

Development of fermented *Momordica charantia* and analysis of biochemical properties.

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Abstract - The experiment was conducted to investigate the effect of natural fermentation on physiochemical and sensory properties of bitter gourd slices. Initially three varieties of bitter gourd *Thinnaveli white* [V₁], *Mathale green* [V₂] and *Thumba karavila* [V₃] were taken. Preliminary investigation was carried out to select the best variety. Sensory analysis was conducted using 30 untrained panelists and the results were analyzed using Kruskal-Wallis test using MINITAB 17. The best variety was treated with four treatments (treatment 01 [T₁] = 3% (w/w) dry salts, treatment 02 [T₂] = 3% (w/w) saturated brine solution, treatment 03 [T₃] = 3% (w/w) dry salts with 1% (w/w) saturated brine solution). To select the best treatment sensory evaluation was conducted. Bitter gourd was fermented for four days and total soluble solids, pH, titratable acidity and vitamin C were analyzed on 1st four days and 7th day of the fermentation.

The results indicated that respondents had a favorable attitude towards fermented *Matale green* V₂ variety and 3% dry salt T₁ treatment. The pH value was significantly declined ($p < 0.05$) from 5.9 to 3.7 during fermentation period. No significance change of TSS ($p < 0.05$) in the sap and a gradual increase of the fruit pieces were observed with time. Total acidity was increased during fermentation while percentage of lactic acid increased from 0.009% to 0.06% within the 7 days of fermentation. There was no significant change in salinity ($p = 0.326$). Microbiological properties (total plate count, yeast and mould count, coliform and e-coli) of the product were acceptable within the 7 days after fermentation. Results revealed that Bitter gourd can be developed as a fermented ready to eat product and storage of the finish product was four weeks in refrigerated conditions without changing the color and the overall acceptability.

Index terms: Fermented bitter gourd, bitter melon, lactic acid fermentation, ready to eat

1 Introduction

Momordica charantia which is also known as balsam pear or bitter gourd is an important functional food crop grown in low lands in Sri Lanka [1]. Bitter gourd is a highly nutritive plant composed of a complex array of beneficial compounds. These include bioactive chemicals, vitamins, minerals and antioxidants which all contribute to its remarkable versatility in treating a wide range of illnesses. The fruits contain high amounts of vitamin C, vitamin A, vitamin E, vitamins B1, B2 and B3, as well as vitamin B9 (folate). The caloric values for leaf, fruit and seed were 213.26, 241.66 and 176.61 Kcal/100 g respectively [2]. The fruit is also rich in minerals including potassium, calcium, zinc, magnesium, phosphorus and iron, and is a good source of dietary fiber (bitter melon "monograph", 2008).

Bitter gourd is anti-diabetic, stimulant, stomachic, laxative, blood purifier and control diabetes [3]. Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates, all of which confer a bitter taste [4]. The main constituents of bitter melon which are responsible for the antidiabetic effects are triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds [5],[6].

As it is a seasonal vegetable, steps should be taken to preserve them to make them available for consumption in off season as well. This could be achieved by extending the shelf life in fresh form or in the processed form [7]. Much of the work is done for preservation of bitter gourd by different methods such as steeping preservation, processing of bitter gourd into rings [8], sun drying and dehydration of bitter gourd [9], hot air drying of bitter gourd slices[10]etc.

Changes in lifestyle patterns has leads to increased demand for cut vegetables as the people do not have time to prepare vegetables at home as well as in hotels. Because of these factors, consumption of minimally processed products has significantly increased [11].

Lactic acid fermentation is one of the commonly practiced methods in food preservation that includes selective control of microorganisms in the fermentation process to stabilize the treated material by salting. It prolongs the availability period of the produce while contributing certain desirable physical and flavor characteristics [12].

Salting provides a suitable environment for lactic acid bacteria to grow which impart the acid flavour to the vegetable [13]. During the fermentation process, vegetables are placed in a jar and salt is added and then mechanical pressure is applied to the vegetable to expel the juice, which contains fermentable sugars and other nutrients suitable for microbial activity. The first microorganisms to start acting are the gas producing cocci (*L. Mesenteroides*). These microbes produce acids. When the acidity reaches 0.25 to 0.3% (calculated as lactic acid), these bacteria slow down and begin to die off, although their enzymes continue to function. The activity initiated by the *L. mesenteroides* is continued by the lactobacilli (*L. plantarum* and *L. Cucumeris*) until an acidity level of 1.5 to 2% is attained. The high salt concentration and low temperature inhibit these bacteria to some extent. Finally, *L. pentoaceticus* continues the fermentation, bringing the acidity to 2 to 2.5% thus completing the fermentation [14].

Though many vegetables are part of our dietary habit, the technology of ready to eat or ready to cook form of minimal processing is available only for few vegetables. Thus it is necessary to develop techniques to preserve bitter gourd. This study has been carried out to develop a product by fermentation of bitter gourd from the endemic micro flora in it and determination of physicochemical, sensory & nutritive quality of the product for a period of time.

2 Material and methods

2.1 Raw material preparation

Bitter gourd varieties were purchased from the local market Sri Lanka. Three different varieties of *Momordica charantia* available in Sri Lanka were selected for the experiment. Varieties were Thinnaveli white [V1], Mathale green [V2], Thumba karavila [V3] and identified at the Horticultural Crop Research & Development Institute, Gannoruwa, Sri Lanka. Fresh good quality, matured and uniformly sized bitter gourd were selected and washed with chlorinated tap water thrice, & seeds were removed. Then they were prepared by slicing with sharp sterile stainless steel knives and sliced into 3 cm* 1 mm thick cubes.

2.2 Selection of best variety

Cubes from all three varieties were mixed homogeneously with 3% (w/w) iodine free powdered salt and stored in a sterile glass jars. They were kept for one week time period at room temperature for the fermentation. Fermented products of the three varieties were introduced to the sensory panel to evaluate the best variety for the final product. The untrained thirty panelists were participated to the sensory evaluation and the samples were analyzed by sensory panel for the; texture, taste, odor, color and Overall Acceptability. According to the results obtained from sensory analysis, the most palatable variety was chosen for the further development.

2.3 Selection of best treatment

Selected best variety was treated with three different treatments using non-iodide powdered salt. Treatment conditions were as follows; treatment 01 [T1] = 3% (w/w) dry salts, treatment 02 [T2] = 3% (w/w) saturated brine solution, treatment 03 [T3] = 3% (w/w) dry salts with 1% (w/w) saturated brine solution. Samples were fermented at 27 °C in sterile glass jars for 7 days. The most favorable treatment condition was selected by using sensory evaluation with untrained thirty panelists. Samples were analyzed by sensory panel for the; texture, taste, odor, color and Overall Acceptability. According to the results obtained from sensory analysis, the most palatable treatment was chosen for the further development and studies.

2.4 Determination of total soluble solids and pH

Total soluble solids (TSS) and pH of the juice and the pieces were determined separately every day for 7 days of time

period. pH of the sample was determined according to the method SLS-144; 1972 using digital PH meter (pHep HI 98107, Hanna Instruments, Portugal). TSS was determined by using the hand refractometer (ATC-1E, ATAGO Co. Ltd., Japan).

2.5 Titratable acidity

Titration acidity was determined by using method described by Kohajdová & Karovičová; with slight modification. For each sample 1ml of juice was diluted up to 40ml with distilled water and titrated with 0.001M NaOH by using Phenolphthalein as an indicator. The NaOH required to neutralize the juice and the titratable acidity was calculated and expressed as % lactic acid.

2.6 Analysis of Vitamin C

Vitamin C content of sap and pieces were determined separately by visual titration using 2,6-dichlorophenol-indophenol (Ranganna, 1995). One gram of bitter gourd pieces were made in to pulp by crushing and one milliliter of sap was taken directly from the product for the titration. Quantity of vitamin C (mg) present in 1 g of sample was calculated for the pieces and Quantity of vitamin C (mg) present in 1ml of sample was calculated for the sap.

2.7 Microbial analysis

The qualities of fermented bitter gourd were based on the number and kind of microorganisms present, which were assessed by yeast and mould content (according to the SLS 516: Part 2:1991), Total plate count (according to the SLS 516 Part 1 :1991) and Presumptive Coliform test (according to the SLS 516 Part 3 - 1982). From each sample 1 ml of juice was taken and diluted from 10⁻¹ -10⁻⁵.

2.8 Statistical analysis

All the analysis was carried out using MINITAB version 17. Sensory characteristics were analyzed by using 5 point rating system with kruskal-wallis test and significances of the samples were measured by using analysis of variance (ANOVA).

3 Results and Discussion

3.1 Variety selection & Treatment selection

The sensory acceptability of Thinnaveli white [V1], Mathale green [V2] and Thumba karavila [V3] has been illustrated in Figure 01. The results revealed high level of acceptance for V2 (Mathale green) obtaining the highest scores for colour, taste, odour, texture and overall acceptability. Lower level of acceptability obtained for V1 & V3 varieties may due to the inability of carrying out the fermentation process on the presence of antibacterial substances and/or unavailability of sufficient level of nutrients for the growth of LAB. As Drewnowski.A. and Gomez. C., (2000) [15] showed, many vegetables contain glycosides that hamper

efficient fermentation and high solanin content of unripe fruit might inhibit the growth of LAB.

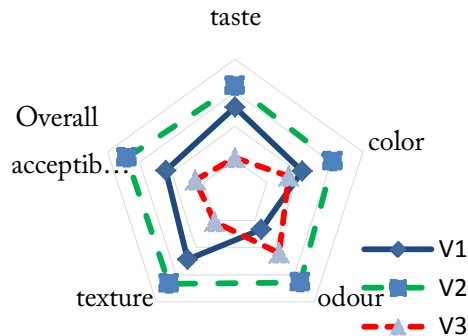


Figure 01-sensory profile of different varieties.

Sensory analysis was done to select best treatment condition out of 4 different treatments. According to results obtained as shown in figure 02, the highest level of acceptance was recorded for T1. This may be due to the fully anaerobic conditions in the dry salt treatment. The odour was reported as ripened Ceylon Olive (weralu) in sensory evaluation which may be resulted from a better combination of flavors due to the hetero fermentative by products. Because Lactobacillus grow well in anaerobic condition and strictly fermentative in nature, homofermentative Lactobacillus species, converting sugars

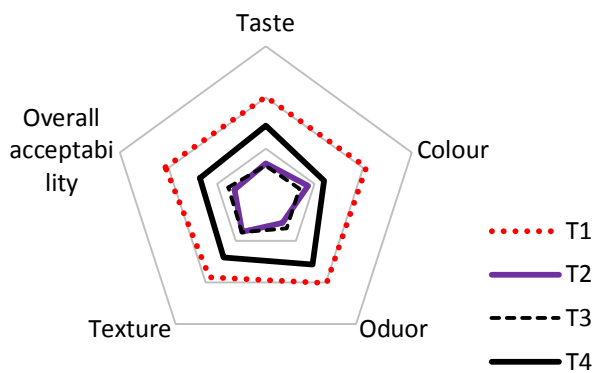


Figure 02- Sensory profile of different treatments.

mostly into lactic acid and heterofermentative species, converting sugars into lactic acid, acetic acid and CO₂ [16].

Considering the above sensory data Mathale Green variety and 3% dry salt treatment was selected for further research activities.

3.2 pH

The pH of the sap reduced from 5.9 to 3.8 from the initial level to the seventh day. Gradual reduction in pH was observed within the tested period (Table 01). The pH reduction may be due to utilization of sugar extracted from the bitter gourd by lactic acid bacteria to produce lactic acid and acetic acid and that are inhibitory to competing bacteria, including psychrotrophic pathogen [17].

3.3 TSS

No significance change of TSS ($p < 0.05$) in the sap and a gradual increase of the fruit pieces were observed with time. The initial high TSS of sap (5.920 ± 0.310) is attributed to the process of leaching of solutes into the brine. The addition of salt to the shredded bitter gourd, extracts out water and sugars besides other nutrients from the shreds into the sap that might have provided a favourable growth medium for lactic acid bacteria [18]. Thus, the extraction of soluble solutes from the shreds is apparently responsible for the subsequent increase of TSS.

3.4 Salinity

No significance change of the salinity ($p > 0.05$) was observed (Table 01). Initial slight reduction of salinity in the 1st day may be due to the osmotic dehydration of the fruit pieces and the significantly unchanged salinity level in later few days may be due to the equilibration of the fruit pieces with the external environment. Salts plays a very important role in lactic acid fermentation of vegetables and sufficient amounts must be added to extract from the plant cells the nutrients required to support growth of lactic acid bacteria and salt also serves to inhibit the growth of undesirable microorganisms and serve as a flavor ingredient in the final product [19].

3.5 Titratable acidity

Titrateable acidity (TA) value is a quantitative measure of the organic acids in a food substrate which is a useful measurement to monitor the progress of acid producing fermentations [20]. Titratable acidity is often expressed in terms of the predominant acid present and presented as % of Lactic Acid. Percentage lactic acid increased from 0.009% to 0.06% within the 7 days of fermentation. Reduction of pH and increment in the percentage lactic acid during the fermentation period coincide together is due to the anaerobic respiration of the lactic acid producing bacteria supported by salt conditions. Wang et al. (2007) also reported that cutting of bitter gourd lead to the accumulation of titrateable acidity [21].

3.6 Vitamin C

The bitter gourd fruits are known as vegetables which are a good source of Vitamin C [22], [23]. The amount of vitamin C in raw bitter gourd was reported as 1.4 mg g⁻¹ & was reduced up to 0.49 mg g⁻¹. Although the Vit C was expected to be preserved in the anaerobic condition where oxygen was not available, the reduction of the Vit C content

may due to the solubility of Vit C in the sap and also

Time (Day)	pH of the sap	TSS of the sap (OBrix)	TSS of pieces (OBrix)	Salinity of sap	Lactic acid % (g per 1mL)	Vitamin C mg/g
Initial	5.900 ± 0.04	5.920 ± 0.310	4.04 ± 0.11	55.00 ± 0.70	0.009 ± 0.00	1.407 ± 0.009
1	4.446 ± 0.198	5.200 ± 0.682	5.120 ± 0.130	51.000 ± 1.414	0.01 ± 0.00	1.001 ± 0.002
2	4.388 ± 0.248	5.430 ± 0.292	5.200 ± 0.122	50.800 ± 2.049	0.03 ± 0.00	0.842 ± 0.004
3	4.080 ± 0.034	5.540 ± 0.313	5.380 ± 0.130	50.800 ± 2.168	0.04 ± 0.00	0.531 ± 0.006
4	3.868 ± 0.008	5.560 ± 0.054	5.840 ± 0.204	51.400 ± 1.342	0.05 ± 0.00	0.487 ± 0.006
7	3.776 ± 0.165	5.568 ± 0.211	5.874 ± 0.150	51.200 ± 1.231	0.06 ± 0.00	0.496 ± 0.005

degradation of ascorbic acid due to the presence of light, oxygen & enzymes of the LAB [24].

Table 01: Physicochemical parameters of fermented bitter gourd (Matale green)

Values are mean ± SD, n = 6

3.7 Microbial analysis

According to the guidelines of Centre for Food Safety, Food and Environmental Hygiene Department, evaluating of total plate count isn't applicable for fermented foods for assessing the quality of such foods. According to the Food and Drug Administration Manual, yeast and mould should be lower than 1.0×10^2 cfu/g. Therefore in the present study, yeast and mould content and total coliform content have been analyzed. For the enumeration of total presumptive coliform, MPN test was used and after incubation period no positive tubes were present. Yeast and mould content was lower than 1.0×10^2 cfu/g within seven days. Therefore the results suggest that this product is safe for human consumption.

4 Conclusion

Considering the physicochemical and microbial properties of the fermented bitter gourd, it can be recommended that Mathale green variety treated with 3% dry salt was in highly satisfactory levels as a ready to eat fermented vegetable. Further analysis can be carried out to analyze the nutritional profile of the product.

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