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PHYSICO-CHEMICAL AND NUTRITIONAL ANALYSIS OF HERBAL BEVERAGE FORMULATED USING HOLY BASIL, MINT AND WHEATGRASS

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ABSTRACT

A functional beverage is one that offers the consumer additional perceived benefits besides its primary function which is hydration. Plants are potent biochemists and have been components of phyto-medicine. Plant based natural components can be derived from any part of the plant. Herbs are present ubiquitously but they are not yet used commercially in combination for formulation of an herbal beverage, but as people are getting more health conscious there is a need of formulating a natural beverage. The medicinal effects of herbstypically result from the secondary metabolites present in the plant. The main focus was Holy basil, mint and wheatgrass so as to formulate a healthy option to all the artificial beverages. The main objective was to analyse the nutritional benefits of the herbal extracts of basil leaves, mint leaves and wheatgrass that could therapeutically help in improving the health of the consumer. In 100 ml sample, it was found that carbohydrate content was 2.66 grams, Energy value was 20.6 KCal, Proteins were 2.37 gram, Sugars were 1.9 gram, Fats were 0.052 gram, Titrable acidity ranged from 0.03-0.12 % while pH was found to be alkaline (6.8-7.5) whereas total soluble solids was found to be 3^0Brix.

Keywords: Holy basil, Mint, Wheatgrass, Stevioside, Nutraceuticals, Functional beverage.

INTRODUCTION

A functional beverage can be defined as one that offers additional perceived benefits besides its primary function, which is hydration (Whitehead, J. 2006). Herbal extracts can be difficult to formulate, especially in liquid oral dosage forms, which are prone to be contaminated with microorganisms that survive in the final formulation. Thus, microbial bio burden is always a risk (Kamil and Lupuliasa, 2011). Preservatives are often connected to the leading causes of adverse reactions. For example, parabens interact with mitochondrial cells and induce male infertility; sodium benzoate causes hyperactivity in children; potassium sorbate cause mutagenic effects in lymphocytes (Mamur *et al*., 2010; McCann *et al*., 2007; Tavares *et al*., 2009). Hence a new product was formulated without any use of preservative which could be consumed in a short span. Focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems (Sirkar, N. 1989).Herbal medicines are widely used for treatment of many diseases and a lot of medicinal plants with anti-tumor activities are available (Kamatenesi *et al*., 2011; Afolayan *et al.*, 2010; Gathirwa *et al*., 2011). The protective effect of herbs rich diets against various cancers has been proposed by several epidemiological studies (Kaefer and Milner, 2008; Mehta *et al*., 2010). Cytotoxic effects of numerous herbal extracts against cancerous cells have been reported (Bisi-Johnson *et al*., 2011). In the Indian system of medicine, plants occupy a predominant place in the therapeutic field. So in this present investigation, three herbs that is Basil, Mint and wheatgrass were used.Basilextracts are used in Ayurveda remedies for heart disease,colds, headaches, stomach disorders, and inflammatory and allergic disorders (Kalabharti *et.al*. 2011). Several medicinal properties have been attributed to this plant in ancient Indian and other systems of medicine like Ayurveda, Siddha, Greek, Roman and Unani. The leaves have been used as expectorant, diaphoretic, antiemetic, anti-rheumatic, anticancer, anthelmintic, antiseptic, and antipyretic and in relieving various gastric disorders. Hence holy basil was selected as main base for the formulation.

Mentha spp. is herb belonging to the *Mentha* genus in the Labiatae family (Choudhury *et.al*., 2006). The beneficial effects of this plant in treatment of many gastro-intestinal disorders have been well documented (Rokaya *et al*., 2010). Also, the anti-microbial, anti- inflammatory and anti-tumoral properties of *M. spp.* have been studied (Pearson *et al*., 2010; Zu *et al*., 2010; Hussain *et al*., 2010). The cytotoxic effects of essential oils from *Mentha spp.* leaves on some cancer cell lines have been revealed in vitro (Zu *et.al*., 2010; Hussain *et.al*., 2010). In addition the antigenotoxic effects of aqueous fraction of M. spp. have been attributed to its modulatory actions on lipid peroxidation (LPO) and antioxidant enzymes (Arumugam and Ramesh, 2009). Moreover the cytotoxicity of essential oil of another Mentha specious (Menthapulegium) on some cancer cell lines has been shown (Shirazi *et.al*., 2004).

There are many reports on biological activities of some compounds derived from (Bisi-Johnson *et.al*., 2011; Ma *et al*., 2011). Accordingly, many biological activities including antioxidant (Agata *et.al*., 2010), anti-tumor (Furtado *et.al*., 2008), immunomodulatory (Yun *et.al*., 2003) and anti-inflammatory (Swarup *et.al*., 2007) effects of Rosmarinic acid (a polyphelonic carboxylic acid) is found in *Mentha spp*. So the second element used was the mint.

Wheatgrass is of particular interest because it is already known to have beneficial health properties. Wheatgrass contains vitamin C, E, Beta-carotene, ferulic acid, vanilic acid, and chlorophyll, some of which have been shown to inhibit the metabolic activation of carcinogens. Wheatgrass extracts have also been shown to contain phenolic compounds, which are responsible for antioxidant activity (Aydos, O. *et al*, 2011) so wheatgrass was also used in formulation.

Consumption of sugar-sweetened beverages may be one of the dietary causes of metabolic disorders, such as obesity. Therefore, substituting sugar with low calorie sweeteners may be an efficacious weight management strategy. So steviol glycoside was used to reduce the calorific intake. Stevia, the common name for the extract stevioside from the leaves of *Stevia rebaudiana Bertoni*, is a natural, sweet-tasting calorie free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners. Stevia has been found to increase insulin sensitivity in rodent models (Chang, Wu, Liu, & Cheng, 2005) and to have beneficial effects on blood glucose and insulin levels in human studies (Curi 1986; Gregersen, Jeppesen, Holst, & Hermansen, 2004), which suggests it may have a role in food intake regulation. In safety studies, no negative side effects were reported (Barriocanal, 2008). Stevia was recently approved for use as a sweetener by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (Joint Food and Agriculture Organization/ World Health Expert Committee on Food Additives, 2005), and has also recently received GRAS approval from the Food and Drug Administration. Stevia is inexpensive and available to most consumers; thus, it has the potential to be widely used and may assist individuals in regulating their weight if it has a positive effect on caloric substitution. So it was selected for formulating the beverage. Tenth Meeting of Food Authority held on 20th September, 2012 at 11:00 hours at FDA Bhavan, New Delhi approved the use of steviol glycoside as an artificial sweetener in various foods. Currently, The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a thorough scientific review of all the available scientific data and concluded Stevia sweeteners are safe for use in foods and beverages, an acceptable daily intake of steviol glycoside of up to 4 mg/kg of body weight was recommended (Gupta *et al*. 2013). So due to all the above health benefits, these four components were selected to be formulated as a single herbal beverage and its nutritional value was estimated by analysing its physical, chemical and nutritional value.

MATERIALS AND METHODS

Present study was conducted in 2015 at K.K. Wagh College of Food Technology, Nasik. Fresh herbs were procured from local market while Steviol glycoside was purchased from Janus Life sciences, West Bengal, India. Other raw material like sucrose was also procured from the local market and the fresh beverage was used for all the estimation.

TITRABLE ACIDITY

The beverage used for estimation was shaken thoroughly and filtered through previously washed and dried muslin cloth. 10 ml of sample was diluted with 40 ml distilled water to make up the volume upto 50 ml and 10 ml was taken for estimation. This diluted aliquot was then titrated against 0.1 N Sodium hydroxide using 1% phenolphthalein solution as indicator and the results were calculated as percent anhydrous citric acid (Ranganna, S. 2012).

pH

pH is defined as the logarithm of the reciprocal of hydrogen ion concentration in grams per litre of the sample and it was calculated using pH meter. It is of importance as the measure of active acidity which influences the flavour or palatability of a product and affects the processing requirements.

REFRACTIVE INDEX

The TSS of sample was measured using hand refractometer (0-32^{0}brix range)(Erma inc. Japan). The refractive index or brix reading was measured by placing a drop of the sample on the refractometer prism.

CARBOHYDRATE ESTIMATION

Total soluble carbohydrates were estimated quantitatively by using Anthrone's method. Total soluble carbohydrate was calculated with the help of a reference curve using D-glucose as standard. In this method, Carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose gets dehydrated to hydroxyl methyl furfural. This compound forms a green coloured product with anthrone and absorption was recorded at 630 nm. In this 100 ml of sample was taken for estimation, in which 5 ml of 0.25N hydrochloric acid was added and was boiled on hot water bath to carry out hydrolysis for 3 hours. The solution was then neutralized using solid sodium carbonate until the effervescence was ceased. The volume was made upto 100 ml and the sample was centrifuged and the supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis. Standard dilutions were carried out using 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard where 0 served as blank and all others were made up the volume to 1 ml in all the tubes including the sample tubes by addingdistilled water. Then 4 ml of anthrone reagent was added and it was heated for ten minutes in a boiling water bath. The sample was cooled rapidly and the absorption was read at 630 nm (USFDA Title 21, Food and Drugs).

TOTAL ASH CONTENT

Two silica plates were weighed and 20 ml sample was introduced to each. The content was evaporated on boiling water bath at 110^0C for 1 hour and then the content was ignited within muffle furnace at 525^0C for 6 hours. The dishes were cooled and weighed again. The difference in weights gave the total ash content and was expressed as percentage (Ranganna, S. 1986).

PROTEIN ESTIMATION

Protein was estimated using macro-kjeldahl method which is based on the determination of the amount of reduced nitrogen present in the sample. (IS 7219:1973, Reaffirmed 2010)

FATS ESTIMATION

Fat was estimated using gas chromatography. (AOAC, 19th Edition. 2012, method no. 922.06, Ch-32, Page no.5)

Energy value

Energy value was calculated using method prescribed by Codex Alimentarius, 3.3.1

RESULTS AND DISCUSSION

TITRABLE ACIDITY

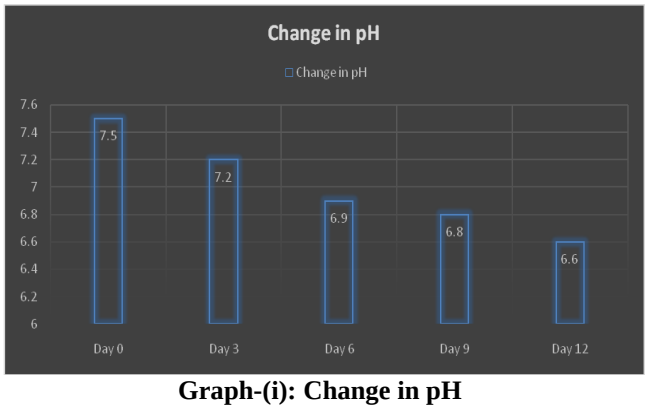
Titrable acidity was found by using the following formulae:

$$\% \text{ Total acid} = \frac{\text{Titre volume} \times \text{Normality of alkali} \times \text{Volume madeup} \times \text{Equivalent wt.of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{Weight of sample} \times 1000}$$

Triplicate reading were taken and an average was taken for calculation accordingly. % acidity was found to be ranging from 0.03 to 0.12 % within a span of 10 days. 0.03 % was the initial reading of day 1 of the freshly prepared sample and there was an initial increase in acidity due to absence of preservative but still it was acceptable for consumption up to 7 days.

pH

pH was measured using pH meter and the readings were ranging from 6.8 to 7.5 and was went from alkaline to acidicover a period of 12 days and result has been shown in bar graph-I.



Graph-(i): Change in pH

TOTAL SOLUBLE SOLIDS

TSS was found to be 3^0Brix using a handheld refractometer.

CARBOHYDRATE CONTENT

To prepare standard curve, 0.5,1 and 1.5 ml of the dextrose standard solution was taken in 100 ml volumetric flask and made up to the volume with water. Then 5 ml of each solution was used and it was plotted as concentrationagainst absorbance to get the standard curve absorbance and the concentration was calculated using following formulae:

$$\text{Concentration of unknown sample} = \frac{\text{Absorbance of unknown sample} \times \text{Concentration of standard sample}}{\text{Absorbance of standard}}$$

Carbohydrates concentration was found to be 2.66 gram /100 gram of the sample.This shows basically shows that the beverage had a low amount of carbohydrate value.

PROTEIN CONTENT

Protein content was measured using macro-kjeldahl process

$$\% \text{ Nitrogen} = \frac{(\text{Sample titre} - \text{blank titre}) \times \text{Normality of HCl} \times 14 \times \text{Volume madeup of the digest} \times 100}{\text{Aliquot of digest taken} \times \text{Wt. of the sample taken} \times 1000}$$

% Protein = % Nitrogen x 6.25
So Nitrogen was found to be 0.3792% and protein was estimated to be 2.37%.

FAT ESTIMATION

% Fat was calculated using gas chromatography and it was estimated to be 0.052 %

ENERGY VALUE

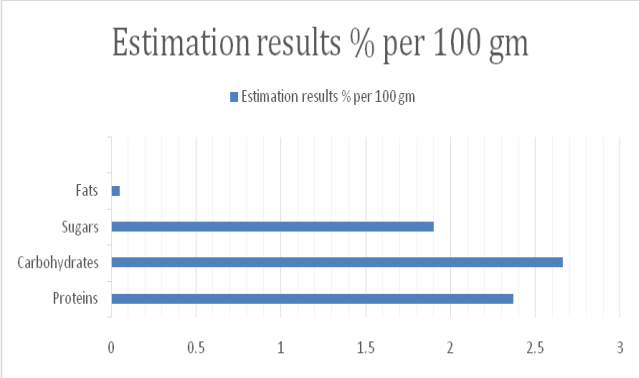
Energy value was found using bomb calorimeter and the energy value was found to be 20.6 Kcal/ 100gm

TOTAL ASH CONTENT

Total ash content was found to be 0.066 %

Table-I: Results of estimation

Sr. No.	Parameter	Result
1	Energy value	20.6 KCal/ 100 gm
2	Proteins	2.37 gm/100 gm
3	Carbohydrates	2.66 gm/ 100 gm
4	Fats	0.052 gm/ 100 gm
5	Titrable acidity	0.03 %
6	pH	Alkaline (6.8-7.5)
7	Total soluble solids	3^0Brix
8	Ash content	0.066 %



Graph-II: Comparison of estimation

CONCLUSION

All the results obtained have been illustrated in table –I and its estimation results can be compared in graph-II. This study clearly shows that the formulated beverage not only was lower in its calorific value but also was low in fats and did not provide any unwanted sugars. The formulated beverage was palatable for a longer period of seven days without any added preservatives in it. So it can be concluded that the beverage was acting as a functional beverage and help in maintaining health and could be used as a substitute to other artificial beverages.

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