



Shelf-life characteristics of Bambara groundnut (*Vigna subterranea* (L.) Verdc) probiotic beverage

Yvonne Yeukai Murevanhema and Victoria Adaora Jideani*

Department of Food Science and Technology, Cape Peninsula University of Technology, P. O. Box 1906, Bellville 7535, Cape Town, South Africa

*Corresponding author. Email: jideaniv@cput.ac.za

Two Bambara groundnut probiotic beverages (BGNPB), fermented with (1) *Lactobacillus bulgaricus* and (2) *Lactobacillus bulgaricus* and *Lactobacillus plantarum* were assessed for their shelf-life characteristics. During the shelf-life study, sampling of the BGNPB was done at two-days interval for 28 days for pH, total titratable acidity (TTA), microbial load and colour. Shelf-life data was modelled with Mitscherlich's law of diminishing returns model and used to estimate the shelf-life of the BGNPB. After the 28 days of the storage pH was pH 4.30 and pH 4.25 at 5°C; pH 3.94 and pH 3.72 at 15°C and pH 3.78 and pH 3.45 at 25°C for (1) *L. bulgaricus* and (2) *L. bulgaricus* and *L. plantarum*, respectively. TTA was 1.8% and 2.0% at 5°C; 2.0% and 2.2% at 15°C while at 25°C it was 2.6% and 2.6% for (1) *L. bulgaricus* and (2) *L. bulgaricus* and *L. plantarum*, respectively. Microbial load and colour were significantly affected by the storage temperatures and time. Shelf-life was estimated as 28 days (right censored) for both BGNPB stored at 5°C. The shelf-life of the BGNPB under refrigeration (5°C) was longer when compared to the dairy counterparts with shelf-life of 14 days at 4°C. Therefore, the longer shelf-life of BGNPB makes the beverage a suitable vehicle to transport probiotics to humans.

Keywords: Bambara groundnut, Mitscherlich's law, probiotic beverage, right censored, shelf-life

Introduction

The time a fermented product remains safe to eat determines its shelf-life. Probiotics are defined as 'live microorganisms that, when administered in adequate amounts confer a health benefit on the host' (Champagne and Gardner 2008; Prado et al. 2008; Gupta and Sharma 2016). The viability of probiotics in products has been cited as an important prerequisite for achieving the beneficial health effects (Bao et al. 2010; Lupien-Meilleur, Roy, and Lagace 2016).

Poor storage conditions can lead to spoilage of fermented products, including probiotic beverages, within a short period of time (Mataragas et al. 2011). A discernible reduction in sensory characteristics is associated with indices of failure of probiotic beverages. Spoilage of probiotic beverages is as a result of changes in its physical, chemical and organoleptic characteristics (Shimakawa et al. 2003; Madora, Takalani, and Mashau 2016), and its microbial load (Ng, Yeung, and Tong 2011), making it unsuitable for human consumption (Mataragas et al. 2011) and diminishing the potential health benefits conferred by the products (Ng, Yeung, and Tong 2011). Maintaining a high load of viable probiotics is threatened by a number of factors, strain variation, acid accumulation and acidity of the product, levels of oxygen in the products and permeability of oxygen through packaging (Biorollo, Reinheimer, and Vinderola 2000; Oliveria et al. 2001; Shimakawa et al. 2003; Lupien-Meilleur, Roy, and Lagace 2016). Champagne and Gardner (2008) stated that probiotic bacteria lose viability during storage in many fermented milk types with pH values below 4.0. Nevertheless, viability during storage and conferring health benefits are of primary importance although there are instances where non-viable cells have shown health benefits (Ouwehand and Salminen 1998). However, it is still desirable to have live cultures in the product, to comply with the regulated specifications.

Fermented plant products are nutritious foods containing high amounts of nutrients and minerals (Wang et al. 2007; Granato et al. 2010; Vijayendra and Halami 2015). Legumes contain bioactive substances (Berk, Tepe, and Arslan 2011; Bouchenak and Lamri-Senhadjji 2015) that protect food quality by retarding oxidative breakdown of lipids (White and Xing 2008). Human consumption of probiotic fermented food has been associated with tremendous health benefits due to improvements of gastrointestinal functions (Granato et al. 2010; Thapa and Tamang 2015).

Bambara groundnut (*Vigna subterranea* (L.) Verdc) (BGN) is an indigenous African crop that has been cultivated in Africa for years. It is a highly nutritious plant that plays a crucial role in people's diets (Murevanhema and Jideani 2015). Like soybean yoghurt, fermented BGN milk (BGNM) may be a suitable probiotic bacterial strain carrier to the host because of its inherent presence of antioxidants, chemical and nutritional composition. It has been established that BGNM could support the growth of lactic acid bacteria (Murevanhema 2013). BGNM contains beneficial nutrients, such as minerals, amino acids (Murevanhema and Jideani 2015) vitamins and bioactive components (Nti 2009). A consumer acceptable BGN probiotic beverage (BGNPB) was successfully developed. However, as far as we know, the shelf-life characteristics of BGNPB has not been documented. Therefore, the objective of this study was to establish the shelf-life characteristics of BGNPB.

Materials and methods

Source of Bambara groundnuts, starter cultures

Bambara groundnuts were purchased from Thusano Products, Louis Trichardt, Limpopo; seeds were sorted into varieties, according to different testa and hilum colours: black; red; brown; black-eye and brown-eye. Bacterial

strains *Lactobacillus bulgaricus* and *Lactobacillus plantarum* were purchased from Danisco and Quantum Bio-technologies, Cape Town.

Production of Bambara groundnut flour and Bambara groundnut milk

The red BGN seeds were chosen on the basis of being better in supporting the growth of *L. bulgaricus*, and consumer preference (Murevanhema 2013). The red variant BGN flour and BGNM were prepared following the method reported by Murevanhema and Jideani (2015).

Production of Bambara groundnut probiotic beverage

Bambara groundnut milk (BGNM) (100 mL x 2) in 250 mL schott bottles were warmed to 45°C in a water bath. The BGNM was then inoculated with 3% of a mother culture of *L. bulgaricus* and another with *L. bulgaricus* and *L. plantarum* and incubated at 35°C for 24 h without stirring as estimated in the study by Murevanhema (2013). The fermented BGNM designated as BGN probiotic beverage (BGNPB) was then cooled in an ice bath and stored in a refrigerator at 5°C.

Shelf-life stability of Bambara groundnut probiotic beverage

The Bambara groundnut probiotic beverage (BGNPB) cold-filled in 250 mL polyethylene terephthalate (PET) (rectangular 127 H x (113 x 50), mass 15 g) plastic bottles (Hilfort plastics, Cape Town, South Africa) were stored in a refrigerator at 5°C, while the other two sets were stored in two Memmert incubators at 15°C and 25°C, respectively. The samples were monitored for pH, total titratable acidity, microbial load and colour each second day for 28 days. The methodologies are described below.

The pH of Bambara groundnut probiotic beverage during storage

The pH of the BGNM (20 mL) was measured at room temperature (21 ± 2°C) using a Knick 766 laboratory pH meter (Elscolab, Netherland).

Total titratable acidity of Bambara groundnut probiotic beverage during storage

Total titratable acidity (TTA) was determined according to the South African legislation on dairy products (Anon 1997). Fermented BGNM (9 ml) was pipetted into an Erlenmeyer flask (250 mL). To it 0.5 mL of a 1.6% phenolphthalein indicator solution in 50% ethanol was added. The fermented BGNM was then titrated against 0.1 M NaOH solution until the first tinge of pink that persists for 30 s appeared. To express the total titratable acidity of the BGNPB as the percentage of lactic acid, the number of millilitres of 0.1 M NaOH used in the test was divided by 10. Percentage of lactic acid, the number of millilitres of 0.1 M NaOH used in the test was divided by 10, as show in equation 1:

$$\% \text{ Lactic acid} = \frac{\text{ml of NaOH used}}{10} \quad (1)$$

Microbial load of Bambara groundnut probiotic beverage during storage

The microbial load was determined according to the method described by Wang et al. (2007), with minor adjustments. BGNPB inoculated with *L. bulgaricus* and another with *L. bulgaricus* and *L. plantarum* (1.0 mL each) were mixed with 9.0 mL of 0.1% sterile peptone solution, serial dilution up to nine were made. Aliquot (1.0 mL) of dilutions (3, 6 and 9) were plated out on 90 mm petri dishes and poured de Man, Rogosa and Sharpe (MRS) agar (12 ± 2 mL). When the agar had solidified, they were double layered with MRS agar (9 ± 2 mL), placed in an anaerobic jar and incubated at 37°C for 48 h.

Colour of Bambara groundnut probiotic beverage during storage

The colour of the BGNPB (50 mL) samples was measured using a Hunterlab Colorflex Spectrophotometer 45°/0° standard, set at standard observer 10° and D65 and standardized with white tile ($L^* = 93.41$, $a^* = -1.18$, $b^* = 0.75$). The BGNPB samples were placed in glass samples holder and reflectance measured for $L^*a^*b^*$ and L^*C^*h colour scales. L^* is lightness with 100 as maximum indicating a perfect reflecting diffuser and the minimum is zero, which is black. The a^* and b^* axes have no specific numerical limits, negative a^* is green, positive is red. Negative b^* is blue, positive b^* is yellow (Sahin and Samnu 2006). Chroma (C) is the quality that distinguishes a pure hue from a grey shade and describes hue saturation or purity; its axis extends from the values (lightness) axis towards the pure hue (Sahin and Samnu 2006).

Data analysis

All results were reported as mean of three independent trials. Analysis of variance (ANOVA) was used to determine the differences between treatments. Duncan's multiple range tests was used to separate means where differences existed (IBM SPSS 2010).

To estimate the shelf-life of BGNPB the Mitscherlich's law of diminishing returns model ($pH = b_1 + b_2 \exp^{-b_3 t}$) was used to model the effect of storage time on the pH of the BGNPB. In this model b_1 is the lower asymptote; b_2 is the difference between the maximum pH and the lower asymptote; and b_3 is the rate at which the minimum pH is reached known as the rate constant (day^{-1}). The model parameters were estimated using the non-linear regression model following the sequential quadratic programming estimation method. The coefficient of determination (R^2) for each regression equation, a measure of the variability in the dependent variable accounted by the model, was calculated as shown in equation 2 (IBM SPSS 2010):

$$R^2 = 1 - \frac{\text{residual sum of squares}}{\text{corrected total sum of squares}} \quad (2)$$

The effect of temperature on the beverage was modelled with Arrhenius equation as shown in equation 3 (Jideani

et al. 2002):

$$\ln(k_q) = \ln(k_{qref}) + \left[\frac{E_a}{R} \left(\frac{1}{T_{ref}} - \frac{1}{T} \right) \right] \quad (3)$$

where k_q = reaction rate constant; k_{qref} = quality rate constant at reference temperature (T_{ref}); T = absolute temperature (in degrees K); E_a = apparent activation energy (in J/mol); R = universal gas constant (8.314 kJ/mol K). The reference temperature (288 K or 15°C) was the mean of the value of the temperature studied.

Results

Effect of storage time and temperature on the pH, TTA and microbial growth in Bambara groundnut probiotic beverage during storage

The pH of BGNPB with *L. bulgaricus* ranged from pH 4.39 to pH 4.3, pH 4.39 to pH 3.94 and pH 4.39 to pH 3.64 at 5, 15 and 25°C storage temperatures respectively (Figure 1). While the beverage with *L. bulgaricus* and *L. plantarum* pH ranged from pH 4.30 to pH 4.25, pH 4.30 to pH 3.75 and pH 4.30 to pH 3.23 at 5, 15 and 25°C storage temperatures respectively. The pH of the two BGNPB were significantly different ($p=0.0001$) from each other. The storage temperature and time had a

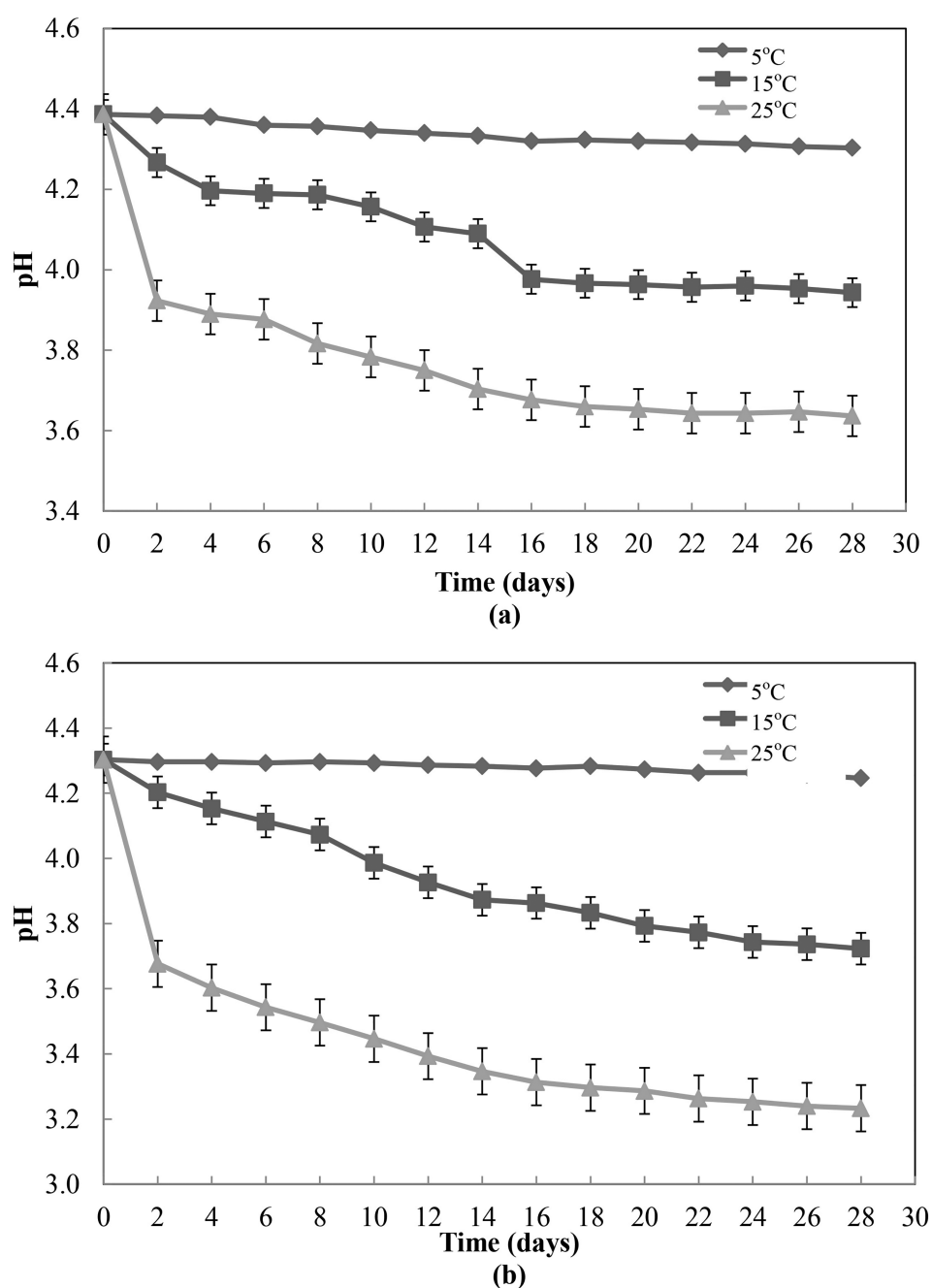


Figure 1: Effect of storage time and temperature on the pH of (a) *L. bulgaricus* and (b) *L. bulgaricus* and *L. plantarum* BGNPB.

significant ($p=0.0001$); ($p=0.0001$) effect, respectively on the pH of the BGNPB. Interaction of the main effect (temperature*time) had a significant ($p=0.00001$) effect on the pH of the BGNPB. BGNPB stored at 5°C had a fairly stable pH. However, storage at 15°C and 25°C resulted in a significant decrease in pH over time.

The total titratable acidity (TTA) (Figure 2) of the BGNPB with *L. bulgaricus* ranged from 1.8–1.87%, 1.8–2.25 and 1.8–2.57% at 5, 15 and 25°C storage temperatures, individually. The beverage with *L. bulgaricus* and *L. plantarum* ranged from: 1.87% to 2%, 1.87% to 2.4% and 1.9% to 2.6%, at 5, 15 and 25°C, respectively. The TTA of the two beverages was significantly

($F(1.254,0.007)=189.433$; $p=0.0001$) affected during the period of study. The storage temperature, storage time, and their interaction significantly ($p=0.001$); ($p=0.0001$) and ($p=0.00001$), affected the TTA of the BGNPB, respectively. The TTA of BGNPB at the 5°C, increased gradually, while an exponential increase was noted for the BGNPB stored at 15 and 25°C.

BGNPB with *L. bulgaricus* had a microbial load range of 7.05–7.88 Log cfu/mL, 7.13–7.59 Log cfu/mL and 7.04–7.59 Log cfu/mL at 5, 15 and 25°C storage temperatures, respectively (Figure 3). Meanwhile the BGNPB with *L. bulgaricus* and *L. plantarum* ranged from 7.06–7.52 Log cfu/mL, 7.08–7.52 log cfu and 7–7.52 Log

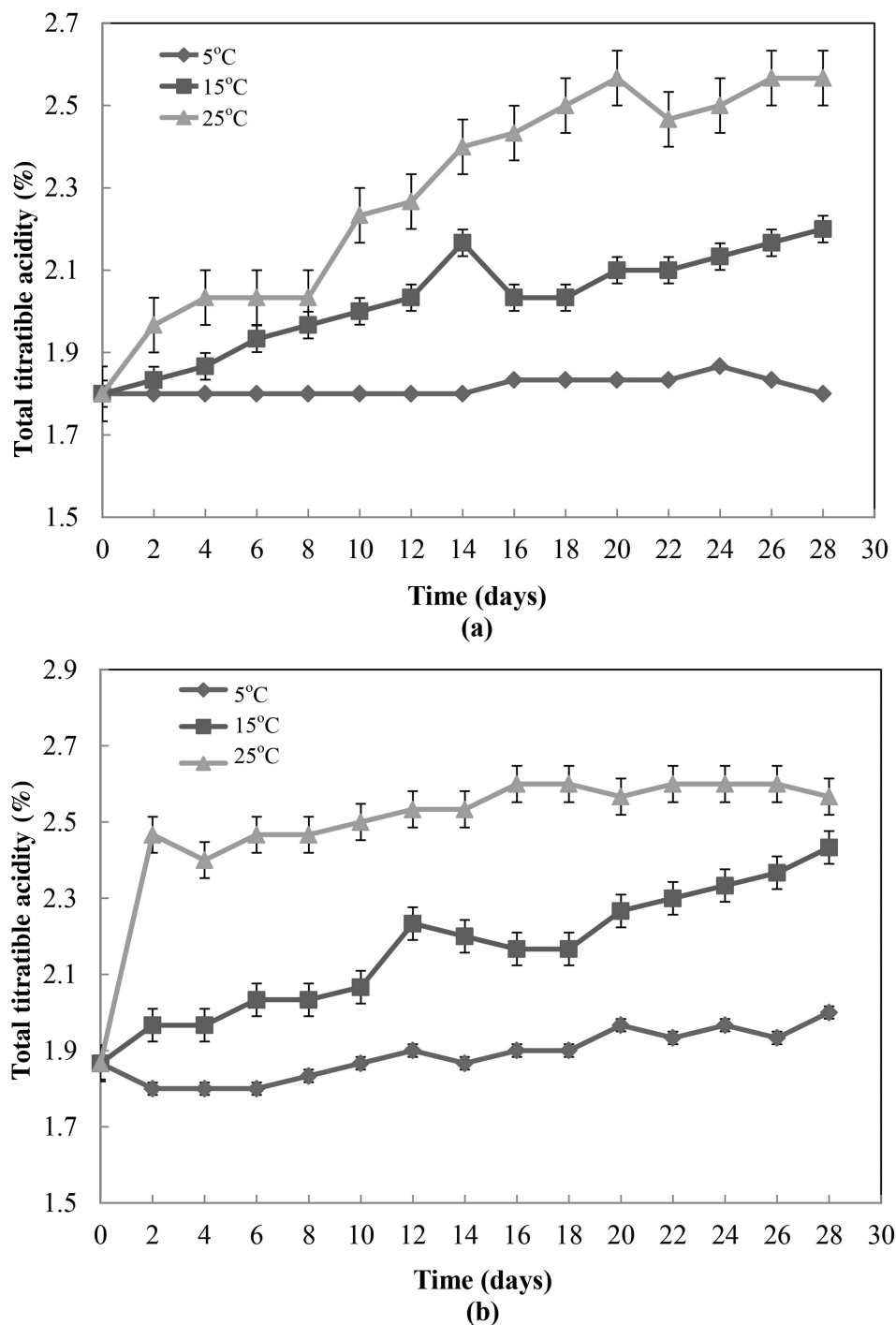


Figure 2: Effect of storage time and temperature on the TTA of (a) *L. bulgaricus* and (b) *L. bulgaricus* and *L. plantarum* BGNPB.

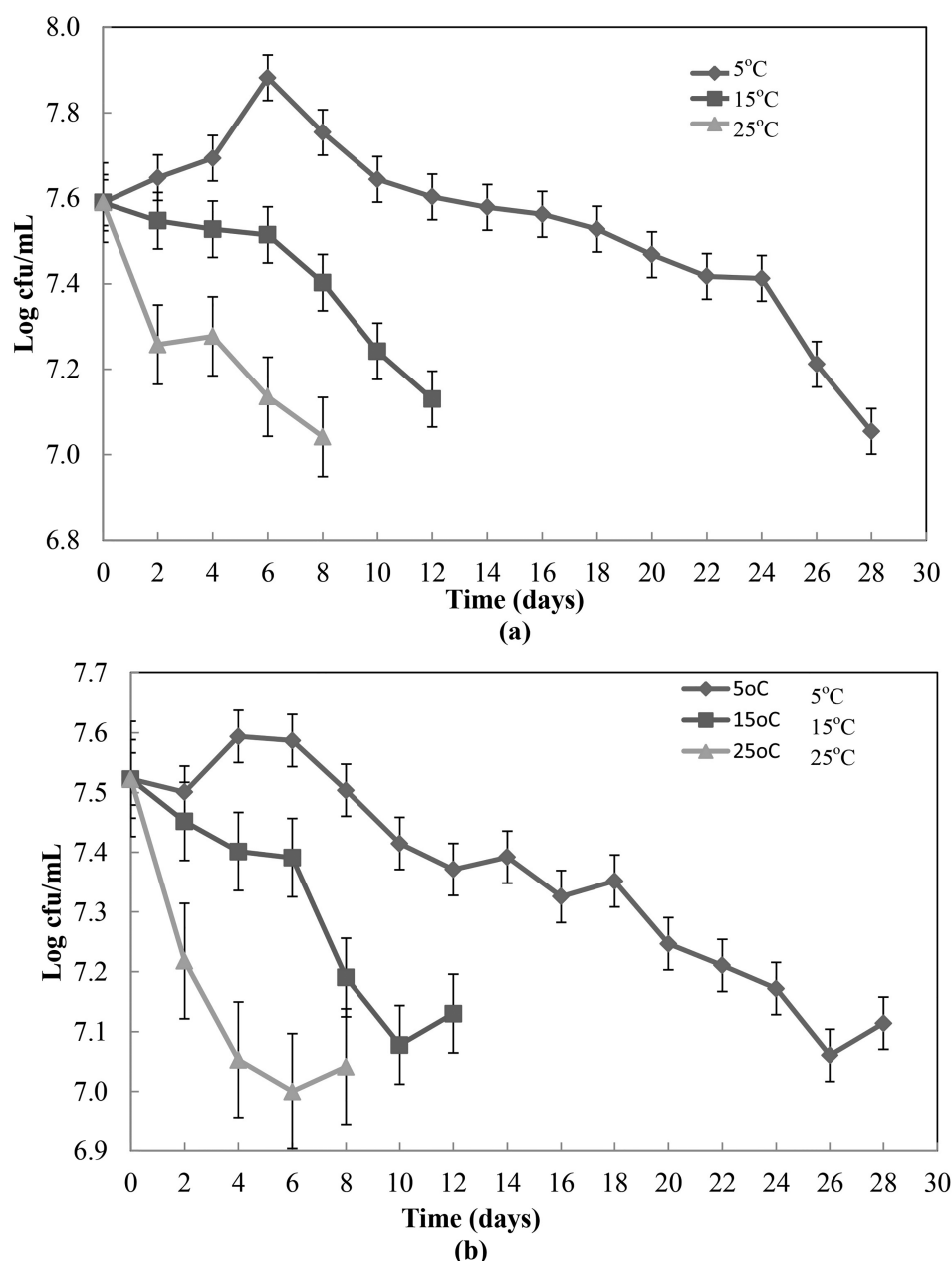


Figure 3: Effect of storage time and temperature on the microbial load of (a) *L. bulgaricus* and (b) *L. bulgaricus* and *L. plantarum* BGNPB.

cfu/mL at 5, 15 and 25°C storage temperatures respectively. Storage temperature ($p=0.0001$), time ($p=0.0001$) and the interaction of the main effect (temperature * time) [$p=0.0001$] had a significant effect on the microbial load, respectively. Microbial load decreased gradually for the BGNPB stored at 5°C, while the BGNPB kept at 15 and 25°C showed a sudden decrease during the storage period.

Effect of storage temperature and time on the colour of Bambara groundnut probiotic beverage

The effect of storage temperature and time on the colour of BGNPB is illustrated in Figure 4. The lightness of the BGNPB with *L. bulgaricus* ranged from 43.2–55.3, 43.2–63.5 and 43.2–67.0, at 5, 15 and 25°C storage temperature, respectively. The lightness of the *L. bulgaricus* and *L. plantarum* ranged from 43.4–56.2,

43.4–64.4 and 43.4–68.0 at 5, 15 and 25°C, storage temperatures respectively. Temperature and time had significant ($p=0.0001$) and ($p=0.0001$) effects on the lightness of the BGNPB. The interaction of time and temperature also had a significant ($p=0.0001$) effect on lightness of the BGNPB. The two BGNPB were also significantly different in lightness; there was an increase in lightness with time. The increase was relatively gradual for the BGNPB stored at 5°C, while a faster increase was observed for BGNPB at 15°C and 25°C.

Redness, yellowness, and hue angle of the BGNPB, were significantly ($p<0.05$) affected by storage temperature, time and the interaction (temperature*time). Redness had a mean range of 4.2–6.4; yellowness 7.2–13.6, and hue angle 45.9–58.7. The redness (a^*) and yellowness (b^*) of the BGNPB were positive which implied that the beverages are in the red and yellow colour space. This

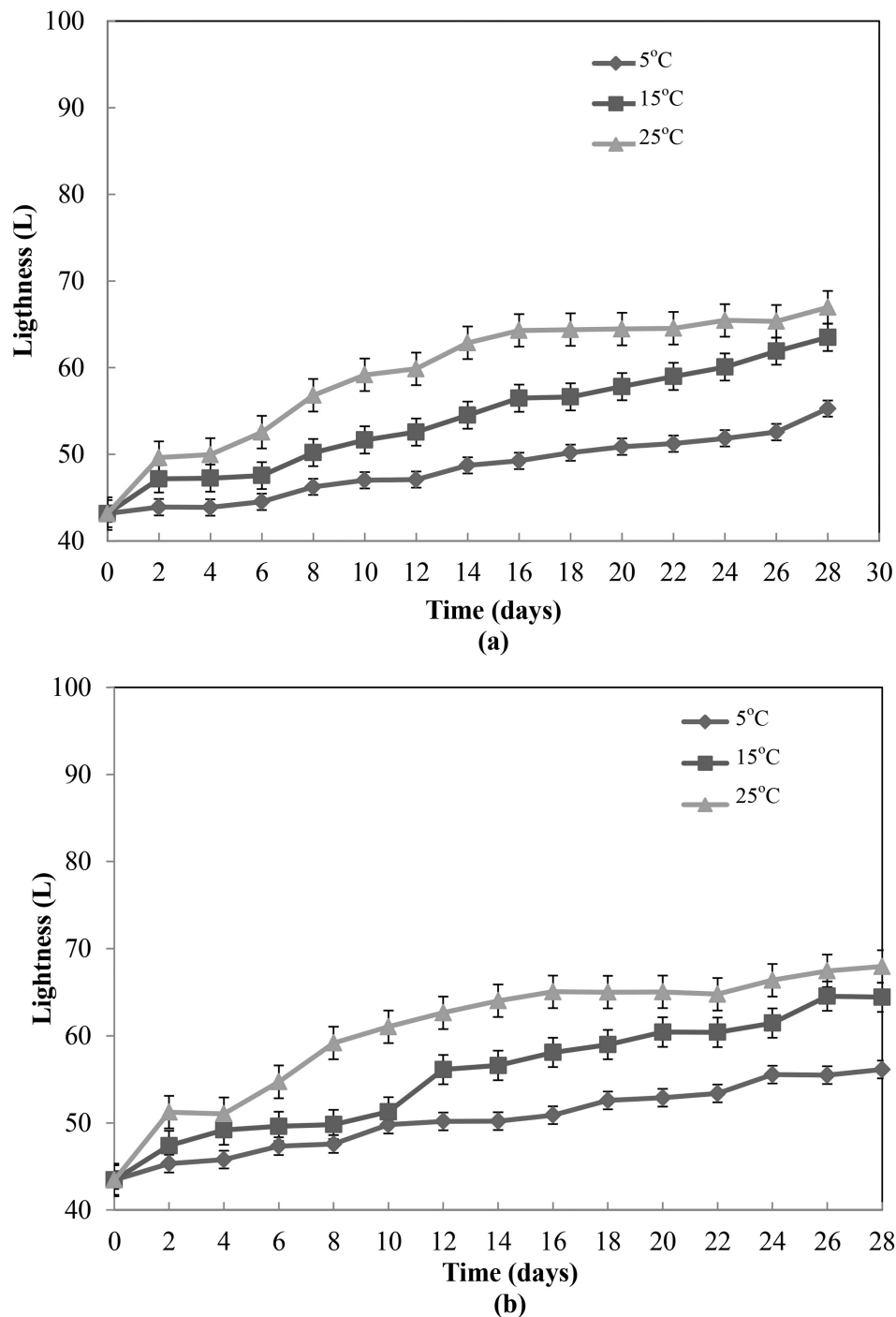


Figure 4: Effect of storage time and temperature on the lightness of (a) *L. bulgaricus* and (b) *L. bulgaricus* and *L. plantarum* BGNPB.

was further confirmed by the hue angles which were within the zero and 90° colour space, which covers the red and the yellow space (Bible and Singha 1993).

Chroma was not significantly affected by the temperature and time during the shelf-life study. A range of 6.3–11.3 was observed for the BGNPB. The two BGNPB were not significantly different in terms of chroma. The hue of the BGNPB was less saturated.

Modelling of shelf-life of BGNPB using pH as index of deterioration

Mitscherlich's law of diminishing returns model was used to model the effect of storage time on the pH of the probiotic beverage. Table 1 indicates the parameters of the model

for *L. bulgaricus* BGNPB and *L. bulgaricus* and *L. plantarum* BGNPB. Index of deterioration below which the probiotics are unable to survive in the BGNPB was chosen as pH 4. The lower asymptote (b_1) decreased with an increase in storage temperature reaching below pH 4 at the end of the storage period in both beverages. The difference between the maximum pH and the lower asymptote (b_2) in both beverages increased with an increase in temperature. The time which the minimum pH or the rate constant (b_3) was reached decreased as the storage temperature increased for both beverages. The rate of deterioration appeared to be faster for *L. bulgaricus* at 15 and 25°C compared to that of *L. bulgaricus* and *L. plantarum*. The rates of deterioration

Table 1: Parameters of the Mitscherlich's law of diminishing returns model for pH of BGNPB^{1,2}.

Temperature (°C)	b ₁	b ₂	b ₃ (day ⁻¹)	R ²	Estimated shelf-life (days)
<i>L. bulgaricus</i>					
5	4.271	0.133	0.093	0.93	28*
15	3.836	0.591	0.125	0.94	18
25	3.664	1.040	0.470	0.92	2
<i>L. bulgaricus</i> and <i>L. plantarum</i>					
5	3.374	0.937	0.004	0.85	28*
15	3.543	0.846	0.108	0.99	10
25	3.288	1.525	0.497	0.94	2

¹BGNPB = Bambara groundnut probiotic beverage

²($pH = b_1 + b_2 \exp^{-b_3 t}$) where b₁ = lower asymptote; b₂ = difference between the maximum pH and the lower asymptote and b₃ = rate at which the minimum pH is reached known as the rate constant (day⁻¹). *Right-censored value – failure time was not observed at the end of the shelf-life

were inserted into the Mitscherlich's law of diminishing returns model ($pH = b_1 + b_2 \exp^{-b_3 t}$) to estimate the time it would take the product to reach the pH of 4. This estimated shelf-life in days is indicated in Table 1 with the three different storage temperatures and the pH.

Effect of temperature on the rate constant of BGNPB during storage

The natural logarithm of the rate constants (b₃ in Table 1) were regressed against the inverse of the temperature using linear regression in order to estimate the model parameters of the Arrhenius equation as illustrated in Figure 5, and Table 2 details the estimated parameters, the activation energy (E_a), the quality rate constant at reference temperature (k_{qref}) and the coefficient of determined (R²). The high values of R² (0.80-0.89), indicates that the effect of temperature on the rate constants during storage could be described by the Arrhenius equation. Activation energy (E_a) of 12.8 kJ/mol was estimated for BGNPB with *L. bulgaricus*, while a higher value of 16.9 kJ/mol was obtained for the BGNPB with *L. bulgaricus* and *L. plantarum*.

Discussions

A significant decrease in pH over time at storage 15°C and 25°C was observed. The same trend was observed by Bensmira and Jiang (2011) and explained as post acidification, which is the reduction of pH and accumulation of organic acids during refrigerated storage (Ng, Yeung, and Tong 2011). The pH of the BGNPB at 5°C was within 4.3 and 4.2; these pH values are within the pH of yoghurt as documented by Tamime and Robinson (1999). The pH range was suitable for restraining pathogenic growth (Yoon, Woodams, and Hang 2004; Bilgidi et al. 2006).

TTA increased with time and was coupled with a drop in pH. Similar trends were observed by Bensmira and Jiang (2011) and Mataragas et al. (2011). Growth rate of microbes is linked to acidification and depends on the culture used (Zare et al. 2012). The increase in TTA and drop in pH trend could be attributed to the speed of acidification which was relatively higher at the higher storage temperatures. Bensmira and Jiang (2011) stated that lactic acid was the common end product of bacterial fermentation, thus the increasing TTA with storage temperature and time could have been as a result of the reduction in pH, post acidification, or further growth of the bacteria

involved. Sadeghi, Pourahmad, and Mokhtare (2017) concur that the increase in acidity shortens the shelf-life of yoghurt by triggering syneresis or resulting in a too acidic environment which can be lethal to the probiotics, thus reducing the microbial load.

Microbial load decreased gradually for the BGNPB stored at 5°C, while the ones kept at 15 and 25°C showed a sudden decrease as illustrated in Figure 3 during the storage period. Similar trends were reported by Ng, Yeung, and Tong (2011) where the microbial load decreased with increase in storage temperature and time. The storage temperature had an effect on microbes' enzymes and their activity, leading to a slower or faster metabolism; hence, the difference in growth rates.

The increase in lightness was in agreement with the observation of Aidoo et al. (2010). The implication is that the BGNPB were becoming lighter with time. There was a negative relationship between pH and lightness of the BGNPB, and a decrease in pH resulted in increased lightness. A similar trend was reported by Allen, Russell, and Fletcher (1997).

The shelf-life study was terminated after 28 days. The shelf-life decreased with an increase in temperature. At refrigeration temperature, the beverages lasted for 28 days, right-censored value. The shelf-life was estimated to be 18 days for *L. bulgaricus* BGNPB and 10 days for *L. bulgaricus* and *L. plantarum* BGNPB at 15°C. Shelf-life at 25°C for the two beverages was estimated to be two days. Hence, it is imperative that BGNPB must be stored at refrigeration temperature in order to maximize shelf-life.

The lower activation energy for *L. bulgaricus* BGNPB further confirms that its rate of reaction was faster. The activation energy of 106 kJ/mol for lactic acid bacteria was reported by Vaikousi, Biliaderis, and Koutsoumanis (2009). The values obtained in this study suggest that the pH drop was not only attributed by the lactic acid bacteria. The difference in activation energy between the two beverages could have been caused by the different strains in each beverage.

Conclusion

The change in pH was used as the index of quality; kinetics of pH was successfully modelled using Mitscherlich's law of diminishing returns and Arrhenius equation. Kinetics studies showed that pH of BGNPB was relative stable under refrigeration (5°C), resulting in

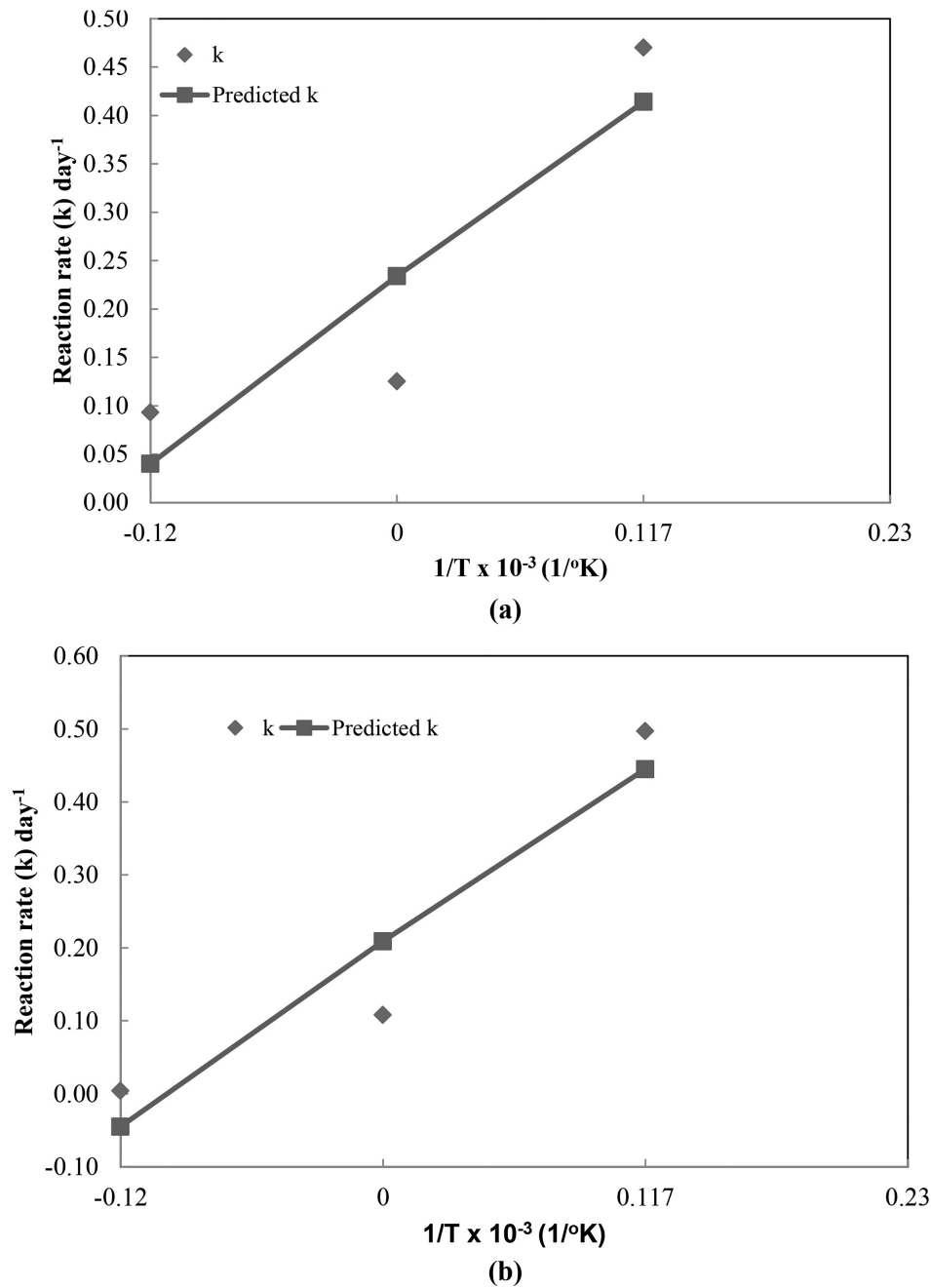


Figure 5: Arrhenius plot of reaction rate constant for pH changes during storage (a) *L. bulgaricus* and (b) *L. bulgaricus* and *L. plantarum* BGNPB.

Table 2: Parameters of Arrhenius equation for the pH of BGNPB during storage.

Probiotic	Temperature (°C)	E_a (kJ/mol)	k_{qref}	R^2
<i>L. bulgaricus</i>	5–25	12.8 ± 6.5	1.26 ± 1.08	0.80
<i>L. bulgaricus</i> and <i>L. plantarum</i>	5–25	16.9 ± 6.0	1.23 ± 1.07	0.89

BGNPB – Bambara groundnut probiotic beverage.

a right-censored shelf-life of 28 days for the two BGNPB. Meanwhile, 18 days for *L. bulgaricus* and 10 days for *L. bulgaricus* and *L. plantarum* BGNPB under abused temperature (15°C) were observed. The two beverages had a shelf-life of two days at accelerated storage 25°C. The shelf-life of the BGNPB under refrigeration (5°C) was longer when compared to the dairy counterparts with

shelf-life of 14 days at 4°C. Hence, BGNPB can be a vehicle to transport probiotics to humans and has a long shelf-life; BGNPB can safely be stored up to 28 days at refrigeration temperature (5°C) with a probiotic load of 10⁷ cfu mL⁻¹. BGNM is an alternative probiotic carrier and can be a solution to consumers demanding health foods that taste good and have an appropriate shelf-life.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Aidoo, H., E. Sakyi-Dawson, K. Tano-Debrah, and F. K. Saali. 2010. "Development and Characterisation of Dehydrated Peanut-Cowpea Milk Powder for use as a Dairy Milk Substitute in Chocolate Manufacture." *Food Research International* 43: 79–85.
- Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. "The Relationship of Broiler Breast Meat Colour and pH to Shelf-Life and Odour Development." *Poultry Science* 76: 1042–1046.
- Anonymous. 1997. Regulations Relating to Milk and Dairy Products, Regulation No. R1555, 21 November 1997. URL <http://www.doh.gov.za/docs/regulations/1997/reg1555.pdf>. 19 May 2011.
- Bao, Y., Y. Zhang, Y. Zhang, Y. Liu, S. Wang, X. Dong, Y. Wang, and H. Zhang. 2010. "Screening of Potential Probiotic Properties of *Lactobacillus* Fermentum Isolated From Traditional Dairy Products." *Food Control* 21: 695–701.
- Bensmira, M., and B. Jiang. 2011. "Organic Acid Formation During the Production of a Novel Peanut Milk Kefir Beverage." *British Journal of Dairy Sciences* 2 (1): 18–22.
- Berk, S., B. Tepe, and S. Arslan. 2011. "Screening of the Antioxidant, Antimicrobial and DNA Damage Protection Potentials of the Aqueous Extract of *Inula Oculus-Christi*." *African Journal of Pharmacy and Pharmacology* 5 (14): 1695–1702.
- Bible, B. B., and S. Singha. 1993. "Canopy Position Influences CIELAB Coordinates of Peach Colour." *HortScience* 28: 992–993.
- Bilgidi, N., A. Elgun, E. N. Herken, S. Turker, N. Ertaş, and S. İbanoglu. 2006. "Effect of Wheat Germ/ Bran Addition on the Chemical, Nutritional, and Sensory Quality of Tarhana, a Fermented Wheat Flour Yoghurt Product." *Journal of Food Engineering* 77: 680–686.
- Birollo, G. A., J. A. Reinheimer, and C. G. Vinderola. 2000. "Viability of Lactic Acid Microflora in Different Types of Yoghurt." *Food Research International* 33 (9): 799–805.
- Bouchenak, M., and M. Lamri-Senhadj. 2015. "Nutritional Quality of Legumes and Their Role in Cardiometabolic Risk Prevention; A Review." *Journal of Medicinal Food* 16 (3): 185–198.
- Champagne, C., and J. N. Gardner. 2008. "Effect of Storage in a Fruit Drink on Subsequent Survival of Probiotic Lactobacilli to Gastro-Intestinal Stresses." *Journal of Food Research International* 41: 539–543.
- Granato, D., G. F. Branco, F. Nazzaro, A. G. Cruz, and A. F. Faria. 2010. "Functional Foods and Non-Dairy Probiotic Food Developments". Trends." *Concepts and Products* 9: 292–302.
- Gupta, M., and S. Sharma. 2016. "Probiotics in Limelight." *Journal of Innovative Biology* 3 (1): 276–280.
- IBM SPSS. 2010. *IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0*. Armonk, NY: IBM Corp.
- Jideani, V. A., I. Nkama, E. B. Agbo, and I. A. Jideani. 2002. "Mathematical Modelling of Odour Deterioration of Millet (*Pennisetum Glaucum*) Dough (*Fura*) as Affected by Time-Temperature and Product Packaging Parameters." *Cereal Chemistry Journal* 79 (5): 710–714.
- Lupien-Meilleur, J., D. Roy, and L. Lagace. 2016. "Viability of Probiotic Bacteria in a Maple sap Beverage During Refrigerated Storage." *LWT-Food Science and Technology* 74: 160–167.
- Madora, E. P., T. K. Takalani, and M. E. Mashau. 2016. "Physicochemical, Microbiological and Sensory Properties of low-fat Yoghurt Fortified with Carrot Powder." *International Journal of Agricultural and Biological Engineering* 9 (1): 118–120.
- Mataragas, M., V. Dimitriou, P. N. Skandamis, and E. H. Drosinos. 2011. "Quantifying the Spoilage and Shelf-Life of Yoghurt with Fruits." *Food Microbiology* 28: 611–616.
- Murevanhema, Y. Y. 2013. "Evaluation of Bambara Groundnut (*Vigna Subterranea* (L.) Verdc.) Milk Fermented with Lactic Acid Bacteria as a Probiotic Beverage." (M.Tech Thesis). Faculty of Applied Sciences, Cape Peninsula University of Technology.
- Murevanhema, Y. Y., and V. A. Jideani. 2015. "Production and Characterization of Milk Produced From Bambara Groundnut (*Vigna Subterranea*) Varieties." *Journal of Food Processing and Preservation* 39: 1485–1498.
- Ng, E. W., M. Yeung, and P. Tong. 2011. "Effects of Yoghurt Starter Cultures on the Survival of *Lactobacillus Acidophilus*." *International Journal of Food Microbiology* 145: 169–175.
- Nti, C. A. 2009. "Effects of Bambara Groundnut (*Vigna Subterranea*) Variety and Processing on the Quality and Consumer Appeal for its Products." *International Journal of Food Science and Technology* 44: 2223–2242.
- Oliveria, M. N., I. Sodini, F. Remeuf, and G. Corrieu. 2001. "Effect of Milk Supplementation and Culture Composition on Acidification, Textural Properties and Microbiological Stability of Fermented Milks Containing Probiotic Bacteria." *International Dairy Journal* 11: 935–942.
- Ouwehand, A. C., and S. J. Salminen. 1998. "The Health Effect of Cultured Milk Products with Viable and non-Viable Bacteria." *International Dairy Journal* 8: 749–758.
- Prado, F. C., J. L. Parada, A. Pandey, and C. R. Soccol. 2008. "Trends in non-Dairy Probiotic Beverages." *Food Research International* 41: 111–123.
- Sadeghi, A. R., R. Pourahmad, and M. Mokhtare. 2017. "Enrichment of Probiotic Yoghurt with Broccoli Sprouts Extract and its Effect on Helicobacter Pylori." *Applied Food Biotechnology* 4 (1): 55–59.
- Sahin, S., and S. G. Samnu. 2006. "Electromagnetic Properties." In *Physical Properties of Foods*, 157–192. Food Science Text Series. New York, NY: Springer.
- Shimakawa, Y., S. Matsubara, N. Yaki, M. Ikeda, and F. Ishikawa. 2003. "Evaluation of *Bifidobacterium Breve* Strain Yakult - Fermented Soymilk as a Probiotic Food." *International Journal of Food Microbiology* 81: 131–136.
- Tamime, A. Y., and R. K. Robinson. 1999. "Science of Yoghurt Production." In *Yoghurt Science and Technology*, 23–43. Boca Raton, USA: Elsevier.
- Thapa, N., and J. P. Tamang. 2015. "Functional and Therapeutic Values of Fermented Foods." In *Health Benefits of Fermented Foods and Beverages*, edited by J. P. Tamang, 111–168. Boca Raton: Taylor and Francis.
- Vaikousi, H., C. G. Biliaderis, and K. P. Koutsoumanis. 2009. "Applicability of a Microbial Time Temperature Indicator (TTI) for Monitoring Spoilage of Modified Atmosphere Packed Mincemeat." *International Journal of Food Microbiology* 133: 272–278.
- Vijayendra, S. V. N., and P. M. Halami. 2015. "Health Benefits of Fermented Vegetable Products." In *Health Benefits of Fermented Foods and Beverages*, edited by J. P. Tamang, 297–324. Boca Raton: Taylor and Francis.
- Wang, F. N., Y. H. Shi, J. Sun, and G. W. Le. 2007. "Evaluation Peanut Flour Fermented with Lactic Acid Bacteria as a Probiotic Food." *Food Science and Technology International* 13: 469–475.
- White, P. J., and Y. Xing. 2008. "Antioxidants in Food". *Source of Natural Antioxidants*. Boca Raton: Woodhead publishing limited.
- Yoon, K. Y., E. E. Woodams, and Y. D. Hang. 2004. "Probiotication of Tomato Juice by Lactic Acid Bacteria." *The Journal of Microbiology* 42: 315–318.
- Zare, F., C. P. Champagne, B. K. Simpson, V. Orsat, and J. I. Boye. 2012. "Effect of the Addition of Ingredients to Milk on Acid Production by Probiotic and Yoghurt Starter Culture." *LWT – Food Science and Technology* 45: 155–160.