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Influence of *Allium sativum* or *Cinnamomum verum* on Physicochemical Characteristics of Yogurt

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ABSTRACT
Enhancement of yogurt texture is become important factor to improve the quality of yogurt. This study was aimed to evaluate the effect of *Allium sativum* or *Cinnamomum verum* water extracts on some physicochemical parameters of the yogurt. Water extract of *A. sativum* or *C. verum* was incubated in yogurt during fermentation and the changes of production of exopolysaccharides (EPS), Water Holding Capacity (WHC), Susceptibility to Syneresis (STS) and rheological properties of yogurt during 21 days of refrigerated storage were investigated. The presence of herbal extracts in yogurt enhanced the yield of EPS as compared to the absence during storage up to 21 days. WHC of yogurt significantly increased in the presence of herbal extract compared to the absence overall storage period for *A. sativum*-yogurt and on day 14 and 21 of storage for *C. verum*-yogurt. STS of fresh plain-, *A. sativum*-and *C. verum*-yogurts were 38.1, 37.8 and 35.5%, respectively. However, refrigerated storage decreased STS ($p<0.05$) to 33.9, 23.6 and 24.5% for plain-, *A. sativum*-and *C. verum*-yogurts respectively after two weeks of storage. Rheological analysis showed that plain yogurt have higher values of G' (elastic modulus) and G'' (viscous modulus) than herbal yogurt. The apparent viscosity of yogurt indicated shear thinning behaviour. In conclusion, although the presence of *A. sativum* or *C. verum* water extract in yogurt did not improve the final texture of yogurt however such additives that were included into yogurt upon preparation could improve the physicochemical properties of yogurt such as EPS production, WHC and STS.

Key words: Yogurt, *Cinnamomum verum, Allium sativum,* exopolysaccharides, rheological properties

INTRODUCTION
Rheometry is a useful technique for measuring the quality of a product in terms of its shelf life, texture etc. Viscoelastic properties in the food industry play the role of evaluating the gelation system by determining the extent and strength of internal structures (Sendra *et al.*, 2010). The development of dairy products with new flavors and health benefits has the potential to increase sales and consumers' satisfactions. Yogurt a popular product throughout the world is a fermented dairy product made from lactic acid fermentation of milk by the action

of yogurt starter bacteria. Texture is very important factor that define the quality of yogurt and as well, the defects in the texture might lead to consumer rejection (Benezech and Maingonnat, 1994). Whey separation is one of the problems that need to be, considered in yogurt industry because its effects on the rheological characteristics of yogurt gels resulting in low quality of yogurt. Susceptibility to Syneresis (STS) provides an indication of the non-homogeneities in the gel system of yogurt in which higher whey separation correlates highly with gel instability (Peng *et al*., 2009).

Bacterial exopolysaccharides (EPS) are regarded as more proper substitute for food additives as biothickeners or biostabilizers. EPS can easily solubilize in water and this increase the viscosity of the milk serum surface, thus reducing syneresis and improve the texture and mouthfeel of yogurts (Khurana and Kanawjia, 2007). Bacterial EPS has the potential to be developed as functional food ingredients with favourable impact on both health and economic benefits. Increase viscosity of EPS containing food was reported useful in assisting transient colonization of probiotics in the gastrointestinal tract (Khurana and Kanawjia, 2007). The improvement of yogurt texture by incorporation dietary fibres during fermentation have been studied using gelatine (Fiszman *et al*., 1999), pectin (Ramaswamy and Basak, 1992), k-carragenean (Xu *et al*., 1992) oat, rice, soy and maize fibers (Fernandez-Garcia and McGregor, 1997). However, the use of plant materials with medicinal properties has not been attempted to similar extent. It is anticipate that phytochemicals present in those plants would adversely affect physical properties of yogurt such as water holding capacity, syneresis, EPS and rheology. Nevertheless in view of the enormous potential of increase nutritional and therapeutical values by addition of medicinal plants, thus it is important to establish the changes in physical properties of yogurt in the presence of plants extracts. Recently, *in vitro* studies have shown that *A. sativum* and *C. verum* water extracts enriched yogurt have anti-diabetic and antioxidant activity (Shori and Baba, 2011a, b) as well as satisfied consumers' taste (Shori and Baba, 2012). Therefore, the present work was carried out to study the effect of *A. sativum* and *C. verum* water extracts on the changes of water holding capacity, susceptibility to syneresis, exopolysaccharides production and rheological properties in yogurt during 21 days of refrigerated storage.

MATERIALS AND METHODS

Materials and chemicals: Commercial fresh and pasteurized full cream cow milk (Dutch Lady, Malaysia) was, purchased from local supermarket. The herbs used in this present study were *C. verum* powdered bark and *A. sativum* powder (McCormick®, Malaysia) purchased from local store. Other supplies were commercial yogurt bacteria mixture (Chris-Hansen, Denmark) 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) which 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. All chemicals used in the proximate analysis were analytical grade and purchased from Sigma Chemical Co. (USA).

Water extraction of herbs: Powdered *C. verum* bark or *A. sativum* (10 g) was mixed thoroughly with 100 mL of distilled H_2O. The mixture was incubated for 12 h in a water bath at 70°C (Haake Model SWD 20) followed by centrifugation (Eppendoft 5804 R; 10000 rpm) for

15 min at 4°C. The pure supernatants harvested and used as *C. verum* or *A. sativum* water extracts in preparation of traditional yogurt.

Preparation of starter culture: Fresh and pasteurized full cream milk 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 preheated milk prior to an overnight incubation at 41°C. The yogurt formed was refrigerated (4°C) and used as starter culture within 3 days.

Preparation of yogurts: *C. verum*-or *A. sativum*-yogurt was prepared by mixing 10 mL of each herbal water extract with 85 mL of pasteurized full cream milk and 5 g of starter culture (Shah, 2003). The mixture was mixed thoroughly and incubated at 41°C until the pH of yogurt reached 4.5. Then, the incubation was terminated by placing the yogurts in ice-bath for 60 min. Yogurts were kept in the refrigerator (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_2O was used in place of herbal-water extract.

Isolation and quantification of exopolysaccharides: Exopolysaccharides (EPS) was isolated from yogurt samples using a modified procedure as described by Sawen *et al.* (2010). Yogurt sample (25 mL) diluted with dH_2O in the ratio of 1:1. The mixture boiled at 100°C for 10 min and cooled down for another 10 min. Then, 4 mL of 20% (w/v) trichloroacetic acid (TCA) added to the solution and allowed to rest for 2 h at room temperature. After centrifugation (10000×g for 30 min at 4°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 mixed with a double volume of cold ethanol and then stored at 4°C for 24 h. The precipitated EPS was collected by centrifugation (10000×g for 30 min at 4°C), dissolved in 10 mL dH_2O and mixed with a double volume of cold ethanol and stored at 4°C for 24 h. The precipitate EPS with ethanol was recovered by centrifugation at 4°C (10000×g for 30 min). Total EPS (expressed as mg L^{-1}) estimated in each sample by phenol sulphuric method (DuBois *et al.*, 1956) using glucose as a standard (Torino *et al.*, 2001).

Water holding capacity (WHC): The water holding capacity of yogurts samples was determined as described by Harte *et al.* (2003) with slight modifications. The yogurt (10 g) was subjected to centrifugation (10000 rpm, 15 min, at 4°C) in a centrifuge tube. The separated whey pipetted out and weighed. The WHC% was calculated by this equation:

$$STS(\%) = 1 - \left(\frac{W_1}{W_2}\right) x100$$

where, W_1 is weight of whey after centrifugation, W_2 is yogurt weight.

Susceptibility to syneresis (STS): Susceptibility to syneresis was determined as described by Isanga and Zhang (2009). Yogurt sample (10 g) placed on a filter paper and placed on top of a funnel. After 6 h of drainage, the volume of the whey collected in a beaker was measured and used as an index of syneresis. The STS was calculated by this equation:

$$STS(\%) = 1 - \left(\frac{V_1}{V_2}\right) x100$$

where, V_1 is volume of whey collected after drainage, V_2 is volume of yogurt sample.

Rheological measurements: The oscillation and viscometry measurements were, performed to determine the rheological behaviour of the set yogurt using Bohlin VOR controlled strain Rheometer (Malvern Instrument UK) with cone plate measuring geometry (4° 40 mm^{-1}). The geometry consists of a rotating lower plate and a fixed upper cone with a measurement gap of 0.150 mm. Temperature controlled at 20±0.1°C with Peltier Plate system (-40 to +180°C, Peltier Plate system from Bohlin Instrument Ltd.) that acts as temperature controller. A controlled shear/strain rate applied to the yogurt sample and the resulting stress response monitored. This shear rate maintained within a range of 0.3%. Prior to rheological analysis, yogurt samples were, gently stirred 10 times by spoon to avoid differences due to structural breakdown during handling of the yogurt.

OSCILLATION MEASUREMENT

Viscoelastic properties of yogurts were, monitored by dynamic oscillatory assays. Two types of oscillation test were run amplitude sweep and frequency sweep which depended on the comparison between the elastic modulus G' and the viscous modulus G" and the loss tangent is then calculated as:

$$\text{Tan } \delta = G''/G'$$

Amplitude sweep: This test first performed at a controlled strain mode with applied strain at a fix unit from range 0.0005 to 0.1 with a constant frequency of 0.5 Hz.

Frequency sweep: The frequency sweep performed at a controlled strain mode (a low deformation strain was chosen from the linear viscoelastic profile from amplitude sweep) with frequency varying from 0.01 to 10 Hz.

Apparent viscosity: The flow curves of the yogurts obtained by varying the shear rate from 0.01 to 80 sec^{-1} and the corresponding apparent viscosity values measured. These values represent the approximate viscosity felt in the mouth (Bourne, 2002).

Statistical analysis: All experiments performed in three batches. However, rheological measurements were determined in two batches. Subsequently, the average taken and data expressed as Mean±standard. The significance was established at $p<0.05$. Data analysis was, done using SPSS® version 17.0.

RESULTS AND DISCUSSION

Exopolysaccharides (EPS) production: The presence of *A. sativum* or *C. verum* water extract in fresh yogurt showed no significant differences ($p>0.05$) in EPS (95.6±0.4 and 85.4±0.2 mg L^{-1}, respectively) as compared to plain yogurt (87.2±0.3 mg L^{-1} Fig. 1). Cold storage of yogurt at 4°C increased ($p<0.05$) EPS in *C. verum* yogurt (176.8±0.7 mg L^{-1}) and

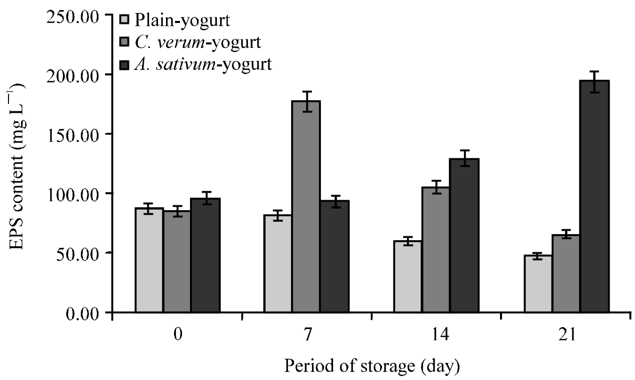

Fig. 1: Changes in exopolysaccharides (mg L^{-1}) of yogurt during 21 days of refrigerated storage (4°C). Values are presented as Mean±SEM (n = 3)

A. sativum yogurt (93.6±0.5 mg L^{-1}) as compared to plain yogurt (81.0±1.4 mg L^{-1}) on 7 days of storage. Prolonged storage of yogurt for two more weeks reduced ($p<0.05$) EPS of plain-and *C. verum*-yogurts (47.4±0.6 and 66.6±1.3 mg L^{-1} respectively) on day 21 of storage (Fig. 1). However, EPS of *A. sativum* yogurt showed significant increased (129.2±0.2 and 193.6±1.2 mg L^{-1}) on 14 and 21 days, respectively compared to plain yogurt. In yogurt, EPS produced in small amount but it does critically influence the development of texture of the final product because of their interaction with the free water in the gel-like structure (Girard and Schaffer-Lequart, 2007; Purwandari *et al.*, 2007). The EPS yield in yogurt found to be affected by strain and species of cultures used and growth condition (Aslim *et al.*, 2005; Degeest *et al.*, 2002; Mozzi *et al.*, 2003). The higher yield of EPS in presence of *A. sativum* and *C. verum* extracts in yogurt than in absence could be due to enhanced numbers ($p<0.05$) of yogurt culture (Shori and Baba, 2012). The differences in the types of EPS might be other reasons for the differences observed (Ramchandran and Shah, 2010). It can be speculated that *A. sativum* and *C. verum* water extracts containing different types of polysaccharide exerts the same synergistic effect on yogurt culture and induce higher EPS production. The decrease in EPS of *C. verum* yogurt on 14 and 21 days of storage, suggested to be attributed to reduction in strain of *lactobacillus* spp. (Shori and Baba, 2012).

Water holding capacity and susceptibility to syneresis: Whey separation is a critical parameter in yogurt manufacturing. WHC in fresh plain yogurt was (26.4±3.1%). The presence of *A. sativum* or *C. verum* in yogurt increased WHC to 33.9±2.8 and 26.6±1.2%, respectively (Fig. 2a). All yogurts tended to show persistent increase in WHC during storage. Refrigerated storage of yogurt over 21 days increased ($p<0.05$) WHC for *A. sativum* yogurt (38.1-41.5%) and *C. verum* yogurt (26.1-35.9%) as compared to plain yogurt (25.9-29.9%, Fig. 2a).

STS in fresh *A. sativum*-and *C. verum*-yogurt was 37.8 and 35.5% respectively whereas plain yogurt showed 38.1% of STS (Fig. 2b). STS of *C. verum* yogurt reduced to almost 24% on 7 and 14 days of storage. However, extended storage to 21 days resulted in increase ($p<0.05$) STS to 34%. On the other hand, *A. sativum* yogurt showed the highest value of STS (44%) on day 7 of storage, this followed by decreased ($p<0.05$) to about 23% on day 14 and 21 of storage (Fig. 2b). Harwalkar and Kalab (1986) and Hess *et al.* (1997) reported that increasing total

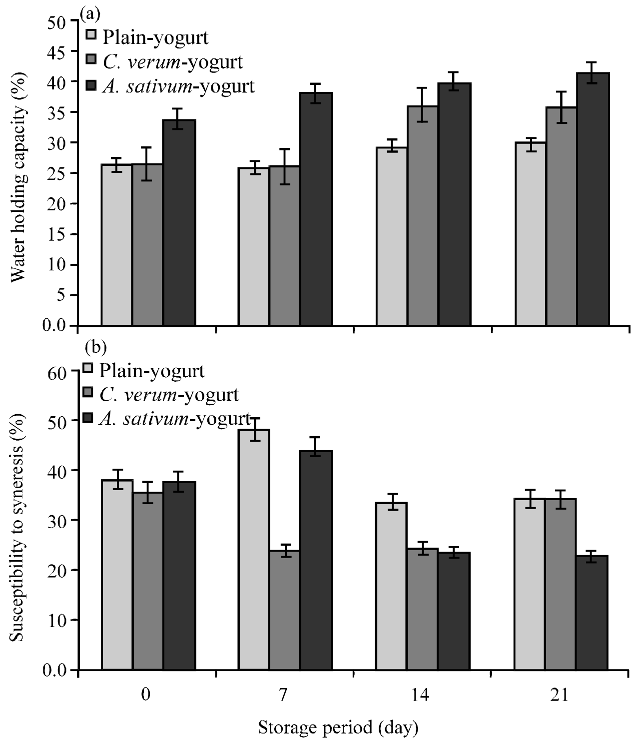

Fig. 2(a-b): Changes in water holding capacity% (a) and susceptibility to syneresis% (b) of yogurt during 21 days of refrigerated storage (4°C), Values are presented as Mean±SEM (n = 3)

solids can resulted in higher water holding capacity and can be further associated with reduced syneresis. Such phenomenon was only observed in *C. verum*-yogurt. Since the latter had higher ($p<0.05$) level of total solids during 21 days of storage (data not shown) demonstrated higher ($p<0.05$) percentage of WHC on day 14 and 21 days of storage and lower degree ($p<0.05$) of syneresis on 7 and 14 days of storage as compared to plain yogurt. In addition it can be suggested that higher EPS in presence of *A. sativum* or *C. verum* than plain yogurt (Fig. 1) whic acts as a hydrocolloids, may be one of the reasons of increase in WHC followed by lower STS over storage. Stabilizers such as pectin and gelatin usually added to the yogurt to prevent or decrease the separation of whey (Lucey, 2001). However, increasing consumer demand for more natural products (without additives or stabilizers) lead to search for another an alternative thus, addition of *A. sativum* or *C. verum* in yogurt could be a good substrate to decrease the whey separation during storage.

DYNAMIC RHEOLOGY

Amplitude sweep: Figure 3 shows the variations of elastic modulus (G') and viscous modulus (G'') of the yogurt subjected to strain% during 21 days of storage. Both G' and G'' were within the linear viscoelastic domain whereby G' had higher values than G'' for both plain-and herbal-yogurts. During refrigerated storage over 21 days plain yogurt showed a linear response (constant G') over a range of strain (0.008-0.009%) with G' values range from 76.98 to 105.43 Pa (Fig. 3a). The presence of *A. sativum* or *C. verum* in yogurt increased the linear

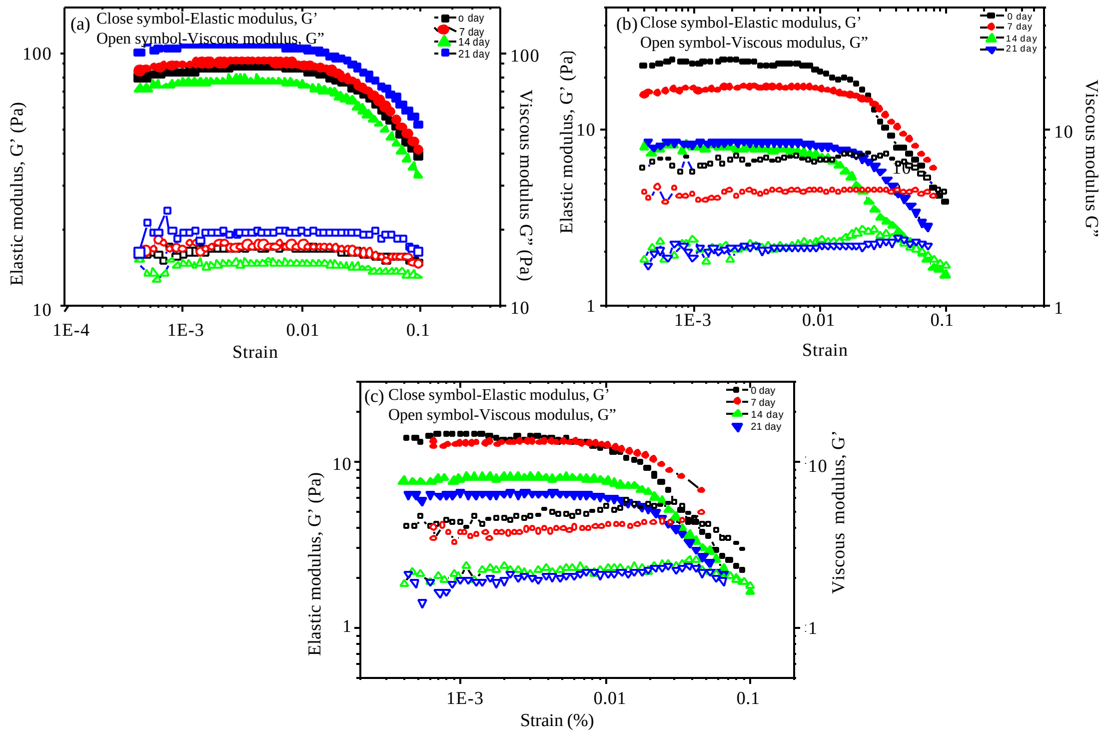

Fig. 3(a-c): Amplitude sweep: Elastic modulus (G') and viscous modulus (G") vs. strain% during 21 days of refrigerated storage at 4°C. (a) Plain yogurt, (b) *A. sativum* yogurt and (c) *C. verum* yogurt, Values are presented as mean (n = 2)

region over a wider range of strain (0.006-0.01%) with the highest G' values showed at 0 day of storage (23.85 and 13.96 Pa, respectively). The lowest G' value for *A. sativum* yogurt was 7.85 Pa on 14 days (Fig. 3b) and for *C. verum*-yogurt was 6.29 Pa; on 21 days (Fig. 3c). However, these linear region in yogurt in presence and absence of *A. sativum* or *C. verum* followed by non-linear downward inflexion at larger strain% until G' and G" crossed over. This indicated that there was ability of yogurts to become more fluid by increase structure breakdown with strain% increase over 21 days of storage. Tunick *et al.* (1990) suggested that the inflexion of the G' values is related to structure breakdown and the amount of stress that the sample can withstand before breaking down.

Frequency sweep: The frequency sweep test of all yogurts' samples at strain 0.01-1 Hz showed that both G' and G" were frequency depended which increased as frequency increases (Fig. 4). During storage, the elastic modulus (G') were higher than the viscous modulus (G") over the whole frequency range measured for plain-and herbal-yogurts. However, G' and G" values were higher in plain yogurt (Fig. 1a) than in *A. sativum* yogurt (Fig. 1b) and *C. verum* yogurt (Fig. 1c). In addition, the highest G' values of *A. sativum*-or *C. verum*-yogurt were observed on 0 and 7 days of storage but prolonged storage to the next two weeks resulted in reduction in the values. All yogurts' samples showed elastic behaviour (G'>G") over the whole range of frequencies tested which indicated solid like behaviour. The lower values of G' and G"

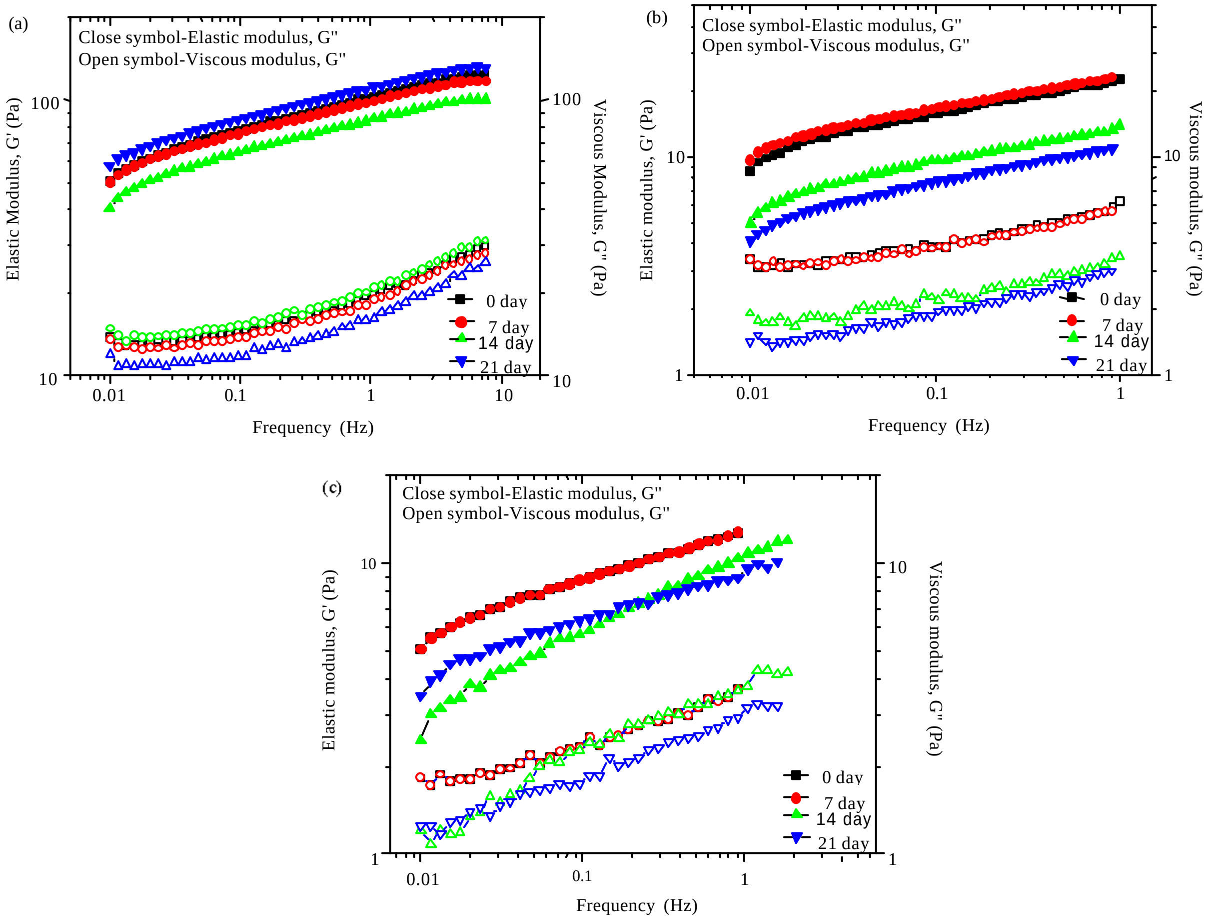

Fig. 4(a-c): Frequency sweep: Elastic modulus (G') and viscous modulus (G") vs. strain% during 21 days of refrigerated storage at 4°C. (a) Plain yogurt, (b) *A. sativum* yogurt and (c) *C. verum* yogurt, Values are presented as mean (n = 2)

in the presence of *A. sativum* or *C. verum* extracts in yogurt could possibly contributes to the disruptive effect of these herbs that causes a decrease of interactions between the protein aggregates (Hassan *et al*., 2003). In fact, the reactive phenolic compounds presence in herbs found to interact with milk protein (casein) in green tea, red wine and fruit beverage when supplement with milk (Alexandropoulou *et al*., 2006; Argyri *et al*., 2006; Cilla *et al*., 2008). Tan (δ) values in yogurts' samples ranged between 0.2-0.3 overall the frequency range during 21 days of storage (data not shown). This indicated that both plain-and herbal-yogurts were acting as concentrated amorphous polymer more than a gel (Steffe, 1996). Moreover, the constant values of tan delta for all yogurt samples refer to constant solid-like behaviour over the entire frequency range whic means these yogurts could behave more than a concentrated solution (Steffe, 1996).

Apparent viscosity: Viscosity profiles of yogurts' samples during refrigerated storage measured as a function of shear rate sweep (Fig. 5). The apparent viscosity of plain-and herbal-yogurts showed shear rate depended which decreased with shear rate increase. In

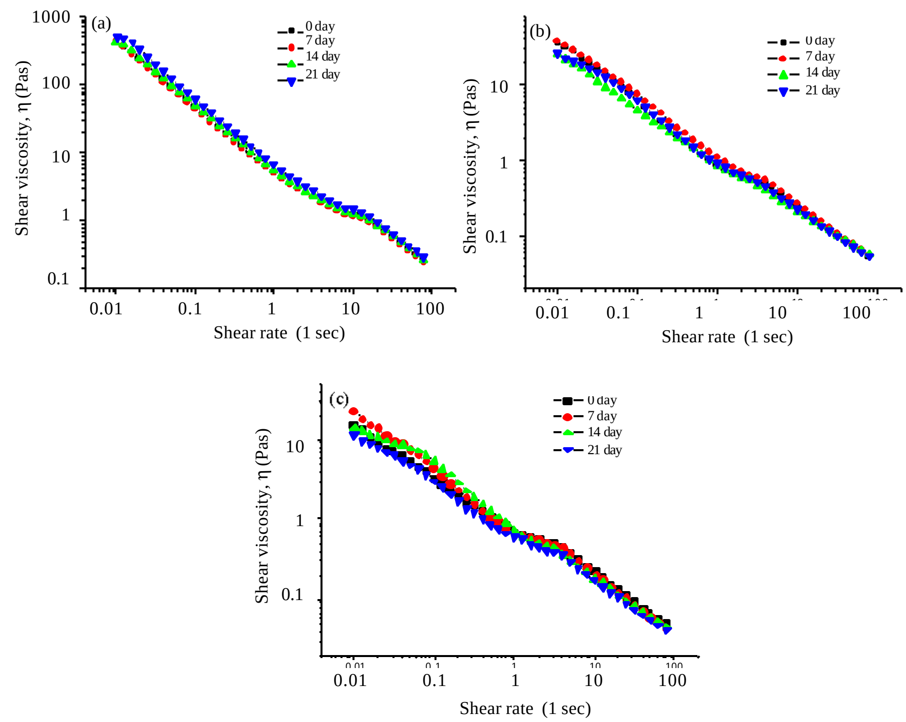

Fig. 5(a-c): Apparent viscosity vs. Shear rate (1 sec) during 21 of days refrigerated storage at 4°C. (a) Plain yogurt, (b) *A. sativum* yogurt and (c) *C. verum* yogurt, Values are presented as mean (n = 2)

addition, both yogurts in presence and absence of *A. sativum* or *C. verum* exhibited characteristic shear thinning behaviour. Refrigerated storage had slight effect on the viscosity profiles of plain-and herbal-yogurts. The shear thinning behaviour of yogurt samples was expected since the behaviour showed weekly-gel structure.

CONCLUSION

The presence of *A. sativum* or *C. verum* in yogurt improved some physicochemical parameters such as total acids, moisture content, total solids, exopolysaccharides and water holding capacity, syneresis as compared to plain yogurt during 21 days of storage. On the other hand, these herbal extracts exhibited negative effect on the yogurt viscosity and showed shear thinning behaviour over storage period.

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