Production and Quality Characteristics of a Probiotic Beverage from Watermelon (*Citrullus lanatus*)

Katlego M. Masebe[1], and Oluwafemi A. Adebo[1*]

Abstract**—** Watermelon is a tropical fruit indigenous to Africa and prone to quick deterioration due to prevailing environmental conditions in Africa. Processing such fruit can mitigate post-harvest losses, increase its shelf life as well as to add variety in products made from it. Functional foods (particularly probiotics) are gaining more recognition due to their potential positive effects on human health and are promoted as gut-friendly products. A probiotic beverage was thus produced using a probiotic lactic acid bacteria strain. Fresh watermelon was cut and juice extracted was then aliquoted into two parts of which one was pasteurized. Both aliquots were differently fermented and analyzed for quality indices. Using the 72 h fermented aliquot, a beverage was developed and subsequently analyzed, this had a pH, viscosity, total dissolvable solid and titratable acidity of 3.68, 0.314 Pa/s, 7.6 °Brix and 0.66 g/L, respectively. This novel beverage suggests an alternative source of probiotics to intending consumers and could help reduce wastage associated with this produce.

Keywords**—** *beverage, lactic acid bacteria, probiotics, watermelon*

I. Introduction

Watermelon (*Citrullus lanatus*) is a fairly utilized fruit [1]. It is a member of the Cucurbitaceous family, thus making it closely related to vegetables such as pumpkin, cucumber, squash and cantaloupe [2]-[5]. Watermelon is extremely juicy as about 90% of its content is water [3], [6]. It has a variety of colours ranging from orange, yellow, red/pink and white. The name watermelon is associated with the deep red/pink coloured fruit [3]. The fruit is consumed worldwide, with China being the world largest producer with approximately 79 million tonnes [7]. Besides consuming watermelon as a fresh fruit, there are some food products that contain the fruit or its extract. The fruit flesh can be added to a fruit salad or its extract can be found in products such as flavoured gummy candy, flavoured soft drinks, wine, flavoured tea, flavoured chewing gums, fruit concentrate, juice and pickled watermelon rind [8].

Fermentation is a process where carbohydrates are converted to alcohol, carbon dioxide and organic acids carried out by microorganisms. Fermentation results in preservation, where the product lasts longer than the initial substrate, i.e. its natural state (e.g. grape fruit and wine). Depending on the mode of the process, fermentation can either be natural (spontaneous) or induced (controlled) [9], [10]. Natural (spontaneous) fermentation can both be desirable (when it is intended) and undesirable (spoilage; when not intended). In spontaneous fermentation, it is difficult to understand the capability and requirement of each species involved in the process. The disadvantages associated with natural/spontaneous fermentation have led to the need for a better, safer and efficient way for the fermentation process. This has thus led to the development of specific strains and starter cultures for the fermentation of foods to obtain desired products [11]-[13]. Sequel to their prevalence and effectiveness in fermented foods, lactic acid bacteria (LABs) are the most significant strains used for controlled fermentation. While other LABs are known, *L. fermentum* have been specifically shown to possess probiotic characteristics and have suitable fermenting properties [14]–[17], and can therefore be used to produce probiotic drinks.

According to [18] concerted efforts are being geared towards the search for probiotics as an effective strategy for health promotion and disease prevention. As defined by Food and Agriculture Organization (FAO), a probiotic is "a live microorganism which confers a health benefit to the host, when administered in adequate amounts" [19]. While there are various available probiotics in the market, this study explored the possibility of developing a probiotic product from watermelon and investigating its properties thereof.

II. Materials and Methods

Ripe watermelons (*Citrullus lanatus*) were purchased at the City Deep Fresh Produce Market in Johannesburg, Gauteng, South Africa. Upon arrival the fruit was washed with distilled water and sanitized with 70% ethanol to reduce chances of cross contamination from wash water to the mesocarp during cutting. Washed watermelon was cut and the exocarp, mesocarp and seeds were separated. Seeds and exocarp were discarded while the mesocarp was juiced using juice extractor (DEFY Model JE210S, Japan). The mesh was discarded while the pink/red juice was further used in this study. The aliquot was then divided into two parts, one was pasteurized and the other was left raw. The pasteurization was carried out for 30 min at 63 °C. Both raw and pasteurized juices were further divided into two

K. Masebe is with the Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, P.O. Box 17011, Doornfontein Campus, Gauteng, South Africa.

O. A. Adebo is with the Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, P.O. Box 17011, Doornfontein Campus, Gauteng, South Africa.

parts, one was naturally fermented while the other was fermented using a probiotic strain (*Lactobacillus fermentum*) obtained from Anatech Instruments (Pty) Ltd, Johannesburg, South Africa. The extracted juice were inoculated with a probiotic *L. fermentum* starter culture and incubated at 37 °C for 24 (day 1), 48 (day 2), 72 (day 3), 96 (day 4) and 120 hr (day 5). Natural fermentation was done in a similar way, but without the *L. fermentum* strain.

A. Analytical Tests

1. Titratable Acidity (TTA)

Using the method described by [20], the TTA of all liquids (raw, naturally fermented and LAB fermented) was analyzed by pouring 25 mL of the juice into 250 mL volumetric flask and filling to the mark with distilled water. The solution was vigorously shaken and transferred into a 100 mL beaker, which was then titrated with 0.119 M NaOH until it reaches the pH of 8.1. The results were expressed as lactic acid concentration (g/L), the process was performed in triplicate.

2. Total Dissolve Solid (TDS)

The Hanna digital refractometer for brix analysis in foods (HI96801, South Africa) was used to analyze the soluble solid content of the raw juice, fermented juice and the final product. The juice was well shaken and a drop of this was added to the stage of the refractometer and result obtained was expressed as °Brix.

3. pH Measurement

Measurements of pH were conducted using a pH meter from Hanna Instrument HI8424 portable pHmeter, South Africa. The pH of raw, naturally fermented, LAB fermented aliquots and final product were all measured in triplicate.

4. Fourier Transmission-Infrared (FTIR) Spectroscopy

Using the method of [21], infrared spectra of the freeze-dried juice samples were obtained using a FTIR spectrophotometer (Thermo Fisher Scientific Inc. USA]. Before the samples was mounted on the instrument, background spectra were collected before samples and recorded with characteristic peaks in wave numbers from 450 to 4000 cm^{-1} at 32 runs per scan.

5. Microbiological Analysis

Into a sterile test-tube containing 9 ml sterile distilled water, 1 mL of each sample (natural and controlled fermented aliquots) was added and mixed using a vortex (Model K-550-GE, Scientific Industries, Inc, Bohemia, NY, USA). Tenfold serial dilutions were performed on the fermented aliquots [22] and inoculated onto Nutrient agar and MRS agar plates. These were respectively incubated for 24 and 48 h at 37 °C [20]. After incubation, colonies were counted and colony forming units were estimated by: CFU/mL= (Number of colonies × Dilution factor)/(Volume of culture plated)

6. Protein, Fat, Dietary Fibre and Carbohydrate

The [23] method was used for the determination of protein, fat, dierary fibre and carbohydrate.

7. Total Sodium

For total sodium, the automatic potentiometric titrator was used. 10 mL of sample was measured into a 200 mL flask and 90 mL of distilled water was added and a stirred with magnetic stirrer. The sample was titrated with 0.1 mol/L$AgNO_3$ [24]. This was done in triplicate.

8. Viscosity Determination

Using the Thermo Scientific HAAKE Falling Ball Viscometer (Model: 8000009, EW-08708-51 USA), a sample of 200 mL was cooled to 26 °C and poured into a viscometer cylinder, then a ball was released, and the time taken for the ball to reach the lower part of the cylinder using gravitational force was recorded and viscosity recorded.

B. Sensory Evaluation of Formulated Beverage

A beverage was formulated from the fermented juice and this was used to determine if the product was accepted by prospective consumers. Consumer acceptability test was done by using a total of 50 panelists comprising of staff and students of the Department of Biotechnology and Food Technology, University of Johannesburg, South Africa. A hedonic scale was used to assess the acceptability of the product based on color, sweetness, flavor, taste, overall likeness, and satisfaction to consumers. Products was rated from 1-5, with 1 being extremely dislike and 5 being extremely like.

C. Statistical Analysis

All other experiments were performed in triplicates and subsequent data generated were expressed as average ± standard deviation. Duncan test was used to determine significant differences among the means. Principal Component Analysis (PCA) and Cluster Analysis (CA) on the FTIR data were done on Unscrambler X statistical software version 10.4.2 (Camo software, Oslo, Norway) [25].

III. Result and Discussion

A. pH and Titratable Acidity (TTA)

Table I indicates the average value of the changes that occurring in pH on both raw, natural and LAB fermented juice. The pH of naturally fermented juice has decreased from 6.19 to 3.73 on day 3 but started increasing from day 4 to pH 5 on day 5. This is an indication of the end of fermentation that fermentable sugars are depleted, and fermenting microorganism are dying off [26]. On a contrary, both the pasteurized and unpasteurized juice fermented with *L. fermentum* showed a steady decrease in the pH, from 6.19 to 3.65 on day 5, thus indicating that fermentation was still taking place till the last day of fermentation. While continuous reduction of the pH values in the *L. fermentum* samples could suggest the viability of these strains during the fermentation process, decreased at longer fermentation times, can be attributed to the fact that the fermenting microorganisms in the naturally fermented samples have entered their decline phase, causing a reduction in microbial activity and subsequent compounds liberated. Such observation with naturally fermented samples have also been ascribed to initially low numbers of LABs causing longer lag periods, consequently resulting in reduced metabolic and microbial activity [27].

TABLE I

CHANGES OCCURRING IN PH, TDS AND TOTAL PLATE COUNT (TPC) ON RAW, NATURAL AND LAB FERMENTED JUICE

Samples	pH	TDS (^{o}Brix)
Laf$^{\phi}$		
Day 1	5.10^{k}±0.00	8.53^{j}±0.06
Day 2	3.71^{d}±0.00	8.03^{i}±0.06
Day 3	3.69^{d}±0.00	7.77^{h}±0.06
Day 4	3.63^{b}±0.00	4.00^{b}±0.00
Day 5	3.48^{a}±0.00	3.60^{a}±0.00
LafP$^{\alpha}$		
Day 1	4.19^{g}±0.01	10.50^{q}±0.00
Day 2	3.75^{e}±0.00	10.20^{p}±0.00
Day 3	3.70^{d}±0.01	8.73^{k}±0.12
Day 4	3.69^{d}±0.01	8.47^{j}±0.06
Day 5	3.64^{c}±0.01	5.63^{c}±0.06
NF$^{\beta}$		
Day 1	5.14^{l}±0.01	7.80^{h}±0.00
Day 2	3.75^{e}±0.01	7.67^{g}±0.06
Day 3	3.68^{d}±0.01	7.57^{f}±0.06
Day 4	4.96^{i}±0.00	7.20^{e}±0.00
Day 5	5.11^{k}±0.00	7.03^{d}±0.06
NFP§		
Day 1	5.61^{m}±0.01	9.93^{o}±0.06
Day 2	3.89^{f}±0.00	9.37^{n}±0.06
Day 3	3.73^{e}±0.01	9.13^{m}±0.12
Day 4	4.32^{h}±0.02	8.90^{l}±0.00
Day 5	5.00^{j}±0.01	8.10^{i}±0.06
Raw	6.19^{n}±0.06	8.67^{k}±0.06
Pasteurized	6.20^{o}±0.00	10.13^{p}±0.12

Each value is a mean ± standard deviation of triplicates. Means with no common letters within a column significantly differ ($p<0.05$). $^{\phi}$Laf – lactic acid fermented; $^{\alpha}$LafP –pasteurized lactic fermented; $^{\beta}$NF –natural fermented; §NFP–pasteurized natural fermented.

As observed in Fig. 1, TTA of the *L. fermentum* juices kept increasing from 0.36 (on day 0) to 0.95 g/L (on day 5) and a similar trend was also observed for the naturally fermented juice, but with a lower TTA value (least was 0.26 g/L on day 5). There was a relationship between the pH values earlier obtained and the TTA values, as increase in acidity was observed to be inversely proportional to the decrease in pH. This could thus suggest that as fermentation of watermelon with or without the probiotic *L. fermentum* strain resulted in the production of organic acids. The increase of acidity in lactic acid fermented aliquots suggest that there are more organic acids formed in the controlled fermentation than in natural fermentation, a similar trend observed for the pH values. This is because the *L. fermentum* strains are hetero fermentors, this suggest that they produce other organic material beside lactic acids, such as acetic acids, ethyl alcohol and carbon dioxide which also affect the value of acidity in the aliquots.

Fig. 1. Titratable acidity for fermented juice in different days
Laf – lactic acid fermented; LafP –pasteurized lactic fermented; NF –natural fermented; NFP –pasteurized natural fermented.

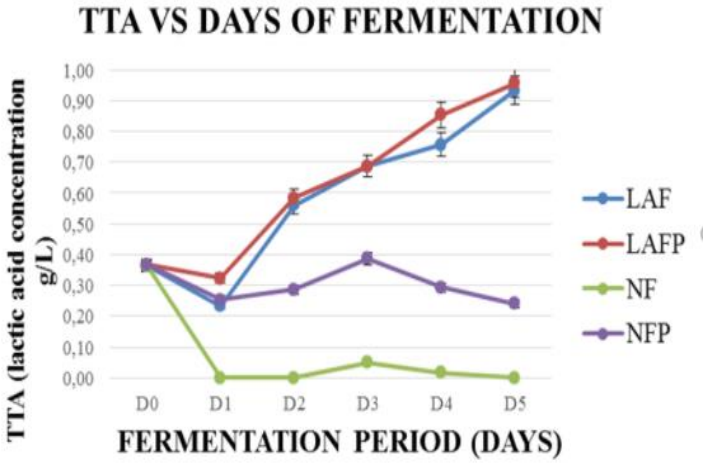

Fig. 1. Titratable acidity for fermented juice in different days
Laf – lactic acid fermented; LafP –pasteurized lactic fermented; NF –natural fermented; NFP –pasteurized natural fermented.

B. Total Dissolve Solids

According to [28], decrease in TDS indicates the utilization of fermentable sugars by the microorganism as carbon and energy sources, to subsequently produce metabolites. Significantly ($p\leq0.05$) lower TDS values of the LAB-fermented samples suggest an increased utilization of available sugars in the aliquots. The least TDS of 3.60 was obtained after the 5th day of fermentation, with a corresponding significantly ($p\leq0.05$) lowest pH value of 3.48. This further suggests increased microbial activities and utilization of the carbon sources by the *L. fermentum* strain during the production of the aliquot.Available fermentable sugars in watermelon include fructose, fructan, glucose and polyol [29], all of which could have been effectively utilized by the probiotic strain. Furthermore, it could be suggested that the presence of other microorganisms dominating the microflora of the naturally fermented samples caused relatively higher TDS values (Table I). While the use of *L. fermentum* would have led to better adaptability and dominance from the beginning of the fermentation producing, leading to significantly lesser residual fermentable sugars, the controlled fermentation process, competitive action of the plethora of microorganisms during the natural fermentation hindered this, resulting in higher amounts of residual sugars.

C. Microbiological Study

Microbial analysis was performed on the different aliquots to monitor the microbial growth or otherwise with fermentation days. As shown in Table II, as the day progresses the amount of CFU/mL decrease due to the depletion of sugar, aging of microorganism and accumulation of waste in the medium. The relatively higher microbial counts obtained on the plate count agar suggests a broad diversity of bacteria in the fermented aliquots. This also indicates that natural (spontaneous) fermentation results from a competitive action of different endogenous microorganisms [30].The higher mean counts of the viable bacteria and LAB of samples fermented with starter cultures suggest that the starter culture dominated and

constituted major portion of the viable bacteria growing on the samples.

TABLE II
MICROBIOLOGICAL ANALYSIS OF FERMENTED JUICE

Days	Type of fermentation	Total plate count CFU/mL ($\times 10^{10}$)	Anaerobic plate count (MRS) CFU/mL ($\times 10^{10}$)
1	NF$^{\beta}$	$5^{b}\pm0.03$	$3^{a}\pm0.16$
	NFP§	$3^{a}\pm0.25$	$4^{ab}\pm0.07$
	Laf$^{\phi}$	$7^{bc}\pm0.11$	$5^{b}\pm0.05$
	LafP$^{\alpha}$	$6^{b}\pm0.34$	$5^{b}\pm0.00$
2	NF$^{\beta}$	$14^{f}\pm0.04$	$11^{d}\pm0.00$
	NFP§	$9^{d}\pm0.00$	$7^{bc}\pm0.00$
	Laf$^{\phi}$	$11^{e}\pm0.01$	$9^{c}\pm0.01$
	LafP$^{\alpha}$	$9^{d}\pm0.01$	$8^{c}\pm0.00$
3	NF$^{\beta}$	$19^{g}\pm0.00$	$15^{f}\pm0.01$
	NFP§	$12^{ef}\pm0.00$	$9^{c}\pm0.01$
	Laf$^{\phi}$	24 ± 0.09	$18^{gh}\pm0.25$
	LafP$^{\alpha}$	$20^{gh}\pm0.12$	$15^{f}\pm0.00$
4	NF$^{\beta}$	28 ± 0.21	$17^{g}\pm0.00$
	NFP§	$16^{fg}\pm0.00$	$13^{de}\pm0.01$
	Laf$^{\phi}$	$26^{j}\pm0.01$	$24^{j}\pm0.00$
	LafP$^{\alpha}$	$22^{h}\pm0.05$	$20^{h}\pm0.00$
5	NF$^{\beta}$	$20^{gh}\pm0.05$	$17^{g}\pm0.01$
	NFP§	$15^{f}\pm0.00$	$12^{d}\pm0.00$
	Laf$^{\phi}$	$24^{hi}\pm0.00$	$26^{j}\pm0.00$
	LafP$^{\alpha}$	$23^{h}\pm0.01$	$21^{hi}\pm0.00$

Each value is a mean ± standard deviation of triplicates. $^{\beta}$NF –natural fermented; §NFP –pasteurized natural fermented; $^{\phi}$Laf– lactic acid fermented; $^{\alpha}$LafP–pasteurized lactic fermented.

D.Fourier Transmission Infrared (FTIR) Spectroscopy

FTIR analysis was performed on the obtained juices to identify possible variations in their spectra. It was envisaged that the obtained spectra would provide a wider spectrum of chemical analysis of all present metabolites in the fermented juice. Computation and chemometric steps of data pre-treatments were followed for the accumulated spectra using PCA and CA. This assisted with the interpretation and comparison of the FTIR spectral data set with the aim of evaluating biochemical events that occurred during fermentation and to discriminate and classify the samples according to clusters and groupings.

All the obtained spectra data were in the FTIR spectroscopic ranges of 3000–600 cm^{-1} wave number [31]. As observed from the mean plots of the average spectra of each sample (Fig. 2 (a), the significant peaks of the product after fermentation were at 774.67 cm-1 and 816.44 cm-1 which lies in the C–Cl stretch alkyl halides peak of 850-550 cm^{-1}, 865.36 cm^{-1} represent 1°, 2° amines (910–665 cm-1) N–H, carboxylic acids group 911.56 and 1038.68 cm^{-1} are aliphatic amines (1250–1020 cm^{-1}), alkyl halides by 1250.64 and 1353.22 cm^{-1} are nitro compounds and aromatic amines, aromatics, alkanes and 1°, 2° amines, amides while the last day of fermentation indicated development of aldehyde, saturated aliphatic and alkynes terminal. During natural fermentation there are alkyl halides and aromatic, carboxylic acids, aliphatic amines, nitro compounds, aromatic, ester saturated aliphatic (which lactic acid fermentation didn't contain in day 1 of the fermentation), alkanes and carboxylic acids. The last day of natural fermentation the aliquot developed the following functional groups aromatic amide, and alkyne amine. Thus, indicating that during fermentation there are functional groups forms and others terminated depending on the type of fermentation, sugar substrate and microorganism responsible for fermentation.

Using PCA, the spectra of the fermented juice samples were transformed into principal components (PCs). The first two PCs attributed for 97% of the variation (Fig. 2 (b) and differentiated the samples. From the left to the right sample are day 4 of natural fermentation, day 3 of lactic acid fermentation, day 4 lactic acid fermentation, day 5 natural fermentation, day 5 and 2 lactic acid fermentation, day 3 natural fermentation, day 1 natural fermentation, raw (un-pasteurize), pasteurize, day 2 natural fermentation and day 1 lactic acids fermentation. To further differentiate the fermented watermelon aliquots samples from each other, a third PC were used which provided an additional 2% variation in the data obtained. Cluster analysis (CA) was further used to classify the samples into groups, as a function of their clusters. This was performed to group sample based on proximity of the characteristic, to give a descriptive, theoretical and non-inferential analysis. As shown in Fig. 2 (d), the samples were classified into two major clusters, a group with red colour and the other with blue. The red colour represent the lactic acid fermented aliquots of Day 3 and the natural fermented aliquots of day 4 this two pose similar behaviour their TDS are between 8.7-8.9 which fall under the same range, pH is also not different from each other 3.70 and 4.32, which means it takes 3 days for the lactic acid bacteria to lower the pH and use up the same nutrients as the mixture of organism in the natural fermented aliquots. The blue are the rest of the days and type of fermentation from unpasteurized, pasteurized, day 1 till day 5 of both fermentations. Even though the range of investigated parameters in the days is wide they were clustered together because they contain similar functional groups and bands. The red cluster falls under the same section in the PCA graph (left hand side) while the rest are on the far-right hand side.

E. Nutritional, Physiochemical Information and Sensory Evaluation

After subsequent analysis on the fermented juice, a product was developed from the pasteurized juice fermented with the LAB strain for 5 days. This was particularly selected due to its reduced pH and relatively high TTA values. Pasteurization is also particularly important and an important component in food processing. The resulting product was also evaluated for nutritional properties.

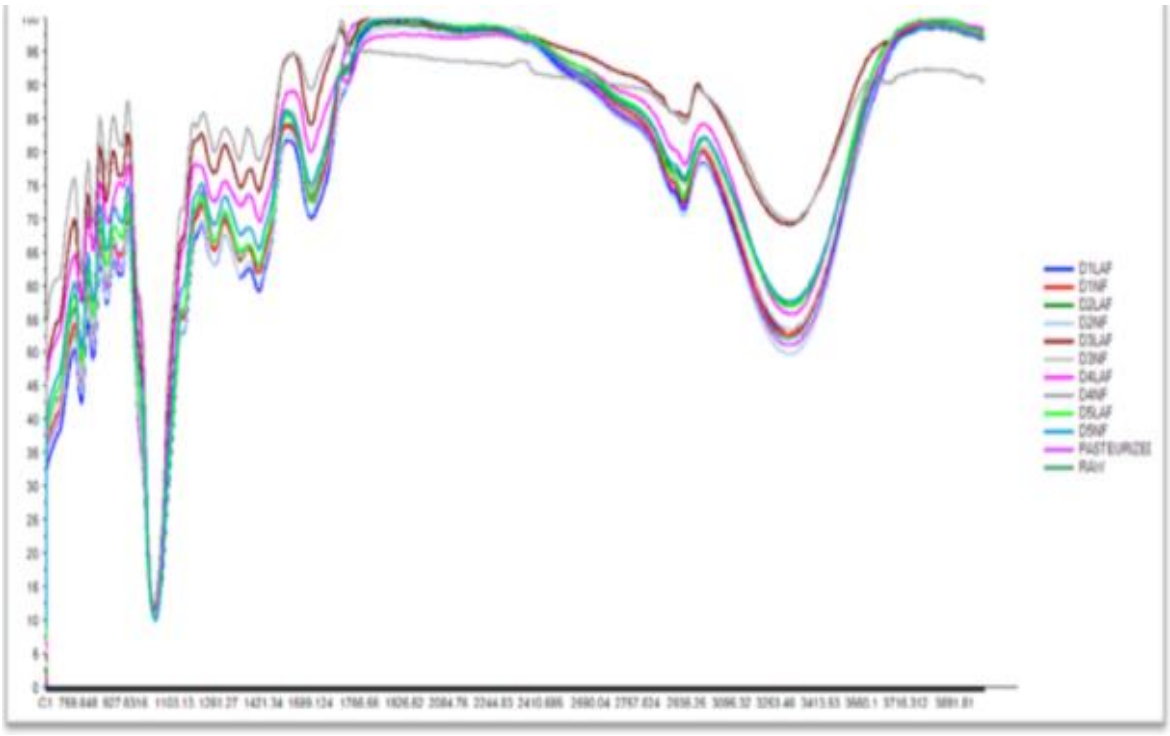

Fig. 2 (a) Average FTIR plot of fermented watermelon juice

D1LAF-Day 1 Lactic acid fermentation, D1NF-Day 1 Natural fermentation, D2LAF-Day 2 Lactic acid fermentation, D2NF-Day 2 Natural fermentation, D3LAF-Day 3 Lactic acid fermentation, D3NF-Day 3 Natural fermentation, D4LAF-Day 4 Lactic acid fermentation, D4NF-Day 4 Natural fermentation, D5LAF-Day 5 Lactic acid fermentation, D5NF-Day 5 Natural fermentation, RAW-Raw Aliquot, PASTEURIZED-Pasteurized aliquot.

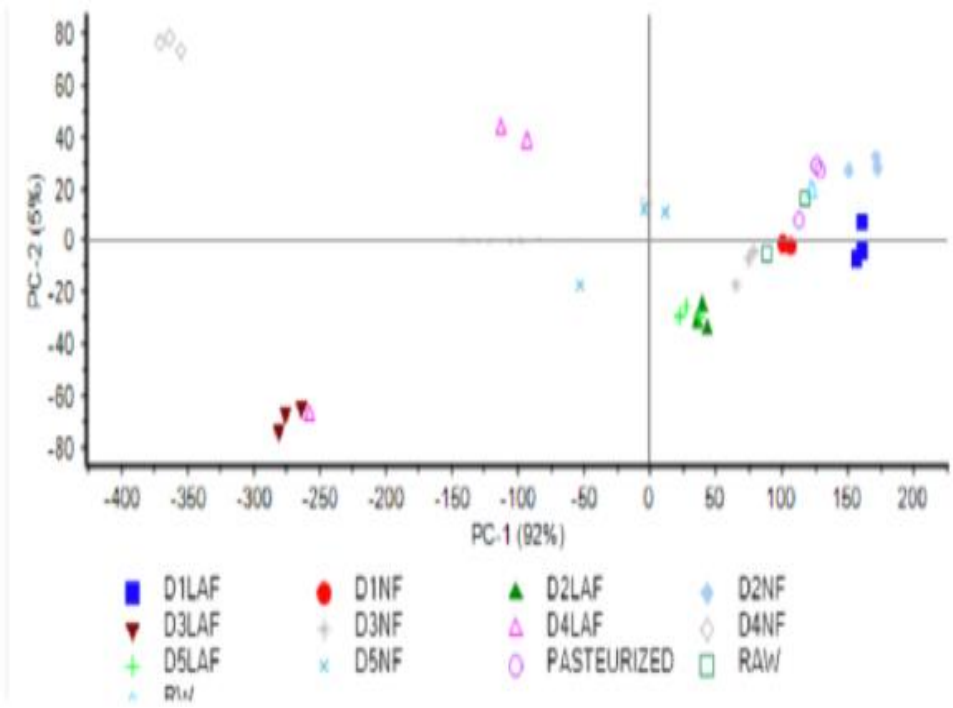

Fig. 2 (b) PCA Score plot of the fermented watermelon juice showing PC1 and PC2

D1LAF-Day 1 Lactic acid fermentation, D1NF-Day 1 Natural fermentation, D2LAF-Day 2 Lactic acid fermentation, D2NF-Day 2 Natural fermentation, D3LAF-Day 3 Lactic acid fermentation, D3NF-Day 3 Natural fermentation, D4LAF-Day 4 Lactic acid fermentation, D4NF-Day 4 Natural fermentation, D5LAF-Day 5 Lactic acid fermentation, D5NF-Day 5 Natural fermentation, RAW-Raw Aliquot, PASTEURIZED-Pasteurized aliquot.

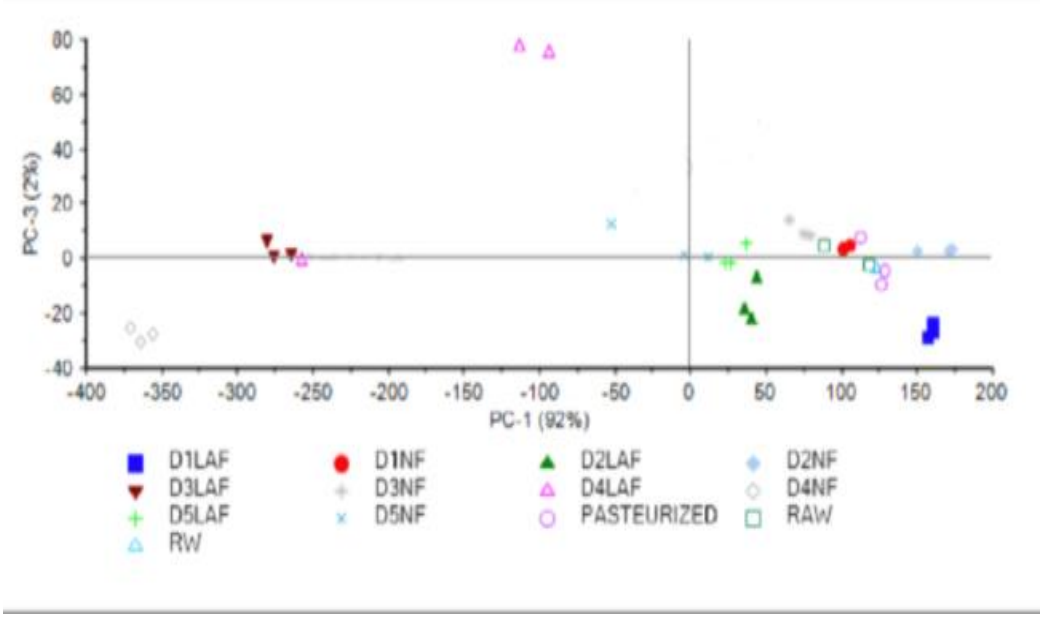

Fig. 2 (c).PCA Score plot of the fermented watermelon juice showing PC1 and PC3

D1LAF-Day 1 Lactic acid fermentation, D1NF-Day 1 Natural fermentation, D2LAF-Day 2 Lactic acid fermentation, D2NF-Day 2 Natural fermentation, D3LAF-Day 3 Lactic acid fermentation, D3NF-Day 3 Natural fermentation, D4LAF-Day 4 Lactic acid fermentation, D4NF-Day 4 Natural fermentation, D5LAF-Day 5 Lactic acid fermentation, D5NF-Day 5 Natural fermentation, RAW-Raw Aliquot, PASTEURIZED-Pasteurized aliquot.

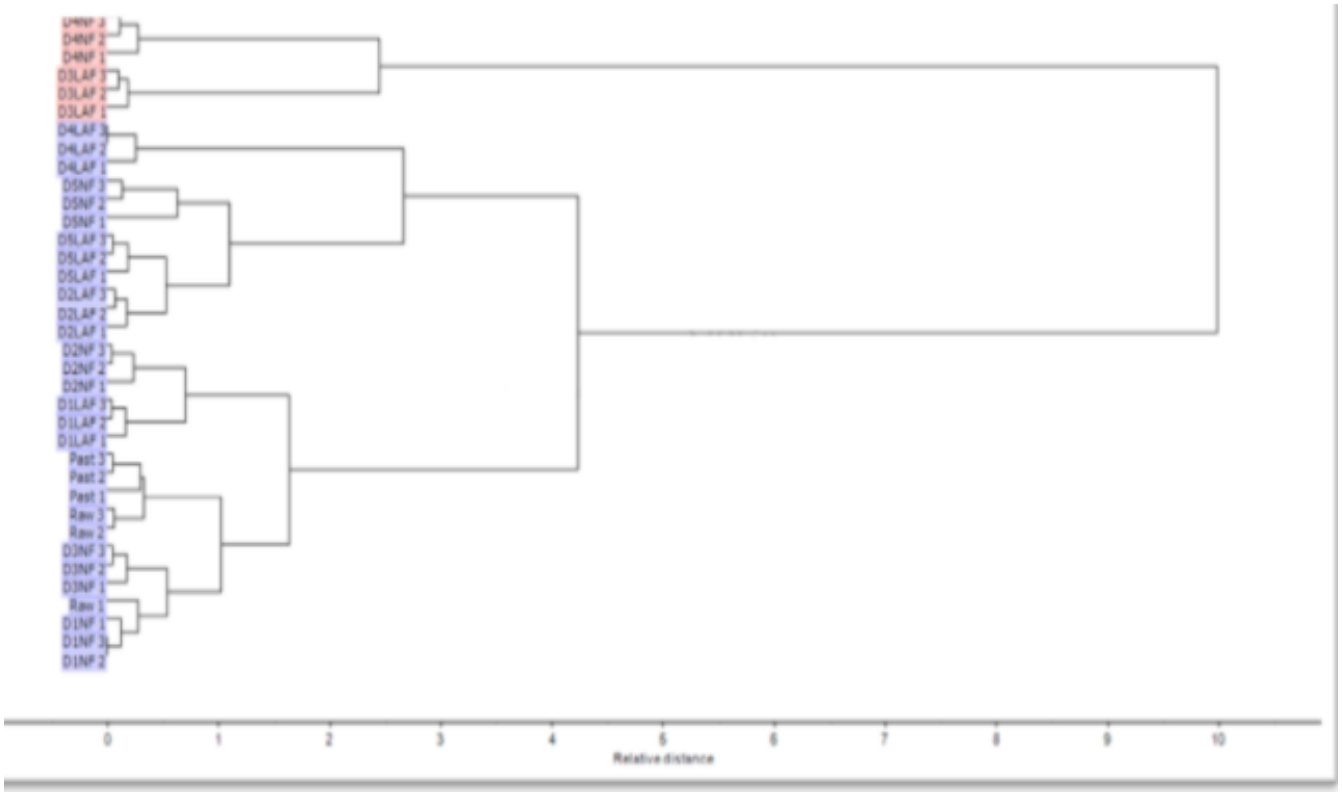

Fig. 2 (d) Cluster analysis of the fermented watermelon juice

D1LAF-Day 1 Lactic acid fermentation, D1NF-Day 1 Natural fermentation, D2LAF-Day 2 Lactic acid fermentation, D2NF-Day 2 Natural fermentation, D3LAF-Day 3 Lactic acid fermentation, D3NF-Day 3 Natural fermentation, D4LAF-Day 4 Lactic acid fermentation, D4NF-Day 4 Natural fermentation, D5LAF-Day 5 Lactic acid fermentation, D5NF-Day 5 Natural fermentation.

As observed in Table III, the product contributes an energy value of 488 kJ per serving and a glycaemic carbohydrate of 14 g per 100 mL serving. Other physicochemical properties of the drink are presented in Table IV. Results from the sensory

evaluation revealed that the panellists generally accepted the beverage (Table V). Although the score was less than 5 (the maximum), the least score was for the taste and overall acceptability of 3.84 each. This could be due to the "newness" of this product, as watermelon is not a common probiotic drink or juice available in the market. Although the product was not subjected to comparison test (in comparison with other juices/probiotic products), the developed probiotic beverage still has a competitive advantage.

TABLE III
NUTRITIONAL INFORMATION OF THE PROBIOTIC WATERMELON BEVERAGE

Typical nutritional information (as packed) Serving size (200ml)		
	Per 100 mL	Per serving size
Energy (kJ)	244	488
Protein (g)	1	2
Glycaemic carbohydrate (g)	14	28
Of which total sugar (g)	6	12
Total fat (g)	0	0
Of which saturated fat (g)	0	0
Dietary fibre (g)	0.4	0.8
Total sodium (mg)	1	2

TABLE IV
PHYSICOCHEMICAL PROPERTIES OF THE DEVELOPED BEVERAGE

Product characteristics	Value
pH	3.68
Viscosity	0.314 Pa s
Total dissolvable solid	7.6° Brix
Titratable acidity	0.66 Lactic acid concentration (g/L)

TABLE V
SENSORY TEST RESULTS FOR THE PROBIOTIC WATERMELON BEVERAGE

Parameters	Score
Color	4.32± 0.62
Sweetness	4.06± 0.65
Flavor	3.88± 0.48
Taste	3.84±0.71
Overall acceptance	3.84± 0.55

IV. CONCLUSION

The study was carried out to investigate and produce a probiotic beverage using underutilized fruit "watermelon", that is generally known to go to waste, after sometime on the shelves. Results obtained showed a promising prospect of this beverage for reduction of wastage of the water melon fruit and for the delivery of probiotic bacteria. Not only is the need to reduce food waste increasingly becoming vital, the demand for functional foods (such as probiotic) is gradually increasing due to the ever-growing pattern of health conscious consumers. Further studies are still however needed to substantiate the probiotic potential of this drink using both *in vitro* and *in vivo* techniques and further characterization of the health benefits and other nutritional potentials in the beverage.

REFERENCES

[1] Tropical fruits (May 2016). Watermelon marketing general information. Available: http://www.itfnet.org/gfruit/Templates%20English/watermelon.market.info.htm.

[2] George Mateljan Foundation (August 2019). The world's healthiest foods – watermelon. Available: http://www.whfoods.com/genpage.php?tname=foodspice&dbid=31.

[3] O. A. Oseni, and V. I. Okoye, "Studies of phytochemical and antioxidant properties of the fruits of watermelon (*Citrullus lanatus. Thunb*),"*Journal of Pharmaceutical Biomedical Science,* vol. 27, pp. 508-514, 2013.

[4] G. Wu, K. J. Collins, P. Perkins-Veazie, M. Siddiq, K. D. Dolan, K. A. Kelly, C. L. Heaps, and C. J. Meininger, "Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic Ssyndrome in zucker diabetic fatty rats1, 2," *Journal of Nutrition*, vol. 137, pp. 2680-2685, 2007. https://doi.org/10.1093/jn/137.12.2680

[5] A. J. Edwards, B. T. Vinyard, E. R. Wiley, E. D. Brown, J. K. Collins, P. Perkins-Veazie, R. A. Baker, and B. A. Clevidence, "Consumption of watermelon juice increases plasma concentrations of lycopene and β-Carotene in humans," *Journal of Nutrition,* vol. 133, pp. 1043-1050, 2003. https://doi.org/10.1093/jn/133.4.1043

[6] J. Axe. (October 2016). Heath benefits of watermelon and recipe. Available: https://draxe.com/wp-content/uploads/2015/05/bigstock-Sliced-Ripe-Watermelon-72055993.jpg.

[7] FAOSTAT (Food and Agriculture Organization of the United Nations Statistics) (August 2019).Crops statistics: watermelons. Available: http://www.fao.org/faostat/en/#data/QC.

[8] Bradford Watermelons (August 2014). Our Products. Available: http://bradfordwatermelons.com/2014/08/our-products/.

[9] M. P. Tarazona-Diaz, and E. Aguayo, "Influence of acidification, pasteurization, centrifugation, storage time and temperature on watermelon juice quality," *Journal of the Science of Food and Agriculture*, vol. 93, pp. 3863-3869, 2013. https://doi.org/10.1002/jsfa.6332

[10] L. Alba-Lois, and C. Segal-Kischinevzky, "Beer and wine makers," *Nature Education*, vol. 3, pp. 17, 2010.

[11] P. K. Sarkar, and M. J. Robert-Nout, *Handbook of indigenous foods involving alkaline fermentation*, CRC Press, USA, 2014, ch. 1, pp. 1-6. https://doi.org/10.1201/b17195

[12] O. A. Adebo, P. B. Njobeh, A. S. Adeboye, J. A. Adebiyi, S. S. Sobowale, O. M. Ogundele and E. Kayitesi, "Advances in Fermentation Technology for Novel Food Products," in: *Innovations in Technologies for Fermented Food and Beverage Industries*, S. Panda and P. Shetty Eds. Switzerland: Springer, 2018a, pp. 71-87. https://doi.org/10.1007/978-3-319-74820-7_4

[13] J. A. Adebiyi, A. O. Obadina, O. A. Adebo, and K. Eugenie, "Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value added products," *Critical Reviews in Food Science and Nutrition*, vol. 58, pp. 463-474, July 2018.

[14] E. F. Reque, A. P. Ashok, S. G. Franco, and C. R. Socco, "Isolation, identification and physiological study of *Lactobacillus fermentum* LPD for use as probiotic in chickens," *Brazilian Journal of Microbiology*, vol. 31, pp. 303-307, 2000.
https://doi.org/10.1590/S1517-83822000000400012

[15] G. E. Gardiner, C. Heinemann, A. W. Bruce, D. Beuerman, and G. Reid, "Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA," *Journal of Clinical and Vaccine Immunology*, vol. 9, pp. 92-96, 2002.
https://doi.org/10.1128/CDLI.9.1.92-96.2002

[16] G. A. Gerbaldo, C. Barberis, L. Pascual, A. Dalcero, and L. Lucila-Barberis, "Antifungal activity of two Lactobacillus strains with potential probiotic properties," *FEMS Microbiology Letters*, vol. 332, pp. 27-33, 2012.
https://doi.org/10.1111/j.1574-6968.2012.02570.x

[17] O. A. Adebo, E. Kayitesi, F. Tugizimana, and P. B. Njobeh, "Differential metabolic signatures in naturally and lactic acid bacteria (LAB) fermented *ting* (a Southern African food) with different tannin content, as revealed by gas chromatography mass spectrometry (GC–MS)-based metabolomics," *Food Research International*, vol. 121, pp. 326-335, 2019.
https://doi.org/10.1016/j.foodres.2019.03.050

[18] S. Sampaolesi, R. R. Gamba, G. L. De Antoni, and A. M. L. Pelaez, "Potentiality of yeasts obtained as beer fermentation residue to be used as probiotics," *LWT-Food Science and Technology*, vol. 113, 108251, 2019.
https://doi.org/10.1016/j.lwt.2019.108251

[19] FAO/WHO. Guidelines for the evaluation of probiotics in food, 2002, London, Ontario, Canada.

[20] A. Chwastek, E. Klewicka, R. Klewicki, and M. Sójka, "Lactic acid fermentation of red beet juice supplemented with waste high bush blueberry-sucrose osmotic syrup as a method of probiotic beverage production," *Journal of Food Processing and Preservation*, vol. 40, pp. 780-789, 2016.
https://doi.org/10.1111/jfpp.12659

[21] J. A. Adebiyi, A. O. Obadina, A. F. Mulaba-Bafubiandi, O. A. Adebo, and K. Eugenie, "Effect of fermentation and malting on the microstructure and selected physicochemical properties of pearl millet (*Pennisetum glaucum*) flour and biscuit," *Journal of Cereal Science*, vol. 70, pp. 132-139, 2016.
https://doi.org/10.1016/j.jcs.2016.05.026

[22] J. J. Z. Liao, and F. Duan, "Calibrating the concentration from a serial dilution process," *Journal of Chemometrics*, vol. 20, pp. 294-301, 2006
https://doi.org/10.1002/cem.1021

[23] AOAC (Association of Official Analytical Chemists) (2016). Official Methods of Analysis of AOAC International, 20th Ed. (editor, Latimer, G.W).

[24] KEM Kyoto Electronics Manufacturing (May 2016). Automatic potentiometric titrator. Available: https://www.mt.com/int/en/home/products/Laboratory_Analytics_Browse/Product_Family_Browse_titrators_main/Compact_Titration.html.

[25] O. A. Adebo, P. B. Njobeh, A. F. Mulaba-Bafubiandi, J. A. Adebiyi, Z. S. C. Desobgo, and E. Kayitesi, "Optimization of fermentation conditions for *ting* production using response surface methodology," *Journal of Food Processing and Preservation*, vol. 42, pp. 1-10, April 2018b.
https://doi.org/10.1111/jfpp.13381

[26] A. Rault, M. Bouix, and C. Béal, "Fermentation pH influences the physiological-state dynamics of *Lactobacillus bulgaricus* CFL1 during pH-Controlled Culture," *Applied Environmental Microbiology*, vol. 75, pp. 4374-4381, 2009.
https://doi.org/10.1128/AEM.02725-08

[27] O. A. Adebo, P. B. Njobeh, and E. Kayitesi, "Fermentation by *Lactobacillus fermentum* strains (singly and in combination) enhances the properties of ting from two whole grain sorghum types," *Journal of Cereal Science*, vol. 82, pp. 49-56, May 2018c.
https://doi.org/10.1016/j.jcs.2018.05.008

[28] T. Ghaffara, M. Irshada, Z. Anwara, T. Aqilb, Z. Zulifqara, A. Tariqa, M. Kamrana, N. Ehsana, and S. Mehmooda, "Recent trends in lactic acid biotechnology: a brief review on production to purification," *Journal of Radiation Research and Applied Sciences*, vol. 7, pp. 222-229, 2012.
https://doi.org/10.1016/j.jrras.2014.03.002

[29] B. Ozcelik, and M. Yavuz, "Watermelon juice: Handbook of Functional Beverages and Human Health. F. Shahidi, and C. Alasalvar, Ed. USA: CRC Press, 2016, ch. 45, pp. 553-562.
https://doi.org/10.1201/b19490-48

[30] O. A. Adebo, P. B. Njobeh, J. A. Adebiyi, and E. Kayitesi, "Co-influence of fermentation time and temperature on physicochemical properties, bioactive components and microstructure of *ting* (a Southern African food) from whole grain sorghum," *Food Bioscience*, vol. 25, pp. 118-127, August 2018d.
https://doi.org/10.1016/j.fbio.2018.08.007

[31] R. M. Pop, A. D. Buzoianui, I. V. Rati, and C. Socaciu, "Untargeted metabolomics for sea buckthorn (*Hippophae rhamnoides ssp*. carpatica) berries and leaves: fourier transform infrared spectroscopy as a rapid approach for evaluation and discrimination. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 42, pp. 545-550, 2014.
https://doi.org/10.15835/nbha4229654