Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 3, Issue, 9, pp.037-041, August, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STUDIES ON THERMAL PROCESSED PROBIOTIC ENRICHED BERMUDA GRASS JUICE

***Sudha, P., Kavitha, K ., Suganya, P., Reni, A. and Hemaprabha, P.**

Department of Food Processing and Preservation Technology, Avinashilingam University for Women, Tamilnadu, India

ARTICLE INFO

Article History:
Received 5th April, 2011
Received in revised form
9th June, 2011
Accepted 28th July, 2011
Published online 23rd August, 2011

Key words:
Probiotics (Lactobacillus Acidophilus),
Bermuda grass (Cynodon dactylon),
Bermuda grass juice,
Thermal processing Quality attributes,
Shelf life.

ABSTRACT

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". The rationale for probiotics is that the body contains miniature ecology of microbes, collectively known as the gut flora. At present it is generally recognized that an optimum 'balance' of microbial population in our digestive tract is associated with good nutrition and health. Bermuda grass is abundantly found in our country. Its botanical name is *Cynodon dactylon.* Bermuda grass juice has wonderful medicinal properties. This herbal juice is rich in vitamin A, fibre, minerals and other nutrients. A study was conducted to incorporate probiotic bacterium into Bermuda grass juice thereby increasing its health benefits and also to increase the shelf life of the juice .The Bermuda grass juice is prepared and additives are added to increase the acceptability and value of the product. The probiotic bacterium *Lactobacillus Acidophilus* is incorporated into the juice. Various quality attributes like TSS, pH, acidity and sensory evaluation are done. The juice with optimized parameters is packed and thermally processed based on the Ball's process time calculation. The storage studies are then conducted on the products at ambient conditions and quality attributes are also verified during the storage.

INTRODUCTION

India is famous for its medicinal plant wealth and the tradition of indigenous system of therapy, specifically the Ayurveda, Jiny Varghese *et al.* [1]. In Ayurveda, Bermuda grass (*Cynodon dactylon)* is well known for its excellent functional properties to resist against various ailments. It belongs to family *Poacea* grass native to North Africa, Asia, Australia and Southern Europe. It has erect stems, can grow 1 to 30 cm tall. The stems are slightly flattened, often tinged purple in colour. The seed heads are produced in a cluster of 3 to 7 spikes together at the top of the stem, each spike 3 to 6 cm long and have a deep root system. The optimum growth condition is between 24 °C to 37°C, Mohammed Shabi *et al* [2]. The leaf juice has also been used in the treatment of hysteria, epilepsy and insanity, Jiny Varghese *et al* [1]. The plant is a folk remedy for headache, haemorrhage, hypertension, measles, snake bite, uro-genital disorders warts and wounds. Auddy [3] has explained that ethanolic extract of *Cynodon dactylon* is found to have most potent antioxidant capacity. Balasubramanian [4] has re-ported the strongest antiviral activity against white spot syndrome virus. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". The first probiotic species introduced into research were *Lactobacillus acidophilus* and *Bifido bacterium bifidum*, Tanboga [5]. At present it is generally recognized that an optimum 'balance' of microbial population in our digestive tract is associated with good nutrition and health, Kailasapathy and Chin [6]. The acid tolerance of the organism varies from 0.3 to 1.9 percent titrable acidity, with an optimum pH at 5.5 to 6.0, Curry and Crow [7]. Juice has been recently suggested as a good medium for functional ingredients such as probiotics, Tuorila and Cardello [8] because it has a wide consumer appeal and is generally recognized as a healthy product. Incorporation of probiotic bacterium into the Bermuda grass extract may add on the value of the juice. Proper preservation technique must be done so as to ensure the extended shelf life and to increase commercial availability of the product. The thermal processing is one of the main food preservation techniques, which intends to guarantee the product's final quality in terms of the consumer's health. The thermal sterilization of canned foods using retort equipment has been one of the most utilized preservation techniques for the last 200 years, Teixeira and Tucker [9]. It is an important method of food preservation in the manufacture of shelf-stable canned foods and has been the cornerstone of the processed food industry for more than a century, Simpson *et al* [10]. The Ball's, Stumbo's, and Pham's methods are also utilized for calculating the process times and lethality, Afaghi *et al* [11].

The slow heating zone (SHZ) refers to a core region in the can that takes the longest time relative to the other regions to reach the final sterilization temperature and hence represents the rate limitation. Pflug [12] defined the SHZ as the region in the food product that receives the least sterilization during the heat transfer process. In thermal processing it is important to provide adequate thermal treatment to the slowest heating zone and estimate its location with time in the food can. The rate of heat penetration is measured by placing a thermocouple

Corresponding author: sudha.raj2000@gmail.com

at the thermal centre of a container to record the temperature of the food during sterilization, assuming that all other points in the container receive more heat and are, therefore, adequately processed. Fellows [13] stated that in cylindrical containers, the thermal centre is at the geometric centre of the cylinder for conduction heated foods and approximately one third up from the base of the container for convection-heated foods. This is in agreement with the finding of Barbosa Canovas *et al* [14], who also stated that the coldest point for a solid product would be at the centre of the can, whereas in liquid-type products it is usually at a lower location. The phrase "minimal thermal process" was introduced by the U.S. Food and Drug Administration in 1977 and it is defined as the application of heat to food, either before or after sealing in a hermetically sealed container, for a period of time and at a temperature scientifically determined to be adequate to ensure destruction of microorganisms of public health concern, Lopez [15].

Thereby to maintain the product stability and considering consumer's demand of nutritious functional foods, the commercial availability of probiotic Bermuda grass juice can be guaranteed using minimal thermal processing method. In India, very little information on Bermuda grass and its products is available in research papers and awareness about its excellent properties is limited. In this endeavour, we would throw some light to popularize a healthy product, probiotic incorporated Bermuda grass juice which would be a remedy various ailments faced in day today life and increasing its commercial value of the product by improving its shelf life using Ball's process time calculation.

MATERIALS AND METHODS

Raw materials

Fresh Bermuda grass (*Cynodon Dactylon)* was collected from the farm as it is abundantly available in nature. Probiotic bacterium (*Lactobacillus acidophilus* -015) used for the study was obtained from NDRI karnal, India. Ginger, Palm Sugar, Honey and citric acid was added to the juice so as to increase the sensory characteristics of the juice. Retortable pouches made up of 12.5 *µ polyester / 12.5 µ aluminium foil / 80 µ polypropylene* layers of 20 × 12 cm size were purchased from M/s Paper Products Limited, Bangalore and used in the present study.

Extraction of Bermuda grass juice

The juice was extracted by taking 100 gm of grass and grinded using the mixer-grinder with 500 ml of water and strained through the previously washed and dried muslin cloth, additives were added in different ratios and sensory evaluation was done by a panel of 10 untrained judges using 9 point hedonic scale so as to optimize the sensory attributes of the product. Thus the ingredient ratio was optimized as 2 % lime, 5% ginger, 10 % honey and 10 % palm sugar in 100 ml of juice (T1) with sensory score of 8.1 and recorded as 'like very much' and pH of optimised product is made to below 4 prior to processing ,Ramasamy [19].

Incorporation of probiotics in Bermuda grass juice

The probiotic culture was grown at 30 ºC for 24 hours used as an inoculum, Yoon *et al* [16]. Probiotic bacterial culture was pipetted out using a micropipette in various proportions of 0.5 % (T2), 1 % (T3), 1.5 % (T4) and 2 % (T5) and distributed into juice and mixed thoroughly. The probiotic Bermuda juice was incubated at 37 ºC, Prajapati *et al* [17] by keeping it in a BOD incubator for 24 hours for encouraging faster multiplication of probiotic bacteria.

Physio-chemical analysis of Bermuda grass juice

Total soluble solids

The total soluble solids present in the juice were determined by using ERMA hand refractometer ranging from 0 to 32 °Brix.

Acidity

Acidity was measured as per the method described by Ranganna [18].

$$\text{Acidity} = \frac{\text{Titre value x 0.1 N (NaOH) x Volume made up x Weight of phenolic acid}}{\text{Volume of the sample x Weight of the sample.}}$$

pH

The pH was determined by using a digital type pH meter. The pH meter was standardized with double distilled water of pH 7.0 and buffers at pH 4.0.

Evaluation of thermal process time for Bermuda grass juice

Thermal processes for high acid and medium acid foods are designed based on the destructions of heat resistant spoilage type vegetative bacteria, yeasts, moulds or the inactivation of enzymes present in the food at temperatures below 100^{o}C. Thus, thermal processes for such foods are normally carried out in boiling water, Ramasamy [19].

Ball's process time calculation

The Euckland thermocouple was fixed at the cold point of the retort pouch and the process time was calculated using Ball's formula method. This method is based on the criterion of achieving a certain minimal centre point temperature at the coldest spot in the product for 85 °C as outlined by Ramasamy [19]. The process time was calculated using the following equation

$$B = f_h \log (j_{ch} I_h / g)$$

Where,

T_{ih} Initial food temperature at start of heating period
T_r Retort temperature
T_c Target temperature
I_h Difference between the retort temperature and food temperature at the start of the heating process (T_r-T_{ih})
g_C The value of g at the end of heating or beginning of cooling period (T_r-T_c)
f_h Heating rate index. It is required for the straight line portion of the heating curve to pass through one complete log cycle. It is also equal to the negative reciprocal slope of the heating rate curve.
l Come up period. In batch processing operations, the retort requires some time to reach the operating

condition. The time from steam on to when the retort reaches, Tr is called the come up period.

T_{pih} Pseudo initial temperature during heating.Temperaure indicated by the intersection of the extension of the cooling curve and the vertical line representing the effective beginning of the process.

j_{ch} Heating rate lag factor. A factor which when multiplied by I_h, locates the intersection of the extension of straight line portion of the semi log heating curve and the vertical line representing the effective beginning of the process = $(T_r-T_{pih}) / (T_r-T_{ih})$.

BPT Thermal process time. This is equal to Ball's process time. Ball's process time (BPT) Corrected for come up time 58 % (steam on to steam off time).

Table . Calculation for Ball's process time calculation

f_h	x minutes
$j_{ch}= \log(T_r-T_{pih}) / \log(T_r-T_{ih})$	j_{ch}
Retort Temperature	T_r ° F
Initial Temperature	T_i °F
I_h	(T_r-T_i) °F
$j_{ch} \times I_h$	Y
Target Temperature	T_c°F
$g_c = T_r-T_c$	g °F
Ball's process time (BPT)	f_h (log ($j_{ch}I_h/ g_c$)) = BPT minutes

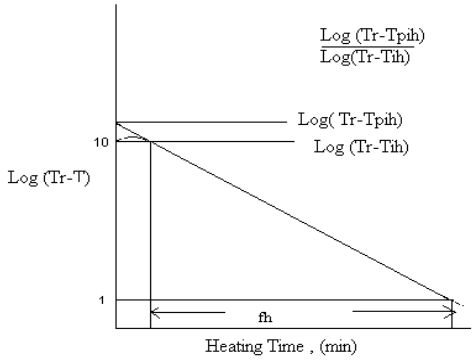

Fig. 1 : Heating curve and heating parameters

Sensory evaluation

Sensory evaluations of Bermuda grass juice were carried out by 10 panellists. They were asked to evaluate colour, odour, taste, mouth feel, and texture for juice. The maximum score for each attribute was 10. While the overall acceptability attributes were 50 scores.

Shelf life studies of the Bermuda grass juice

The Bermuda grass juice samples were packed and stored at 37 □C in retort pouches which is thermally processed based on Ball's process time calculation. Thus the effectiveness of processing and standardized product was optimized.

RESULTS AND DISCUSSIONS

Physical characteristics of the Bermuda grass

The average length of the grass samples was found to be 14.48cm.The moisture content is found to 10 to 12 % (wb). Volume was found by Platform scale method and the average was found to be 0.25 m^3. The bulk and true density was found to 0.0559 kg m^{-3} to 0.1539 kg m^{-3} respectively by tapping method, Ranganna [18].

Chemical characteristics of Bermuda grass Juice

The total solids and total soluble solids contents are important factors in the production of juice. It is well established that the higher the total solids, the better is the quality of juice. The results show that the TSS value of the raw Bermuda grass juice lies in the range of 1.8 to 2.1 ^{0}Brix. After the addition of other ingredients, the optimized sample before the addition of bacterium got increased at the range of 12.5 to 12.7 ^{0}Brix this may be because of the presence of palm sugar and honey. After the incubation of bacterium in various proportions, there is decrease in value from 11 to 9.8 ^{0}Brix due to the fermentation of juice by the probiotic bacteria, fig 2.

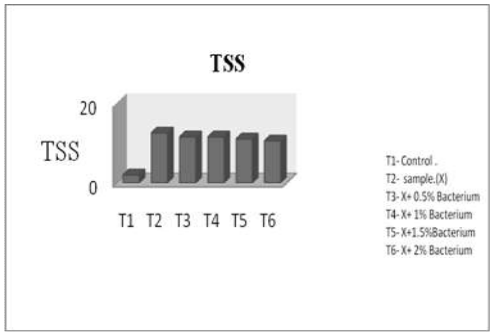

Fig. 2: TSS Characteristics of Bermuda grass juice

Titrable acidity and pH value would be of greater importance because the ratio of total soluble solids to acidity will affect flavor. The acidity and pH of the control was recorded at ranges of 0.137 to 0.138 % and 6.5 to 6.6 respectively. After the addition of all the ingredients there was a gradual increase in acidity due to the presence of citric acid and the value increased as 0.274 to 0.277 % and simultaneously the pH value ranges from 5.3 to 5.5. When the probiotic bacterium was incorporated into the juice, the acidity value ranges for these samples increases from 0.318 to 0.386 % and pH from 4.5 to 3.9. It is mainly due to the gradual growth of micro organisms in the juice, Fig 3 and Fig 4.

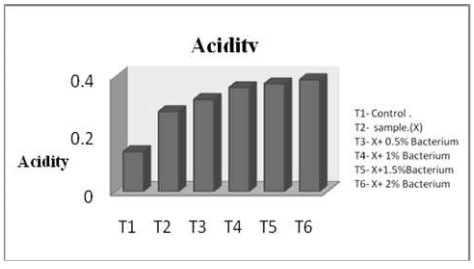

Fig. 3: Acidity Characteristics of Bermuda grass juices

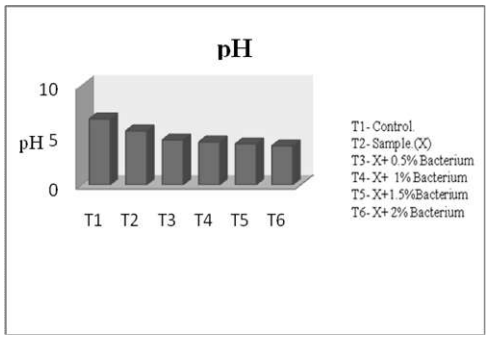

Fig. 4: pH Characteristics of Bermuda grass juices

Evaluation of thermal processing time

The heat penetration data was plotted on a semi log graph sheet with (T_r-T) on the Y axis and time in min on X axis based on the criterion of achieving a certain minimal centre point temperature at the coldest spot in the product at 85 °C as shown in the fig 5.

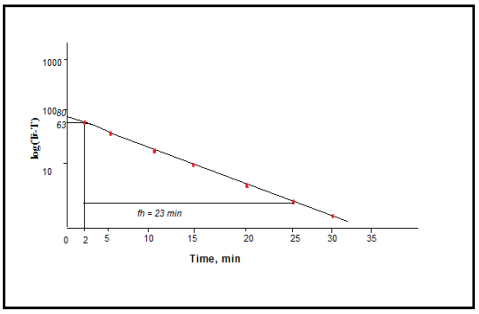

Fig. 5: Heat Penetration Curve for Bermuda grass juice

Thus the process time of Bermuda grass juice was calculated as 9 minutes approximately for 85 °C. The juice samples are packeted in retort pouches and heat treated by keeping it in water bath and the effectiveness of thermal processing on quality attributes was analysed.

Table 2: Ball's Process Time Calculation for Bermuda grass Juice

1.	f_h	23min.
2.	j_{ch}	0.433
3.	Retort temperature (T_r)	93 °C
4.	Initial temperature (T_i)	30 °C
5.	$I_h = T_r - T_i$	63 °C
6.	$J_{ch}\ I_h$	27.279
7.	Log $j_{ch}\ I_h$	1.44
8.	Target Temperature (T_c)	85 °C
9.	$g = T_r - T_c$	11
10.	Log g	1.04
11.	log ($j_{ch}\ I_h$)-log g	0.39
12.	B = f_h (log ($j_{ch}\ I_h$)-log g)	9.07 minutes.

Storage studies based of quality attributes

Shelf life is considered to be the important parameter so as to ensure quality and consumer approval.Studies are undergone by storing the samples under the room temperture and quality attributes are monitored till the day of spoilage. It shows that the fresh unprocessed bermuda grass juice sample have only lasted 5 to 6 hours after the prepartion maintaining all the sensory qualities of the product and it started to be unaccepatable with pH ranging 3 to 3.5 and acidity 0.482 to 0.483 %.In case of thermally processed samples, the acidity and pH variations are very slow because of the retardation for the growth of micro organisms due to heat treatments. When we consider the acidity of control sample, it ranges from 0.138 to 0.483 % within 2 to 3 days thereafter a decrease in sensory quality is observed. In T2 sample, the range was between 0.276 to 0.482 % and chances of spoilage were only 4 days after the storage. In case of bacterium incorporated samples, the slight spoilage was only shown after 7 to 10 days; the acidity range was from 0.318 to 0.414 %, fig 6 and fig 7.

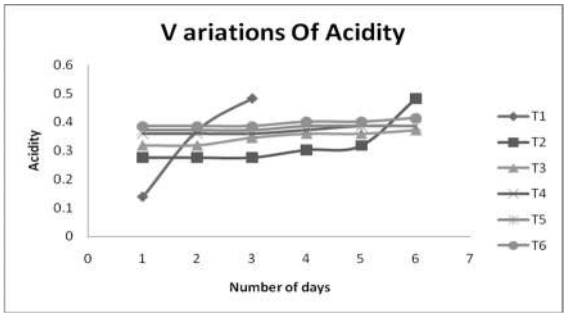

Fig. 6: Variations in acidity during storage

When we consider pH, it also showed very slow reduction due to destruction spoilage micro organisms. In control sample, it ranges from 6.6 to 3.5. In T2, it ranges from 5.4 to 3.4 till the day of spoilage. In bacterium incorporated samples the pH reduction was from 4.5 to 3.7 within 7 to 10 days. The TSS remains almost in constant because of reduced utilization of sugars by the microbes as they are thermally killed. Similar results have been reported by Gardiner *et al* [20].

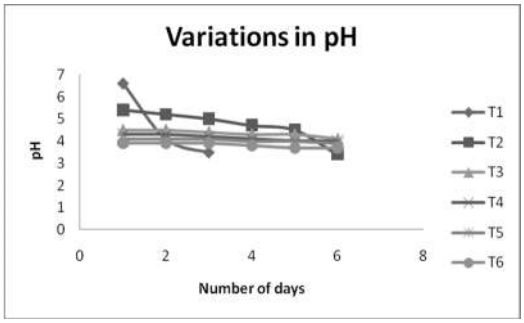

Fig. 7: Variations in pH during storage

Sensory evaluation for optimization of Bermuda grass juice

The thermally processed probiotic juices of various proportions were subjected to sensory evaluation by a panel of 10 untrained judges using 9 point hedonic scale. Probiotic juice sample of 1 % probiotic bacterium showed higher sensory score of above 6 for different sensory attributes. Qualitatively, this value was recorded as 'like slightly'. The

sample with 1.5 % of bacterium added showed lower acceptability of 6.3 compared to other juices.

Conclusions

Determination of optimum thermal process time and thereby extending the storage period of qualitatively enriched juices is very useful and acceptable for the consumers. Thus ,from the above results we have concluded that, the Bermuda grass juice with 1 % probiotic bacterium have extended shelf life more than 7days when it is processed for 9mins for 85 ^{0}C based on Ball's process time calculation.

REFERENCES

[1] Jiny Varghese.K, Anila.J, Nagalekshmi.R, Resiya.S and Sonu.J., Dasapushpam: The traditional uses and the therapeutic potential of ten sacred plants of Kerala state in India. *International journal of Pharmaceutical Sciences and Research,* 1(10), pp. 50-59, 2010.

[2] Mohamed Shabi.M, Sasikala.S, Gayathri.K, Sreelakshmi B. S., Cynodon *dactylon:* A Review. *International journal biomedical research and analysis*, 1(2) , pp .65-67 ,2010

[3] Auddy.B, Ferreira.M, Blasina.F, Lafon.L, Arredondo.F, Dajas.F, Tripathi.C, Seal .T, Mukherjee .B., Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *Journal of ethnopharmacol*, 84(2-3), pp.131-8, 2003.

[4] Balasubramanian .G, Sarathi.M, Venkatesan.C, John Thomas and Sahul Hameed.A.S., Oral administration of antiviral plant extract of *Cynodon dactylon* on a large scale production against White spot syndrome virus (WSSV) in *Penaeus monodon* , *Aquaculture* , 279(1-4), pp. 2-5,2008.

[5] Tanboga.I, Caglar.E and Kargul.B., Campaign of probiotic food consumption in Turkish children, oral perspectives 'Probiotics for your child'. *International Journal of Paediatric Dentistry,* 13(1), pp. 59, 2003.

[6] Kailasapathy.K and Chin.J., Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium spp*, *Immunology and Cell Biology*. 78, pp. 80-88, 2000.

[7] Curry.B and Crow.V., *Lactobacillus spp*.: General characteristics. *Encyclopedia of Dairy Sciences*, 3(1), pp. 1479-1484.

[8] Tuorila.H and Cardello.A.V., Consumer responses to an off flavor in juice in the presence of specific health claims. *Food Quality and Preference*, 13, pp.561-569, 2002.

[9] Teixeira A.A, and Tucker G.S., On-line retort control in thermal sterilization of canned foods. *Food Control*, (8), pp.13-20, 1997.

[10] Simpson.R, Figueroa.I, and Teixeira.A., Simple practical, and efficient on-line correction of process deviations in batch retort through simulation. *Food Control*, 18, pp.458-465, 2007.

[11] Afaghi.M, Ramaswamy.H.S. and Prasher.S.O., Thermal process calculations using artificial neural network models. Food Research International, 34(1), pp. 55-65, 2001.

[12] Pflug.I.J., A textbook for introductory course in microbiology and engineering of sterilization,Minneapolis, Minnesota: Environmental Sterilization Laboratory, 1987.

[13] Fellows, P.J., *Food processing technology (principles and practise)*: Woodhead Publishing Series in Food Science and Technology, UK, 1996.

[14] Barbosa Canovas G.V, Ma.L and Barletta B., Technomic calculations using artificial neural network models. *Food Research International*, 34, pp.55-65, 1997.

[15] Lopez. A., *A complete course in canning and related processes*, The canning trade: Baltimare, MD,1987.

[16] Yoon K.Y, Woodams E.E and Hang Y.D., Fermentation of beet juice by beneficial lactic acid bacteria. *Lebensmittel-Wissenschaft und-Technologie*, 38, pp. 73-75, 2005.

[17] Prajapati.J.B, Shah R.K and Dave J.M., Survival of Lactobacillus acidophilus in blended-spray dried acidophilus preparations. *Australian Journal of Dairy Technology,* 42, pp. 17-21, 1987.

[18] Ranganna.S., *Manual of Analysis of fruit and vegetable products*, Tata Mc Graw Hill Publishing Company Limited: New Delhi, 1979.

[19] Ramaswamy.H.S., Thermal Processing of Fruits: Principles and Process Calculations, *Phalasamskrana,* pp.107-117, 1995.

[20] Gardiner. G, Sullivan. E, Kelly .J, Auty, M.A.E, Fitzgerald. G.F, Collins. J.K, Ross. R.P and C. Stanton., Comparative survival of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying, *Applied and Environmental Microbiology*, 66(6),pp. 2605-2612.

****