

**Development of a probiotic enriched bottle gourd
(*Lagenaria siceraria*) beverage from local cultivar of
Assam and evaluation of its health benefits**

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Manas Jyoti Das

Registration Number TZ189895 of 2018



DEPARTMENT OF FOOD ENGINEERING AND TECHNOLOGY

SCHOOL OF ENGINEERING

TEZPUR UNIVERSITY

TEZPUR-784028

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CHAPTER 7

Conclusions and future scope of work

Conclusions

The study revealed the effectiveness of a combined microwave-ultrasonic pasteurization system on *Listeria monocytogenes* in bottle gourd juice. Response surface methodology based on the face centered composite design was employed for optimization with microwave power (W), microwave-induced temperature (°C), ultrasound amplitude (%) and ultrasound exposure time (min) as the independent factors. Microbial cell viability, total phenolic content, total terpenoid content, and antioxidant activity were considered as the dependent factors. Microwave-induced temperature, ultrasound amplitude percentage as well as ultrasonic exposure time were found to be most effective in reducing *L. monocytogenes* count. The ideal condition was 70°C temperature; 750 W microwave yield power, 80% amplitude, and 15 minutes of ultrasound exposure time in which about 5 log decrease of *L. monocytogenes* count was observed. Furthermore, microwave and ultrasound processed juice showed superior antioxidant activity, vitamin content, storage activity and physicochemical parameters as compared to conventionally processed juice.

The toxicity studies of microwave with ultrasound treated *Lagenaria siceraria* juice and its health beneficial effects.

The effects of different concentration of *Lagenaria siceraria* juice on % inhibition of haemolysis of erythrocytes incubated in hypotonic solution was observed. An MTT based cell viability assay in HPBMC was conducted using different concentration of *Lagenaria siceraria* juice.

Cell viability assay of *Lagenaria siceraria* juice was again checked in THP-1 human monocyte cell line for 24 h and was measured by MTT based method.

Acute and Subacute Oral Toxicity of lyophilized *Lagenaria siceraria* juice was studied in Experimental Wistar Rats (*Rattus norvegicus*). All experimental procedures followed the Animal Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by Institutional animal Ethical Committee of Defence Research Laboratory, Tezpur, Assam India. with a registration number 1227/GO/Rbi/S/08/CPCSEA and protocol number 17/IAEC/DRL/25/2/2022. Effect of the lyophilized *Lagenaria siceraria* juice on haematological parameters, biochemical parameters and histopathology was conducted of the vital organs of the

animal models in both acute and subacute oral toxicity study. No signs of toxicity were noticed in the experimental animal models.

The health beneficial effects of *Lagenaria siceraria* juice was investigated for its antidiabetic potential using *in vitro* enzyme inhibitory assays. 50µl of *Lagenaria siceraria* juice showed the highest inhibition of α Glucosidase enzyme. Similarly, 200µg of lyophilized *Lagenaria siceraria* juice showed inhibition of DPP4 enzyme activity over 60%. Insulin mediated 2NBDG uptake was checked using lyophilized *Lagenaria siceraria* juice on L6 rat skeletal muscle cell line at different concentration. 10µg/mL concentration of *Lagenaria siceraria* juice were found to be effective on 2NBDG uptake.

The present study demonstrates the antidiabetic effects of lyophilized *Lagenaria siceraria* juice on streptozotocin induced diabetic rats with improved glucose tolerance activities. These findings give credence to the claim that lyophilized *Lagenaria siceraria* juice could be used as a medicinal plant for the treatment of diabetes.

Real Time PCR analysis was done of TNF α and IL-1 β mRNA level in THP-1 macrophage pre-treated with or without lyophilized *Lagenaria siceraria* juice in varied concentration, in presence or absence of LPS. *Lagenaria siceraria* juice was seen to attenuate LPS induced inflammation in THP-1 macrophage, further luciferase activity assay reported about the that NF-kB gene expression of THP-1 cells in presence of LPS were reduced in presence of *Lagenaria siceraria* juice.

Sensory evaluation of BGJ samples, acquired from microwave-ultrasound based combined treatment was performed in this investigation. For the assessment raw (sample-1) and conventionally treated (sample-2) samples alongside microwave-ultrasound treated (sample-3) were considered. An innovative approach of hybrid fuzzy logic and proportional odd modelling (FL-POM) was implemented for the analysis of the sensory scores. From the results of fuzzy logic, the similarity values for the juice samples and their associated QA were resolved. These values were considered as input for hybridization with the POM approach. The assessed coefficients obtained from the results of POM were considered for the ranking of the samples and quality traits. The ranking of the BGJ samples was observed in the order of sample-1>sample-3>sample-2, while for their related quality credits the ranking was in the order of Color>Taste>Aroma>Mouth feel. The

microwave-ultrasound treated BGJ was derived as the best sample as compared to the raw sample owing to its higher inclination and agreeableness.

In the present study a probiotic bottle gourd beverage was developed. Isolation and identification of lactic acid bacteria (LAB) from rice beer (zutho) prepared in Nagaland, India and fermented Bamboo shoot from Manipur, India was performed, and their growth associated, and functional properties were studied. LAB strains were identified as *Lactobacillus plantarum* and *Limosilactobacillus fermentum* based on 16s rRNA sequencing. Dried bottle gourd pomace was used for the extraction of fiber. The soluble dietary fiber was found to be 3g/100g of dry bottle gourd waste powder. *In vitro* fermentation of the dietary fibers using carbohydrate free MRS media showed the significant survival and growth of *L. plantarum* strain showing the potentiality of bottle gourd fiber as prebiotic. The isolated bacterial strains and bottle gourd fibers were incorporated in the pasteurized bottle gourd juice. The storage study and the survivability of the strains in bottle gourd juice achieved the required probiotic survivability of around 10^7 c.f.u/ ml in the beverage.

Summary and future scope

The research work presents a bottle gourd beverage pasteurized by the combined treatment of microwave with ultrasound and with minimal denaturation of natural qualities. The pasteurized bottle gourd juice was found to be non-toxic and with health beneficial properties like anti-diabetic and anti-inflammation. Isolated probiotic strains with bottle gourd fiber survived in the bottle gourd juice.

Further *in vivo* studies can be conducted for type II diabetes and anti-inflammatory properties of the juice.