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# Enrichment of yogurt made from camel milk with *Allium sativum* and *Cinnamomum verum*: Influence on syneresis, water holding capacity, exopolysaccharides and rheological properties

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#### Abstract

The influence of Allium sativum and Cinnamomum verum water extracts on the water holding capacity (WHC), susceptibility to syneresis (STS), exopolysaccharides (EPS) and rheological properties of yogurt during 0, 7, 14 and 21 days of refrigerated storage  $(4^{\circ}C)$ were investigated. The WHC of yogurt in presence of C. verum or A. sativum was significantly higher than plain-vogurt overall storage period. The highest WHC showed on day 21 of storage with 24.5±1.6% and 17.3±1.5% for C. verum- and A. sativumyogurts respectively. The STS uniformly reduced (p<0.05) in presence of herbs compared to in absence. ESP in plain- and herbal-yogurts increased during refrigerated storage. However, both plain- and A. sativum-yogurt decreased (p<0.05) on day 21 of storage. Amplitude sweep showed liquid like behaviour for both herbal and plain-yogurt during storage. However, addition of C. verum or A. sativum in yogurt showed remarkable improvement in the structure of yogurt on day 21 of storage. Both herbal- and plain-yogurts showed shear thinning behaviour. Refrigerated storage up to 21 days showed less shear thinning behaviour

as compared to 0 day for both plain- and *C*. *verum-* yogurt but not for *A. sativum-*yogurt.

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*Keywords:* Yogurt, Cinnamomum verum, Allium sativum, Water holding capacity, Susceptibility to syneresis, Rheology.

#### Introduction

Yogurt is a healthy dairy product produced by bacterial fermentation of milk. The potential to increase consumer's satisfaction by development of yogurt products with new flavors, health benefits and good quality has been increased.

Viscoelastic properties of yogurt are one of the main rheological parameters use to characterise the texture that may lead to define the quality of yogurt and M Shori et al., Enrichment of yogurt made from camel milk with Allium sativum and C. verum:

ensure the consumer acceptability (Benezech and Maingonnat, 1994).

Milk coagulation during fermentation by acid with consequence of removal of calcium bond between casein micelles causes destabilization of casein lead to aggregates and form a curd (Shaker et al., 2000). This gelation process starts with the aggregation of whey proteins associated with caseins (â-lactoglobulin) that denatured to a certain degree by fermentation (Heertje et al., 1985 and Lee and Lucey, 2010). The mechanism of gelation process as described by Xu et al. (2008) suggests two main interactions that occur between whey proteins and casein micelles after fermentation that is a direct interaction of â-lactoglobulin with casein micelles via ê-casein binding and a reaction between á-lactalbumin and âlactoglobulin with the micelles through an intermediate formed between the two whey proteins in solution. Thus, crosslinking or bridging of denatured whey proteins associated with the casein micelles results in an increase in number and strength of bonds between protein particles (Corredig & Dalgleish, 1999; Schorsch et al., 2001; Guyomarc'h et al., 2003; Lee and Lucey, 2004).

Camel's milk is different from other ruminant milk and it does not form coagulum in acidic environment (Wangoh, 1993). Thus, fermented camel milk products are difficult to produce because of the problem in milk coagulation. Mohamed and Larsson-

Raznikiewicz et al. (1990) found that camel milk coagulum failed to reach a gel-like structure even after 18 h incubation with lactic acid culture. Likewise, Jumah et al. (2001) study the effect of milk source including milk from ovine, caprine, bovine and camel on the rheological properties of curd during the gelation process of yogurt and found that the lowest value for viscosity was showed by yogurt made from camel milk. Attia et al. (2001) reported that the anti-coagulation factors in camel milk is lead to difficulty of producing firmed structure of fermented camel milk because the starter culture showed a longer lag phase and an earlier decline phase resulting in a fragile coagulum. Another study by Hashim et al. (2009) showed that camel milk yogurt fortified by 1% gelatin or 0.75% alginate + 0.075%calcium has the highest intensities for firmness and body. Recently in vitro study, camel milk yogurt enriched with A. sativum or C. verum water extracts showed therapeutic values such as antioxidants and anti-diabetic activity (Shori and Baba, 2011a; b) and improved lactic acid bacteria (LAB) growth (Shori and Baba, 2012). However, to the best of our knowledge there are no researches on the effect of physical and rheological properties of camel milk yogurt during refrigerated storage. Therefore, the aim of this research was to study the effect of A. sativum or C. verum water extracts on the physical and rheological properties of camel milk yogurt during refrigerated storage at 4°C.

#### **Materials and Methods**

## 1 Materials and chemicals

Commercial pasteurized full cream camel milk (Al-Turath) was purchased from local supermarket in Saudi Arabia. C. verum bark and A. sativum powder (McCormick<sup>®</sup>, Malaysia) were from local purchased store. Commercial yogurt bacteria mixture (Chris-Hansen, Denmark) was purchased from a local distributor. Each sachet of yoghurt mix contains Lactobacillus acidophilus LA-5 (4 billion), Bifidobacterium Bb-12 (4 billion), Lactobacillus casei LC-01 (1 billion) and Streptococcus thermophilus Th-4 (1 billion). Probiotic mixture (Bio-Life, Malaysia) was purchased from a local. One capsule contained 5 billion cfu of mix probiotic bacteria such as Lactobacillus acidophilus, L. delbrueckii ssp. Bulgaricus, L. casei, L. rhamnosus, Bifidobacterium bifidum, B. infantis, B. longum in the ratio of 1:1:1:1.

# 2 Water Extraction of herbs

The mixture of *C. verum* or *A. sativum* powder (10g) in 100 mL of distilled  $H_2O$  was incubated overnight in a water bath at 70 °C (Haake Model SWD 20). The mixture was centrifuged (Eppendorf 5804 R; 10000 rpm) for 15 minutes at 4°C and carefully remove the supernatant to use as *C. verum* or *A. sativum* water extract in preparation of yogurt.

# **3** Preparation of Starter Culture

The pasteurized full cream milk (1 L) was pre-heated to 41°C. One sachet of yogurt bacteria mixture and a capsule of probiotic mix were added and mixed thoroughly with the pre-heated milk prior to an overnight incubation at 41°C. The yogurt formed was refrigerated (4°C) and used as starter culture within 3 days (Rashid *et al.*, 2007).

## **4** Preparation of Yogurts

To prepare 100 mL of C. verum- or A. sativum-yogurt: 85 mL of pasteurized full cream milk was heated to 41 °C followed by addition of 10 mL of herbal water extract from C. verum or A. sativum and 5 g of starter culture (Shah, 2003). The mixture was mixed thoroughly prior to incubated at 41°C. The fermentation process was stopped at pH 4.5 by placing the yogurts in icebath for 60 minutes. Yogurt was then kept in the refrigerator at 4°C for predetermined period (21 days) prior to analysis. The same procedures were carried out to prepare plain yogurt (control) except 10 mL of distilled H<sub>2</sub>O was used in place of C. verum or A. sativum water extract.

#### **5** Water holding capacity

The water holding capacity (WHC) of yogurts samples was determined as described by Harte *et al.* (2003) with slight modifications. Yogurt sample (10 g) was placed in a centrifuge tube and centrifuged at 4°C (10000 rpm) for 15 minutes. The separated whey was

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pipetted out and weighed. The following formula was used to calculate WHC%:

WHC(%) = 
$$\left(1 - \frac{W_1}{W_2}\right) \times 100$$

Where:  $W_1$  = Weight of whey after centrifugation,  $W_2$  = Yogurt weight.

#### 6 Susceptibility to syneresis (STS)

Susceptibility to syneresis was carried out as described by Isanga & Zhang, (2009). Yogurt sample (10 g) was placed on a filter paper (Whatman No. 41) placed on top of a funnel. After 6 hours of drainage, the volume of the whey collected in a beaker was measured and used as an index of syneresis. The following formula was used to calculate STS:

$$\mathrm{STS}(\%) = \frac{V_1}{V_2} \times 100$$

Where: V1 = Volume of whey collected after drainage; V2 = Volume of yogurt sample.

# 7 Isolation and quantification of exopolysaccharides

Exopolysaccharides (EPS) was isolated from yogurt samples using a modified procedure as described by Elin et al. (2010). Yogurt sample (25ml) was diluted with  $dH_2O$  in the ratio of 1:1. The mixture was boiled at 100°C for 10 min followed by cooling down for another 10 min. Then, 4ml of 20% (w/ v) trichloroacetic acid (TCA) was added to the solution and kept for 2 hour at room temperature. After centrifugation  $(10000 \times \text{g for } 30 \text{ min at } 4^{\circ}\text{C})$  to remove the precipitated proteins and bacterial cells, the pH of the supernatant was adjusted to 6.8 using 40% (w/v) NaOH. The solution was subjected to centrifugation again (10000×g for 30 min at 4°C). The supernatant was mixed with a double volume of cold ethanol and then stored at 4°C for 24 hour. The precipitated EPS was collected by centrifugation (10000×g for 30 min at 4°C), dissolved in 10 ml dH<sub>2</sub>O and mixed with a double volume of cold ethanol and stored at 4°C for 24 hour. The precipitate EPS with ethanol was recovered by centrifugation at 4°C  $(10000 \times g \text{ for } 30 \text{ min})$ . Total EPS (expressed as mg/L) was estimated in each sample by phenol sulphuric method (Dubois et al., 1956) using glucose as a standard (Torino et al., 2001).

#### 8 Rheological properties of yogurt

Dynamic oscillatory measurements of yogurts were carried out using Bohlin VOR controlled strain Rheometer (Malvern Instrument UK), with a coneplate geometry, in which the rotating cone was 40 mm in diameter, and cone angle of  $4^{\circ}$  with a gap of 0.150 mm. Temperature maintenance at  $20\pm0.1$  °C which achieved with Peltier Plate system (-40 to +180°C, Peltier Plate system from Bohlin Instrument Ltd.) that acts as temperature controller. Yogurt samples were gently stirred 10 times by spoon prior to rheological analysis in order to avoid the differences due to structural breakdown during handling of the yogurt. Amplitude sweeps were carried out at 20°C to characterize the viscoelastic linear region of yogurt samples. Strain ranging from 0.0005 to 0.1% was used at a constant frequency of 0.5Hz. The elastic modulus (G'), the viscous modulus (G'') and the loss tangent defined as tan d = (G''/G') were obtained from the equipment software in all cases. Shear viscosity profiles of yogurt samples were measured as a function of shear rate sweep (0.010 - 80.000 s<sup>-</sup> <sup>1</sup>). All the measurements were performed on day 0, 7, 14, 21 days.

#### **Statistical Analysis**

All Analyses were performed in three batches for WHC and STS analysis and two batches for rheological measurements during 0, 7, 14 & 21 days of storage at 4 °C. The average was taken and data were expressed as mean  $\pm$  standard. The significance was established at p<0.05. Data analysis was done using SPSS<sup>®</sup> version 17.0.

#### Results

#### 1 Water holding capacity (WHC)

The presence of *A. sativum* or *C. verum* in yogurt resulted in higher WHC than plain yogurt during storage at  $4^{\circ}$ C (Figure. 1). The WHC in fresh plain yogurt was  $0.41\pm 0.3\%$  whereas this value showed increased in the presence of *C. verum* (p<0.05) or *A. sativum* to 2.7±0.1% and 0.57±0.1% respectively (Figure 1). Refrigerated storage to 21 days showed higher increase (p<0.05) of WHC both in presence of *A. sativum* and *C. verum* than plain yogurt. The



**Fig.1:** water holding capacity (%) vs storage period (day) during 21 days refrigerated storage at 4 °C. Values are presented as mean ± SEM (n=3).

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highest WHC was recorded on day 21 of storage with  $24.5\pm1.6\%$  and  $17.3\pm1.5\%$  for *C. verum-* and *A. sativum-*yogurts respectively.

#### 2 Susceptibility to syneresis (STS)

The presence of *A. sativum* and *C. verum* in yogurt uniformly reduced STS compared to plain yogurt both in fresh (0 day) and refrigerated stored yogurt. Refrigerated storage increased (p<0.05)

STS both in plain- and *A. sativum*yogurts whereas the reverse was true for *C. verum*- yogurt. However, the latter showed the highest increased (p<0.05) on day 21 of storage with 23.5±3.1%.

# **3** Microbial exopolysaccharides production

The EPS isolated from fresh plain



**Fig.2:** Susceptibility to syneresis (%) vs storage period (day) during 21 days refrigerated storage at 4 °C. Values are presented as mean ± SEM (n=3).



**Fig. 3:** Exopolysaccharides (EPS) concentration (mg/L) vs storage period (day) during 21 days refrigerated storage at 4 °C. Values are presented as mean ± SEM (n=3).

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yogurt was 278.6± 0.3 mg/L (Fig. 3). The presence of A. sativum or C. verum in yogurt non significantly increased EPS to 297±0.2 mg/L and 296.4±0.3 mg/L respectively. The first week of refrigerated storage increased (P<0.05) EPS of A. sativum- and C. verumyogurts  $(418.2\pm0.4 \text{ mg/L} \text{ and})$ 472.2±0.2 mg/L respectively) compared to plain-yogurt (283.8 ±0.4 mg/L; Fig. 3). Prolonged storage of yogurt to more two weeks had significant effect on EPS of plain- and A. sativum-yogurts but not in C. verum-yogurt. However, both plain and A. sativum-yogurts decreased (p<0.05) on day 21 of storage (Fig. 3).

## 4 Dynamic rheology

Yogurt samples in the presence or absence of A. sativum or C. verum showed a drop in both elastic modulus (G') and viscous modulus (G'') over the whole strain% range measured during the 21 days of storage (Figure 4). The viscous modulus (G'') was dominant over the elastic modulus (G')during throughout the storage period. However, plain yogurt showed higher G' values than G'' on 0 day over short range of strain (0.05-0.08%) prior to G' and G'' crossed over (Fig. 4a). In the presence of A. sativum or C. verum in yogurt refrigerated storage up to 14 days showed no effect on the changes of yogurt structure. However, extend the storage to 21 days enhanced the structure in A. sativum yogurt only over 0.05-0.17% of strain (Fig. 4b) and in C. verum yogurt over the whole strain range (Fig. 4c).

# **5** Apparent viscosity

Viscosity profiles of yogurts during storage were measured as a function of shear rate sweep (Fig. 5). Both plainand herbal-yogurts exhibited shearthinning behaviour, i.e., the apparent viscosity decreases with shear rate. Refrigerated storage had remarkable effect on the viscosity profiles of plain yogurt (Fig. 5a) that showed increase in viscosity with extended storage period. Viscosity profiles for C. verum yogurt showed improved during refrigerated storage compared to 0 day (Fig. 5c) whereas A. sativum yogurt showed no changes during storage (Fig. 5b). However, A. sativum- and C. verum-yogurt showed the highest viscosity on day 14 of storage.

# Discussion

The increase of water holding capacity of yogurt in the presence of A. sativum or C. verum extract was lead to decrease undesirable syneresis. A higher concentration of EPS in herbal-yogurt than plain-yogurt could be related to increase of WHC and decrease of STS because EPS produced by LAB can help prevent whey separation of yogurt (Girard and Schaffer-Lequart, 2007; Purwandari et al., 2007). Recently, Shori and Baba, (2012) reported that, presence of A. sativum or C. verum in yogurt made from camel milk has enhanced the growth of LAB which could lead to increase the concentration of EPS in yogurt. In addition, the interaction between EPS and free water





**Fig. 4:** Elastic modulus (*G*') and viscous modulus (*G*'') vs. strain % during 21 days refrigerated storage at 4 °C. (a) plain yogurt, (b) *A. sativum* yogurt , and (c) *C. verum* yogurt. Values are presented as mean (n=2).

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**Fig. 5:** Apparent viscosity vs. Shear rate  $(1^{-s})$  during 21 days refrigerated storage at 4 °C. (a) plain yogurt, (b) *A. sativum* yogurt , and (c) *C. verum* yogurt. Values are presented as mean (n=2).

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in yogurt may cause texture improvement (De Vuysi and Degeest, 1999 and Hassan *et al.*, 2002).

Shear strain sweep in yogurts showed viscous behaviour (G' < G'') over the whole range of strain% (Fig. 4) indicated liquid like behaviour. Lower G' values than G" in camel milk yogurts through the 21 days of storage suggested due to extensive particle rearrangement during structure formation lead to creation large pores associated with greater whey separation (Skriver, 1995 and Lucey et al., 2004). The increase in whey separation (syneresis) causes the linear decrease in both G' and G" values during increase the strain% overall the storage period resulted in fragile structure that showed to breakdown easily even at lower strain% (Tunick et al., 1990). However, the dominate of elastic modulus over viscous modulus in A. sativum- and C. verum- yogurts on day 21 of storage indicated that the presence of these herbal extracts in yogurt could improve the structure (solid like behaviour) by decreasing the syneresis in yogurt (Figure 2). In addition, increase EPS in presence of herbal extract in yogurt could be attributed to increase colloidal linkage between milk proteins micelles resulted in more intense network of yogurt (Pyo and Song, 2009). Moreover, the higher EPS in C. verum yogurt than A. sativum yogurt on day 21 of storage could explain the improvement of C. verum yogurt texture that can withstand the

breaking down during increase of strain%. However, this indicated that both yogurts behave as a very weak gel, much closer to a concentrated solution than to a true gel. A. sativum- yogurt showed ability to turn into liquid like behaviour by increased the tan d values to more than 1 (data not shown). The weakness in the network of yogurt increase the sedimentation of casein aggregates that lead to syneresis occurred and causes slippage in the modulus (Haque et al., 2001). This can be overcome by strengthening the yogurt structure with stabilizers such as gelatin or alginate (Hashim et al., 2009).

The linear decrease of the elastic and viscous modulus corresponds to a typical shear thinning profile. The low viscosity in yogurt is in agreement with Jumah *et al.* (2001) who related that to small amount of casein which is not sufficient to make a complete matrix. Al-Alawi and Laleye (2011) found that the amount of different proteins in pasteurized market camel milk such as ê-casein and â-lactoglobulin is very low (744.00 and 248.00 mg/L respectively) compared to pasteurized market cow milk (3,554.67 and 2,397.33 mg/L respectively). These two proteins are important in gelation process of yogurt (Xu et al., 2008). However, the improvement of herbal-yogurt viscosity during refrigerated storage suggested refers to release of more â-lactoglobulin and ê-casein during proteolysis activity of yogurt starter culture. Further Shori et al., Enrichment of yogurt made from camel milk with Allium sativum and C. verum: N

studies need to be carried out for isolation and quantification the protein of milk present in yogurt during refrigerated storage. Moreover, the further reduction in pH of herbal-yogurt compared to plain-yogurt during refrigerated storage (Shori and Baba, 2011a,b) might enhance further the rearrangement of the protein network (Ross-Murphy, 1990) because of removing calcium and neutralizing the negative charges of casein micelles which aggregates and forms a curd (McMahon *et al.*, 1984).

#### Conclusion

The WHC of yogurt in presence of C. *verum* or *A. sativum* was significantly higher than plain-yogurt overall storage period. In addition, increase the concentration of EPS in the presence of herbal extract can lead to significant STS reduction compared to in the absence. Rheological properties showed liquid like behaviour for both herbal and plain-yogurt during storage. However, addition of C. verum or A. sativum in yogurt enhanced the structure of yogurt on day 21 of storage. The shear thinning behaviour of yogurt improved during refrigerated storage for plain- and C. verum- yogurt but not for A. sativumyogurt compared to fresh yogurt.

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