Utilization of lactic acid bacteria combinations in the development of low lactose yoghurt

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Abstract

This study was intended to develop low lactose yoghurt for lactose intolerant individuals (LIIs). In this study, five starter culture combinations were formulated using *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, to select the most effective lactose converting combinations for developing a yoghurt safe for LIIs. A risk assessment on the developed product was run to ensure its suitability for the LIIs using HACCP approach. Physicochemical, sensorial, and microbiological properties of the developed product were determined at 0, 5, and 10 days of storage. For risk assessment, critical control points (CCPs) in the process of yoghurt production were determined. For each CCP critical limits (CLs) were identified particularly for lactose content. Four CCPs in the manufacturing of low lactose yoghurt were identified. Our findings indicated that the combination of *Lactobacillus casei* with *Bifidobacterium lactis* reduced the lactose content to 0.35%, 0.25%, and 0.23% at day 0, 5, and 10 of storage, respectively. The amount of lactose in the developed yoghurt was less than the 1% level that considered to be safe for most LIIs. *Lactobacillus casei* and *Bifidobacterium lactis* combination is recommended as a starter culture for manufacturing probiotic yoghurt low in lactose content destined for LIIs.

Keywords: Critical limits; HACCP approach; Lactose intolerant; Risk assessment; Starter culture.

1. Introduction

Milk and milk products are part of a healthy diet and daily consumption of them is widely recommended. Yoghurt has become a popular fermented dairy product, with unique properties that enhance the bioavailability of some nutrients and health (Han *et al*., 2014). Milk and milk products consumption is not recommended for persons suffering from lactose intolerance (LI) or Lactose maldigestion. It is a condition resulting from the inability to digest lactose and has been attributed to insufficient amounts of lactase in the small intestine to metabolize lactose (Zahid *et al.*, 2010; Ojetti *et al.*, 2013). LI is a common medical problem that affects a large proportion of the world population and significantly affects the lives of affected individuals (Zahid *et al*., 2010; Ojetti *et al.*, 2013; Savaiano *et al*., 2013; Strand *et al.*, 2014). Consumption of milk or other dairy products by lactose intolerant individuals (LIIs) can cause symptoms including: diarrhoea, bloating, flatulence, abdominal pain, cramps and nausea, which occur between 30 min and 2 hours after the ingestion of lactose (Mummah *et al*., 2014). Many LIIs are left with no other choice than to restrict consumption of dairy foods containing lactose (Shaukat *et al.*, 2010; Alexandre *et al*., 2013). Avoidance of dairy foods may lead to adverse health problems, including poor bone health, higher blood pressure, higher body weight, a higher incidence of colon cancer, and a higher risk of developing diabetes (Nicklas *et al*., 2011).

Moreover, dairy avoidance leads to reduced intake of protein and calcium (Savaiano *et al*., 2013; Setty-Shah *et al*., 2013).

The successful manufacture of yoghurt is enshrined in two compatible and, to some extent, overlapping concept i.e. good manufacturing processing (GMP) and the hazard analysis critical control point (HACCP) system (Hoolasi, 2005). HACCP is a systematic scientific approach, which designed to prevent the occurrence of the physical, chemical, and biological hazards of food products. In addition, it deal with the whole production process from primary production to final consumption by identifying, evaluating, preventing, eliminating, controlling or reducing hazards to acceptable levels, and specifying their critical control point at the earliest possible point, to avoid the distribution of unsafe food to consumers (Massimo *et al*., 2013; Yamina *et al*., 2014).

Production of low lactose product for LIIs is one of the major challenges for dairy product producers and traders and is highly demanded by consumers. Therefore, this study aims to develop low lactose yoghurt intended for LIIs consumption by using combinations of various lactic acid bacteria.

2. Materials and methods

2.1. Materials

Cow milk samples were collected from University of Khartoum Farm, Khartoum North, Sudan. Skim milk powder and pectin stabilizer were obtained from Saputo Company (Canada). *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from CHR Hansen Middle East and Africa FZ-LLC (Dubai, UAE). All chemicals were of analytical grade and were obtained from Sigma Aldrich (Sigma, MO, USA).

2.2. Starter culture combination

Five starter cultures combinations were formulated as follow:
T0: *S. thermophilus + L. delbrueckii* subsp. *Bulgaricus* (1:1)
T1: *L. acidophilus + B. lactis* (1:1)
T2: *L. casei + B. lactis* (1:1)
T3: *B. lactis + S. thermophilus + L. delbrueckii* subsp. *bulgaricus* (1:1:1)
T4: *L. casei + S. thermophilus + L. delbrueckii* subsp. *bulgaricus* (1:1:1)

2.3. Yoghurt manufacture

The yoghurt was manufacture as depicted in the flow diagram (Fig. 1) following the method described by Nguyen *et al*. (2014). In this process, raw bovine milk was standardised with 0.2% skim milk powder and 0.2% of pectin stabilizer. After that, mixture was homogenized, and pasteurized at 90°C for 15 seconds, then cooled to 44°C. Pasteurized milk was divided into five equal portions, inoculated randomly with one of the starter culture combinations (T0, T1, T2, T3, or T4). The mixtures were stirred, poured into plastic cups and incubated at 43°C for about 4 h until pH 4.6 was reached (Han *et al.* 2014). Then, a portion of the fermented products was immediately transferred to an ice bath to stop the fermentation, and the remaining products were kept at 4°C for 10 days. Physicochemical, sensorial, and microbiological properties of the developed product were determined at 0, 5, and 10 days of storage.

2.4. Characterization of the developed yoghurt

2.4.1. Physicochemical Properties

The lactose, galactose, glucose, total solid (TS), fat, protein, viscosity, pH, and titratable acidity (TA) were determined according to standard official methods (AOAC, 2003). Solids non-fat (SNF) was determined by difference as reported by Jayeola *et al*. (2010). Lactose, galactose and glucose contents were determined using the Milko-Scan Milk Analyzer (FOSS Company, Hillerod, Denmark). Viscosity was measured with a viscometer (Haake Viscotester, Thermo Electron Corporation, Dreiech, Germany). The pH was determined at room temperature (27°C) using a

digital pH meter (Model L. Pusl muchen 15-1260/7, Germany). The titrable acidity was calculated as lactic acid (%) of an equivalent weight of 90 mg (1 ml 0.1 N NaOH ≡ 0.09 g lactic acid).

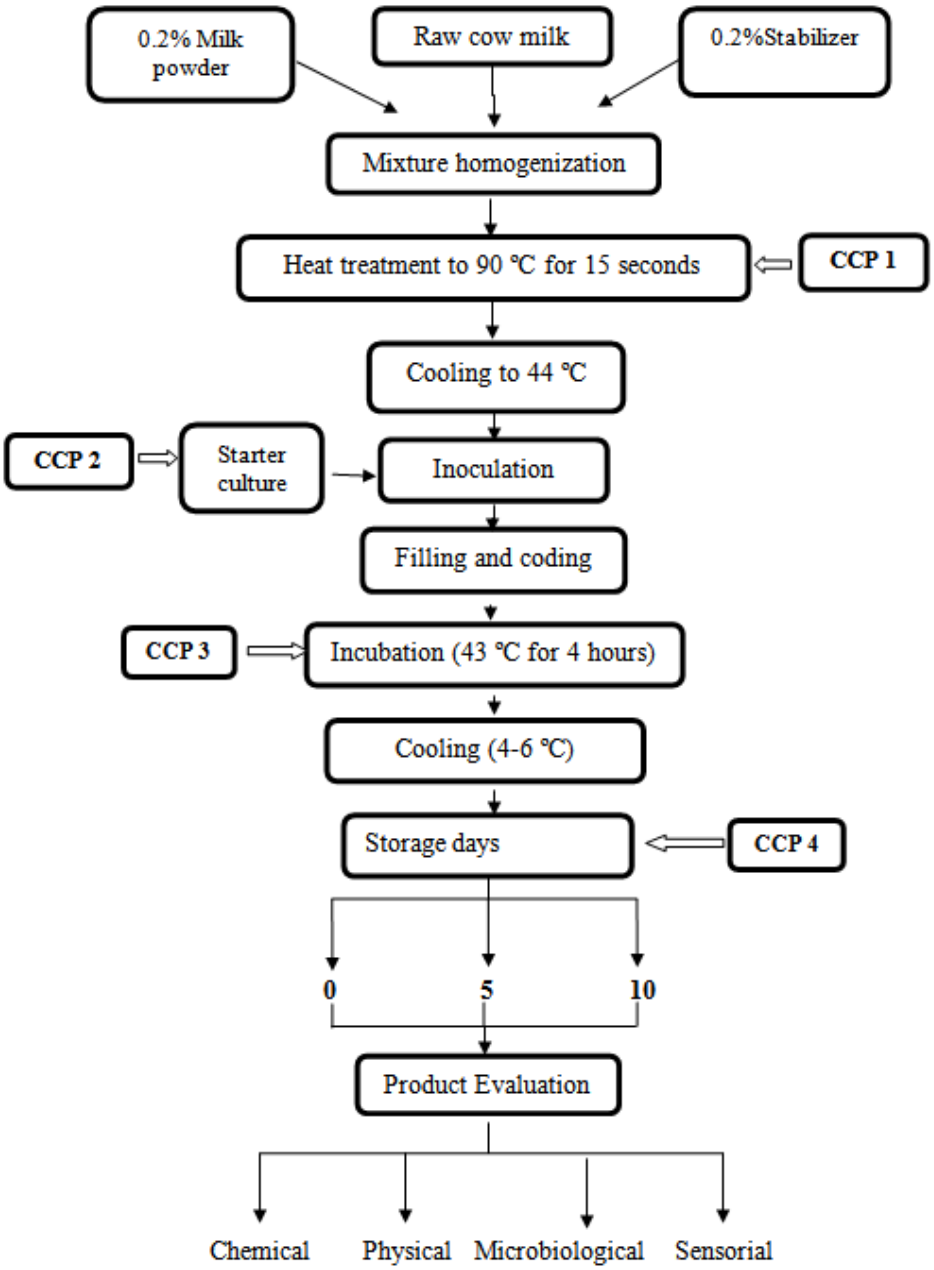

Fig.1. Flow diagram for the probiotic yoghurt manufacture and evaluation

2.4.2. Microbiological analysis

At each appropriate storage period, 10 g yoghurt samples were taken aseptically, diluted in 90 mL of sterile Ringer's solution (0.1%, w/v), and then serially diluted using 9 mL of sterile Ringer's solution up to a dilution of 10^{-3}. Plates with 25–250 colonies were selected for manual counting (Harrigan, 1998; Nguyen *et al*., 2014). Plate count agar medium, and yeast and mold agar medium were used to determine the total bacterial count and yeast and mould counts, respectively (Harrigan, 1998). For bacterial count, the plates were incubated at 37°C for 48 h, whereas, for yeast and mold counts the plates were incubated at 28°C for 5-7 days. Coliforms counts were determined according to the method described by Falade et al. (2015).

2.5. Determination of the critical control points

Critical control points (CCPs) and their critical limits were identified throughout the production chain (Fig. 1). Lactose contents were particularly considered as the most important CCPs. The CL of lactose content was monitored carefully and considered crucial for the safety of the developed yoghurt.

2.6. Sensory evaluation

The sensory attributes of all yoghurt samples were evaluated at 5 days intervals throughout the storage period by a semi-trained panellists using scoring methods as described by Han et al. (2014). The ratings were presented on a 9-point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely). Results were given as averages of the three trials for each types of yoghurt.

2.7. Statistical analysis

The microbial count data were transformed to log before submitted to analysis (Kiros *et al*., 2016). The physicochemical, microbiological, and sensory data were statistically analyzed using SAS package (SAS Institute, Cary, USA). Least significant difference (LSD) was used for mean comparison and significance was accepted at $P \leq 0.05$.

3. Results and Discussion

3.1. Raw milk analysis

Prior to manufacturing yoghurt, raw milk was analysed for lactose content, titratable acidity (TA) and pH to confirm its freshness and suitability for yoghurt production. The milk had lactose, TA, and pH of 5.75%, 0.176%, and 6.58, respectively (data not shown). The results were in the normal range for cow milk as reported previously for cow milk from different countries (Hossain *et al*., 2012; Kiros *et al*., 2016). While lactose content was higher than that reported by Chen *et al*. (2014) who found that milk lactose contents were 4.59% and 4.7%, respectively. The milk used in this study was thus regarded as suitable for yoghurt manufacturing.

3.2. Physicochemical characteristics of the developed yoghurt

As shown in Tables 1-3 lactose contents in batches T0, T1, T3, and T4 were higher than the standard level according to Dinçel (2012), who reported that milk had 4.8% lactose, which decreased to 2.5- 2.6% in yoghurt. Also, it was observed that lactose level of batch T2 at day 10 of storage was lower than that reported by Alm (1982) who found that after 11 days storage of yoghurt the lactose content decreased to about 2.3 g/100 g by enzymatic methods. On day zero, batch T2 had the lowest lactose content followed by batches T3, T1, T0 and T4.. The

Table 1. Physicochemical characteristics for processed yoghurt on zero-day

Parameter	Starter Culture Combination				
	T0	**T1**	**T2**	**T3**	**T4**
Fat content (%)	3.74^{b}+0.04	3.35^{e}+0.01	3.45^{d}+0.02	3.93^{a}+0.02	3.52^{c}+0.03
Crude protein (%)	4.65^{a}+0.02	4.16^{d}+0.03	4.24^{c}+0.03	4.67^{a}+0.02	4.54^{b}+0.01
Lactose content (%)	3.74^{a}+0.02	2.92^{b}+0.02	0.34^{d}+0.02	2.82^{c}+0.03	3.77^{a}+0.02
SNF (%)	12.92^{a}+0.03	12.24^{e}+0.02	12.54^{d}+0.02	12.65^{c}+0.01	12.72^{b}+0.02
Total solids (%)	15.86^{a}+0.01	14.66^{d}+0.02	14.96^{c}+0.02	15.23^{b}+0.02	14.92^{c}+0.03
Titratable acidity (%)	0.66^{a}+0.01	0.54^{b}+0.02	0.67^{a}+0.02	0.56^{b}+0.01	0.69^{a}+0.01
Galactose (%)	0.36^{c}+0.01	0.95^{b}+0.01	1.91^{a}+0.03	0.96^{b}+0.02	0.23^{d}+0.01
Glucose (%)	0.41^{d}+0.01	1.06^{c}+0.01	2.06^{a}+0.02	1.17^{b}+0.02	0.36^{e}+0.01
pH-value	4.26^{b}+0.01	3.98^{c}+0.01	4.24^{b}+0.01	4.31^{a}+0.01	4.26^{b}+0.01
Viscosity (c.p)	87.81^{b}+0.20	93.23^{a}+1.03	85.33^{b}+0.76	87.16^{b}+0.26	85.08^{b}+0.56

Mean values (n=3) having different superscript letters in a row are significantly different ($P \leq 0.05$).
T0 = *S. thermophilus+ Lactobacullus bulgaricus;* **T1***= L. acidophilus + B. bacterium lactis;*
T2 *=L. casei + B. bacterium lactis;* **T3***=B. bacterium lactis + S. thermophilus +L Bulgaricus*
T4*= L. casei + S. thermophilus + L. Bulgaricus*

Table 2. Physicochemical characteristics for processed yoghurt on 5th day

Parameter	Starter Culture Combination				
	T0	**T1**	**T2**	**T3**	**T4**
Fat content (%)	3.67^{a}+0.07	3.27^{c}+0.03	3.19^{c}+0.02	3.28^{c}+0.03	3.48^{b}+0.02
Crude protein (%)	4.16^{ab}+0.04	4.07^{b}+0.04	4.18^{a}+0.03	4.19^{a}+0.04	4.27^{a}+0.02
Lactose content (%)	3.54^{a}+0.03	2.66^{c}+0.05	0.25^{d}+0.02	2.66^{c}+0.25	3.41^{b}+0.25
SNF (%)	12.68^{a}+0.03	12.06^{d}+0.05	12.37^{b}+0.04	12.18^{c}+0.04	12.71^{a}+0.04
Total solids (%)	15.36^{a}+0.14	14.47^{c}+0.04	14.57^{c}+0.81	14.26^{d}+0.14	14.76^{b}+0.13
Titratable acidity (%)	0.67^{a}+0.05	0.55^{b}+0.02	0.68^{a}+0.02	0.57^{b}+0.01	0.69^{a}+0.03
Galactose (%)	0.47^{c}+0.04	0.96^{b}+0.08	1.92^{a}+0.09	0.97^{b}+0.02	0.28^{d}+0.03
Glucose (%)	0.49^{c}+0.03	1.27^{b}+0.04	2.10^{a}+0.05	1.27^{b}+0.02	0.46^{e}+0.03
pH-value	4.22^{a}+0.05	3.91^{b}+0.04	4.20^{a}+0.05	3.91^{b}+0.01	4.17^{a}+0.03
Viscosity (c.p)	90.90^{a}+2.70	82.77^{s}+1.05	88.38^{b}+1.76	88.61^{b}+1.36	87.46^{c}+1.56

Mean values (n=3) having different superscript letters in a row are significantly different ($P \leq 0.05$).
T0 = *S. thermophilus+ Lactobacullus bulgaricus;* **T1***= L. acidophilus + B. bacterium lactis;*
T2 *=L. casei + B. bacterium lactis;* **T3***=B. bacterium lactis + S. thermophilus +L Bulgaricus*
T4*= L. casei + S. thermophilus + L. Bulgaricus*

Table 3. Physicochemical characteristics for processed yoghurt on 10th day

Parameter	Starter Culture Combination				
	T0	**T1**	**T2**	**T3**	**T4**
Fat content (%)	2.48^{a}+0.01	2.56^{a}+0.01	2.31^{d}+0.07	2.28^{b}+0.04	2.45^{a}+0.04
Crude protein (%)	2.79^{a}+0.08	2.31^{b}+0.03	2.70^{a}+0.10	2.18^{b}+0.03	2.24^{b}+0.06
Lactose content (%)	2.89^{a}+0.44	2.59^{a}+0.04	0.22^{b}+0.02	2.64^{a}+0.29	2.95^{a}+0.05
SNF (%)	7.44^{a}+0.51	7.81^{a}+0.07	7.84^{a}+1.01	7.15^{a}+0.29	7.42^{a}+0.57
Total solids (%)	8.97^{a}+0.15	8.56^{a}+0.66	9.64^{a}+1.11	9.25^{a}+0.25	9.80^{a}+0.35
Titratable acidity (%)	0.68^{a}+0.09	0.59^{a}+0.09	0.69^{a}+0.02	0.62^{a}+0.03	0.71^{a}+0.02
Galactose (%)	0.95^{b}+0.04	1.01^{b}+0.04	1.99^{a}+0.17	0.99^{b}+0.05	0.29^{c}+0.01
Glucose (%)	1.06^{c}+0.04	1.36^{b}+0.03	2.12^{a}+0.01	1.29^{b}+0.09	0.54^{d}+0.01
pH-value	4.19^{a}+0.01	3.90^{b}+0.09	4.13^{a}+0.03	3.84^{b}+0.14	3.93^{b}+0.07
Viscosity (c.p)	91.53^{a}+2.56	78.20^{b}+2.08	90.22^{a}+1.07	88.90^{a}+1.10	89.56^{a}+0.51

Mean values (n=3) having different superscript letters in a row are significantly different ($P \leq 0.05$).
T0 = *S. thermophilus+ Lactobacullus bulgaricus;* **T1***= L. acidophilus + B. bacterium lactis;*
T2 *=L. casei + B. bacterium lactis;* **T3***=B. bacterium lactis + S. thermophilus +L Bulgaricus*
T4*= L. casei + S. thermophilus + L. Bulgaricus*

decreasing trend of lactose had been reported in Labneh during 21 days of storage (Bano et al., 2011). Initially, yoghurt from batch T2 had way lower ($P \leq 0.05$) lactose content than all that from batches T0, T1, T3, and T4. Apparently, such variation in lactose content is attributed to the different strains used in the starter culture combinations. For all batches, lactose content of yoghurt continued to decrease as the storage period increased. Fat, protein, SNF and TS results in all samples were in agreement with that reported previously in many reports (Codex, 2011; Dinçel, 2012; Erkus *et al*., 2014; Falade *et al*., 2015; Kiros *et al*., 2016). Values of TA of batch T3 was lower than the range 0.90- 1.07% reported by Kiros et al. (2016). This could be attributed to milk composition variation. However, TA results of batches T0, T2, T4, and T3 were found above the minimum recommended limit of 0.6% by Codex (2011). The formation of a coagulum is reported to start at this level of acidity (Mukisa et al., 2010). The pH values in batches T0 and T2 were in alliance to other authors have presented an optimum final pH of 4.1 to 4.4 is desirable, this not only facilitates flavour production by the *Lactobacilli* and development of a good coagulum but also prevents the growth of spoilage and pathogenic microbes. Also, pH values of all batches except for batch T1 were in accordance with those observed by Han *et al*. (2014). The growth rate of microbes has been associated with acidification and depended on the culture used (Mukisa *et al*., 2010; Falade *et al*., 2015). The low pH of the samples (below 4.4) must certainly have prevented most pathogens and other acid-susceptible microbes from growing as indicated in all batches (Falade *et al*., 2015). Viscosity results of batches T0, T2, T4, and T3 were within the values reported by Erkus et al. (2014) in different yoghurt samples. While viscosity results were found high in the case of batch T1. Fat, protein, SNF and TS results of all batches decreased significantly ($P \leq 0.05$) over the storage period. The decline in fat and protein content may be attributed to lipolytic and proteolytic changes. In addition, the decreasing trend of fat, protein, SNF, and TS during storage had been reported in previous studies (Ismail *et al*., 2006; Bano *et al*., 2011; Falade *et al*., 2015). The TA values of all batches increased significantly ($P \leq 0.05$) over the storage period with the exception of batch T1. TA results of batches T0, T2, T4, and T3 were consistent with previous studies, which found that the concentration of lactic acid also increasing on storage (Bano *et al*., 2011; Nguyen *et al*., 2014). The pH values of all batches decreased significantly ($P \leq 0.05$) over storage period. During the storage period, a stable decrease of the pH values and increase in the TA was observed in batches T0, T2, T4, and T3, and this could be ascribed to the starter culture's activity. A similar trend was observed for a study reported that decreased pH values resulted in simultaneous increased titratable acidity values over the storage period (Falade *et al*., 2015). While no direct relationship was observed between pH and TA in batch T1, this result was similar to those reported by Olugbuyiro (2011). Galactose and glucose contents of all batches increased significantly ($P \leq 0.05$) over the storage period. These results were in alliance with the result reported by Alm (1982). Viscosity results of batches T0 and T1 decreased significantly ($P \leq 0.05$) over storage period. While, results of viscosity in batches T2, T0, T4, and T3 increased significantly ($P \leq 0.05$) over the storage period. Results of viscosity for batches T2, T0, T4, and T3 were in alliance with the result of (Falade *et al*., 2015), and different from those reported by (Hanif *et al*., 2012).

3.3. Determination of the critical control points (risk assessment and hazard analysis)

Raw milk, inoculation, incubation, filling, and storage are the critical control points (CCPs) during manufacturing of yoghurts (Fig. 1). Raw milk is the first CCP as it constitutes a suitable substrate for dangerous pathogens and other microorganisms, which may contaminate milk after collection (Chountalas *et al*., 2009). To control these hazards, milk was chilled at 0-4 ºC immediately after collection and maintained at this temperature during transportation. Air-borne pathogens may contaminate milk powder after its production (Ali and Fischer, 2005). To control these hazards, milk powder was mixed with other ingredients and subjected to heat treatment during yoghurt manufacture (Fig. 1). Starter culture should be of high vitality and free from foreign bacteria. Culture viability was measured by the amount of lactic acid produced (Tamime and Robinson, 1999). Inoculation is the second CCP and the only hazard at stage relates to inefficient heat treatment since already existing pathogens are not destroyed. This can happen if heat treatment temperature drops below 75 ºC for at least 20 sec, to control that milk was pasteurized at 90 °C for 15 min. Provided that all raw materials are safe before use, no other hazards are present (Chountalas *et al*., 2009). Incubation and filling are the third CPP. During incubation, airborne pathogens such as coliforms, molds, and yeasts constitute possible hazards, and to control these hazards incubation process was done under aseptic conditions. Packaging or filling materials could also be a source of microbial contamination, which was effectively eliminated under aseptic packaging conditions. Storage is the last CPP as the product storage period provides the time necessary for detecting hazards introduced at previous production stages (Chountalas *et*

al., 2009). Owing to the acidic nature of yoghurt, the spoilage microbes that may appear are coliforms, yeasts, and molds for which critical limits have been defined (Mayoral *et al*., 2005). In the storage stage, long exposure to high temperature (above 68 ºC) favours the growth of yeasts and molds (Viljoen *et al*., 2003), and to avoid that the manufactured yoghurt were immediately stored after fermentation process in a refrigerator at 4-6 °C as described in the previous section.

3.4. Microbiological analysis

The microbial counts of the different yoghurts (T0, T1, T2, T3, and T4) during cold storage for 0, 5, and 10 days are shown in Fig. 2A, B, & C. According to the Codex standard for fermented milks (Codex, 2011), yoghurt should contain a minimum of 7 log CFU/ml as the total of lactic acid bacteria microorganisms constituting the starter culture. All the yoghurt samples in all batches satisfied this requirement. The presence of coliform in food is an indication of poor hygiene and possible contamination with microorganisms of faecal origin (Mukisa *et al*., 2010; Falade *et al*., 2015). Coliforms were not detected in all batches. This is in agreement with the study of Sengupta *et al*. (2013), where an absence of coliform was reported in fresh and fortified soy yoghurts at zero time and on 7th day of storage. Coliforms disappearance is attributed to the combined effect of pH dropping below 4.5 and the action of antimicrobial substances produced by lactic acid bacteria (Mukisa *et al*., 2010). Yeasts and moulds were not detected in all batches. This could be attributed to the high hygienic conditions followed in the laboratory that prevented post-production contamination since the main factors for yeast and mould growth in yoghurt production are microbiological quality of any ingredients introduced into yoghurt after heat treatment (85°C for 30 min) of the milk (Kiros et al. 2016). Several studies have reported contamination of processed yoghurts by yeasts and molds (El Bakri and El Zubeir, 2009). Furthermore, starter cultures are widely known for the production of organic acids and other secondary metabolites such as bacteriocins that act against the growth of spoilage and pathogenic bacteria during fermentation (Jayeola *et al*., 2010).

3.5. Sensory evaluation

Significant differences were observed for mean flavour, consistency, colour, taste, and overall acceptability scores among T0, T1, T2, T3, and T4 yoghurt sample (Fig. 3). The means flavour, consistency, taste, colour and overall acceptability scores of yoghurts of all samples decreased significantly during storage. At the beginning of storage (day 0, significantly high mean flavour score (8) was recorded in yoghurts obtained from T2, while minimum flavour score (5) was found in control yoghurts obtained from T0 treatment. Yoghurts obtained from combination T2 exhibited significantly highest consistency and colour scores (9) at start of storage, while lowest mean scores (5) of these attributes were recorded in T3 yoghurt at end of storage. T0 yoghurts exhibited significantly highest mean taste score (9) at the beginning of storage, while lowest mean score (5) was recorded in yoghurts obtained from T3 yoghurt at end of storage. Non-significant differences were observed for mean overall acceptability scores among control (T0) yoghurts and T2 yoghurts during cold storage.

4. Conclusion

The combination T2 (*Lactobacillus casei* and *Bifidobacterium lactis*) was efficient to substantially decrease lactose content of yoghurt and developed a product with good overall characteristics compared to T0 (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*), T1 (*Lactobacillus acidophilus and Bifidobacterium lactis*), T3 (*B. lactis*, *S. thermophilus*, and *L. bulgaricus*), and T4 (*L. casei*, *S. thermophilus*, and *L. bulgaricus*) containing yoghurts. Therefore, the selected T2 combination could be considered potentially appropriate for producing a functional probiotic yoghurt with low lactose that suitable for lactose intolerant individuals.

Conflict of interest

The authors declare that they have no conflict of interest.

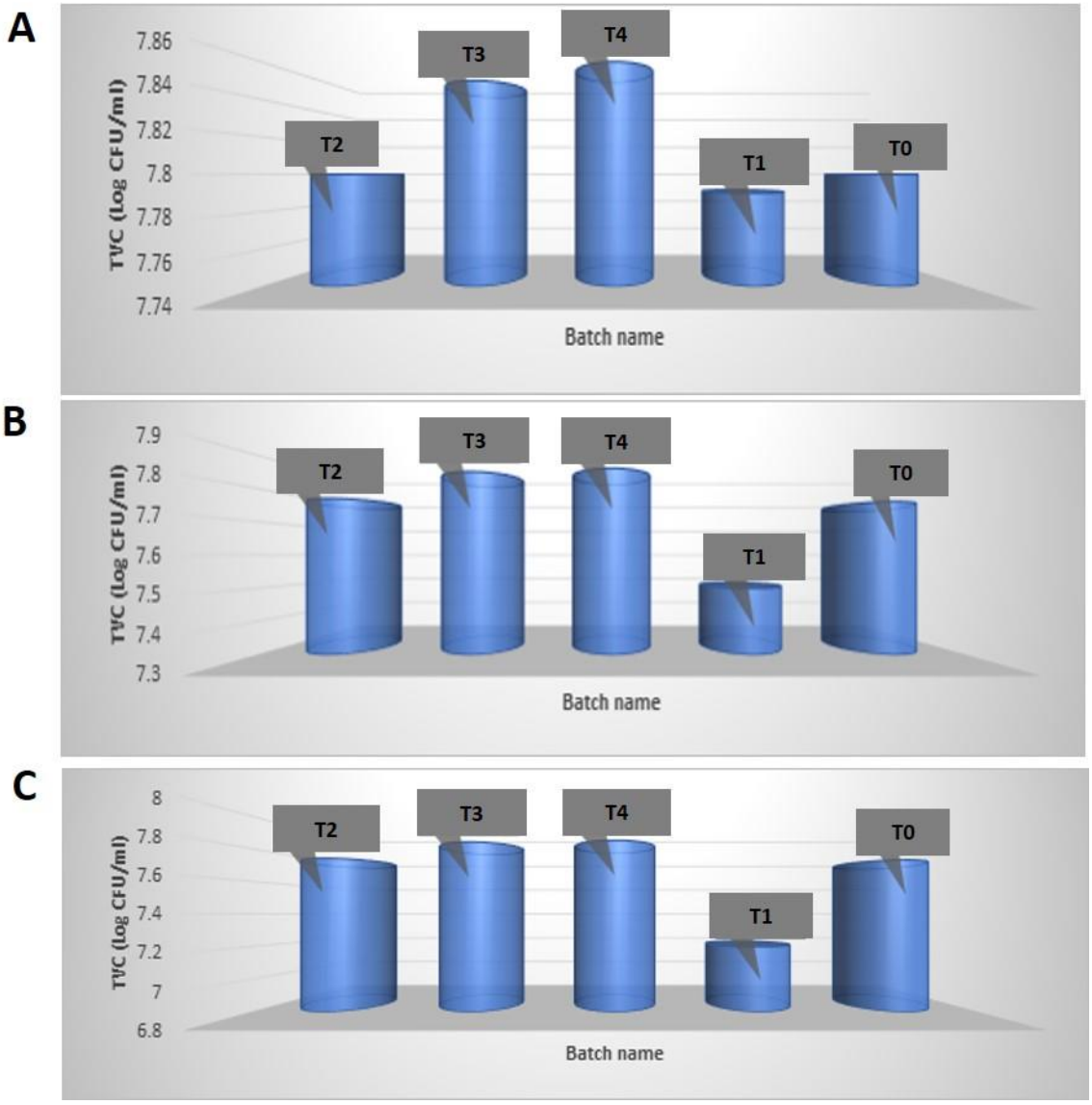

Fig. 2. Total viable count (log_{10} CFU/ml) of the processed yoghurt (T0, T1, T2, T3, and T4) at day 0 (A), day 5th (B), and day 10th (C) of storage.

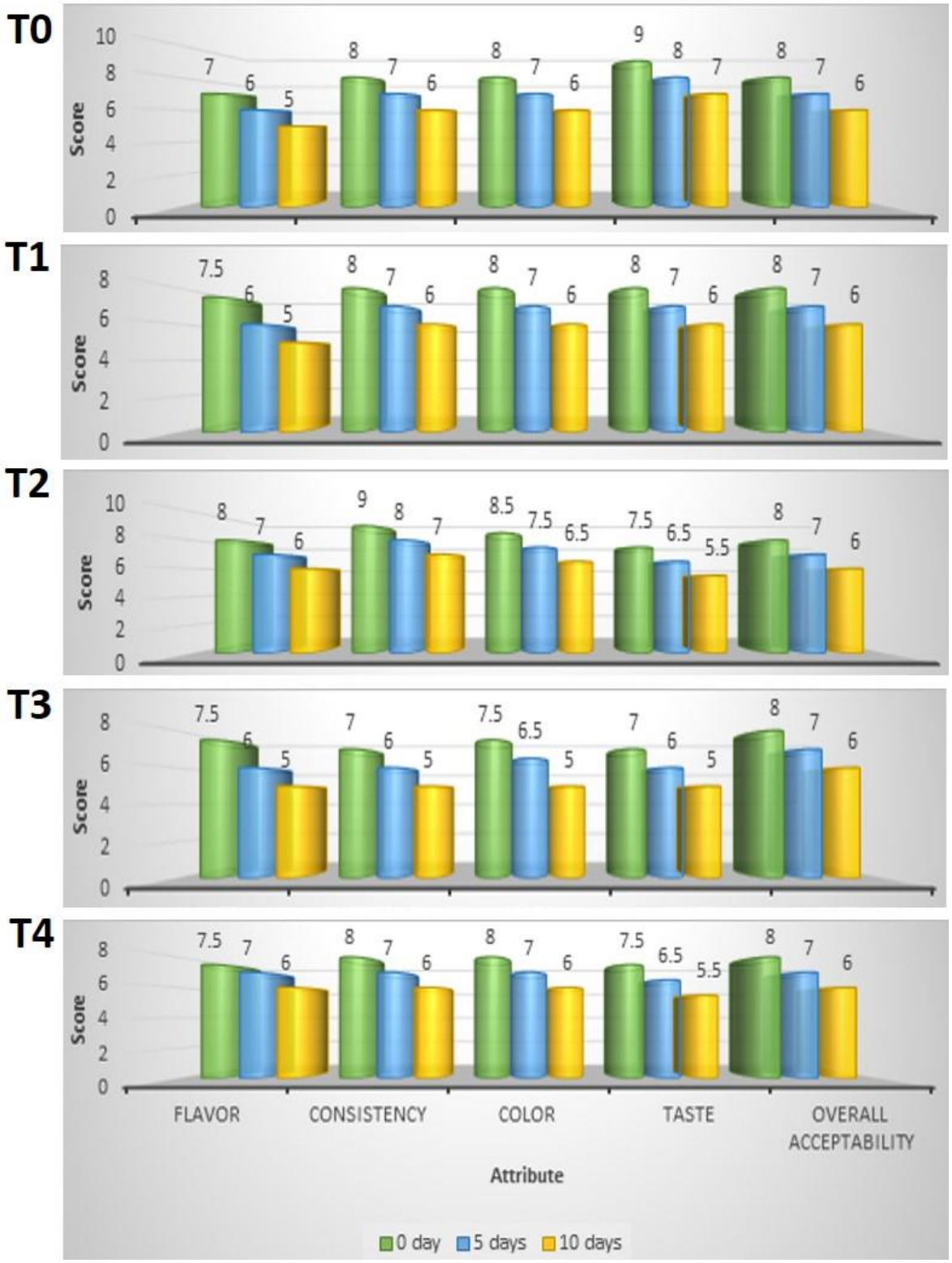

Fig.3. Sensory quality attributes (flavor, consistency, color, taste and overall acceptability) of yoghurt samples (T0, T1, T2, T3, and T4) during 0, 5 and 10 days of storage.

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