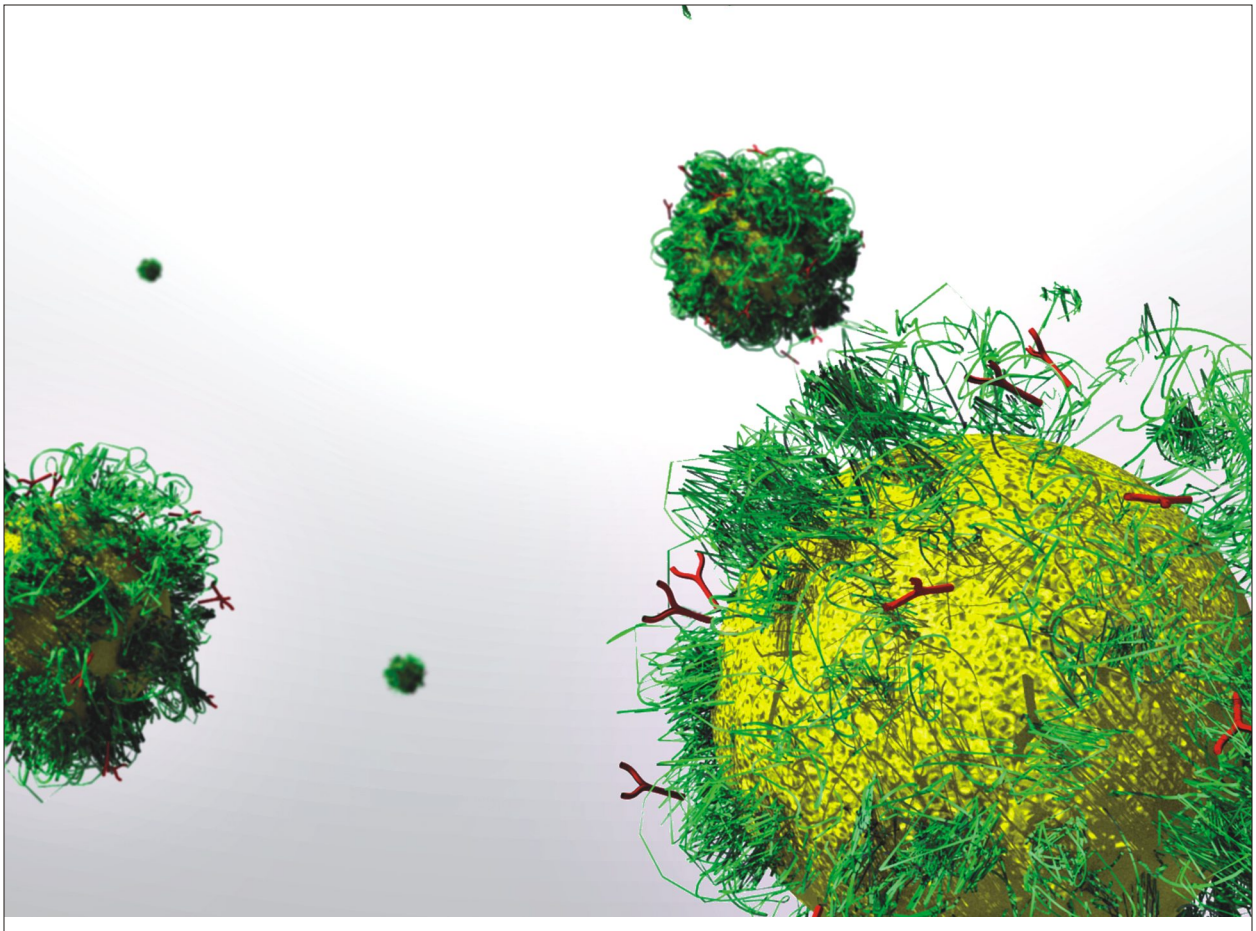

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Nutritional and Therapeutical Values of Chickpea Water Extract Enriched Yogurt Made from Cow and Camel Milk

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ABSTRACT

Chickpea has long been consumed as source of dietary protein and it has also been linked to reduced risk of several diseases. In the present study, the effect of chickpea water extract addition into cow and camel milk-yogurt on acidification activity, Total Phenolic Content (TPC), antioxidant activity (1, 1-diphenyl-2-picrylhydrazyl (DPPH) inhibition) and the viability of lactic acid bacteria (LAB) during 0, 7, 14 and 21 days of refrigerated storage (4°C) were investigated. In addition, sensory analysis of yogurt was evaluated after first day of storage. The pH values showed slightly decreased in chickpea-cow-and camel-milk yogurt during refrigerated storage compared to control (Plain-yogurt). The Total Acid (TA) enhanced ($p<0.05$) in presence of chickpea water extract into cow-and camel-milk yogurt more than respective control. TPC and antioxidant activity of yogurt were higher ($p<0.05$) in presence of chickpea water extract in both types of yogurt than control. In addition, there were significant losses in the cell numbers of *Lactobacillus* spp. in both presence and absence of chickpea water extract in camel milk-yogurt more than in cow milk-yogurt. The growth of *S. thermophilus* in chickpea-cow milk yogurt improved significantly during refrigerated storage. However, the viable cell counts of *S. thermophilus* in chickpea-camel milk yogurt reduced after 7 days of storage. The presence of chickpea water extract showed more affected on the sensory evaluation in cow milk yogurt than in camel milk yogurt. In conclusion, the inclusion of chickpea water extract into cow-and camel-milk yogurt could improve nutritional and biological quality of yogurt which could be utilized by the food industry as functional yogurt.

Key words: Yogurt, chickpea, antioxidant activity, total phenolic content, *S. thermophilus*, *Lactobacillus* spp.

INTRODUCTION

Biotechnological research on food has played important role in the development and creation of products enriched with nutrients (Sloan, 2002) and therapeutic benefits (Hasler, 2002). Yogurt is a fermented milk product often regarded as a nutritious food because of the fermentative action of Lactic Acid Bacteria (LAB) and their metabolites (Lourens-Hattingh and Viljoen, 2001). In addition, it is able to enhance digestion (Gibson *et al*., 1997; Halliwell, 1992), vitamin and mineral absorption and can be taken daily to boost the body health (Isolauri *et al*., 2001).

Yogurt manufacture from cow's milk is the most widely used in many countries. Other milk types such as camel, buffalo and goat are becoming increasingly available because of their unique taste and therapeutic values (Ebing and Rutgers, 1991). Camel milk besides being part of the staple diet in parts of Africa and Asia is also considered as health promoting. The

health benefits associated with camel milk consumption was suggested due to the presence of high concentration of insulin/insulin like protein (~52 units L^{-1}; Agrawal *et al*., 2005). It contains all essential nutrients and the composition is similar to that of cows' milk (Yagil, 1982). However, camel milk has special properties not found in cow milk including lower cholesterol (Abu-Lehia *et al*., 1989), higher antibacterial and antiviral properties (El Agamy *et al*., 1992), higher vitamin C content (Kumar *et al*., 2009; Wernery *et al*., 2005), higher levels of immunoglobulin, lactoferrin, lysozyme, lactoperoxidase a nd peptidoglycan recognition protein (Agrawal *et al*., 2004). In addition, no allergic responses were reported in camel milk due to low amount of β-lactoglobulin which is one of the major allergens in cow's milk (Al-Alawi and Laleye, 2011; Shabo *et al*., 2005).

Recently, several studies showed that the inclusion of fruits and medicinal herbal extracts into milk during fermentation have notable changes in yogurt properties such as organoleptic (Zainoldin and Baba, 2009; Shori and Baba, 2011a), microbial content (Behrad *et al*., 2009; Shori and Baba, 2012) and improve the therapeutical values of the final products (Zainoldin and Baba, 2009; Behrad *et al*., 2009; Shori and Baba, 2011a, b, 2012; Amirdivani and Baba, 2011; Shori *et al*., 2012, 2013). Chickpea is one of the most important legumes (FAO, 1994) and it was originated in the Middle East about 7500 years ago. It has been considered as a balanced diet not only because it is a valuable source of protein (15-30%) (Chavan *et al*., 1987; Fernandez and Berry, 1988) but it is also a good source of carbohydrates, minerals and trace elements (Huisman and Van der Poel, 1994; Williams and Singh, 1988). Fermented chickpea with different microorganism has been studies in several researches to improve the microbial growth (De Leon *et al*., 2000; Hatzikamari *et al*., 2007; Emerging Food R and D Report, 2004) and antioxidant properties (Fernandez-Orozco *et al*., 2009). Therefore, the objective of this research was to study the effect of addition of chickpea water extract in yogurt made from cow and camel milk on acidification activity, total phenolic content, antioxidant activity and viability of lactic acid bacteria (*Lactobacillus* spp. and *Streptococcus thermophilus*) during 0, 7, 14 and 21 days of refrigerated storage at 4°C. In addition, sensory evaluation of yogurt was investigated after first day of storage.

MATERIALS AND METHODS

Materials and chemicals: Chickpea used in the current study obtained from local store in Malaysia. Commercial fresh and pasteurized cow milk (Dutch Lady, Malaysia) and camel milk (Al-Turath, Saudi Arabia). Further supply incorporated in the present study was commercially available yogurt bacteria mixture (Chris-Hansen, Denmark) containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* Bb-12, *Lactobacillus casei* LC-01 and *Streptoccus thermophilus* Th-4 in the ratio of 4:4:1:1. In addition, probiotic mixture (Bio-Life, Malaysia) was purchased from local store in Malaysia. One capsule contents 5 billion cfu of probiotic bacteria including *L. bulgaricus*, *L. rhamnosus*, *B. infantis* and *B. longum* in the ratio of (1:1:1:1). The chemical compounds used in this study were 1, 1-Diphenyl-2-Picrylhydrazyl Radical (Sigma, St. Louis, MO), Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), MRS and M17 agar and buffered peptone (Oxoid, Basingstoke, Hampshire, England).

Chickpea water extracts preparation: The yogurt water extract was prepared according to Martini *et al*. (1987). Chickpea powder (10 g) was dissolved in 100 mL distilled water and incubated overnight in water bath at 70°C (Julabo, Model Sw-21 c). The mixture centrifuged at 10000 rpm for 15 min. The supernatant was used in the preparation of chickpea-yogurt.

Preparation of starter culture: Commercial fresh and pasteurized full cream milk (1 L) content 3 and 4% fat for both camel and cow-milk, respectively were heated to 41°C. Subsequently, a mixture of yogurt bacteria mix and a capsule of probiotic mix were added and the mixture incubated for 12 h at 41°C in water bath. The yogurt formed refrigerated at 4°C and used as yogurt starter culture within 3 days (Rashid *et al.*, 2007).

Preparation of yogurt: Chickpea-yogurt was prepared by mixing 10 mL of chickpea water extract, 85 mL of commercial pasteurized full cream milk and 5 g of starter culture (Shah, 2003). The mixture was homogenized thoroughly and incubated at 41°C. The pH was measured every 30 min until pH reached 4.5. Chickpea-yogurt was placed in ice-bath to cool for 60 min. Then, yogurt was kept in the refrigerator for up to 21 days. The plain yogurt (control) was prepared following the same methodology except that 10 mL of distilled water was used in place of chickpea water extract. The all analysis was carried out every 0, 7, 14 and 21 days of storage at 4°C.

Preparation of yogurt water extract: Ten gram of yogurt was added to 2.5 mL of distilled H_2O and the pH was adjusted to 4.0 with 1 M HCl. The mixture was incubated in water bath at 45°C for 10 min. After centrifuged at 4°C (10000 rpm, for 10 min) the supernatant was adjusted to pH 7.0 by NaOH (0.5 M) followed by second centrifugation at 4°C (10000 rpm, for 10 min). The supernatant was used for analysis within 12 h of preparation.

Measurement of pH and total acid (TA): The pH of all yogurt samples was determined at room temperature with a digital Metler Toledo 320 pH meter (Kailasapathy, 2006). The total acid in yogurt was measured.

Total phenolic content assay: Total phenolics were determined as gallic acid equivalents (Shetty *et al.*, 1995). One milliliter of yogurt water extract was transferred to a test tube and 1 mL of 95% ethanol; 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent 50% v/v were added and the mixture was allowed to stand at room temperature for 5 min. Thereafter, 1 mL of 5% Na_2CO_3 was added and the mixture was kept in the dark for an hour. The absorbance measured at 725 nm using Spectrophotometer (Shimadzu UV Mini 1240). The standard calibration curve was plotted using gallic acid at the concentrations of 10-60 μg mL^{-1}. The TPC was expressed in micrograms equivalents of gallic acid per gram (μgGAE g^{-1}) sample.

Measurement of antioxidant activity by DPPH inhibition assay: The antioxidant activities in yogurt were measured by using DPPH method that measuring the free radical scavenging ability of yogurt water extract (Shetty *et al.*, 1995). To 3 mL of 60 μm DPPH in ethanol, 250 μL of water yogurt extract was added and the mixture was allowed to stand in the dark at room temperature for several min. The absorbance was monitored at 517 nm against controls, which contained 250 μL of ethanol instead of the extract. The percentage of inhibition was calculated (Shetty *et al.*, 1995) as follows:

$$\text{Inhibition}\,(\%) = \frac{A_{517}^{\text{control}} - A_{517}^{\text{extract}}}{A_{517}^{\text{control}}} \times 100$$

Viability determination of LAB: Yogurt sample prepared in buffered peptone water by mixing 1 mL of yogurt with 9 mL of 0.15% sterile buffered peptone water. The mixture was thoroughly

stirred and serial dilutions were prepared by using buffered peptone water. The viability of *S. thermophilus* and *Lactobacillus* spp. were enumerated as described by Kailasapathy *et al.* (2008) and Rybka and Kailasapathy (1995). The agar were selected as suitable for the viability study included de Man Rogosa and Sharpe agar (MRS) for *Lactobacillus* spp. enumeration and M17 agar containing lactose solution (10% w/v) for *S. thermophilus* enumeration. Both *Lactobacillus* spp. and *S. thermophilus* were anaerobic (Kailasapathy *et al.*, 2008) and aerobic (Rybka and Kailasaphaty, 1995) incubated, respectively at 37°C for 48 h.

Sensory analysis: The sensory evaluation of yogurt was performed after first day of refrigerated storage. An untrained panel of 12 assessors recruited from students of University of Malaya. Their age range between 19 and 24 years old (mean age was 22). The evaluation form was given to each panel included description of 7 attributes i.e., texture, color, taste (sour, sweet, bitter), aroma and overall preference. The panels evaluated 2 groups of yogurt (cow milk yogurt and camel milk yogurt) and each group contained 2 coded yogurt samples served in plastic cups (10 mL for each). The first and second groups contained plain-and chickpea-yogurt made from cow and camel milk, respectively. Water was available for panel members to rinse their mouth between samples. The evaluation was scored on 1-10 point hedonic scale according to Larmond (1970) where 1-2 = extremely poor, 3-4 = poor, 5-6 = fair, 7-8 = good, 9-10 = excellent.

Statistical analysis: The conducted experiments were carried out in three different batches of yogurt (n = 3). Data were expressed as Mean±SME. All the data obtained were subjected to ANOVA using SPSS data analysis software system, version 17.0. Significant differences for mean comparison were determined by using Duncan's *post hoc* test at $p<0.05$.

RESULTS AND DISCUSSION

Acidification activity measurement: No significant differences in pH values of fresh yogurt (0 day) both in presence and absence of chickpea water extract (Fig. 1). Refrigerated storage

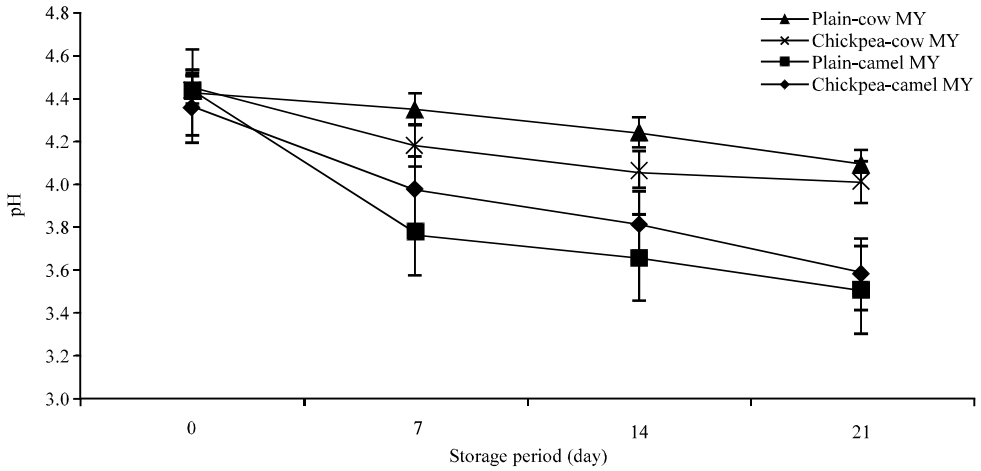

Fig. 1: Changes of pH in yogurt during refrigerated storage at 4°C, Values are presented as Mean±SEM (n = 3), For all treatment, ANOVA showed no significant effect at 5% level during all period of storage, *MY = Milk yogurt

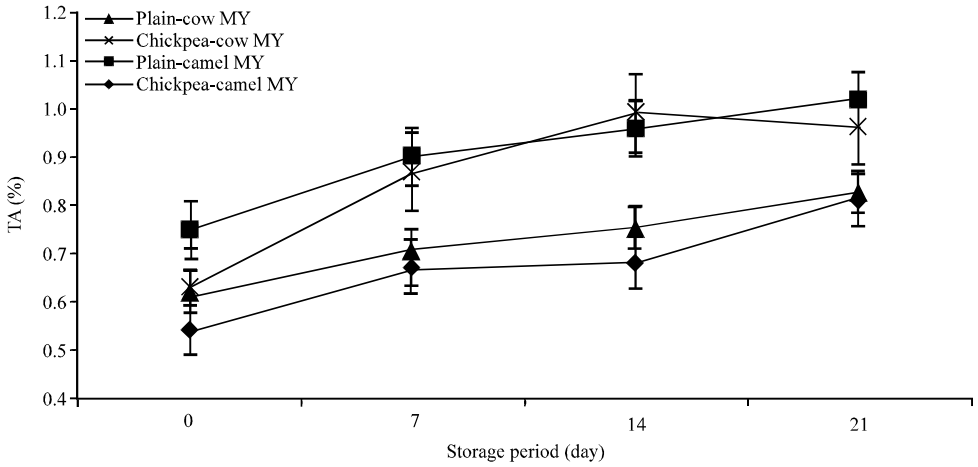

Fig. 2: Changes of total acid (TA%) during refrigerated storage at 4°C, Values are presented as Mean±SEM (n = 3), For all chickpea-cow milk yogurt (overall period of storage) and chickpea-camel milk yogurt (7, 14 and 21), ANOVA showed a significant effect at 5% level, *MY = Milk yogurt

slightly decreased pH values in both types of yogurt. However, the presence of chickpea water extract into yogurt showed no significant difference in pH reduction compared to control yogurt overall storage period (Fig. 1). In the other hand, TA showed significant increased in chickpea-cow milk yogurt compared to control (Fig. 2). Although fresh chickpea-camel milk yogurt showed almost same TA value compared to control (0.6%) however, storage at 4°C increased ($p<0.05$). TA values in chickpea-yogurt more than in control overall storage period (Fig. 2). Besides, the presence of chickpea water extract in yogurt enhanced the increase in TA of yogurt made from cow milk more than camel milk. Besides, the presence of chickpea water extract in yogurt enhanced the increase in TA of yogurt made from cow milk more than camel milk (i.e., lactose) and protein products (Lourens-Hattingh and Viljoen, 2001; Papadimitriou *et al*., 2007) during fermentation and refrigerated storage (Saint-Eve *et al*., 2008).

Total phenolic content: Folin-Ciocalteu assay was used to measure the total phenolic content in yogurt water extract. This assay is one of the methods widely used for quantitative analysis of total phenols in foods and any oxidisable group, principally phenolic hydroxyl can be determined (Asami *et al*., 2003). In the present study, the inclusion of chickpea water extract into yogurt increased ($p<0.05$) TPC compared to control in both types of yogurt (Fig. 3). TPC in fresh plain-cow-and camel-milk yogurt were 31.1±0.01 and 60.0±0.01 μg GAE g^{-1}, respectively. However, the presence of chickpea water extract increased ($p<0.05$; 0 day) TPC to 38.0±0.1 and 124.7±1.8 μg GAE g^{-1} of chickpea-cow-and camel-milk yogurt, respectively (Fig. 3). Refrigerated storage had minimum increased in TPC of chickpea-cow milk yogurt (41.6±1.8-47.2±0.7 μg GAE g^{-1}; from 7 to 21 days of storage) whereas chickpea-camel milk yogurt showed small reduction ranged from 118.7±0.7 to 100.6±0.6 μg GAE g^{-1} (Fig. 3). In addition, TPC in the presence of chickpea water extract in camel milk yogurt was about 3 folds higher than in cow milk yogurt during the first two weeks of storage.

The present study showed that chickpea water extract had TPC about 127 μg GAE g^{-1} (data not shown). In addition, previous study reported that chickpea flour has high content of

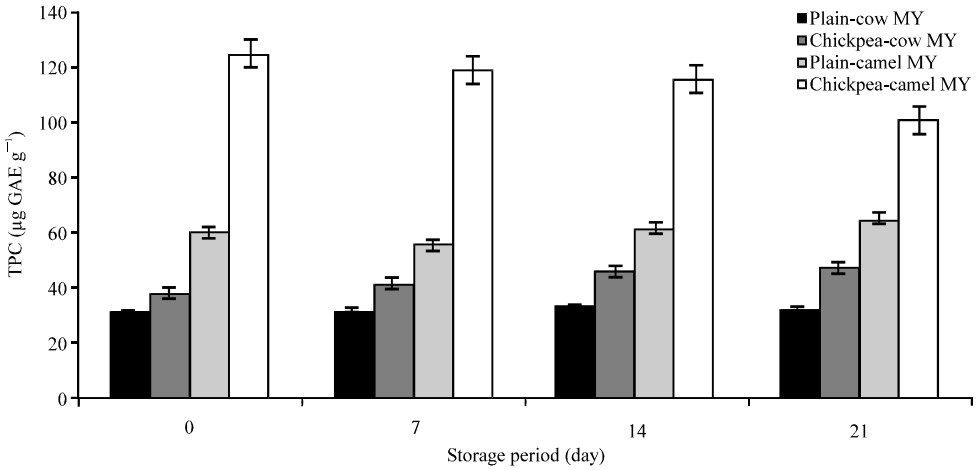

Fig. 3: Changes of total phenolic content (µg GAE g^{-1}) in yogurt during refrigerated storage at 4°C, Values are presented as Mean±SEM (n = 3), For both cow and camel-milk yogurt in present of chickpea ANOVA showed a significant effect at 5% level compared to respective control overall period of storage, *MY = Milk yogurt

phenolic ompounds during fermentation process (Fernandez-Orozco *et al.*, 2009). This could explain the higher content of pohenolic compounds in the presence of chickpea water extract in yogurt than in the absence. Besides, the current results are in agreement with other researches that reported higher TPC in presence of some plant water extracts such as *Allium sativum*, *Cinnamomum verum*, *Azadirachta indica*, peppermint, dill and basil than plain yogurt (Shori and Baba, 2011a, b; Amirdivani and Baba, 2011). Consumption of foods with a highly content of phenolic compounds are reported to decrease the risk of some diseases such as cardiovascular and cancer (Kris-Etherton *et al.*, 2002; Remesy *et al.*, 1998).

Antioxidant activity: Fresh plain cow-and camel-milk yogurt showed antioxidant activity about 26 and 15%, respectively (Fig. 4). The inclusion of chickpea water extract increased ($p<0.05$) antioxidant activity to 37 and 56% for cow-and camel-milk yogurt, respectively. The highest increase of antioxidant activity in the presence of chickpea water extract during refrigerated storage was observed after 14 days for cow milk-yogurt (59.8±2.9%) and 7 days for camel milk-yogurt (67.6±1.7%, Fig. 4). However, plain cow-and camel-milk yogurt was showed the highest antioxidant activity on day 14 of storage (30.4±1.8 and 58.53±1.4%, respectively). The high liberation of phenolic compounds in the presence of chickpea water extract in yogurt may be contributed to higher antioxidant activity than in the absence. These results are confirmed with other study such as Shori and Baba (2011a, b), Amirdivani and Baba (2011) and Zainoldin and Baba (2009). Although, the antioxidant activity of chickpea water extract was low about 14.1±2.2% (data not shown) however, nitrogenous compounds from protein breakdown balance between their formation and degradation to volatile and nonvolatile compounds may have related to antioxidant activity (Virgili *et al.*, 2007). Antioxidants in dietary products were found to be positively correlated with anti-diabetic properties (Shetty *et al.*, 2006). Therefore, the consumption of high antioxidant properties present in chickpea-yogurt may be expected to play a role in the management of type 2 diabetes.

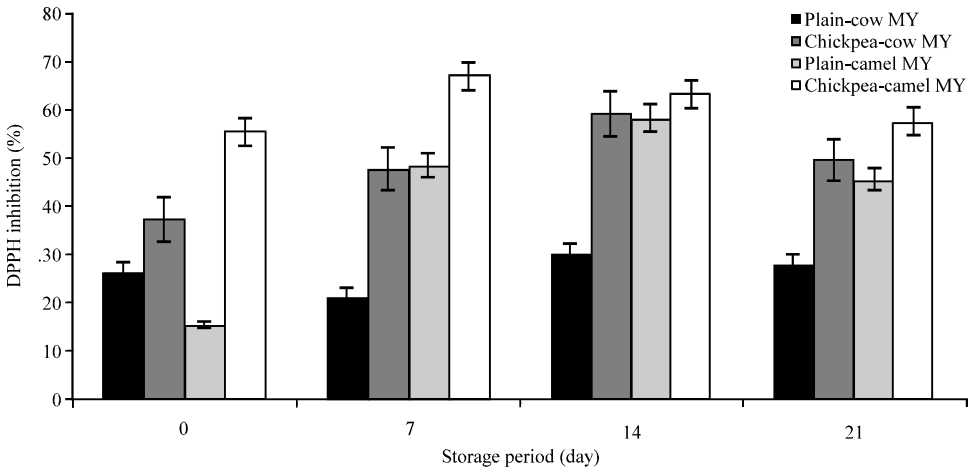

Fig. 4: Changes of antioxidant activity in yogurt (DPPH inhibition%) during refrigerated storage at 4°C, Values are presented as Mean±SEM (n = 3), For both cow-and camel-milk yogurt in present of chickpea ANOVA showed a significant effect at 5% level during all period of storage, *MY = Milk yogurt

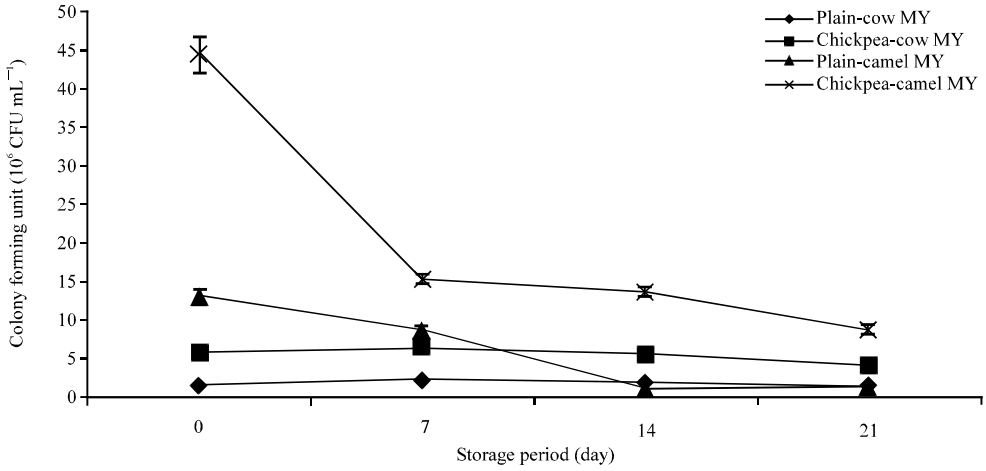

Fig. 5: Changes of bacterial counts of *Lactobacillus* spp. (10^6 CFU mL^{-1}) during refrigerated storage at 4°C, Values are presented as Mean±SEM (n = 3), For both cow-and camel-milk yogurt in present of chickpea ANOVA showed a significant effect at 5% level during all period of storage, *MY = Milk yogurt

Survival of lactic acid bacteria: Fresh chickpea-cow milk yogurt showed higher ($p<0.05$) *Lactobacillus* spp. cell counts (5.83×10^6 CFU mL^{-1}) than control (1.40×10^6 CFU mL^{-1}; Fig. 5). Storage at 4°C showed small increased in viability of *Lactobacillus* spp. on day 7 of storage (2.23×10^6 and 6.53×10^6 CFU mL^{-1}) for plain-and chickpea-cow milk yogurt, respectively. However, slight reduction was showed on the next 2 weeks of storage (Fig. 5). In the other hand, this results showed there were significant losses in the cell numbers of *Lactobacillus* spp. in camel milk yogurt both in presence and in absence of chickpea water extract (Fig. 5). The average viable cell counts

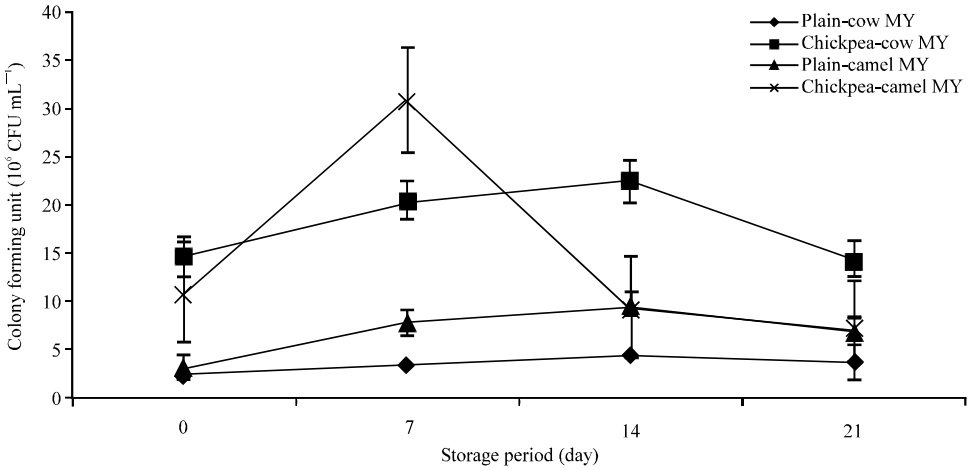

Fig. 6: Changes of bacteria counts of *Streptococcus thermophilus* (10^8 CFU mL^{-1}) during refrigerated at 4°C storage. Values are presented as Mean±SEM (n = 3). For chickpea-cow milk yogurt (overall storage period) and chickpea camel milk yogurt (0 and 7 days), ANOVA showed a significant effect at 5% level. *MY = Milk yogurt

of *Lactobacillus* spp. in fresh plain-camel milk yogurt were 13.19×10^6 CFU mL^{-1}. However, this value decreased ($p<0.05$) rapidly to 8.84×10^6 CFU mL^{-1} on day 7 of storage. Prolonged storage to more two weeks showed reduction in *Lactobacillus* spp. cell counts to almost 1.2×10^6 CFU mL^{-1}. Similarly, the viable cell counts of *Lactobacillus* spp. in the presence of chickpea water extract in camel milk yogurt were 44.37×10^6 CFU mL^{-1} on 0 day. Refrigerated storage had significant effect by decreasing the cell counts of *Lactobacillus* spp. overall storage period up to 8.78×10^6 CFU mL^{-1} on day 21 of storage.

In contrast, the viable cell counts of *S. thermophilus* were higher ($p<0.05$) in fresh chickpea-cow milk yogurt (14.5×10^8 CFU mL^{-1}) than in fresh plain-cow milk yogurt (2.4×10^8 CFU mL^{-1}; Fig. 6). Refrigerated storage increased ($p<0.05$) viability of cells growth to the highest counts were shown on day 14 of storage (4.3×10^8 CFU mL^{-1} for plain-yogurt and 22.3×10^8 CFU mL^{-1} for chickpea-yogurt). However, prolonged storage to 21 days showed reduction in *S. thermophilus* cell counts to 3.7×10^8 and 14.1×10^8 CFU mL^{-1} for plain-and chickpea-yogurt, respectively (Fig. 6). In the other hand, the inclusion of chickpea water extract into camel milk yogurt showed higher *S. thermophilus* cell counts (10.6×10^8 CFU mL^{-1}; 0 day) than control yogurt (3.1×10^8 CFU mL^{-1}; Fig. 6). After the first week of storage the growth of *S. thermophilus* increased ($p<0.05$) in both presence (30.6×10^8 CFU mL^{-1}) and absence (7.7×10^8 CFU mL^{-1}) of chickpea water extract. Prolonged the storage up to 21 days showed reduction ($p<0.05$) in the cells counts of chickpea-yogurt (7×10^8 CFU mL^{-1}; on 21 days).

Djeghri-Hocine *et al.* (2007) found that chickpea is efficient to stimulate LAB growth that related to its protein content. However, during fermentation and refrigerated storage number of *Lactobacillus* spp. and *S. thermophilus* could be affected by the chemical composition of the two sources of milk and pH level (Hassan *et al.*, 2008; Abdelgadir *et al.*, 2008; Ashmaig *et al.*, 2009). Several studies reported that, the survival of *Lactobacillus* spp. are normally reduce to lower counts than in fresh yogurt by the 14th day of refrigerated storage (Kailasapathy and Sultana, 2003; Laniewska-Trokenheim *et al.*, 2010) while, the survival of *S. thermophilus* increase throughout the first 14 days of storage (Dave and Shah, 1997; Birillo *et al.*, 2000). This finding is in agreement to

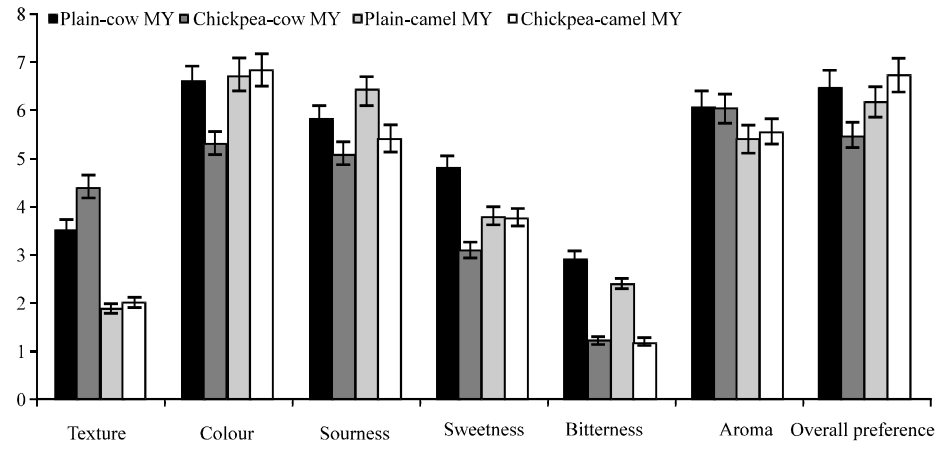

Fig. 7: Mean taste panel scores for chickpea-yogurt versus control (plain-yogurt) made from cow and camel milk during the first day of storage, Values are presented as Mean±SEM (n = 12), A 1-10 point hedonic scale (1-2 = extremely poor, 3-4 = poor, 5-6 = fair, 7-8 = good, 9-10 = excellent)

the present result. Higher viability of LAB in camel milk-than in cow milk-yogurt during storage may be explained by a slower pH reduction in camel milk yogurt than in cow milk yogurt (Fig. 1). Similar observation was obtained by Shori and Baba (2012). Besides, high levels of essential growth factors in the form of peptides and amino acids in camel milk yogurt may have promoted the growth of *Lactobacillus* spp. and *S. thermophilus* compared to cow milk yogurt (Shori and Baba, 2011b).

Sensory evaluation: The sensory evaluation of yogurt has become an important criterion for consumers to making the purchasing decision whereas yogurt type influenced consumers' preferences to a low extent (Majchrzak *et al.*, 2010). The inclusion of chickpea water extract into yogurt showed significant improved in the texture of yogurt made from cow milk but not camel milk (Fig. 7). The panelists observed that less ($p<0.05$) sour and bitter taste in chickpea-yogurt made from both cow and camel milk than respective control. According to the panelists, the presence of chickpea water extract in yogurt reduced ($p<0.05$) the sweetness, colour and overall preference of yogurt made from cow milk but not camel milk (Fig. 7). Chickpea-yogurt had no differences in aroma compared to control both in cow-and camel-milk yogurt. The growth of LAB during milk fermentation may be attributed to the changes in sensory parameters (Donkor *et al.*, 2007). However, chickpea water extract could be a good additive to enhance the quality of yogurt especially in yogurt made from camel milk more than cow milk.

CONCLUSION

Acidification of yogurt was improved in the presence of chickpea water extract which was more in cow milk-than in camel milk-yogurt. Furthermore, this study concluded that inclusion of chickpea water extract into yogurt appeared to increase TPC and antioxidant activity as well as supported the cells growth of *Lactobacillus* spp. and *S. thermophilus* in both cow-and camel-milk yogurt. The addition of chickpea water extract has been affected the sensory evaluation of cow milk yogurt more than camel milk yogurt.

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