and a low pH range of samples from 4.1 to 4.34 (Fig. 2g) and presumably there was no or minor change in color such that couldn't be perceived by the panelists.

## Physicochemical analysis

### *pH*

Response surface plot of pH values are given in Fig. 2g. W/C ratio and its interaction with FC affected the pH value of samples after 3 weeks of storage (Table 1). The higher ionic strength of liquid phase enhances the pH value of samples with high W/C ratio. This aspect could be useful in the shelf life of product that is limited by over-acidification (Gonzalez-Martınez et al. 2002). However whey powder's available nutrients might partly affect the growth of starter cultures (Amatayakul et al. 2006), hence a slight decrease in sample's pH was observed at above 0.58 ratios (Fig. 2g).

According to the result of Yuksel and Erdem (2010) milk fat content decreases the proteolytic activity of *Lactobacillus delbrueckii* subsp. *bulgaricus*, therefore lactic acid production decreases and consequently pH increases with increasing the FC. However increase of fat and WP in yoghurt raise the interaction of WP with fat globules and their combination within the oil droplet membrane (Krzeminski et al. 2011).

In our study, the effect of mTGase on the pH value of samples was not significant (Table 1). Similar results were reported by Gauche et al. (2009) and Şanlı et al. (2011).

### *Syneresis*

FC, W/C ratio and mTGase had highly significant influence on yoghurt syneresis after 21 days of storage (Table 1).

Increasing the PC and WPD with rising in W/C ratio (up to 0.7) reduced the yoghurt syneresis (Fig. 2e). The ability of undenatured and denatured-WP to immobilize water are 0.32 and 2.34 g water per g protein respectively (Jaros and Rohm 2003). Higher isoelectric pH $\sim(5)$ of WP than yoghurt pH $\sim(4)$ leads to increase in the amount of WP with positive charge in WPC fortified yoghurt and thus enhances its water binding capacity (Remeuf et al. 2003).

Figure 2e shows the reductive influence of FC on yoghurt syneresis. Because of protein adsorption on the surface of homogenized fat globules, increasing the FC enhances the protein ability to immobilize water (Keogh and O'kennedy 1998). At low level of total solids (10–14%), the presence of fat (0–4%), changed the microstructure of the network, increased interlinked bonds and reduced mesh size of protein network (Pereira et al. 2006).

According to our statistical results, yoghurt syneresis reduced linearly with increasing the amount of enzyme (Fig. 2f). Enzymatic cross linking of milk proteins by mTGase reduces the size of pores in the protein network, limits gel permeability and improves WHC (Lorenzen et al. 2002; Şanlı et al. 2011). In fact, TGase fixes the protein network and restricts rearrangement of protein matrix causing gel syneresis (Ercili-Cura et al. 2013).

### *Whey protein denaturation*

In our study, change in W/C ratio from 0.22 (in native milk) to 0.7 increased the denatured-WP of prepared mixes

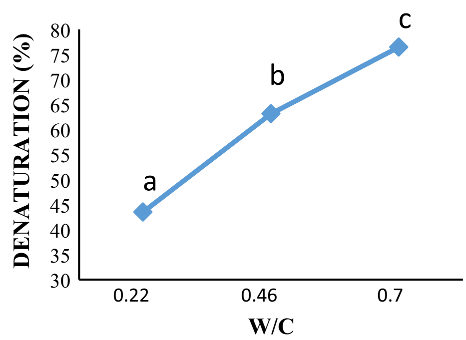


**Fig. 3** Effect of different whey protein to casein ratio (W/C ratio) on thermal denaturation of whey protein

(Fig. 3). This influence could be a result of increase in WP and total protein concentrations, the preventive effect of lactose and different pH of prepared mixes during the heat treatment (Table 2). Anema et al. (2006) reported, rising in the concentrations of WP and TP increase WPD. According to the result of Vasbinder and de Kruif (2003) the amount of denatured-WP (especially $\beta$-lactoglobulin) and their association to the casein micelles increase with reducing of heating pH.

In order to study the lactose influence on denaturation in the constant level of WP concentration, the ratio of lactose to existent-WP (L/WP) was measured for each blend (Table 2). Decreasing the rate of protein denaturation with increasing of L/WP ratio, is due to prioritize protein hydration in the existence of the sugars that explained by preferential hydration theory (Anema et al. 2006).

## Optimization

A response optimizer was used to determine the optimum formulation of yoghurt with W/C ratio and mTGase in ranges and lower FC. The selected parameters consisted of higher consistency coefficient, consistency, texture and mouth feeling and lower syneresis and pH. The optimum ratios were 0.97 (U $g^{-1}$ protein) mTGase, 0.46 W/C ratio and 0.5% FC.

In order to certify the obtained optimum formulation, yoghurt samples with the optimized formulation were prepared in two replications and the validation test was done on them. The predicted and true responses in optimum conditions are shown in Table 3. The validation test was not significant ($P > 0.05$), indicated the credibility of optimum formula.

**Table 3** The predicted and true responses in optimum condition

| parameters | Predicted response | True response |
|---|---|---|
| consistency coefficient ($Pa.s^n$) | 7.25 | 7.98 ± 0.71 |
| Consistency (mj) | 47.18 | 48.30 ± 0.41 |
| pH | 4.30 | 4.30 ± 0.02 |
| Syneresis (%) | 16.8 | 16.62 ± 1.01 |
| Texture | 4.11 | 4.22 ± 0.16 |
| Mouth feeling | 4.12 | 4.25 ± 0.14 |

## Conclusion

Response surface methodology was efficacious to determine the optimum formulation of low fat set type yoghurt with altered W/C ratio. The mixture with 0.97 (U $g^{-1}$ protein) mTGase, 0.46 W/C ratio and 0.5% FC was suggested as the optimum formulation. Alteration of W/C ratio had the most obvious influence and in some cases such as sensory attributes (texture and mouth feeling) and hardness of yoghurt, it could completely cover the effects of the other variables. This influence could be useful in reduction of FC of yoghurt and can prevent texture from weakening. Due to the enzymatic cross-linking of milk protein by mTGase, yoghurt viscosity and yield stress were increased. mTGase, FC and W/C ratio also chiefly reduced the yoghurt syneresis.

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