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Effect of carrot juice and stabilizer on the physicochemical and microbiological properties of yoghurt

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ABSTRACT

Yoghurt fortification with fruits and vegetables has high potential to improve the nutrients and health promoting effects of the yoghurt. The effects of carrot juice (0, 10, 15 and 20%) and gelatin stabilizer (0.5, 0.6 and 0.7%) (w/w, base milk) addition on the properties of 12 yoghurt samples in a 3 × 4 factorial arrangement were investigated. Addition of carrot juice increased pH and syneresis significantly, but decreased titratable acidity (TA) and total viable counts (TVC). The TA and TVC were higher than minimum recommended of 0.6% lactic acid and 6 $\log_{10}$ CFU g^{-1}, respectively for yoghurt. Coliform, yeast and mold counts were <10 CFU g^{-1}. Syneresis decreased with stabilizer addition ($p < 0.01$). With 10 to 20 percent carrot juice addition, the total carotenoid content (mg/kg) increased (6.73 and 10.26, respectively) compared to control (3.05) ($p < 0.05$). However, the effects of carrot juice and stabilizer additions on total phenolic contents and antioxidant ferric reducing power were insignificant ($p > 0.05$). The results showed that yoghurt with suppressed syneresis and improved nutritional and total carotenoids contents can be processed from 10 to 15 percent carrot juice and 0.7 percent stabilizer additions.

1. Introduction

The development of diversified dairy products such as fruits and vegetables fortified yoghurt has a significant contribution for the dairy sector development in Ethiopia. The healthy food image of yoghurt is due to its probiotic effects which include protection against gastrointestinal upsets, enhanced digestion of lactose, decreased risk of cancer, lower blood cholesterol, improved immune response, enhanced short chain fatty acids (SCFAs) production, assimilation of protein and calcium (Granato, Branco, Cruz, Faria, & Shah, 2010; Gahruie, Eskandaria, Mesbahi, & Hanifpour, 2015).

Yoghurt with no added flavor is predominantly sour due to the lactic acid produced by fermentation. For better acceptance, fruits, flavoring agents and sweeteners are added to yoghurt to improve flavor balance and mask partially acetaldehyde flavor of yoghurt (Routray & Mishra, 2011). Various evidences demonstrate that fruits and vegetables intakes are associated with an improved health because of various nutrients and bioactive phytochemicals (Sun-Waterhouse, 2011). Thus, yoghurt are fortified with various fruits (Ayar, Sert, Kalyoncu, & Yazici, 2006; Oliveira et al., 2015), fruit seed extracts (Chouchouli et al., 2013) and vegetables (Puvanenthiran, Stevovitch-Rykner, McCann, & Day, 2014; Gahruie et al., 2015) to enhance positive health promoting effects of the yoghurt.

Carrot is rich in β-carotene and bears ascorbic acid, tocopherol and is classified as vitaminized food (Sharma, Karki, Singh, & Attri, 2012). It also bears carbohydrates, calcium, phosphorus, iron, potassium, magnesium, copper, manganese, sulfur and phenolic compounds, but it is deficient in protein and fat. Yoghurt is rich in protein, fat, calcium, potassium, B vitamins (B1, B2, B6, nicotinic and pantothenic acids) but is deficient in iron, vitamin C, carotenes and dietary fibers (Gahruie et al., 2015). Thus, combination of carrot juice and yoghurt will improve nutritional and functional food characteristics of the yoghurt. Salwa, Galal, and Neimat (2004) have studied the effect of carrot juice blending ratio on the shelf life and

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sensory properties of yoghurt and reported that shelf life and consumer acceptance were improved with 15% carrot juice addition. However, an attempt to reduce syneresis was not made which is a defect in yoghurt processing. To suppress syneresis, addition of stabilizers like gelatin or other hydrocolloids that function as a gelling agent or thickener provide good stability and desirable yoghurt textures (Routray & Mishra, 2011). Ares et al. (2007) showed addition of gelatin at a level of 0.6% (w/w) into yoghurt has suppressed incidence of syneresis. Therefore, in this work, the effects of carrot juice and stabilizer levels on the physicochemical, microbiological and functional (probiotic, total carotenoid, total phenolic and antioxidant ferric reducing power) properties of twelve yoghurt formulations are reported.

2. Materials and methods

2.1. Milk samples and ingredients

Fresh whole cow's milk was collected from Haramaya University dairy farm, Ethiopia. Fresh carrots (*Daucus carota* L. cv. Nantes) were purchased from farmer's field located near Haramaya University, stabilizer (gelatin with 240 Blooms manufactured in Brazil by Bake Mate) and sweetener (cane sugar) were purchased from a supermarket. Freeze-dried yoghurt starter culture (YC-X11 CHR HANSEN) was purchased from chemical suppliers (Nile Star Import and Export, Addis Ababa).

2.2. Experimental design

The experiment was conducted in triplicate in a completely randomized design of 3 × 4 factorial combinations of gelatin (0.5, 0.6 and 0.7 g per 100 g of milk) and carrot juice (0, 10, 15 and 20 g per 100 g of milk).

2.3. Preparation of carrot juice

Carrot roots were washed thoroughly, ends removed, peeled by sharp knife, cut longitudinally into halves and blanched at 90 °C for 5 min to tenderize carrot tissues and inactivate pectinase and peroxidase enzymes (Salwa et al., 2004). Carrot juice was extracted in a mechanical blender with sieves (Type 6001×, model No. 31JE35 6 × .00777, USA) and analyzed for moisture, total soluble solids (TSS), titratable acidity, total sugar, total phenolics and total carotenoid contents.

2.4. Yoghurt processing

Prior to yoghurt processing, all equipment used was sterilized in an autoclave after thorough wash cleaning. Heat sensitive materials such as plastic equipment were placed in boiling water for 30 min to kill vegetative cells on the material surface.

Starter preparation: The yoghurt starter culture was inoculated into fresh milk that was heated at 90 °C for 30 min. The inoculate was incubated at 45 °C until pH 4.6 was attained, stored overnight (4 °C) and then was used in the yoghurt processing.

Twelve yoghurt formulations were processed as described by Ayar et al. (2006). Dry ingredients (gelatin and 4% cane sugar on milk weight basis) were weighed and separately blended into three different gelatin stabilizer levels (0.5, 0.6 and 0.7%, w/w). Each dry ingredient blend was mixed thoroughly with fresh milk, filtered through cheese cloth and preheated to 50 °C to facilitate melting of gelatin and uniform mixing. The resulting premix was heat treated for 30 min at 85 °C and cooled to 45 °C in a 4 °C water bath. Then each of the three premixes was further divided into four equal portions to which carrot juice (0, 10, 15 and 20%, w/w on milk basis) was added. Maintaining a temperature of 43 °C, the resulting yoghurt samples were filled into coded screw capped glass jars, inoculated with 3% (w/w) yoghurt starter culture and incubated (Gallenkamp Incubator Plus Series, England) at 43 °C for a period of 2.5 h. To determine the incubation period and to monitor fermentation progress, a separate control yoghurt (yoghurt without carrot juice and 0.6% gelatin) was prepared to which pH probe was inserted, concurrently incubated in a thermostatically controlled water bath (43 °C). The incubation of the experimental yoghurt was terminated when the pH of the control yoghurt reached 4.7. The yoghurt samples were then immediately cooled by transferring them from the incubator to a refrigerator (4 °C) and then stored for 24 h. The effects of carrot juice and stabilizer addition levels on the physicochemical and microbiological characteristics were reported based on yoghurt samples analyzed in triplicate after 24 h of storage.

2.5. Proximate composition of milk and yoghurt

The total solids content of milk was determined according Richardson (1985) and that of yoghurt according to IDF (1991). The moisture content of both milk and yoghurt samples were determined by difference (AOAC, 1998 Method No. 990.20):

$$\%\text{Moisture} = 100 - \text{Total solids (TS\%)}$$

The fat contents of milk and yoghurt were determined using AOAC (1998) Method No 905.02 and solids-not-fat (SNF) by subtracting percentage of fat from total solids (TS %). Total protein (% N × 6.38) and ash contents were determined as described in AOAC (1998) Methods No. 991.20 and No. 945.46, respectively.

2.6. pH and titratable acidity (TA) of milk and yoghurt

The TA of both milk and yoghurt samples were determined by titrating with 0.1 N NaOH (Richardson, 1985) and TA was expressed as percentage of lactic acid (LA). The pH was determined by a glass electrode attached to the pH meter (model number 510 Cyber Scan, Eutech Instruments) with a temperature probe after calibrating (buffer solutions pH 4 and 7).

2.7. Chemical analysis of carrot juice

The total solids content of carrot juice was determined by oven drying (AOAC, 1998 Method 920.151), total soluble solids (TSS) by hand refractometer (Atago N1, USA) (AOAC, 1998 Method 932.12) and total sugar by colorimetric method (Somogyi, 1945). The TA was expressed as percentage of citric acid equivalent (AOAC, 1998 Method No 942.15), pH by a glass electrode attached to the pH meter (model pH 510, Oakton instruments USA).

2.8. Total phenolics content (TP) from carrot juice and yoghurt

2.8.1. From carrot juice

The TP content was determined by the Folin-Ciocalteu method after extraction from carrot juice with 30 mL of solvent (80% aqueous ethanol, containing 1% conc. HCl) in a conical flask, agitating in an orbital shaker (200 rpm, at 50 °C, for 2 h), filtering (Whatman No. 4) as described in Lima et al. (2005). The filtered extract was used for the determination of TP content and ferric reducing power.

The extract (100 μL) was mixed with 750 μL of Folin-Ciocalteu reagent, allowed to stand at room temperature for 5 min and mixed gently with 750 μL of 6% (w/v) sodium carbonate. A blank was made by mixing distilled water and reagents. After allowing to

stand at room temperature (40 min), absorbance was measured at 725 nm using a UV/Visible spectrophotometer (model 6505 UV/Vis Spectrophotometer, Genway UK) and the result was expressed as milligrams of gallic acid equivalents (GAE) per kg of juice.

2.8.2. From yoghurt

The TP content was extracted as described in Wallace and Giusti (2008) from a yoghurt sample (10 g) after blending with 30 mL of 0.1% HCl acidified methanol for 2 min on laboratory blender and centrifugation (3500 rpm for 15 min) (Model K240 Centurion Scientific Ltd). The supernatant collected was brought to 50 mL with acidified methanol and TP content was determined as described above.

2.9. Total carotenoid contents from carrot juice and yoghurt

Total carotenoid content was determined according to Bandyopadhyay, Chakraborty, and Raychaudhuri (2008). Carrot juice (1 g) or homogenized yoghurt sample (5 g) was saponified after mixing with 37.5 mL methanol and 12.5 mL of 50% potassium hydroxide in a flask to release esterified carotenoids, remove chlorophylls and lipids. The unsaponifiables were extracted with diethyl ether (20 mL × 2), washed twice (40 mL distilled water) and the extract was dried over anhydrous sodium sulfate. Diethyl ether was evaporated on a steam bath and the dried residue was redissolved in petroleum ether (20 mL). The extract color absorbance was measured at 450 nm with a UV–Vis spectrophotometer and total carotenoid content was reported as mg of β-carotene equivalent per kg of carrot juice or yoghurt.

$$Total\ carotenoids\ \left(\frac{\mu g}{kg}\right) = \left[\frac{Abs}{2592}\right] x \frac{V(mL)x10^4}{Sample\ weight\ in\ kg}$$

Where: Abs = is absorbance reading; 2592 = is extinction coefficient of β-carotene in petroleum ether; V is total extract volume (20 mL).

2.10. Ferric reducing power (FRP) of carrot juice and yoghurt

The FRP of carrot juice extract was determined according to Oyaizu (1986). An extract (100 μL) of carrot juice or yoghurt was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6), 1% potassium ferricyanide (2.5 mL) and the mixture was incubated in a water bath at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged (3000 rpm, 10 min). The upper layer (2.5 mL) was mixed with distilled water (2.5 mL) and freshly prepared 0.1% ferric chloride solution (0.5 mL). Absorbance was measured at 700 nm. After subtracting absorbance of a blank sample (prepared from distilled water), FRP was determined from ascorbic acid (AA) calibration standard as AA equivalents in milligram per kilogram of carrot juice or yoghurt.

2.11. Syneresis (whey separation)

Syneresis (%) expressed as volume of separated whey per 100 mL of yoghurt was determined in a triplicate by taking homogeneous five mL of yoghurt in a test tube, centrifugation (5000 rpm for 20 min at 4 °C) and measurement of whey separated 1 min after centrifugation (Bakirci & Kavaz, 2008).

2.12. Microbiological analysis

Sample preparation: yoghurt sample for microbiological analysis was prepared according to the method described by Richardson (1985). From thoroughly mixed sample, 11 g of yoghurt was sampled and mixed with 99 mL of peptone water (40 °C) and the content was mixed (10 min) using a shaker (Grant GL 5400, England) to obtain a homogenous dispersion. This 1:10 dilution was directly used for yeast and mould (YMC), and coliform counts (CC), whereas for total viable count (TVC) a serial dilution was made up to 10^{-7} with peptone water.

2.12.1. Total viable count

The TVC was determined using Hansen's Yoghurt Agar (HYA) as growth media (Keating & White, 1990). From the appropriate dilutions (i.e., 10^{-5}, 10^{-6} and 10^{-7}), 1 mL was pour plated on sterile petri plates and a molten HYA (15–20 mL) was added. Triplicates of appropriate decimal dilutions of the yoghurt sample on HYA were incubated at 37 °C for 72 h. Results were reported in $\log_{10}$ (CFU g^{-1} of yoghurt). Counts on two consecutive dilutions, which gave less than 300 colonies per plate were used to calculate the weighted average of colony forming units per gram of yoghurt sample (N):

$$N = \frac{C}{[Vx(n_1 + 0.1n_2)xd]}$$

Where: C = sum of colonies on all plates counted, V = volume applied to each plate, n_1 = the number of plates counted at the first dilution, n_2 = the number of plates counted at the second dilution and d = the dilution from which the first count was obtained.

2.12.2. Coliform count

The CC was determined using Violet Red Bile Agar (VRBA) by the pour plate technique (Richardson, 1985). Plates were incubated at 30 °C for 24 h, typical dark red colonies (>0.5 mm in diameter) were considered as coliforms and results were reported as counts per gram of yoghurt.

2.12.3. Yeast and mould counts

The YMC was determined by incubating 1 mL of the 1:10 dilution of yoghurt by the pour plate method on Acidified Potato Dextrose Agar (APDA) medium at 25 °C for 5 days and results were expressed as yeast and mould count per gram of yoghurt (Richardson, 1985).

2.13. Statistical data analysis

The data from proximate composition analysis, physicochemical and microbiological analyses of yoghurt samples were analyzed using a general linear model (PROC GLM) of the SAS software. In the event of a significant interaction, the combinations of levels of factors involved in the interaction were investigated for significant differences using least square means. Significance was established at $p < 0.05$. The microbial count data were $\log_{10}$ transformed before submitted to SAS for statistical analysis.

3. Results and discussion

3.1. Composition of milk and carrot juice used

The milk total solids, water, solids-not-fat, protein, fat and ash contents were 12.4, 87.6, 9.1, 3.0, 3.3 and 0.7 (%w/w, fresh basis), respectively and are typical values reported for cow milk (Claeys et al., 2014). The milk TA was 0.12 (%w/w LA) and pH was 6.68 which are in the normal range for cow milk. The base milk used was thus regarded as suitable for yoghurt processing. The carrot juice total solids (%w/w), moisture (%w/w), total soluble solids (% w/w), total sugars (as dextrose % w/w), total carotenoids (mg kg^{-1}), total phenolics (mg kg^{-1}) and TA (as citric acid% w/w) analyzed on fresh juice weight basis were 8.5, 91.5, 7.6, 6.0, 48.5, 46.3 and 0.23,

respectively and are similar to those reported for carrot juice by Sharma et al. (2012).

3.2. Proximate composition of the yoghurt

The total solids content, crude protein, fat, ash and solids-not-fat of yoghurt samples ranged from 16.2 to 17.4%, 2.8–3.1%, 2.8–3.3%, 0.74–0.77%, and 13.4–14.1%, respectively (Table 1). Proximate composition of the yoghurt was significantly ($p < 0.01$) affected by carrot juice addition but not by stabilizer (Table 2). The interaction effect of carrot juice and stabilizer on the proximate composition was not significant ($p > 0.05$). Increasing carrot juice significantly lowered TS, SNF and fat mass fraction but increased the moisture content of the yoghurts. Crude protein content was significantly lowered as the concentration of carrot juice increased but the difference between yoghurt samples with 15% and 20% carrot juice was not significant ($p > 0.05$). The ash content of yoghurt also significantly decreased with increasing carrot juice even though ash contents of yoghurt with 15% carrot juice was similar to the 10% and 20% carrot juices.

Variation in yoghurts proximate composition was due to differences in formulation and the compositional difference between carrot juice and base milk used. Addition of carrot juice had a dilution effect on yoghurt composition and this was due to high moisture content of the carrot juice. Information regarding proximate composition of yoghurt is mandatory from legal requirement since it gives information on the product nutritional quality. In addition, microbiological and sensory properties of the product are dependent on proximate composition. According to COMESA/ECA (2004) on East African Standard, yoghurt should have a minimum total SNF content of 8.2% (w/w). Whereas, in Codex (2011) was stated a minimum of 2.7% protein and a milk fat (%w/w) content of less than 15. Hence, all yoghurt samples in this study satisfy these requirements.

3.3. Total carotenoids, total phenolics (TP) and ferric reducing power

The total yoghurt carotenoids content was significantly ($p < 0.01$) increased with increased carrot juice (Table 1). However, no significant ($p > 0.05$) variation in TP content and FRP were observed. Amount of stabilizer added did not affect ($p > 0.05$) total carotenoids (TC), TP and FRP of yoghurt samples (Table 2). Minimum average TC of 3.05 ± 0.04 mg kg^{-1} was observed in yoghurt sample without carrot juice (control) while maximum of 10.26 ± 0.06 mg kg^{-1} was observed in yoghurt with 20% carrot juice. The TP content and FRP of yoghurt samples were in the range of 35.49 ± 1.20 to 37.61 ± 0.82 mg GAE kg^{-1} and 106.02 ± 2.94 to 113.06 ± 0.56 mg AAE kg^{-1} of yoghurt, respectively.

The occurrence of phenolic compounds in milk and dairy products are a consequence of several factors, e.g., consumption of particular fodder crops by cattle, catabolism of proteins by bacteria, contamination with sanitizing agents, process-induced incorporation or by deliberate addition as specific functional ingredients (O'Connell & Fox, 2001). Increase in the TP content and consequently in FRP could be expected in yoghurt with higher levels of carrot juice. However, due to the relatively lower TP content (46.25 ± 1.27 mg GAEkg^{-1}) in the carrot juice used in this research, increase in the TP content and FRP of the yoghurt through carrot juice supplementation was subtle. This is also in part contributed by high binding of phenolic compounds to milk casein proteins before its gel formation on fermentation. Similar insignificant change in the total phenolic content and FRP in yoghurt was reported when grape seed extract phenolics was supplemented into milk before inoculation-fermentation (Chouchouli et al., 2013). The TP content of carrot can vary depending on the cultivar, pre-harvest management, postharvest handling, storage and processing methods (Sharma et al., 2012). The results showed, due to increased carotenoids and a supply of other possible phytonutrients like ascorbic acid, phenolics, tocopherols and fibers from carrot juice, that there is a possibility to improve yoghurts with potential to supply antioxidant, pro-vitamin A and dietary fibers.

Table 2
Analysis of variance (ANOVA) p-values on the effects of stabilizer and carrot juice levels on proximate compositions, total carotenoids, total phenolics, ferric reducing power, pH, titratable acidity, syneresis and total viable counts of yoghurts.

Properties	Sources		
	STL	CJL	STL*CJL
Total solids	0.8238	0.0001	0.9996
Moisture	0.8238	0.0001	0.9996
Solids not fat	0.8292	0.0001	0.9884
Crude protein	0.5843	0.0001	0.9947
Fat	0.8516	0.0001	0.9987
Ash	0.6572	0.0001	0.9049
Total carotenoids	0.1610	0.0001	0.1885
Total phenolics	0.2626	0.1032	0.3026
Ferric reducing power	0.1308	0.1504	0.5927
pH	0.4831	0.0001	0.3062
Titratable acidity	0.6891	0.0001	0.7798
Syneresis	0.0001	0.0001	0.0001
Total viable counts	0.5468	0.0001	0.2371

Where: STL = stabilizer level, CJL = carrot juice level, STL*CJL = interaction.

Table 1
Proximate compositions, total carotenoids, total phenolics, ferric reducing power, pH and titratable acidity of yoghurt manufactured using various carrot juice levels.

Parameters	Carrot juice (%, w/w)				Range
	0	10	15	20	
Moisture content (%)	82.58 ± 0.06^{d}	83.29 ± 0.07^{c}	83.56 ± 0.06^{b}	83.85 ± 0.04^{a}	82.58–83.85
Total solids (%)	17.42 ± 0.06^{a}	16.71 ± 0.07^{b}	16.44 ± 0.06^{c}	16.15 ± 0.04^{d}	16.15–17.42
Solids-not-fat (%)	14.12 ± 0.04^{a}	13.71 ± 0.03^{b}	13.55 ± 0.02^{c}	13.39 ± 0.02^{d}	13.39–14.12
Fat %	3.30 ± 0.02^{a}	3.00 ± 0.04^{b}	2.89 ± 0.04^{c}	2.76 ± 0.05^{d}	2.76–3.30
Crude protein (%)	3.14 ± 0.04^{a}	2.86 ± 0.05^{b}	2.79 ± 0.05^{c}	2.77 ± 0.04^{c}	2.77–3.14
Ash %	0.77 ± 0.01^{a}	0.76 ± 0.01^{b}	0.75 ± 0.01^{bc}	0.74 ± 0.01^{c}	0.74–0.77
TC (mg kg^{-1})	3.05 ± 0.04^{d}	6.73 ± 0.05^{c}	8.71 ± 0.03^{b}	10.26 ± 0.06^{a}	3.05–10.26
TP (mg GAE kg^{-1})^{NS}	37.00 ± 1.06	36.49 ± 0.97	36.58 ± 1.12	35.75 ± 1.09	35.75–37.00
FRP (mg GAE kg^{-1})^{NS}	110.53 ± 3.53	109.37 ± 4.26	107.66 ± 2.75	107.21 ± 2.67	107.21–110.53
pH	4.42 ± 0.01^{d}	4.46 ± 0.01^{c}	4.48 ± 0.01^{b}	4.54 ± 0.01^{a}	4.42–4.54
TA (% LA)	0.71 ± 0.01^{a}	0.68 ± 0.01^{b}	0.66 ± 0.01^{c}	0.62 ± 0.01^{d}	0.62–0.71

Results are mean ± standard deviation of nine samples. Means across a row with different superscript letters are different ($p < 0.05$); TC = Total carotenoids; TP = Total phenolics; GAE = Gallic acid equivalent; FRP = Ferric reducing power; TA = Titratable acidity; LA = Lactic acid; NS = non-significant.

3.4. pH and titratable acidity

Yoghurts pH significantly ($p < 0.01$) increased with increased carrot juice levels but not ($p > 0.05$) with the amount of added stabilizer (Table 2). The interaction effect of carrot juice and stabilizer was not significant ($p > 0.05$). The yoghurt pH ranged from 4.42 ± 0.01 to 4.55 ± 0.01 which is lower than the minimum acidity (pH 4.6 or lower) recommended by Frye (2013) for yoghurt. The higher pH values observed in carrot-supplemented yoghurt (Table 1) was due to lower activity of yoghurt bacteria during the incubation period compared to the control that had higher total solids content especially higher solids-not-fat content and similar was reported when total solids content of milk was enhanced (Mahdian & Tehrani, 2007).

The TA ranged from $0.71 \pm 0.01\%$ LA for control yoghurt to $0.62 \pm 0.01\%$ LA for yoghurt sample with 20% carrot juice. Increased carrot juice addition significantly ($p < 0.01$) lowered TA of yoghurt samples (Table 1). However, the TA of yoghurt was not significantly affected by the amount of stabilizer addition (Table 2). The TA observed in this study were lower than the range 0.90–1.07% already reported (Bakirci & Kavaz, 2008). This could be attributed to milk composition variation and the presence of skim milk powder in their yoghurt formulations which contributed toward increased TA as compared to this work. For balanced flavor development in yoghurt, TA values should be within certain limits for consumer preferences. The acidity of all yoghurt formulations in this work was above the minimum recommended limit of 0.6% by Codex (2011).

3.5. Syneresis

Syneresis is a major defect in yoghurt production that could limit the shelf life and acceptability because of undesirable appearances. Both carrot juice and gelatin stabilizer addition main effects and their interaction significantly ($p < 0.01$) affected yoghurt syneresis (Table 2). The syneresis of yoghurt samples was in a range 36.39 ± 0.26 to $57.64 \pm 0.14\%$ (Table 3). Syneresis significantly ($p < 0.05$) decreased with the increase of added stabilizer, but it increased significantly ($p < 0.05$) with the increase of added carrot juice. For each carrot juice level, the syneresis of yoghurt decreased with increasing stabilizer addition ($p < 0.05$). Increased carrot juice in yoghurt samples at 0.5% stabilizer resulted in a significant ($p < 0.05$) increase in syneresis but the difference in syneresis value between yoghurt samples with 10 and 15% carrot juice was not significant. Similarly, yoghurt samples with 0.7% stabilizer level showed a significant increase ($p < 0.05$) in syneresis with increasing carrot juice addition except no significant difference was observed with 15 and 20% carrot juice addition. What is also interesting is that the syneresis observed at 0.7% stabilizer and with the highest added carrot juice (15 and 20%) is similar to the one observed at 0.5% stabilizer and 0% carrot juice addition (Table 3). Stabilizer increase counteracted against syneresis and lowest syneresis was observed at 0.7% level. The syneresis found in this work with 0.5% gelatin use was similar with the results reported by Ayar et al. (2006) who used gelatin at the same level. Salwa et al. (2004) reported that the syneresis of yoghurt was increased due to carrot juice addition, which is in agreement with this study. However, our results showed that the addition of stabilizer greatly reduced syneresis as opposed to the results reported by Salwa et al. (2004) where a visible whey separation was observed with added carrot juice starting from the day fresh yoghurt (just after manufacture) processed. In this work, there was no visible serum separation due to spontaneous syneresis in the yoghurt with carrot juice added at any concentration showing improvement by gelatin stabilizer. Gelatin is known to enhance the water holding capacity of the gels and in other work where milk protein concentrate and skim milk powder used no serum expulsion was observed for gels containing $\geq$1% gelatin (Pang, Deeth, Sharma, & Bansal, 2015).

Table 3
Effect of carrot juice and stabilizer on syneresis (%, v/v) of yoghurt.

Carrot juice (%, w/w)	Stabilizer (%, v/v)		
	0.5	0.6	0.7
0	45.01 ± 0.31^{f}	43.36 ± 0.47^{g}	36.39 ± 0.26^{h}
10	51.64 ± 0.31^{c}	47.86 ± 0.10^{e}	42.66 ± 0.55^{g}
15	51.90 ± 0.19^{c}	49.16 ± 1.89^{d}	44.84 ± 0.33^{f}
20	57.64 ± 0.14^{a}	54.45 ± 0.55^{b}	45.33 ± 0.89^{f}

Values are mean ± standard deviation of three replicate samples; Means in a row or column with different superscript letters are different ($p < 0.05$).

3.6. Microbiological analysis

3.6.1. Total viable count (TVC)

The TVC was significantly affected ($P < 0.01$) by the carrot juice levels in the yoghurt (Table 4) but the main effect of stabilizer and interaction effects of carrot juice and stabilizer addition were not significant (Table 2). The control (without carrot juice) yoghurt had the highest TVC (8.54 ± 0.01 $\log_{10}$ $CFUg^{-1}$) and the lowest (8.24 ± 0.05 $\log_{10}$ CFU g^{-1}) was found in yoghurt with 20% carrot juice. The TVC of yoghurt samples in this work are in the range of 7.7–8.6 logs $CFUg^{-1}$ reported for yoghurt (Keating & White, 1990). The decrease in TVC with higher carrot juice levels may be because of the lower total solids content compared to the control. Mahdian and Tehrani (2007) showed that growth and activity of starter bacteria improved in samples with higher amounts of total solids.

Yoghurt samples with or without carrot juice, had a total viable count of >8 $\log_{10}$ CFU g^{-1} which is the minimum limit recommended by National Yoghurt Association of the USA. The presence of a large number of live and active bacterial cells and/or metabolites formed during yoghurt fermentation has beneficial effects on human health.

3.6.2. Yeast, mould and coliform counts

Yeast and mould (YMC) and coliform counts (CC) were below 10 CFU g^{-1} (i.e., below the detection limit) in all treatments. Total yeast and mould counts recommended are <10 CFU g^{-1} (Mostert & Jooste, 2002). This could be attributed to the high hygienic conditions followed in the laboratory that prevented post-production contamination since the main factors for yeast, mould and coliforms growth in yoghurt production are microbiological quality of any ingredients introduced into yoghurt after heat treatment (85 °C for 30 min) of the base milk.

4. Conclusions

Yoghurt samples at each carrot juice level have fulfilled the requirements of COMESA standard in terms of its physicochemical and microbiological qualities. Moreover, addition of gelatin stabilizer has effectively reduced syneresis. Carrot juice addition into yoghurt reduced the total viable yoghurt bacteria count even though for all carrot juice levels used the count were higher than 10^{8} $CFUg^{-1}$ after one day of product making which is above the minimum recommended by *Codex* standard. Yoghurt samples with 10–15 percent carrot juices and 0.7 percent stabilizer showed improved nutritional and total carotenoid contents with suppressed syneresis.

Table 4
Effect of carrot juice and stabilizer on total viable count in (Log_{10} CFU g^{-1}) yoghurt.

Carrot juice (%, w/w)	Stabilizer (%, w/w)			CJL main effect
	0.5	0.6	0.7	
0	[2]8.55 ± 0.01[A]	8.54 ± 0.01[A]	8.53 ± 0.01[BA]	[1]8.54 ± 0.01[a]
10	8.52 ± 0.04[BA]	8.51 ± 0.01[BA]	8.52 ± 0.01[BA]	8.52 ± 0.02[a]
15	8.48 ± 0.01[B]	8.50 ± 0.01[BA]	8.48 ± 0.01[B]	8.49 ± 0.01[b]
20	8.25 ± 0.07[DC]	8.21 ± 0.04[D]	8.27 ± 0.02[C]	8.24 ± 0.05[c]
STL main effect [NS]	8.45 ± 0.13	8.44 ± 0.14	8.45 ± 0.11	

CJL = Carrot juice level (%, w/w); STL = Stabilizer level (%, w/w); NS = non-significant ($p > 0.05$).Values are mean ± standard deviation of three replicate samples. [1]Means with different lower case superscripts in a column are statistically different ($p < 0.05$). [2]Means in a row with common upper case superscript letters are not different ($p > 0.05$).

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References

AOAC. (1998). *(Association of official analytical chemists). Official methods of analysis.* Method No 942.15, Method No 990.20, Method No. 991.20, Method No. 945.46, Method No 905.02, Method 920.151 (16th ed.). Washington, DC, USA: AOAC, 1998 Method 932.12.

Ares, G., Gonçalvez, D., Pérez, C., Reolón, G., Segura, N., Lema, et al. (2007). Influence of gelatin and starch on the instrumental and sensory texture of stirred yogurt. *International Journal of Dairy Technology, 60*, 263–269.

Ayar, A., Sert, D. I., Kalyoncu, H., & Yazici, F. (2006). Physical, chemical, nutritional and organoleptic characteristics of fruit added yogurts. *Journal of Food Technology, 4*, 44–49.

Bakirci, I., & Kavaz, A. (2008). An investigation of some properties of banana yoghurts made with commercial ABT-2 starter culture during storage. *International Journal of Dairy Technology, 61*, 270–276.

Bandyopadhyay, M., Chakraborty, R., & Raychaudhuri, U. (2008). Effect of beet and honey on quality improvement and carotene retention in a carrot fortified milk product. *Innovative Food Science and Emerging Technologies, 9*, 9–17.

Chouchouli, V., Kalogeropoulos, N., Konteles, S. J., Karvela, E., Makris, D. P., & Karathanos, V. T. (2013). Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts. *LWT- Food Science and Technology, 53*, 522–529.

Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., et al. (2014). Review: consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control, 42*, 188–201.

Codex. (2011). CODEX STAN 243-2003: standard for fermented milks. In FAO/WHO (Ed.), *Milk and milk products* (2nd ed., pp. 6–16) (Rome, Italy).

COMESA/EAC. (2004). *Regional dairy trade policy paper, researched and published under the auspices of COMESA and EAC in collaboration with the RATES Center, Nairobi and ASARECA/ECAPAPA, Entebbe; with support from USAID/REDSO, Nairobi.*

Frye, C. P. (2013). Regulations for product standards and labeling manufacturing. In R. C. Chandan, & A. Kilara (Eds.), *Manufacturing yoghurt and fermented milks.* Oxford, UK: John Wiley & Sons.

Gahruie, H. H., Eskandaria, M. H., Mesbahi, G., & Hanifpour, M. A. (2015). Scientific and technical aspects of yogurt fortification: a review. *Food Science and Human Wellness, 4*, 1–8.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety, 9*, 455–470.

IDF. (1991). *Yogurt: Determination of total solids content (reference method). IDF standard 151.* Brussels, Belgium: International Dairy Federation.

Keating, K. R., & White, C. H. (1990). Effect of alternative sweeteners and fruit-flavored yoghurts. *Journal of Dairy Science, 73*, 54–62.

Lima, V. L. A. G., Melo, E. A., Maciel, M. I. S., Prazeres, F. G., Musser, R. S., & Lima, D. E. S. (2005). Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages. *Food Chemistry, 90*, 565–568.

Mahdian, E., & Tehrani, M. M. (2007). Evaluation the effect of milk total solids on the relationship between growth and activity of starter cultures and quality of concentrated yoghurt. *American-Eurasian Journal of Agriculture and Environmental Science, 2*, 587–592.

Mostert, J. F., & Jooste, P. J. (2002). Quality control in the dairy industry. In R. K. Robinson (Ed.), *Dairy microbiology handbook* (3rd ed., pp. 655–736). New York: John Wiley and Sons.

Oliveira, A., Alexandre, E. M. C., Coelho, M., Lopes, C., Almeida, D. P. F., & Pintado, M. (2015). Incorporation of strawberries preparation in yoghurt: Impact on phytochemicals and milk proteins. *Food Chemistry, 171*, 370–378.

Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315.

O'Connell, J. E., & Fox, P. F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *International Dairy Journal, 11*, 103–120.

Pang, Z., Deeth, H., Sharma, R., & Bansal, N. (2015). Effect of addition of gelatin on the rheological and microstructural properties of acid milk protein gels. *Food Hydrocolloids, 43*, 340–351.

Puvanenthiran, A., Stevovitch-Rykner, C., McCann, T. H., & Day, L. (2014). Synergistic effect of milk solids and carrot cell wall particles on the rheology and texture of yoghurt gels. *Food Research International, 62*, 701–708.

Richardson, G. H. (1985). *Standard methods for the examination of dairy products* (15th ed.). Washington DC: American Public Health Association.

Routray, W., & Mishra, H. N. (2011). Scientific and technical aspects of yogurt aroma and taste: a review. *Comprehensive Reviews in Food Science and Food Safety, 10*, 208–2020.

Salwa, A. A., Galal, E. A., & Neimat, A. E. (2004). Carrot yoghurt: sensory, chemical, microbiological properties and consumer acceptance. *Pakistan Journal of Nutrition, 3*, 322–330.

Sharma, K. D., Karki, S., Singh, N., & Attri, T. S. (2012). Chemical composition, functional properties and processing of carrot-a review. *Journal of Food Science and Technology, 49*(1), 22–32.

Somogyi, M. (1945). A new reagent for the determination of sugars. *Journal of Biological Chemistry, 16*, 61–68.

Sun-Waterhouse, D. (2011). The development of fruit-based functional foods targeting the health and wellness market: a review. *International Journal of Food Science and Technology, 46*, 899–920.

Wallace, T. C., & Giusti, M. M. (2008). Determination of color, pigment, and phenolic stability in yoghurt systems colored with nonacylated anthocyanins from *Berberis boliviana* L. as compared to other natural/synthetic colorants. *Journal of Food Science, 73*, 241–248.