CHAPTER 1

INTRODUCTION

Introduction

The bottle gourd, also known as *Lagenaria siceraria*, is a vegetable that is extensively cultivated in India and is popular since it has a high nutritional content and is inexpensive [3]. It has been stated that the juice of bottle gourd provides a variety of health benefits and therapeutic effects against a number of disorders, including hyperthyroidism, diabetes, flatulence, and piles [3]. It has been shown that bottle gourd contains triterpenoid chemicals such as cucurbitacins B, D, G, and H [8]. These compounds are thought to contribute to the regulation of blood glucose levels [14,24]. Additionally, it has a high choline content compared to other vegetables, and choline is one of the foods that acts as a neurotransmitter to alleviate mental illnesses and sadness [17]. Although it is a popular vegetable in India, the amount of industrial processing that it undergoes in its many forms is restricted. The vegetable has been used in traditional Indian medicine as a cardiac tonic, aphrodisiac, general tonic, hepatoprotective, analgesic, anti-inflammatory, expectorant, and diuretic. It also has a therapeutic benefit in traditional Chinese medicine.

Consumers prefer fruit and vegetable juice with nutritious quality, natural taste, and freshness, preserved without any preservatives as they are perceived as safe. However, the safety and shelf life of bottle gourd juices remains a challenge. Important factors affecting the spoilage of bottle gourd juice includes pH, water activity and its nutritional parameters for microorganism growth [1]. Bottle gourd juice is normally treated for 121, 63, 75ºC for 7, 30 and 10 min, respectively [3]. However, thermal processing can damage the naturally occurring compounds in the food, as long heating process have negative effects on the nutritional qualities of the product [22]. HTST (High temperature and short times) which was considered as a mild heat treatment also showed decrease in the nutritional value by bringing chemical changes in the product [22]. Consumer demand for nutritious foods, which are minimally and naturally processed, has led to processor interest in non-thermal or mild thermal technologies. Microwave heating as a promising alternative to conventional pasteurization has been reported in different studies [15]. Microwaves have the capacity to penetrate and dissipate energy into the food with the interaction of water molecules inside the food, and this property of microwave helps in heating the food rapidly.

Many studies also reported ultrasound as an alternative to conventional thermal processing to decrease microbial population with minimal changes in the available bioactive compounds. Ultrasound has been reported [16] to be useful in sterilization and extraction in reduced processing times and with high efficiency [19]. The lethality of ultrasound can be increased if it is combined with different other techniques like the use of heat, microwave, UV, and high pressure. In recent years, there is a great demand of food which promote health and wellness. The foods that meet this demand are those which have functional properties.

The FAO/WHO definition of a probiotic is "live microorganisms which when administered in adequate amounts confer a health benefit on the host" Probiotic consumption has dramatically grown with most products claim of gastrointestinal health and immune support. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) say that probiotic strains are microorganisms that are good for the health of their host.

In addition, the viability of the bacterium, adequate dosage, and the presence of at least one positive health impact are three requirements that must be met before selecting a probiotic. Probiotics to exhibit the desired beneficial effects on the host's health, they must first overcome the biological barriers that are present in the digestive system of the host. Although there are a few things that have to be taken into consideration as criteria for the selection of new probiotic isolates, such as tolerance to acidic and bile environments and antibacterial activity, among other things.

Most probiotic products are marketed in the form of yogurt and fermented milk. There is a growing interest in developing non-dairy probiotic products. Bottle Gourd could be one of the substrates for probiotic vegetable drink as its production as vegetable is large all over India and is also found almost in all the states of India.

1.1. Hypothesis

1. Pasteurization of *Lagenaria siceraria* juice with a combined semi thermal process of microwave and ultrasound may keep its nutrition and health beneficial values intact.
2. *Lagenaria siceraria* is believed to be nontoxic and safe for consumption.

3. *Lagenaria siceraria* juice might have potential anti-diabetic and anti-inflammatory activity.
4. *Lagenaria siceraria* juice can be a potential substrate for the development of a probiotic vegetable drink.

1.2. Gap of Research

- In recent years, there is a great demand of food having functional properties. Fruits have already taken this path of success, but a scanty amount of work has been done on vegetable drinks.
- Further comparison with other vegetables, bottle gourds having a large production got little attention both in North-east India as well as India as a whole.
- Most probiotic products are marketed in the form of yogurt and fermented milk, though a few probiotic fruit drinks are noticed. Exhaustive literature survey on probiotic vegetable drink clearly reveals that very few works have been carried out in our country.

1.3.Objectives

1. To study the combined effects of microwave with ultrasound treatment on the pasteurization and nutritional properties of bottle gourd (*Lagenaria siceraria*) juice
2. To study the toxicity of microwave combined ultrasound treated *Lagenaria siceraria* juice and its antidiabetic and anti-inflammatory effects
3. To study the sensory quality of bottle gourd juice (BGJ) samples, obtained from microwave-ultrasound based combined treatment
4. To develop a *Lagenaria siceraria* juice enriched with prebiotics and isolated probiotics

Objective 1

To study the combined effects of microwave with ultrasound treatment on the pasteurization and nutritional properties of bottle gourd (*Lagenaria siceraria*) juice.

The bottle gourd was collected from homestead garden of Napaam, Tezpur, Assam, India (latitude 26.651218, longitude 92.783813). The herbarium of the sample was identified by The Department of Botany, Gauhati University, Guwahati, Assam, India (*Lagenaria siceraria*, Accession No: GUBH18494). Good quality bottle gourds were washed, cleaned, and wiped with an absorbent paper, peeled, and cut into uniform cubes ($2 \times 2 \times 2$ cm^3).

Blanching was carried out in distilled water following the protocol described by Bhat, Saini, Kumar, & Sharma (2017) with minimal modification (85 ºC for 5 min).

Strain of *L. monocytogenes* was cultured on *Listeria* Selective Enrichment Broth (HiMedia, M1865) for 24 h at 37ºC. After 24 h growth, the culture containing the cells were centrifuged for 5 minutes at 8,000 rpm. The spiked juice was kept in an incubator for half an hour at 37 ºC before the pasteurization treatments for adaptation purpose. The destruction level of *L. monocytogenes* cell load was considered as an indicator for the effectiveness of the process on microorganisms.

A response surface methodology face centred composite design was employed with microwave power (MP) (250-750W), microwave induced temperature (MT) (30-70ºC), ultrasound amplitude (UA) (20-80%) and ultrasound exposure time (UT) (5-15min) as factors.

Total phenolic contents, total terpenoides and antioxidant activities were taken as positive response while survivability of the microbial culture was taken as the negative response of the design.

The content of total phenolics was determined using Folin-ciocalteu reagent (FCR) [21] with minor modification. The content of total terpenoids was determined using linalool 97% (L2602-100G, Sigma-Aldrich) following standard protocol [7]. DPPH radical scavenging activity of the BGJ was determined according to the previously described method [12] with minor modification.

The extracted bottle gourd juice was conventionally pasteurized at 75 °C for 10 min [3] in a hot water bath (Equitron, India). The temperature of the sample was monitored with a laser point infrared digital thermometer (Bexco, South Korea).

Determination of total solids (TS) and total soluble solids (TSS) were determined by standard method [18]. Titratable acidity (TA) was determined by using the standard protocol [18].

Polyphenols profiling of BGJ was carried out using RP-HPLC (Waters, United States) gradient elution method [20]. The protein content was estimated by Bradford's method using the manufacturer's protocol (Sigma Aldrich, USA). The described protocol was also followed for amino acid compositional analysis [9]. Water soluble vitamins and fat soluble vitamins were quantified using AOAC/relevant official methods after duly validated with Certified Reference Materials / Standard Reference Materials and as explained by [5].

The change in color was checked using Hunter Lab colorimeter (Ultrascan Vis, HunterLab, USA). The antioxidant activity of the juice samples was determined using four different methods namely DPPH free radical scavenging assay, nitric oxide (NO) assay, superoxide radical scavenging (SOD) assay and reducing power (RP) assay.

Objective 2

To study the toxicity of microwave combined ultrasound treated *Lagenaria siceraria* juice and its antidiabetic and anti-inflammatory effects

Toxicity study

Human Blood was collected voluntarily. RBC was isolated from blood samples and washed three times with PBS (pH-7.4) then centrifugation at 2000 rpm for 10 min. 2% Erythrocyte suspension (ES) was re-suspended in saline solution. The reaction mixture contains 100 µL of ES, 0.1% Triton X-100 and 25, 50 and 100 µg/ml of plant extract was added to 96-well microplates after that incubated for 60 min at 37 °C under constant agitation. The release of haemoglobin was determined after centrifugation (2000 rpm for 10 min) by photometric analysis of the supernatant at 576 nm.

The cytotoxic effect if any was investigated in human PBMCs [5]. Isolated PBMC (3 × 103 cells in 200 µL) was seeded in RPMI-1640 supplemented with 10% foetal bovine serum (FBS) in ninety-six well plates.

In vitro cytotoxicity of *Lagenaria siceraria* juice sample was performed using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazoliumbromide) based method in THP-1 cell line. $2X10^3$Cells were seeded RPMI-1640 supplemented with 10% FBS in 96 well plate.

Male wistar rats (*Rattus norvegicus*) weighing 130–160 g was used for the acute and sub acute toxicology studies. Haematological, biochemical analyses and histopathology were performed at Defence Research Laboratory, Tezpur, Assam, India. The study was performed according to the Organization of Economic Co-Operation and Development (OECD) guideline 423 and 407 for testing of chemicals and World Health Organization guideline. 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 1 with a registration number 1227/GO/Rbi/S/08/CPCSEA and protocol number 17/IAEC/DRL/25/2/2022

In vitro anti-diabetic study of *Lagenaria siceraria* juice

The α-amylase and α-glucosidase inhibitory activity of lyophilized *Lagenaria siceraria juice* was determined according to the modified method described by [10].

Determination of DPP-4 inhibition activity DPP-4 inhibition of *Lagenaria siceraria* juice was carried out in a black 96-well plate as previously described by [6].

Glucose uptake assay was performed using glucose uptake cell-based assay kit (Cayman, USA) following manufacturer's instruction. Briefly, L6 myotubes were kept in no glucose DMEM for overnight. Cells were then pretreated with *Lagenaria siceraria* juice in different concentration for 1 h followed by palmitate (0.75 mM) incubation for 4 h and 30 min before the termination of incubations cells were treated with insulin (100 nM).

In vivo anti-diabetic study of *Lagenaria siceraria* juice

Animals in groups were rendered diabetic after overnight fast (8 h) by intraperitoneal injection of a single dose of STZ, 55 mg/kg BW dissolved in 0.2 ml of 0.1M freshly prepared citrate buffer at pH 4.5. Development of diabetes was confirmed by fasting

blood glucose (FBG) test 3 days after induction with STZ solution by tail pricking of blood droplets using a handheld glucometer. Rats with FBG of more than 6 mmol/L were deemed eligible and included in the study [13].

Rats in group I (control) were normal nondiabetic rats. Rats in group II were diabetic rats with no treatment. Rats in group IV received treatment of metformin and rats in group III received Dose of *Lagenaria siceraria* juice of 600mg/kg BW freshly re-constituted in deionized water. The dose of the *Lagenaria siceraria* was chosen in accordance with a previous study by [13] and from the acute toxicity studies.

Oral glucose tolerance test (OGTT)

After 4 weeks of *Lagenaria siceraria* treatment, rats were fasted overnight before performing the test and glucose (2.5 g/kg) was orally administered. The blood glucose levels were determined from the tail vein before and after the glucose challenge (0, 15, 30, 60, 90, 120 min) [13].

Intraperitoneal insulin tolerance test (IPITT)

After 4 weeks of *Lagenaria siceraria* treatment, rats were fasted for 6 h followed by an intraperitoneal administration of insulin. The blood glucose levels were determined from the tail vein at 0 (prior to insulin administration), 30, 60, 90, and 120 min after insulin administration [13].

Animal sacrifice

The animals were humanely euthanized by halothane overdose (5% by volume in oxygen) on day 31 of treatment, and blood was collected by cardiac puncture in lithium heparinized tubes, separated into plasma, and stored at − 80◦C prior to analysis. Gastrocnemius muscles were excised, rinsed in normal saline, snap-frozen in liquid nitrogen, and stored at − 80°C for further study. The pancreas were excised and divided into equal halves, which were either snap-frozen in liquid nitrogen for further biochemical analysis or preserved in phosphate-buffered formalin for histological analysis [13].

Histological analysis

The processing of pancreas tissues with formalin was done according to the standard laboratory protocol for paraffin embedding. The liver and pancreas specimens were washed and fixed in 10% phosphate-buffered formalin, dehydrated in graded ethanol, deparaffinized with p-xylene, and embedded in paraffin wax at 4 μm thickness. This was followed by staining of the cross-sections of tissue with hematoxylin and eosin on coated slides. The slides were then scanned with the photomicrograph taken by Leica Microscope DM 500 that was fitted with a Leica ICC50 HD camera (Leica Biosystems, Germany) [13].

***In vitro* anti-inflammatory study of *Lagenaria siceraria* juice**

THP-1 monocytes were procured from the National Centre for Cell Science, Pune, India and were cultured in RPMI1640 containing penicillin (100 U/ml) and streptomycin (100 mg/ml) and supplemented with 10% FBS in a humidified 5% CO_2 environment at 37 C. *Lagenaria siceraria* juice sample were checked for its property to attenuate LPS induced inflammation in THP-1 macrophage. Real Time PCR analysis showing TNFα and IL-1β (mRNA level in THP-1 macrophage pre-treated with or without *Lagenaria siceraria* Juice in varied concentration was checked, in presence or absence of LPS (100ng/ml) for 4 h.

Objective 3

To study the sensory quality of bottle gourd juice (BGJ) samples, obtained from microwave-ultrasound based combined treatment.

In the present investigation, sensory evaluation of bottle gourd juice (BGJ) samples, obtained from microwave-ultrasound based combined treatment was performed. The raw (sample-1) and conventionally treated (sample-2) alongside microwave-ultrasound treated (sample-3) were considered for the assessment of sensory evaluation. An innovative approach of hybrid fuzzy logic and proportional odd modelling (FL-POM) was implemented for the analysis of the sensory scores. The similarity values for the juice samples and their quality attributes were resolved from the results obtained by fuzzy logic. 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, and their related quality attributes ranked in the order color>taste>aroma>mouth feel. The microwave-ultrasound treated BGJ evinced as the best sample in comparison to the raw BGJ [11,23].

Objective 4

To develop a *Lagenaria siceraria* juice enriched with prebiotics and isolated probiotics.

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 based on 16s rRNA sequencing. Strains were checked for its acid and bile survivability.

Dried bottle gourd pomace was used for the extraction of fiber. The protocol for fiber extraction was used from [2]. *In vitro* fermentation of the dietary fibers was tested using carbohydrate free MRS media in an incubator at 37°C for 48h.

Probiotic strains were added to bottle gourd juice with soluble dietary fiber.

Bibliography

[1]. Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. Microbes associated with freshly prepared juices of citrus and carrots. *International Journal of Food Science*, 2014.

[2]. Begum, Y. A., Baishya, P., Das, M. J., Chakraborty, S., & Deka, S. C. Novel approach for the development of dietary fibre-anthocyanin enriched functional bread from culinary banana bract. *International Journal of Food Science & Technology*, 55(11): 3455-3462, 2020.

[3]. Bhat, S., Saini, C. S., Kumar, M., & Sharma, H. K. Effect of thermal and alternate thermal processing on bottle gourd (*Lagenaria siceraria*) Juice. *Journal of Food Processing and Preservation*, 41(3): e12911, 2017.

[4]. Borah, R., Kumar, A., Das, M. K., & Ramteke, A. Surface functionalization-induced enhancement in surface properties and biocompatibility of polyaniline nanofibers. *Royal Society of Chemistry Advances*, 5(60): 48971-48982, 2015.

[5]. Chyne, D. A. L., Ananthan, R., & Longvah, T. Food compositional analysis of Indigenous foods consumed by the Khasi of Meghalaya, North-East India. *Journal of Food Composition and Analysis*, 77: 91-100, 2019.

[6]. Fujiwara, K., & Tsuru, D. New chromogenic and fluorogenic substrates for pyrrolidonyl peptidase. *The Journal of Biochemistry*, 83(4): 1145-1149, 1978.

[7]. Ghorai, N., Chakraborty, S., Gucchait, S., Saha, S. K., & Biswas, S. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. *Protocol Exchange*, 5(10): 1038, 2012.

[8]. Ghosh, K., Chandra, K., Ojha, A. K., Sarkar, S., & Islam, S. S. Structural identification and cytotoxic activity of a polysaccharide from the fruits of *Lagenaria siceraria* (Lau). Carbohydrate Research, 344(5): 693-698, 2009.

[9]. Gogoi, D., Arora, N., Kalita, B., Sarma, R., Islam, T., Ghosh, S. S., Devi, R., & Mukherjee, A. K. Anticoagulant mechanism, pharmacological activity, and assessment of preclinical safety of a novel fibrin (ogen) olytic serine protease from leaves of *Leucas indica*. *Scientific Reports*, 8(1): 1-17, 2018.

[10]. Jayasri, M. A., Radha, A., & Mathew, T. L. α-amylase and α-glucosidase inhibitory activity of Costus pictus D. Don in the management of diabetes. *Journal of Herbal Medicine and Toxicology*, 3(1): 91-94, 2009.

[11]. Kimura, A., Lee, J. H., Lee, I. S., Lee, H. S., Park, K. H., Chiba, S., & Kim, D. Two potent competitive inhibitors discriminating α-glucosidase family I from family II. *Carbohydrate Research*, 339(6): 1035-1040, 2004.

[12]. Kitts, D. D., Wijewickreme, A. N., & Hu, C. Antioxidant properties of a North American ginseng extract. *Molecular and Cellular Biochemistry*, 203(1-2): 1-10, 2000.

[13]. Mbara, K. C., Rambharose, S., Baijnath, H., Nlooto, M., & Owira, P. M. (2022). Antidiabetic effects of Psidium x durbanensis Baijnath & Ramcharun ined.(Myrtaceae) leaf extract on streptozotocin-induced diabetes in rats. *Journal of Ethnopharmacology, 297*, 115542.

[14]. Nadeem, S., Dhore, P., Quazi, M., Pawar, S., & Raj, N. *Lagenaria siceraria* fruit extract ameliorate fat amassment and serum TNF–in high–fat diet–induced obese rats. *Asian Pacific Journal of Tropical Medicine*, 5(9): 698-702, 2012.

[15]. Peng, J., Tang, J., Luan, D., Liu, F., Tang, Z., Li, F., & Zhang, W. Microwave pasteurization of pre-packaged carrots. *Journal of Food Engineering*, 202: 56-64, 2017.

[16]. Pérez-Grijalva, B., Herrera-Sotero, M., Mora-Escobedo, R., Zebadúa-García, J. C., Silva-Hernández, E., Oliart-Ros, R., Pérez-Cruz, C., & Guzmán-Gerónimo, R. Effect of microwaves and ultrasound on bioactive compounds and microbiological quality of blackberry juice. *LWT-Food Science and Technology*, 87: 47-53, 2018.

[17]. Rahman, A. H. Bottle gourd (*Lagenaria siceraria*) a vegetable for good health, 2003.

[18]. Ranganna, S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products: Tata McGraw-Hill Education, 1986.

[19]. Saeeduddin, M., Abid, M., Jabbar, S., Wu, T., Yuan, Q., Riaz, A., Hu, B., Zhou, L., & Zeng, X. Nutritional, microbial and physicochemical changes in pear juice under ultrasound and commercial pasteurization during storage. *Journal of Food Processing and Preservation*, 41(6): e13237, 2017.

[20]. Saikia, S., Mahnot, N. K., & Mahanta, C. L. Optimisation of phenolic extraction from *Averrhoa carambola* pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chemistry*, 171: 144-152, 2015.

[21]. Singleton, V. L., & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3):1965.

[22]. Sun, D.-W. Thermal Food Processing: New Technologies and Quality Issues: CRC Press, 2012.

[23]. Tarantola, E., Bertone, V., Milanesi, G., Capelli, E., Ferrigno, A., Neri, D.,& Freitas, I. Dipeptidylpeptidase-IV, a key enzyme for the degradation of incretins and neuropeptides: activity and expression in the liver of lean and obese rats. *European Journal of Histochemistry*: EJH, 56(4): 2012.

[24]. Yue, J., Xu, J., Cao, J., Zhang, X., & Zhao, Y. Cucurbitane triterpenoids from *Momordica charantia* L. and their inhibitory activity against α-glucosidase, α-amylase and protein tyrosine phosphatase 1B (PTP1B). *Journal of Functional Foods*, 37: 624-631, 2017.

[25]. Zou, C., Wang, Y., & Shen, Z. 2-NBDG as a fluorescent indicator for direct glucose uptake measurement. *Journal of Biochemical and Biophysical Methods*, 64(3): 207-215, 2005.