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(RESEARCH ARTICLE)

Production of functional fruit juice from water melon (*Citrullus lanatus*) by fermentation of lactic acid bacteria

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Abstract

Fruit juice is an ideal medium in the production of a probiotic drink. Water melon (*Citrullus lanatus*) fruit is a suitable medium for the production of probiotic juice due to its nutritional content. Fermentation using lactic acid bacteria is one of the most conventional and recognized arts of food preservation. In this study microbiological and physicochemical changes during fermentation of water melon juice were investigated. Sensory evaluation of the fermented fruit juice was also carried out. The results showed that total bacterial, total fungal and coliform counts ranged between 0-7.2, 0.6-7.8 and 0-19.2 CFU/ml respectively. *Staphylococusaureus* was not detected in any of the sample. The pH ranges obtained for the various samples are 4.32-5.13, 4.36-5.11, 4.31-5.14 and 4.55-5.14. The titratable acidity (TTA) ranged between 0.22-0.29, 0.16-0.26, 0.20-0.31 and 0.10-0.11. The following ranges were obtained for the proximate analysis: moisture (94.19-95.93), ash (0.87-0.95), crude fibre (0.00-0.00), crude protein (1.33-1.82), fat (0.02-0.03) and carbohydrate (1.70-4.63). The organoleptic properties are appearance (4.4-4.8), sweetness (4.2-4.7), texture (4.2-4.6), colour (4.1-5.0), odour (4.3-4.5) and overall acceptability (4.2-4.72).

It could be concluded from this study that samples of water melon juice fermented with *Lactobacillus fermentum* and *L. plantarum* had the lowest microbial load compared to the control. These samples also had the highest protein contents and received the highest scores in term of overall acceptability.

Keywords: *Lactobacillus*sp; Water melon; Functional beverage; Nutritional property; Antimicrobial activity

1 Introduction

Water melon (*Citrilluslanatus*) is a crop that is grown in the tropical regions all over the world. The fruit is made up of high water content which is about 93%, hence the name water melon (Ike et al., 2020). Watermelon grows throughout all seasons and it is classified in the family curbitaceae (Yau et al., 2010). The juice of watermelon has almost neutral pH (5.2 -6.7 and its sugar content is very high (Yau et al., 2010).

It is consumed in large quantity and has several health benefits, thirst quenching capacity, good nutritional value and antioxidant properties (Lani*et al*., 2022). Watermelon contains large quantity of mineral salts (Potassium, sodium, iron and magnesium and vitamins (A, B, C and E). It is also an excellent source of antioxidants like phenolic compounds and carotenoids and some specific amino acids such as citrullin and arginine (Romdhane*et al*., 2017).

Africa is the number three highest producer of watermelon in the world but large percentage (30-40%) of the harvested fruits are lost in the form of damages, spoilage and decay before reaching the target consumers (Mandha*et al*., 2021).

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In order to minimize the economic loss incurred by food companies and farmers, this study transforms such fruits into products with a longer shelf-life using fermentation.

Fermentation is the metabolism of organic substrate by microbes like lactic acid bacteria to obtain desirable biochemical changes (Ray and Joshi, 2014). As a result of their prevalence and effectiveness in fermented foods lactic acid bacteria (LAB) are the most significant strains used for controlled fermentation (Masebe and Adebo, 2019).

LABs are divided into two groups depending on their fermentation pathway. Lactococcus and streptococcus yield two lactate from one glucose molecule. Heterofermentative such as Leuconostoc and Weissella produce one lactate, ethanol and carbondioxide from one molecule of glucose. In addition, LAB produce small organic compounds that give the aroma and flavor to fermented products. LAB contribute to enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food through the fermentation process and enrichment of food substrate biologically with protein, essential amino acids, essential fatty acids and vitamin (Ike et al., 2020). The aim of this current study is to ferment watermelon juice using lactic acid bacteria.

2 Material and methods

2.1 Sample Collection

Samples of healthy watermelon (*Citrullus lanatus*) with unbroken skin was purchased from local fruits market, in Ibadan Oyo state, Nigeria. LAB strains (L*actobacillus fermentum* and *Lactobacillus plantarum*s strains) were collected from biology department, The Polytechnic Ibadan.

2.2 Sample Preparation

The purchased water melon (*Citrulluslanatus)* fruits were washed with sterile distilled water, so as to remove dirt and pesticides residue. The knife and the glass ware (conical flask) to be used were also sterilized in an autoclave for 45minutes at 121 ºC .The washed fruit samples were carefully cut open with the sterilized kitchen knife and the seeds were removed. The flesh (pulp) was scooped into a blender, and was blended.The homogenized blend was sieved through a two-fold muslin cloth to obtain the juice used in the study. About 1000 g of the homogenized blendedwatermelon was decanted in a transparent bucket.

The juice produced was further pasteurized at 85°C for 15 minutes . About 200 ml of pasteurized sample was poured into 4 different conical flasks, and were allowed to cool.

2.3 Inoculum preparation

The identified lactobacillus (*L. plantarum and L. fermentum)* obtained courteously from biology department, The Polytechnic Ibadan, were activated by culturing a loopful of inoculum in 25ml of a sterile MRS agar for 24hours. The purity of the cultures was confirmed by streaking the MRS agar. The single colony on the media indicates that the cultures were considered pure.

2.4 Fermentation of juice with *Lactobacillus* species

Lactobacillus planetarium was introduced into the first conical flask containing the sample, and was labeled sample A. To the second sample *Lactobacillus fermentum was* added and was also labeled sample B. While, the two LAB strains (*Lactobacillus plantarium and Lactobacillus fermentum*) were added and well label sample C. The fourth sample was fermented without using any organism, and was labeled sample D. All the juice samples were allowed to stand on the shelf at ambient temperature while analyses were carried out on daily basis for three days.

2.5 Determination of physicochemical parameters

2.5.1 pH

pH determination is the measurement of the acidity and the alkalinity level of a product. The pH of the sample was performed every 24 hrs using a pH meter. This was simply measured by immersing the pH electrode in 20 ml of the sample. The readings were taken from the pH meter for 3 days. Before the pH measurements were taken, the pH meter was calibrated and the sample slurry was thoroughly stirred aseptically to homogenize the mixture and achieve uniformity.

2.5.2 Total titratable acidity (TTA)

The TTA analysis was done using AOAC (AOAC, 2010) method. 10 ml of the sample was pipetted into a beaker and 3 drops of Phenolphthalein indicator was added. Titration was done using 0.1M NaOH to a faint pink colour for at least one minute compared against a white background. The titre volume was noted and used to calculate TTA which was expressed as percentage Lactic Acid. The TTA was determined and expressed as follows:

$$\% \text{ Lactic Acid} = A \times 0.009 \times 100/v$$

Where:A= ml of 0.1 NaOH required for the titration and V= ml of sample taken for the test.

The acidity was calculated as lactic acid using the formula

$$\frac{\text{Volume of base used} \times \text{Normality of NaOH} \times 9}{\text{Volume of sample used (average titre)}}$$

2.5.3 Proximate analysis

Moisture content, crude fiber, crude fat, ash and carbohydrate contents of samples were determined using AOAC (2010) method. Crude protein was determined by Kjeldahl method as decribed by Kirk and Sawyer (1991).

2.5.4 Microbiological analysis

Daily changes in the microbial population (cfu/ml) of the total viable bacteria, (lactic acid bacteria (LAB)) was determined using Nutrient agar (NA), De Man Rogosa Sharpe agar (MRSA), Potato Dextrose agar (PDA) and MacConkey agar respectively. Samples were enumerated by using appropriate serial dilution and pour plate method. At every 24 h, samples were aseptically withdrawn from the fermentor, serially diluted and 1ml each from dilution factor 10^{-3} was dispensed in triplicates on nutrient agar, de Man Rogosa Sharpe agar and potato dextrose agar. The fungal plates were incubated at 28ºC for 2 to 3 days while the bacterial cultures were incubated at temperatures ranging between 30 to 35ºC for 1 to 2 days. The result of the respective incubated plates was checked, the colonies that developed on the plates were counted, recorded and expressed as Colony Forming Unit per mill (cfu/ml) and spore forming unit per mill respectively (sfu/ml). The isolated microbes were later sub cultured into their respective freshly prepared media in order to obtain pure strains, the sub culturing was done repeatedly to obtain pure isolate before they were later stored on slant bottles. Pure isolates were streaked on Nutrient agar while the fungi colonies were subcultured on Potato Dextrose agar.

2.6 Sensory analyses

The organoleptic properties of the samples (fermented watermelon juice with *Lactobacillus*) were determined by a set of characteristics such as color, texture, sweetness, acidity and overall flavor. The preferences of the native consumers were achieved using a hedonic test. The hedonic test of these samples were carried out in a clean room using four samples coded A, B, C, and D were presented.

2.7 Statistical analyses

All obtained data in this study were analysed using analysis of variance (ANOVA). Descriptive statistics in form of means and standard deviation and Duncan post hoc were also used to assess the data. The analyses were done using SPSS 21 to determine the level of differences between the control, treated samples and the different treatments

3 Results and discussion

The result of microbial counts during fermentation of watermelon juice is presented in table 1. The result showed that there was no any form of bacterial growth throughout the period of fermentation. Lactic acid bacteria are known to produce antibacterial metabolites which is likely to be responsible for lack of growth of bacteria in the fermenting juice (Lani*et al*., 2022). However, some amount of fungal growth was observed in all the samples. The fungal count obtained for the various samples are: Sample A (3.6 -5.94 x 10^3cfu/ml); sample B (0.6 - 4.8 x10^3cfu/ml); sample C (0.72 - 7.2 x 10^3cfu/ml) and sample D (0.12 - 7.8 x 10^3cfu/ml). In another study Adesokan et al. (2008) reported a reduce microbial load when suya was biopreserveby lactic acid bacteria.

Table 2 shows the results of physicochemical changes during fermentation of watermelon juice. It is observed that there was a slight decrease in pH values with a corresponding increase in titratable acidity. The range of pH obtained are sample A (4.32 to 5.13), sample B (4.36 to 5.11), sample C (4.31 to 5.14) and sample D (4.55 to 5.14). The sample ranges for titratable acidity are: Sample A (0.22 to 0.29), sample B (0.16 to 0.26), sample C (0.20 to 0.31) and sample D (0.10 to 0.11).

The proximate composition of the fermented watermelon juice is presented in table 3. By the third day of fermentation, the following ranges are presented for specific parameter monitored; moisture content (94.85 -95.93); Ash content (0.87 - 0.92); crude fibre (0.00 - 0.00) crude protein (1.33 -1.48); fat (0.02 -0.03) and carbohydrate (1.70 - 2.78).

The result of sensory evaluation is presented in table 4. This result shows that sample A fermented with *L. plantarum* had the highest overall acceptability of 4.72. however, sample D (control) was rated better than samples B and C.

Table 1 Microbial Count of watermelon juice fermented with lactic acid bacteria

	TBC (NA) Day1Day2Day3			**TFC (PDA) Day1Day2Day3**			**MAC Day1Day2Day3**			**MSA Day1Day2Day3**		
Sample code	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$	$\times10^3$
A	0	0	0	4.8	3.6	5.94	0	0	0	0	0	0
B	0	0	0	0.6	4.8	3.6	0	0	0	0	0	0
C	0	0	0	0.72	3.6	7.2	0	0	0	0	0	0
D	0.6	3.6	7.2	0.12	3.0	7.8	0	0.6	19.2	0	0	0

Key: TBC: Total Bacterial Count; NA: Nutrient Agar; TFC: Total Fungal Count; PDA: Potato Dextrose Agar; MAC: MacConkey Agar; MSA:Mannitol Salt Agar; A: Watermelon juice fermented with Lactobacillusplantarum; B: Watermelon juice fermented with Lactobacillusfermentum; C: Watermelon juice fermented with Lactobacillusplantarum and fermentum; D: Watermelon juice with no starter culture (Control)

Table 2 Physicochemical analysis of watermelon samples

DAY 1				
Physicochemical parameters	**Sample A**	**Sample B**	**Sample C**	**Sample D**
pH	5.13±0.00	5.11±0.01	5.14±0.00	5.14±0.00
TTA	0.22±0.01	0.16±0.00	0.20±0.01	0.10±0.0
DAY 2				
physicochemical parameters	**Sample A**	**Sample B**	**Sample C**	**Sample D**
pH	4.76±0.01	5.09±0.01	5.12±0.00	5.11±0.01
TTA	0.24±0.00	0.18±0.01	0.24±0.00	0.11±0.01
DAY 3				
physicochemical parameters	**Sample A**	**Sample B**	**Sample C**	**Sample D**
pH	4.32±0.00	4.36±0.06	4.31±0.01	4.55±0.011
TTA	0.29±0.00	0.26±0.01	0.31±0.00	0.11±0.00

Key: TTA: Titratable Acidity

Table 3 Proximate analysis of watermelon samples

DAY 1				
Proximate parameters (%)	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Moisture Content	94.19±0.02	93.95±0.02	94.07±0.02	93.61±0.01
Ash Content	0.90±0.02	0.94±0.01	0.94±0.02	0.91±0.01
Crude Fibre	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Crude Protein	1.82±0.01	1.81±0.02	1.84±0.02	0.82±0.00
Fat	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01
Carbohydrate	3.06±0.03	3.28±0.01	3.13±0.03	4.63±0.02
DAY 2				
Proximate parameters (%)	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Moisture Content	94.56±0.02	94.77±0.02	94.14±0.03	94.44±0.01
Ash Content	0.88±0.01	0.95±0.01	0.90±0.00	0.91±0.03
Crude Fibre	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Crude Protein	1.65±0.02	1.54±0.01	1.59±0.00	1.57±0.01
Fat	0.02±0.00	0.02±0.00	0.02±0.01	0.03±0.00
Carbohydrate	2.87±0.03	2.73±0.01	3.35±0.03	3.04±0.04
DAY 3				
Proximate parameters (%)	**Sample A**	**Sample B**	**Sample C**	**Sample D**
Moisture Content	94.85±0.18	95.44±0.04	95.93±0.06	95.27±0.10
Ash Content	0.87±0.02	0.92±0.02	0.89±0.01	0.89±0.01
Crude Fibre	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Crude Protein	1.48±0.02	1.33±0.02	1.47±0.06	1.47±00.01
Fat	0.02±0.00	0.03±0.01	0.02±0.01	0.02±0.01
Carbohydrate	2.78±0.19	2.29±0.06	1.70±0.01	2.35±0.08

Table 4 Sensory evaluation of watermelon juice Samples

Sample	**Appearance**	**Sweetness**	**Texture**	**Colour**	**Odour**	**Overall acceptability**
A	4.8^{a}	4.7^{b}	4.6^{a}	5.0^{a}	4.5^{b}	4.72^{a}
B	4.4^{b}	4.2^{a}	4.2^{a}	4.4^{b}	4.4^{b}	4.32^{a}
C	4.4^{a}	4.2^{a}	4.6^{b}	4.2^{c}	4.3^{a}	4.30^{b}
D	4.4^{b}	4.7^{c}	4.6^{c}	4.3^{b}	4.3^{c}	4.50^{b}

4 Conclusion

It could be concluded that fermentation of watermelon juice led to improved nutritional quality and overall acceptability. However, further study should be carried out to determine parameters like shelf life

Compliance with ethical standards

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