INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume 5, Issue 3)
Available online at: www.ijariit.com

Optimization and standardization of lemon grass incorporated into pseudostem and mint extracts based isotonic drink

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ABSTRACT

The Lemongrass based Isotonic Drink was developed with the incorporation of the banana pseudostem and mint extracts using palm sugar syrup as a sweetener. The quality of the product was computed by a series of microbiological tests, proximate analysis, and organoleptic evaluation. The microbial load was evaluated and is found to be <10 which meant that the product has increased shelf life with better taste and color even in the absence of preservatives. Therefore, the aroma of lemongrass, nutrients of pseudostem, freshness, and flavor of mint along with palm sugar elevates its economic importance, consumer awareness and attracts them towards the advent of blended functional drinks. The Response Surface Methodology aims at optimizing the ingredients and designing experiments from which the final product is formulated. This software helps in showing the interaction of the independent variables at each response with the use of 3D graphical representation.

Keywords— *Lemongrass, Isotonic beverage, Retort processing, Box-Behnken design, Shelf-life studies*

1. INTRODUCTION

The word 'herb' is derived from the Latin word 'herba' meaning grass or green crop. India is the largest producer of medicinal herbs and is called as botanical garden of the world. Lemongrass is a perennial grass plant which is commonly available in tropical and sub-tropical countries like South East Asia and Africa but now it is commercially cultivated in different parts of India. *Cymbopogon citranus* has been observed with the properties of being an anti-amoebic, anti-bacterial, anti-diarrheal, anti-filarial and anti- fungal. According to Nambiar and Matela (2012), the leaves of lemongrass contain phenol compounds which are rich on antioxidants. Reinforcing the extract with further advancements in science, it can be used as an anti-inflammatory drug. Thus the potential properties of lemongrass make it noticeable not only as a flavourant but also as a medicinal herb.

Banana (Pseudostem), being a plantation crop belongs to the family Musaceae accounts for about 23% of the world's total fruit population. (Debabandya Mohaptra., 2012). Banana fruit has a protective outer layer and an inner fleshy portion which is edible. It constitutes about 75% of water and 25% dry matter. Banana pseudostem has a curative effect on diabetes and is also a diuretic as it prevents and cures kidney stones. Similar to the fruit, pseudostem rich in potassium and vitamin B6 which helps in the production of haemoglobin and insulin, they tend to maintain fluid and electrolyte balance in the body.

The palm sugar is considered to be a natural sweetener obtained from the sap of nipa palm trees (*Nypa fructicans*) which are indigenous to the coastlines and tropical regions of the Indian and Pacific oceans. The palm sugar is generally made from its sugar rich sap. Food products with a low level of the glycemic index(GI) (55 or less) are recommended for controlling diabetes and hypertension, used in weight reduction and also to lessen the risks associated with the heart. As far as palm sugar is concerned, its GI is 35. Therefore, it qualifies itself as a natural sweetener without any constraint in consumption.

Mint is a perennial herb which acts as a breath freshener and a stomach-soother. Peppermint *(Mentha piperita)* and Spearmint *(Mentha spicata)* are well-known and widely used. The characteristic smell and aroma of mint are due to the presence of a naturally available compound called cyclic terpene alcohol named as menthol. The menthol helps the enzymes necessary for digestion and is also recommended for a gastrointestinal

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disorder, common cold and muscle spasms. (Kodandaramreddy and Kavita. 2013).

An isotonic drink is a beverage which is designed to help athletes rehydrate, as well as replenish electrolytes, sugars and other nutrients that are lost or depleted after rigorous exercise. As the requirement of the fluid is high, the scope of such beverages is highly increasing and demanding because of its shelf-life stability offered during retort processing for perishable food products like banana pseudostem. Therefore, it meets the requirements of a healthy lifestyle in the form of low-cost nutritious food by reducing the underutilization of pseudostem which in turn clears the ignorance of the people.

The Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the effects of several factors and obtaining the optimum condition of factors for desirable responses (F.Sanchez Rojas, 2010). Box Behnken Design is one of the common method employed for optimization. Experiments are often run at different factor values, called levels. The advantage of using BBD over other methods is that it is a special 3-level design that requires only three levels to run an experiment. The factor is placed at 1 to 3 equally spaced coded values. that is, -1, 0, +1. Thus the preparation of our product involves the optimization of ingredients used in it by means of BBD in Response Surface Methodology.

2. MATERIALS AND METHODS

2.1 Raw materials

The *Cymbopogon citratus* were collected from the local market in the Coimbatore. The selection of banana variety for extraction of juice was based on local availability. Among the banana, Musa sepitium are available abundantly in local areas. So the variety was selected for extraction of pseudostem juice and for all the experiments.

2.2 Preparation and extraction of juice

Extraction of banana pseudostem juice was done by simple extraction procedure. Pseudostem of banana (*Musa sepitium*) variety was chopped into small pieces and directly placed into hand driven mechanical juicer. The pseudostem pieces were subjected to high pressure in the juicer and the juice was, coming out of the pseudostem through an outlet located at the bottom of the juicer. The collected juice was then filtered using a muslin cloth to obtain a clean juice. The method of mixing water with the pseudostem pieces and then extraction of juice with the help of a mixer grinder lowered the concentration of the constituents of the juice; thereby reduce the effectiveness of the juice. The present method adopted gave pseudostem juice of uniform quality.

Fresh, green leaves of lemongrass were collected, washed and soaked in water for 3 hours. The leaves were then subjected to drying at ambient temperature for 24 hours. The washed lemongrass was chopped into pieces, the pseudostem extract was added with the chopped lemongrass in a hand driven mechanical mixer to give good blend.

2.3 Preparation of flavouring extracts

Twenty grams of fresh mint leaves were washed, chopped into small pieces and blend with water in hand-driven mechanical mixer. The obtained extract was filtered through a muslin cloth.

2.4 Addition of Sweeteners

Palm sugar crystals were bought from local super markets in packets. It was powdered and added to boiling water to make it as a syrup. The mixture was constantly stirred to get the final thread structure. The desired consistency was obtained at a temperature between 106°C. The syrup was let to cool down in ambient temperature.

3. FINAL PRODUCT PROCESSING

The final composition of the isotonic drink was optimized using RSM. Based on the sensory evaluation of all the 17 trails, the definite proportions of lemongrass and mint extracts along with the palm sugar were obtained to be 120ml, 40ml, 16ml and 70ml respectively. The final product is packed in 250ml retort pouches and stored for further studies.

The processing steps for the formulation and processing of isotonic drink are discussed here. The process flow chart is provided in figure 1.

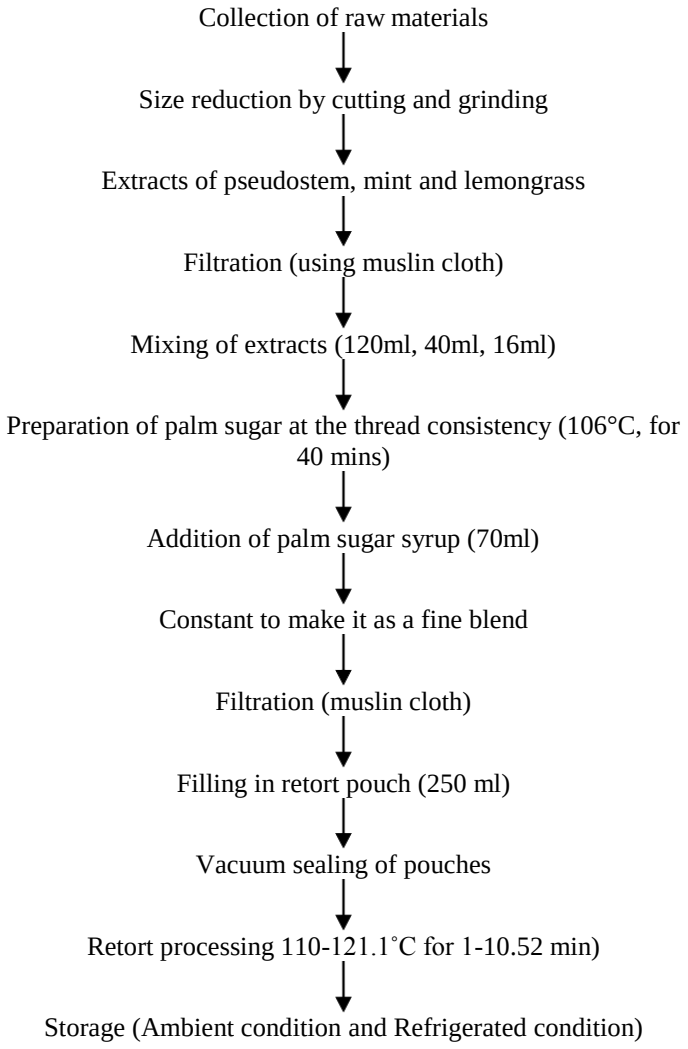

Fig. 1: Flow diagram for isotonic drink preparation and processing

4. EXPERIMENTAL DESIGN

Response Surface Methodology (RSM) was adopted in experimental design. Box-Behnken Design (BBD) is preferred over Central Composite Design (CCD) because of its maximum efficiency for an RSM problem involving three factors and three levels. The number of runs for BBD is less than Central Composite Design (CCD). After determining three preliminary range of various ingredients for flavored Isotonic drink, a Box Behnken experimental design, three variables, was used to study response pattern and to determine the optimum combination of variables

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5. RETORT PROCESSING

The thermal processing of isotonic drink was done in a forced steam/ air type of retort. It is one of the types of "overpressure" retort of 50 pouch capacity producing a pressure of 2kg/cm^2. The temperature can be varied and fixed based on the readings required for research. Sterilization in this type of retort is accomplished by using a steam and air moisture which is circulated within the retort by means of a turbine type fan.

6. QUALITY ANALYSIS

The product was tested for engineering properties such as moisture content, Ash content, Acidity, Bx acid, Viscosity, Turbidity. It also tested for biochemical and microbial properties such as pH, TSS, Total sugar, reducing sugar, None reducing sugar, fat content, potassium content, protein content, Iron, calorific value, fiber, carbohydrates, antioxidant, phenolic content, colour analysis and total plate count.

7. SENSORY ANALYSIS

The prepared sample of the isotonic drink was evaluated for sensory qualities on the basis of colour, taste, flavor, texture, and overall acceptability on a 9-point hedonic scale. A panel of 45 members was used for sensory evaluation throughout the storage.

8. RESULTS AND DISCUSSION

The results of the experiment obtained during the course of investigation have been described in this chapter under appropriate headings. Laboratory experiments were conducted for the preparation of the isotonic drink at different composition and process conditions which were optimized using BBD of RSM. The biochemical analysis, proximate analysis and the storage studies of the isotonic drink were emanated in this chapter. The observations recorded during the study are summarized in the form of tables and illustrated through figures**.**

8.1 Experimental Design for composition optimization of the isotonic drink

The optimization of the isotonic drink depends on the concentration of various ingredients and processing conditions. The results of optimization in terms of its composition and process conditions are discussed in this section. The average response obtained for each experimental combination was fitted in the general form of a quadratic polynomial. Seventeen runs were carried out to select the best composition of the product were given in table 4.1 The values given in the runs 17, 11, 14, and 13 are the repeats of 6. The response fit analysis, regression coefficient estimations, and model significance was conducted. The adequacy of the models was tested using the F-ratio and coefficient of determination (R2). The final equations for composition in terms of coded factors are given in the following equations:

$$a = +8.40 - 0.037 * A - 0.037 * B + 0.075 * C - 0.23 * A2 - 0.18 * B2 - 0.100 * C2 + 0.050A * B - 0.075 * A * C + 0.13 * B * C$$

where a = pH, A= pseudostem extract, B = lemongrass extract, C = mint extract

$$b = +6.594E - 003 + 4.987E - 003 * A + 1.738E - 003 * B + 5.000E - 004 * C$$

where b = Titrable acidity

$$c = +8.98 + 0.53 * A - 0.037 * B - 0.062 * C$$

where c = total soluble solids

$$d = +8.36 + 0.50 * A + 0.18 * B + 0.050 * C$$

where d= sensory evaluation

8.2 Composition Optimization

The composition varies for each run of the experimental design with the ingredients A, B and A Based on these independent variables the response changes that is, the results of sensory evaluation, pH, titrable acidity and total soluble solids. The word lack of fit refers to the fact that the simple linear regression model may not adequately fit the data. While the goodness of fit for the quadratic model implies that the lack of fit of the model is not significant. Fisher's value and p-value indicates that the model system was statistically significant. These values are tabulated in the following tables.

Table 1: Goodness of fit test for composition optimization for pH

Source	Sum of square	DF	Mean square	F-value	prob > F
Linear	8.176E-003	9	9.085E-004	0.45	0.8503
2FI	8.176E-003	6	1.363E-003	0.68	0.6793
Quadratic	5.000E-003	3	1.667E-003	0.83	0.5413
Cubic	0.000	0			

Table 2: Goodness of fit test for composition optimization for titrable Acidity

Source	Sum of squares	DF	Mean square	F value	P-Value	R^2
Probe > F						
Linear	8.569E-007	9	9.521E-008	0.48	0.8368	0.9927
2FI	8.544E-007	6	1.424E-007	0.71	0.6624	0.9927
Quadratic	5.525E-007	3	1.842E-007	0.92	0.5073	0.9940
Cubic	0.000	0				

Table 3: Goodness of fit test for composition optimization for total soluble solids

Source	Sum of squares	DF	Mean square	F value	P-Value	R^2
Probe>F						
Linear	8.324E-003	9	9.248E-004	0.46	0.8453	0.9933
2FI	5.824E-003	6	9.706E-004	0.49	0.7954	0.9943
Quadratic	2.500E-003	3	8.333E-004	0.42	0.7510	0.9957
Cubic	0.000	0				0.9967

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Table 4: Goodness of fit test for composition optimization for sensory evaluation

Source	Sum of squares	DF	Mean square	F value	P-Value Probe>F	R^2
Linear	8.176E-003	9	9.085E-004	0.45	0.8503	0.9929
2FI	8.176E-003	6	1.363E-003	0.68	0.6793	0.9929
Quadratic	5.000E-003	3	1.667E-003	0.83	0.5413	0.9943
Cubic	0.000	0				0.9965

Table 5: Optimized values for composition and process conditions of isotonic drink

Variable name	Value of parameter	Unit
Pseudostem concentration	29.98	ml
Lemongrass concentration	10	ml
Mint concentration	4	ml

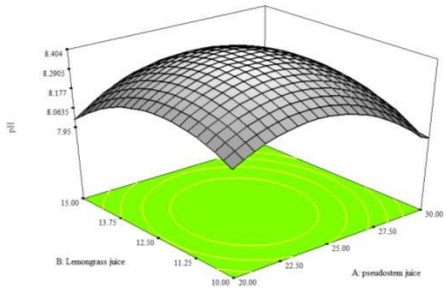

Fig. 2: Response Surface plot for composition optimization as a function of concentration of various ingredients and pH as response

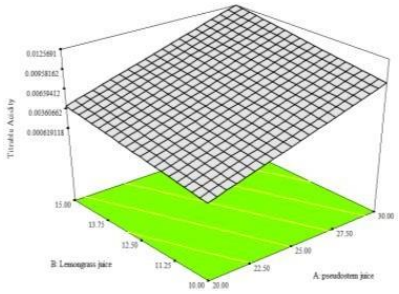

Fig. 3: Response Surface plot for composition optimization as a function of concentration of various ingredients and Titrable acidity as response

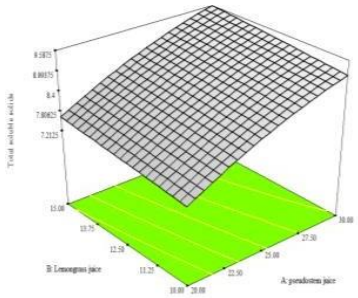

Fig. 4: Response Surface plot for composition optimization as a function of the concentration of various ingredients and Total soluble solids as a response

8.3 Optimization

The optimization part in Design Expert software version 6.0.8 searches for a combination of factor levels that simultaneously satisfy the requirements placed (that is optimization criteria) on each one of the responses and process factors (that is multiple response criteria). Graphical optimization methods were used in this research by selecting desired goals for each factor and response. In a graphical optimization with multiple responses, the software defines the region where requirements simultaneously meet the proposed criteria. The shaded area on the overlay plot figure 4 and 5 is the region that meets the proposed criteria. The optimum value obtained from the overlay plot is reported in Table 5.

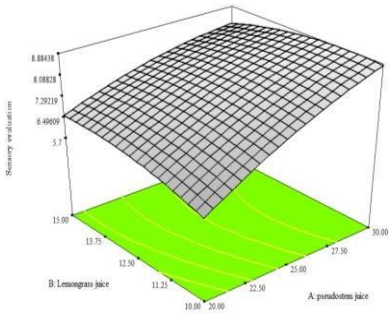

Fig. 5: Response Surface plot for composition Optimization as a function of concentration of various ingredients and sensory evaluation as a response

8.4 Biochemical Analysis

Moisture and ash content were determined by AOAC method, protein by Djeldahl method, total sugars and carbohydrates by biochemical methods. The estimated proximate analysis and their corresponding results are found from Table 6 below.

Table 6: Biochemical Analysis of the Lemongrass based Isotonic Drink

Proximate Analysis	Results
Carbohydrate (%)	11.70
Total Fats (g/100ml)	0.3
Fibre (g/100ml)	0.02
Total Soluble Solids (°Brix)	9.20
Protein (%)	0.06
Total sugars (%)	11.59
Reducing Sugars (%)	0.11
Non- Reducing Sugars (%)	11.48
Calorific Value (kcal/100ml)	47.07
Phenols (mg/100ml)	11.39
Antioxidant activity (mg/100ml)	127.01
Calcium (mg/100ml)	39.08
Total plate count	<10

9. CONCLUSION

The Indian isotonic drinks market is expected to record a CAGR of 11% during the forecast period. In India, the isotonic drinks market occupies a very small portion of 0.03% of the total non-traditional drinks market. The predicted coefficient of determination (R2) was 0.6903 and the adjusted R2 value was 0.9933. This indicates the model data gives the optimized values. The product shows good carbohydrate content of 11.70% per 500ml of the sample. The protein content of 0.06% in the drink, is found to be least of all the nutrients. The

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pseudostem is an excellent source of calcium which is dominating as 39.08mg/100g of the product. The total plate count was found for the 10th and 15th day. It was found to be <10 which showed that the product would still remain fresh for 30-45 days when refrigerated and in ambient room temperature it should be consumed best before 2 weeks without the use of preservatives.

10. ACKNOWLEDGEMENT

The cooperation extended by the staff of the Food Processing and Preservation Technology Department of Avinashilingam Institute for Home Science and Higher Education for women is greatly acknowledged.

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