‘Development of Functional Probiotic Bottle Guard Juice Preparation"

Dissertation II

Submitted by

Maryam Sheikhdon Salad

Programme-M.Sc. (Nutrition and Dietetics)
Section: H1731
School of Agriculture

Lovely Professional University, Phagwara

Under the Guidance of
Er. Poorva

Assistance Professor of Department

Department of Food Technology and Nutrition
School of Agriculture

Lovely Professional University, Phagwara

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CERTIFICATE

This is to certify that Maryam Shikhdon Salad **(Registration NO. 11716545)** has personally completed M.Sc. dissertation II entitled **'Development of Functional Probiotic Bottle Gourd Juice Preparation"**, under my guidance and supervision. To the best of my knowledge, the present work is the result of hid original investigation and study. No part of dissertation-1 has ever been submitted for any other purpose at the university. The project report is appropriate for the submission and the partial fulfillment of the conditions for evaluation leading to the award of Master of Nutrition and Dietetics

Date: 12 May, 2018

Signature of Supervisor

Er. Poorva

Assistant Professor

School of Agriculture

Lovely Professional University

Phagwara, Punjab, India

DECLARATION

I hereby declare that the work presented in the dissertation entitled **'Development of Functional Probiotic Bottle Guard Juice Preparation''** is my own and original. The work has been carried out by me at school of Agriculture, Lovely Professional University, Phagwara, Punjab, India; under the guidance of **Er.Poorva**, Assistant Professor (Food Technology) at school of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Nutrition and Dietetics.

Date: May, 2018 Maryam Sheikhdon Salad (11716545)

I certified that the above statement made by the student is correct to the best of my knowledge and belief.

Date: May, 2018

Er.Poorva

Assistant Professor

School of Agriculture

Lovely Professional University

Phagwara, Punjab, India,

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INTRODUCTION

Bottle gourd belongs to the Cucurbit family (cucumber, squash, etc.) with the scientific name Lagenaria siceraria. Cucurbitaceae family is commonly known as the gourd, melon or pumpkin family among which Lagenaria species is the most famous. The bottle gourd belongs to the genus Lagenaria that is derived from the word galena, meaning the bottle. Bottle gourd is also known as Calabash, Doodah, and Lauki in different parts of India. It is yellowish green, having the shape of a bottle with whiter pulp. India, Sri Lanka, South Africa, Indonesia and Malaysia are the major bottle gourd producing countries in the world. The gourd vegetables are grown on 4.05 lakh hectares in world. The total area under bottle gourd cultivation in India was about 1.17 lakh hectares and total production was 1.43 lakh tonnes . Major bottle gourd growing states of India are U.P., Punjab, Gujarat, Assam, Meghalaya and Rajasthan. Bottle gourd fruits are available in varying shapesand sizes. In recent years, bottle gourd has been used to treat diabetes . Bottle gourd is one of the excellent fruits gifted by the nature to human beings having composition of all the essential constituents that are required for normal and good human health Bottle gourd has long been an important component of indigenous herbal medicines, particularly in Asia. Its leaves, seeds and flowers also have medicinal applications . Its consumption is advocated by traditional healers for controlling diabetes mellitus, hypertension, liver diseases, weight loss and other associated benefits. It is well known that bottle gourd is helpful in constipation, premature graying of hair, urinary disorders and insomnia which reflect significant health-promoting properties . Genus Lagenaria to which bottle gourd belongs is characterized by following key features: The background color is either light green or dark green , regular or irregular stripes. The portion of fruit containing seed can be flat to round, cylindrical, club-shaped or long and narrow. These fruits may be long, oblong or round in shape depending upon the variety.

REVIEW OF LITERATURE

BOTTLE GOURD

Lageneria siceraria popular known as Bottle gourd. It is climbing plant which is bear shaped hard shelled and bottle-shaped grounds as a fruit. It cultivates in India, Japan, Srilanka, china, Thailand and Africa. Bottle gourd is makes excellent diet rich vitamins in and minerals. Bottle gourd has the highest content of choline which serves as procures of neurotransmitter Acetyl choline which in turns crucial of retaining and enhance memory among all the vegetables known to man till date. This vegetable is used for management of many disease like: cardiovascular disorder hepatic disease and ulcer. Bottle gourd juice helps to control blood pressure and hypertensive patient because it has high potassium content. And also helps to lose weight quickly because high dietary fiber, and cholesterol control because low fat content.

Classification of bottle gourd:

Kingdom	Plantae
Division	Magnoliophyta
Classes	Magnoliopsida
Order	Cucurbit ales
Family	Cucurbitaceous
Genes	Lageneria
Species	L.siceraria
Botanical name	Lagenaria siceraria

Parle Milind et al., 2011

Most common region India in bottle gourd

In India bottle gourd found in various regions like : Punjab, Haryana, Delhi, Kerala, Assam, Bengal, Andhra Pradesh, Maharashtra, Karnatka, and Talminadu.

Region	Names
PunjabHaryanaDelhi	Lueki,dhudhi;orghiya
Kerala	Churakka
Assam	Jatilao
Bengal	Lau
AndhraPradesh	Sorakaaya
Maharashtra	Dhudhi-Bhopala
Karnataka	Sorekayi
Talminadu	Suraikkaai

Parle Milind et al 2011

Chemical composition of bottle gourd:

Dietary content of bottle gourd:

Sr.No.	Constituent	With peel(g/100g of dry ghiya)	Without peel(g/100g of dry ghiya)
1	Total sugar	5.870	8.290
2	Reducing sugar	5.220	7,920
3	Non reducing sugar	0.650	0.290
4	Starch	1.310	1.570
5	Curd fiber	4.450	3.400
6	Natural detergent fiber	22.710	21.160
7	Acid detergent fiber	16.260	15.670

8	Hemocellouse	6.450	5.580
9	Cellulose	16.070	16.400
10	Legenin	0.193	0.167

Parle Milind et 2011

Mineral table content of bottle gourd:

The bottle has chemical composition especially mineral include with peal and without peal like iron , zinc, copper etc**.**

Sr.No.	Mineral	With peal(mg 100 of lauki)	Without peal(mg 100 of lauki)
1	Iron	11.87	2.33
2	Phosphorous	240.33	187.33
3	Potassium	3320.00	3356.67
4	Zinc	3.77	3.47
5	Magnesium	162.33	146.33
6	Copper	0.19	0.24
7	Sodium	27.88	36.68
8	Manganese	0.26	0.31

Parle Milind et al 2011

Amino acid present in bottle gourd:

Sr.No.	Amino acid	Fruit(g100/g ghiya)	Seeds(g100/g ghiya)
1	Trytophan	0.003	0.431
2	Threonin	0.018	0.903
3	Isoleucine	0.033	1.246
4	Luecine	0.036	2.079
5	Methionine	0.004	2.079
6	Cystine	---------	0.551
7	Phinaylaline	0.015	1.222
8	Valine	0.027	1.972
9	Argenine	0.14	4.033
10	Histidine	0.004	o.681

Parle Milind et al 2011

Vitamin content of bottle gourd:

Sr.No.	Vitamins	Fruit(g100/g ghiya)	Seeds(g100/g ghiya)
1	Vitamin c	10.100	1.900
2	Thiamine	0.029	0.210
3	Riboflavin	0.022	0.320
4	Niacine	0.320	1.745
5	Vitamin B6	0.040	0.224

6	Pantothenic acid	0.152	0.339
7	Vitamin E	16.02/g	1.000

Parle Milind et al 2011

MEDICINAL PROPERTIES:

1:Anti-oxidant activity:

Acetone extract of fruits show maximum antioxidant activity in model, fresh juice fresh fruits increase health, prevent liver damage maintain level of endogenous Antioxidant enzyme**.**

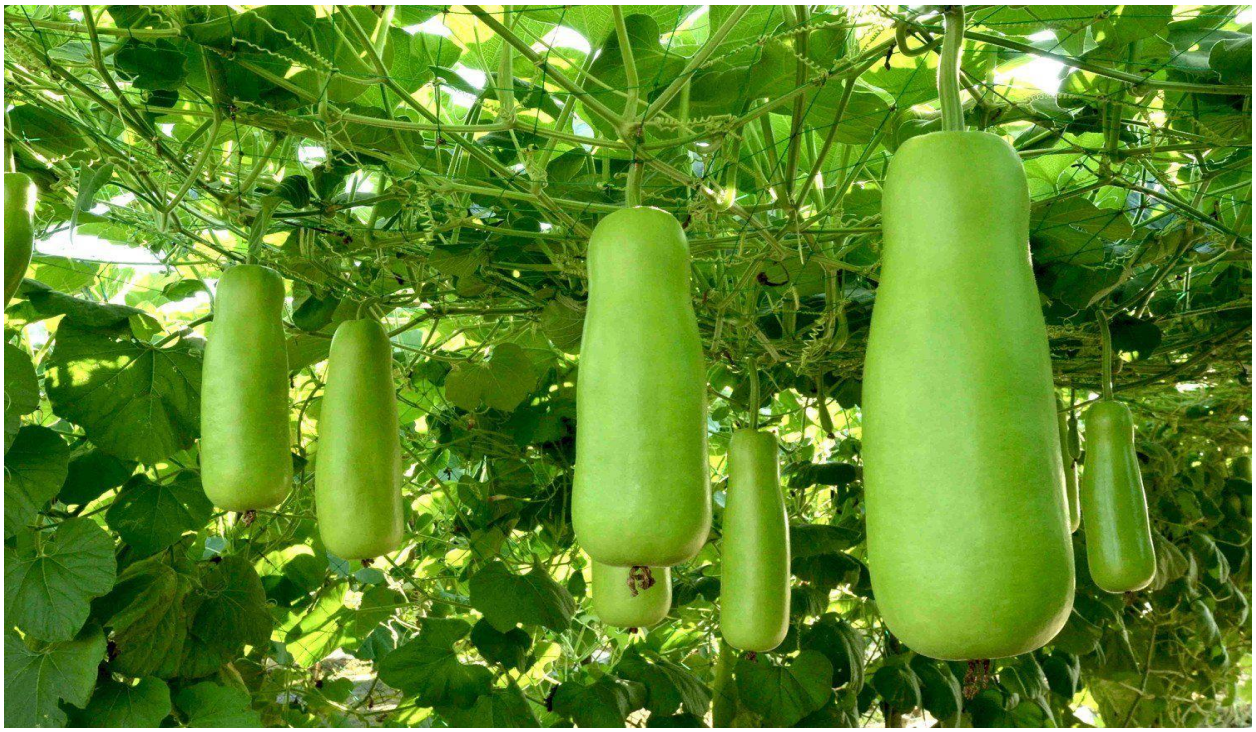
2. Diuretic activity: this fruit we assessed by measuring different diameters like total urine output and volume of concentration urine sodium potassium and chloride.

3. Immunomodulatory activity: prevention and reduction hormonal immune response and also will increase WBC.

Flower

Leaves

Seeds



Fruits

Probiotics

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit on the host (UNFAO/WHO 2001). Probiotics commonly are isolated from human and animal intestinal tracts. Dead bacteria and end product of bacterial growth also may transmit some health benefits but they are not considered as probiotics because they are not alive when administered.

Over the past two decades consumption of probiotic drink is increasing due to the awareness of people about the health benefits of these drinks. (Ostlie et al., 2002). Probiotics can be consumed in any form but mainly they are marketed as capsules, powders, enriched yogurts, yogurt-like products and milks.

2.4.1 Desirable probiotic properties

In order to a potential probiotic strain following properties are expected:

- Acid and bile tolerance.
- Bile salt hydrolase activity.
- Antimicrobial activity against pathogenic microorganisms.
- Adhesion to mucosal and epithelial surfaces (Mercenary et al., 2008).
- Ant mutagenic and Antigarcinogenic properties.
- Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella* sp., *Listeria monocytogenes* and *Clostridium difficile* (Saarela et al., 2000).

2.4.2 Mechanisms of probiotic activity

Exact mechanism of their action is not yet known but they act by the following:

- Production of bacitracin and short chain fatty acid.
- Lowering of gut pH.
- Nutrient competition to stimulation of mucosal barrier function and immunomodulation.
- Induce phagocytosis and IgA secretion.
- Modify T-cell response (Guarner and Malagelada, 2003, McNaught and MacFie, 2001, Isolauri et al., 2001)

2.4.3 Health benefits of probiotic bacteria

The microorganisms present in the colon act as a barrier to pathogenic microorganisms but these microorganisms diminish their integrity due to stress, illness, antibiotic treatment, changes in diet, or physiological alterations in the gut. Consumption of probiotics maintains the balance of microorganisms in the gut and prevents the risk of many diseases such as gastrointestinal disorders including gastrointestinal infections, inflammatory bowel diseases, and even cancer by reinforcing the body's natural defense mechanisms (Macfarlane and cummings, 1999, Saarela et al., 2000). Due to their health benefits probiotic bacteria have been increasingly included in yoghurts and fermented milks during the past two decades.

2.4.3.1 Probiotics as Diarrhea treatment

Rotavirus is the most common cause of severe diarrhea in the infants and young children. *Bifid bacteria* (which constitute the predominant intestinal flora of breastfed infants), as well as other lactic-acid-producing organisms such as *Streptococcus thermophiles* were found to have a protective effect against acute diarrhea disease (Saavedra et al., 1994). *S. thermophiles, L. bulgaricus, L. acidophilus,* and *B. bifidum* were also found to be effective against enter toxigenic *E. coli* a causative agent to travelerdiarrhea (Black et al., 1989). Probiotics microorganisms such as *L. rhamnosus, L. casei*, and the yeast *S. boulardii* was also found to reduce the risk of antibiotic associated diarrhea caused by *Clostridium difficile.*

2.4.3.2 Probiotics for lactose intolerance

Lactose intolerance is the inability to digest and absorb lactose (the sugar in milk) that results in gastrointestinal symptoms such as abdominal discomfort, flatulence and diarrhea when milk or food products containing milk are consumed. It is basically due to the deficiency of beta galactosidase enzyme which is required for the hydrolysis of lactose. Probiotic bacteria such as *S. thermophiles*and *L. delbrueckii*sp. *Bulgaricus have high* beta galactosidase activity and improve the metabolism of lactose in the lactose intolerant people (Kechagia et al., 2012).

2.4.3.3 Probiotics as allergy treatment

Probiotic bacteria also found to be effective against food allergen and atopic dermatitis. *B. lactis*and *L. rhamnosus*GG were found to be effective in decreasing the eczema severity in the infants. *L. rhamnosus*GG has been found successful in preventing the occurrence of atopic eczema in high risk infants, when supplied prenatally to selected mothers who had at least one first degree relative with atopic eczema, allergic rhinitis, or asthma.

2.4.3.4 Probiotic bacteria against cancer

Probiotic bacteria reduce the risk of cancer by decreasing the levels of carcinogenetic enzymes produced by colonic flora through normalization of intestinal permeability and microflora balance as well as production of Ant mutagenic organic acids and enhancement of the host's immune system (Hirayama and Rafter, 1999).

OBJECTIVES

- To check the Growth profiling of probiotic bacteria,
- To formulate of functional Bottle gourd beverage using selective probiotic bacteria,
- To analyse the Nutritional Properties of functional Bottle gourd beverage,
- To analyse Sensory evaluation and the estimation of shelf life of functional Bottle gourd beverage.

PROBLEM BACKGROUND

Probiotic have positive effect on digestive system; immune system like it enhance our specific and non-specific immunity. It helps in the degradation of toxic receptor. It reduces the risk factor of colon cancer etc.and on the other hand bottle gourd is good for brain and cerebral fluid.

MATERIALS AND METHODS

4.1 Chemicals

Chemicals like Hydrochloric Acid, Glucose, Potato Dextrose Agar (PDA), Sodium Chloride, Sodium Hydroxide, Phenolphthalein, Ethanol, Petroleum Ether, Sulphuric Acid, Anhydrous sodium sulphate, Copper, MRS Cysteine Medium (M369) etc. will be used for the preparation of functional bottle gourd beverage

4.2 Plastic Ware and Glasswares

Storage bottles, micro-centrifuge tubes, Petri dishes, Measuring cylinders, beakers, conical flasks, glass bottles, test tubes, Micropipettes, syringes , syringe filters , vials, glass pipettes, aluminium foil etc. will be used during the preparation of functional bottle gourd beverage.

4.3 Growth medium, microorganisms and culture conditions

MRS-Cysteine Medium (M369) will use as growth medium for probiotic strains. Growth medium will be prepared as per manufacturer instructions followed by sterilization by autoclaving at 15 psi for 15 min prior to use. Bottle gourd will be buy from local Market, Jalandhar and used as substrate for fermentation.

4.4 Equipments

The equipments like Bio-safety cabinet, Electronic balance, pH meter, Autoclave , Incubator shaker, Centrifuge, Microwave, Vortex , UV/Visible Spectrophotometer, Digital oven, Magnetic stirrer , Mixer grinder , Water bath and Refrigerator etc. will be used to check the growth profile of selective probiotic strain.

4.5 Preparation of MRS-Cysteine medium

MRS broth will be prepared as per manufacturer's instructions and autoclave at 121°C, 15 psi for 15 min. After autoclaving, medium will cooled down to the room temperature and then 1 % (v/v) of filter sterilized (0.22 µm) Cysteine HCl will aseptically added to the prepared MRS medium and mixed carefully to avoid any gas bubble formation.

4.6 Growth profile of probiotic strains in MRS-Cysteine medium

MRS-Cysteine medium will be used for the revival of lyophilized probiotic cultures. In order to understand the growth pattern of probiotic bacteria in MRS-Cysteine medium, static fermentation will be carried out for 24 h at 37 °C.

Procedure

Bottle gourd Seedculture will prepared by inoculating lyophilized culture (1%) of probiotic in 70 ml MRS-Cysteine broth in 100 ml schott bottle and then incubated statically at 37°C for 24 h.

1. 10% (v/v) of primary seed culture then transferred into 70 ml secondary seed culture (MRS-Cysteine broth) and cultivated for 24 h at 37°C in static condition.
2. Secondary seed culture (10%, v/v) again transferred to 70 ml MRS-Cysteine broth and incubated at 37°C for 24 h and designated as tertiary seed culture. After each 2 h of incubation, whole cell culture fluid (2 ml) willwithdrawn to monitor the growth profile of probiotic bacteria. Optical density will be measured at 600 nm.

4.7 Sensory evaluation of fermented product

The functional pumpkin beverage will be evaluated for their sensory characteristics namely appearance, aroma, flavour, taste, consistency / texture and overall acceptability. The evaluators will ask to record their observation on a sensory data sheet based on 0-3 scale.

0 - Bad

1 - Good

2 - Very Good

3 - Excellent

4.8Physico-chemical analysis functional bottle gourd beverage

Functional bottle gourd beverage will be analyzed for various Physico-chemical parameterslike moisture content, total soluble solids, pH, titrable acidity, ash content, fat, protein, carbohydrates and energy value.

4.8.1 Determination of pH level

The pH of the bottle gourd juice will be measured by withpH meter.

4.8.2 Determination of colour and viscosity

The colour of the bottle gourd juice will be observed by with spectrophotometer and viscosity with rheometer (Jing Zhao et al., 2014).

4.8.3 Moisture content determination

The moisture content of the functional bottle gourd beverage will be determined according to the Association of Official Analytical Chemists method (AOAC, 1995).For this, the sample (250 ml) will placed in an oven at 105 ºC for 3 h. Reading willtaken at a constant weight. The moisture content then expressed as the percentage (%) of the dry weight of the sample (Olugbuyiro and Oseh, 2011).

Moisture content %= W_2-W_1/W×100

Where W_1 and W_2=weights of Petri plates along with the sample before and after drying respectively and W=weight of sample.

4.8.4Titrable acidity

The titrable acidity will determined by measuring the produced lactic acid by titrating 20 ml sample with 0.1 N NaOH to pH 8.2 in the presence of phenolphthalein as anindicator. The used amount of NaOH (milliliter) will multiplied by two, and titratable acidity will thus obtained in Soxhlet-Henkel degrees (ºSH) while the volumetric productivity expressed in g $l^{-1}h^{-1}$, calculated by multiplying the ºSH by 0.225 and dividing by the fermenting time. (Bulatovic et al., 2012, Varga, 2006).

4.8.5 Total soluble solids

Total soluble solids were calculated by using hand Refractrometer.

4.8.6 Fat content

The fat content will be determined by Soxhlet Method.

Reagents

- Petroleum ether

Method

1. Place the bottle and lid in the incubator at 105° C overnight to ensure that weight of bottle is stable.
2. Weigh about 3- 5 g of sample to paper filter and wrap.
3. Take the sample into extraction thimble and transfer into Soxhlet.
4. Fill petroleum ether about 250 ml into the bottle and take it on the heating mantle.
5. Connect the Soxhlet apparatus and turn on the water to cool them and then switch on the heating mantle.
6. Heat the sample about 14 h (heat rate of 150 drop/ min).
7. Evaporate the solvent by using the Vaccum condenser.
8. Incubate the bottle at 80 – 90 ° C until solvent is completely evaporated and bottle is completely dry.
9. After drying, transfer the bottle with partially covered lid to the Desiccator to cool. Reweigh the bottle and its dried content.

4.8.7 Protein content

Protein content was estimated by Kjedahl Method

Reagents

- Kjedahl catalyst: Mix 9 part of potassium sulphate with 1 part of copper sulphate.
- Sulphuric acid
- 40 % NaOH solution
- O.2 N HCL solution

- 4 % Boric Acid
- Indicator solution : Mix 100 ml of 0.1 methyl red (in ethanol) with 200 ml of 0.2 % bromocresol green (in 95 % ethanol)

Method

1. Place sample (0.5 – 1.0) in digestion flask.
2. Add 5g Kjedahl catalyst and 200 ml of conc. Sulphuric Acid.
3. Prepare a tube containing the above chemical except sample as blank. Place flask in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml of distilled water cautiously.
5. Immediately Connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix Content thoroughly, and then heat until all ammonia is distilled.
6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard sodium hydroxide.

4.8.8 Ash Content

Dry Ashing was carried out by incineration of food samples at a very high temperature (525 ^{0}C) in a muffle furnace. Ash is equivalent to the mineral content of the food sample. Accurately weighed samples 3g were taken in a tarred silica dish and ignited over a low flame to char organic matter. After complete charring, the dishes were placed in a muffle furnace and heated at 550 ^{0}C for 3-4 h, till greyish to off white colour ash was obtained. The silica dish containing ash was cooled in Desiccator and weighed. Percentage of total ash calculated as follows:-

$$\textbf{Ash (\%)} = \frac{\textbf{Weight of ash}}{\textbf{Weight of sample}} \times 100$$

4.8.9 Carbohydrate content

Total carbohydrates (%) = 100 - moisture (%) - protein content (%) - crude fat (%) - ash (%) (AOAC method, 2000)

4.8.10 Determination of Carbohydrates

The Carbohydrates like sucrose, glucose and fructose will determine byhigh-performance liquid chromatography (HPLC) system (Jing Zhao et al., 2014).

The energy content was measured in calorific value according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat).

EXPECTED OUTOME

Preparation of bottle gourd using probiotic organism to develop a healthy which would enhance the nutrition value of population.

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