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Formulation and quality evaluation of *spirulina* incorporated ready to serve (RTS) functional beverage

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ABSTRACT

Functional beverages have been gaining importance in recent times. It helps in prevention of diseases and promotion of health. It is the best opportune for delivering the essential vitamins, minerals, phytochemicals and antioxidants as it is convenient and most preferred by consumers. Blending of fruit juices helps enhance the potential of the beverage. This study was carried out to develop and investigate the properties of the spirulina incorporated blends of kokum and sweet lime Ready to serve (RTS) functional beverage. Five ratios of RTS functional beverages were prepared from kokum, sweet lime juice, brown sugar, chia seeds, spirulina and citric acid. The beverages hence prepared along with the control sample were subjected to sensory evaluation by panel members using nine-point hedonic scale. The two best variations chosen by the panelists were further selected for quality evaluation. Nutritional composition studies revealed that the beverage was a good source of vitamin C and protein. The products were considered safe for consumption until five days from the date of preparation. Hence, the functional beverage prepared can be considered an ideal replacement for the commercially available carbonated beverages.

KEY WORDS: RTS FUNCTIONAL BEVERAGE, SPIRULINA, NEW PRODUCT DEVELOPMENT, SENSORY EVALUATION

INTRODUCTION

Humans need enough food to live and the right assortment of foods for optimal health (Mahan *et al.*, 2017). To maintain proper health, the diet should contain required amounts of nutrients to meet the daily requirements (Srilakshmi 2018). Non-communicable diseases (NCD's), also known as chronic diseases, are caused due to unhealthy diet, lack of physical activity, use of tobacco and alcohol, if left unattended it would eventually lead to obesity. Consuming fruits on a regular basis could be one of the most cost-effective methods for preventing chronic

diseases. As fruits provide life enhancing functional properties, they play a crucial role in human diet, providing most of the vital nutrients (Sindumathi *et al.,* 2015).

Functional foods are those which supply health benefits beyond basic nutrition to the population (Swami *et al.*, 2014). Thus, there is a great demand for such foods worldwide, owing to its various health benefits (Motohashi *et al.,* 2017). Intake of fruits in the form of beverages is well enjoyed by all age groups of the society due to their pleasant flavor, taste and nutritional characteristics (Kausar *et al.*, 2019 and Thirukkumar *et al.*, 2015). Fruit beverages are highly beneficial as they are easily digestible and nutritionally far superior compared to commercially marketed beverages. (Divyasree *et al.*,2018).

Blending of fruit juices, help in improving the existing therapeutic properties of a fruit and to overcome the high cost of some exotic fruit juices, scarcity or seasonal availability (Sangma *et al.*, 2016 and Sasikumar *et al.*, 2015). It is also effective in places where there is inadequate nutrient intake, thereby helping overcome various deficiency diseases(Gaikwad *et al.*, 2013). Hence, to encourage robust living, efforts were made to formulate a *Spirulina* incorporated RTS functional beverage.

The ingredients used such as *spirulina*, kokum and chia seeds are known to possess functional properties. *Spirulina*,(*Arthrospira*) is a low-fat, low-calorie, cholesterol-free, source of easily digestible vegetable protein, which contain protein, essential fatty acid (gamma-linolenic acid) , iron, B-complex vitamins, vitamin E, and chlorophyll (Vijayarani *et al.*, 2012 and Kulshreshtha *et al.*, 2008 and Padma *et al.*, 2016). Ayurvedic texts reveal that kokum has been used as traditional medicine owing to its therapeutic effects (Jagtap *et al.*, 2015). It is packed with B- complex vitamins, minerals like potassium, manganese and magnesium, which help control blood pressure (Milind *et al.*, 2013).

On comparison with the commercially available beverages, the newly formulated beverage does not contain any artificial coloring agent(s). The RTS functional beverage, prepared using indigenous ingredients makes it appealing and refreshing for the consumers. The beverage formulated will also be a convenient, healthier alternative for people to carry.

METHODOLOGY

The study protocol was reviewed and approved by the Independent Institutional Ethics Committee of SDNB Vaishnav College for Women, Chennai (Ethical clearance No. SDNBVC/HSC/IHEC/2019/10)

A. SELECTION AND PROCUREMENT

Good quality products and fresh fruits were selected for the preparation of *Spirulina* incorporated RTS beverage. *Spirulina* powder and kokum rind were procured online, fresh sweet lime from local market and brown sugar, chia seeds, citric acid from super market in Chennai.

B. PRE- PREPARATION OF RAW MATERIALS

1. SOAKING

Kokum rinds were soaked in hot water for thirty minutes and chia seeds were soaked in warm water for ten minutes before the preparation of the functional beverage.

2. EXTRACTION OF KOKUM JUICE

Once kokum were soaked well, it was transferred to a blender and blended well into a puree. The blended, kokum puree along with soaked water was transferred to a clean pan and cooked for fifteen minutes in low flame. After fifteen minutes the stove was switched-off and the puree was allowed to cool in room temperature. Later on, it was filtered using a muslin cloth. The procedure was adopted from the work by Chauhan *et al.*, 2018.

3. EXTRACTION OF SWEET LIME JUICE

The fruit was washed thoroughly in running tap water and cut into half. The seeds were removed from the fruit manually. With the help of squeezer the fruits were squeezed to extract the juice. The extracted fruit juice was filtered using steel filter to remove the pulp. The procedure was adopted from the work by Chauhan *et al.*, 2018.

TABLE: 1 DIFFERENT TYPES OF SAMPLE WITH MEASURED INGREDIENTS

SAMPLES	KOKUM JUICE EXTRACT (ml/100ml)	SWEET LIME EXTRACT (ml/100ml)	BROWN SUGAR (g/100ml)	*SPIRULINA* (g/100ml)	CHIA SEED (g/100ml)
Sample A	70	30	10	0.5	3
Sample B	80	20	10	0.5	3
Sample C	60	40	10	0.5	3
Sample D	50	50	10	0.5	3
Sample E	40	60	10	0.5	3

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Control	50	50	10	-	3

Extracted kokum juice and sweet lime juice were added in five varying ratios in separate containers for the preparation of RTS beverage and a fixed amount of brown sugar, *spirulina*, chia seeds and citric acids were added to the extracted juices and stirred well.The glass bottles were allowed to boil in water for 15 to 20 minutes at 85^0 C as suggested by Saranya *et al.*, 2016. Further, the formulated RTS beverage was filled into glass bottles and kept at refrigerated temperature (4^0 C), the procedure was adopted from the work by Chauhan, *et.al*, 2018. The control RTS beverage was prepared without adding *spirulina* in the ratio 50:50 (kokum: sweet lime)

C. NUTRITIONAL COMPOSITION

The formulated RTS functional beverages were analyzed for energy, carbohydrate, protein, fat and vitamin C by standard AOAC methods, 2012.

D. MICROBIAL ANALYSIS

The growth of microbes, storage stability and shelf life were analyzed during 0^{th} day, 3^{nd} day and 5^{th} day of the beverage, using standard total plate count method.

E. SENSORY EVALUATION

The RTS beverage was assessed for acceptability based on appearance, taste, flavor, texture and overall acceptability using the nine-point hedonic scale with 20 semi-trained panel members. The panel members use their sense of sight, taste, smell, feel and hearing to assess the quality of the food**.**

RESULT AND DISCUSSION

Spirulina incorporated RTS functional beverages were formulated in five varying ratios as given in Table 1. The formulated RTS beverages were then evaluated in triplicates to understand its nutrient, microbial and organoleptic properties. The beverages that were given most average scores, sample E and sample B were selected for further test through sensory evaluation.

TABLE: 2 NUTRIENT ANALYSES FOR FORMULATED BEVERAGE

S.No.	ANALYSIS	CONTROL	SAMPLE E	SAMPLE B	P VALUE	LEVEL OF SIGNIFICANCE
1	Energy (kcals)	166.7±2.02	153.7±1.23	143.5±1.44	0.006	S*
2	Carbohydrate	11.13±0.015	10.75±0.053	7.76±0.07	0	S*

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	(g)					
3	Protein (g)	0.95±0.06	1.36±0.2	1.04±0.08	0.0011	S*
4	Fat (g)	NIL	NIL	NIL	NIL	NIL
5	Vitamin C (mg)	134.6±5.13	123±4.58	112±2.64	0.0019	S*

Values are represented as triplicate of mean and standard deviation. Significant difference ($p \leq 0.05$) was shown by Duncan Multiple Range Test (DMRT).

NS- non significant, S^{*}- significant at 5% level, S^{**}- significant at 1% level.

From table 2, we will be able to infer that, among the samples analyzed; the control sample obtained highest energy and carbohydrate values of 166.7±2.02 kcals and 11.13±0.015 g respectively, followed by sample E 153.7±1.23 kcals and 10.75±0.053 g of energy and carbohydrate value. The Protein content of the beverages ranged from 0.95±0.06 to 1.36±0.2 g. Sample E recorded a value of 1.36±0.2 g whereas control received lowest value of 0.95±0.06 for protein. As there was no *spirulina* added to the control sample, its protein content was low. Fat content of *spirulina* incorporated RTS beverages were found to be nil as all the ingredients were poor sources of fat. Upon analyzing the vitamin profile of the beverage, the results revealed that the control received the highest value of vitamin C (134.6±5.13mg), followed by sample E providing 123±4.58 mg of vitamin C. The high values of vitamin C can be attributed to the addition of kokum and sweet lime in the ratio 50:50 as both the ingredients are good sources of vitamin C.

TABLE: 3 MICROBIAL ANALYSES FOR FORMULATED BEVERAGE

S.No.	SAMPLE	0^{th}DAY (cfu/ml)	3^{nd} DAY (cfu/ml)	5^{th}DAY (cfu/ml)
1	Control	1.20×10^{1}	1.25×10^{2}	1.32×10^{3}
2	Sample E	0.65×10^{1}	0.85×10^{2}	0.96×10^{3}
3	Sample B	0.45×10^{1}	0.95×10^{2}	1.13×10^{3}

The Table 3, reveals the results of the microbial analysis of the stored beverage. The *spirulina* incorporated RTS beverage was within the acceptable limit until the 5^{th} day from the date of preparation. It also showed that the Total Plate Count (TPC) increased slightly with the storage period. The result indicates that increase in storage time, increases the microbial count of the beverage. The maximum

bacterial load permitted for fruit beverages are 1.0×10^4 as suggested by Rashed *et al.*, 2013. Hence, it can be concluded that the growth of bacterial count was within limit and safe for human consumption.

TABLE: 4 SENSORY EVALUATION OF FORMULATED FUNCTIONAL BEVERAGE

SAMPLE	APPEARANCE	COLOR	TASTE	TEXTURE	FLAVOR	OVERALL ACCEPTABILITY
Sample A	8.45±0.75	8.3±0.86	7.35±1.08	8.15±0.93	7.4±1.18	7.93±0.85
Sample B	8.45±0.68	8.45±0.68	7.85±0.74	8.1±0.96	7.75±0.96	8.12±0.63
Sample C	8.35±0.67	8.55±0.51	7.85±0.87	8.25±0.78	8±0.97	8.2±0.52
Sample D	8.35±0.74	8.45±0.75	7.45±1.05	8.05±1.05	7.45±1.14	7.95±0.76
Sample E	8.6±0.59	8.7±0.57	8.1±1.11	8.25±1.06	8.1±1.16	8.35±0.77
Control	8.35±0.48	8.05±0.82	8±0.85	7.95±0.68	8.35±0.58	8.14±0.29

Values are represented as triplicate of mean and Standard Deviation

The results as shown in table 4, had recorded the highest score for appearance in sample A (8.45±0.75) and B (8.45±0.68). Sample C scored highest range of 8.55±0.51 for color. The attribute of taste had received the highest score of 8.1±1.11 and 8±085 for sample E and control respectively. Samples C and E recorded highest score of 8.25±0.78 and 8.25±1.06 in similar range for texture. Control received highest score of 8.35±0.58 followed by sample E and C in the similar range of 8.1±1.16 and 8±0.97 for flavor. Overall acceptability indicates the overall preference of the formulated beverage in varying ratios. Sample E received first rating (8.35±0.77) in terms of overall acceptability due to lesser addition of kokum (40%) and higher addition of sweet lime(60%). Sample B received second rating (8.12±0.63), followed by control (8.14±0.29).

CONCLUSION

The synergistic effects of the ingredients incorporated into the RTS functional beverage had contributed to its unique nutrient and flavor profiles. Among the varied ratios of beverages formulated and evaluated, the ratio that had 0.5 g of *spirulina* was found to be most preferred by the panelists. Upon using ingredients like *spirulina* powder and kokum, we will be able to ensure the availability of bioactive compounds to its consumers. It thereby helps in improving the nutritional status of the general population and to overcome the nutritional deficiencies. Blending of different juices from various sources not only enriches quality and nutrition of basic raw material, but also offers to develop a better food product with enhanced nutritive value.

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