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Standardization and Proximate Composition of Fortified Milk Beverage

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ABSTRACT: Micronutrient deficiency is a persistent problem in India mainly due to low dietary diversity and poor nutrient density of staple foods. The trendiest processing technique to overcome nutritional deficiencies was food fortification which considered to be more cost effective and enables improvement of health. "Fortification" means deliberately increasing the content of essential micronutrients in a food so as to improve the nutritional quality of food and to provide public health benefit with minimal risk to health. As India is the largest producer of milk, which is a staple food it can be fortify with essential nutrients such as vitamins, minerals, using fruit and vegetable resources. The main challenges of this research were to standardize the fortified milk beverage using natural resources rich in micronutrients, fatty acids and to study the physico-chemical parameters of the developed beverage. The ingredient levels was selected by performing conjoint analysis and in-bottle sterilization as heat treatment for extending shelf-life of developed fortified milk beverage. The ingredients combination was found that, carrot juice (15 per cent), moringa leaves juice (5 per cent), irradiated mushroom juice (6 per cent), dates juice (2 per cent), encapsulated DHA algal oil (1 per cent), sugar (10 per cent), natural identical colourant (0.06 per cent) and flavourant (0.15 per cent) respectively. These combinations of fortificants were added with skim milk and processed using in-bottle sterilization techniques 120˚C/15mins. The proximate composition of developed beverage contains total calories of 57.80 kcal, moisture 84.64%, total solids 15.36%, total ash 0.89% respectively and observed that highly significant (P<0.01) with control (skim milk).

Keywords: Nutritional Deficiencies, Fortified milk beverage, Conjoint analysis, In-bottle sterilization.

INTRODUCTION

Micronutrients are referred to as vitamins and minerals, they needed only in very small amount for growth and health development, disease prevention and wellbeing, also essential for physiological function of the body. The outcomes of micronutrient deficiencies cause early death, impoverished health, vision effects, stunted growth and learning impairment. The micronutrient deficiencies are tackled by various measures like food fortification, dietary diversification, nutritional education and micronutrient supplementation.

The WHO and UNICEF computed a worldwide assessment nearly 190 million children of pre-school age are deficient in vitamin A with more than 2 million people are deficient of other key micronutrients like iron, iodine and zinc (Venkatesh *et al*., 2021). Food fortification of essential micronutrients like iron, folic acid, vitamin B12, iodine, vitamin A and vitamin D is being done in India. Fortified milk beverage is a drink that designed to help pre-school children health to contribute their growth performance, brain development and to prevent from malnutrition.

For complete well-being of body require vitamins and minerals act as a nurturing agent to curb the micronutrient deficiencies such as iron (anaemia), iodine (goiter and hypothyroidism) and vitamin A (blindness) are considered major public health problems. Micronutrient deficiencies (such as iron, calcium, vitamin A and D) not only affect the health but also projected to cost around 0.8-2.5 per cent of the gross domestic product (Stein and Qaim 2007).

Skim milk is a nutritious by-product from cream separation that containing valuable nutrients like lactose, proteins, minerals and vitamins etc., which have indispensable value as human food. In India, the major source of skim milk is from the production of cream industry, it can be used as supporting material for many food productions because of low fat content. Carrots are good source of β-carotene, a precursor of

vitamin A which is regarded as important for combating vitamin A deficiency (VAD) was reported by Fratianni *et al*. (2010). Mushrooms exposed to sunlight or UV radiation was a good source of vitamin D2 fortification with foods has been shown to be a safe and effective way to increase 25(OH) D levels in children and adults was studied by Keegan *et al*. (2013).

Gopalakrishnan *et al*. (2016) studied nutritive importance and medicinal application of *Moringa oleifera* commonly known as 'miracle tree'. The leaves were rich in minerals, vitamins and other essential phytochemicals also used as potential anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic and anti-microbial agent. The major component of dates was carbohydrates about 70%, also a good source of fiber, vitamins and minerals, calcium, iron, fluorine and selenium. 100 g dates consumption provides over 15% of RDA (Manickavasagan *et al*., 2012). Omega-3 DHA fatty helps in mental health illnesses, bipolar depression, major depressive disorder, etc. Microalgae as the best natural source of preformed omega-3 DHA over fish oil and other essential nutrients such as iron, zinc, magnesium, vitamin B3, B6, C and E was explained by Charles *et al*. (2019).

Conjoint analysis is a multivariate technique used specifically to understand how consumers develop preferences for products or services and to formulate predictions about ingredients levels towards product concepts and it is also called as trade-off analysis. Mostly conjoint analysis used for selecting and characterizing quality attributes of ingredients levels in food product which is based on the multi-attribute product concepts was suggested by Navarasam *et al*. (2021). When a large number of combinations (3 X 3 X 3 X 3 X 3 = 243) given to sensory panel, they may be show off non-response rate (due to fatigue, boredom) becomes very high. So, such condition can reduce by generating number of cards created by an orthogonal array method using SPSS statistical software.

Food fortification as the major approach to the treatment of malnutrition was considered to be more effective method to develop food with nutritional benefits, achievable over a relatively shorter time than other forms of food aid (Singh, 2004).

Hence the development of fortified milk beverage was not only to treat or prevent the specific nutritional deficiencies but also to promote a general state of well-being in all age groups was suggested by Ottaway (2009).This multi-micronutrient fortified beverage was fortified with carrot juice, UV irradiated mushroom juice, moringa leaves juice, dates juice and encapsulated DHA emulsion as a resource of micronutrients such as vitamin A and D, minerals (iron and calcium) and DHA fatty acid which considered overcome nutritional deficiencies in children.

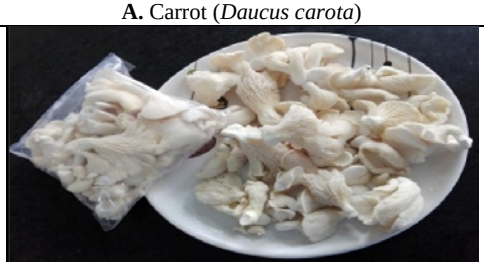
MATERIALS AND METHODS

A. Raw Materials

The raw materials shown in Fig.1. were used for preparing fortified beverage were fresh skimmed milk was obtained from the Livestock farm, College of Food and Dairy Technology, Tamil Nadu, India. Carrot, moringa, mushroom, dates and sugar were purchased from the local market of Koduvalli, Chennai, Tamil Nadu, India. DHA algal oil was purchased from M/s Shiridi Sairam Science Needs, Tamil Nadu, India.

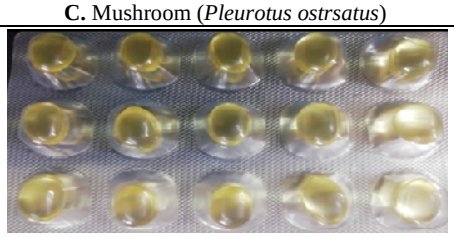
A. Carrot (*Daucus carota*)

B. Moringa leaves (*Moring oleifera*)



C. Mushroom (*Pleurotus ostrsatus*)

D. Dates (*Phoenix dactylifera*)



E. DHA (Docosahexaenoic acid) Algal oil (*Schizochytrium* spp)

F. Skimmed Milk

Fig. 1. Raw materials utilized for the development of fortified milk beverage.

B. Methods

In this study sterilization techniques were used to process the product and to improve their shelf life. Among all the ingredients, DHA algal oil fortified by using novel nanoencapsulation technology through micro fluidizer.

C. Preliminary trials for preparation of fortified milk beverage

Preliminary trials were conducted with the following combinations of ingredients was shown in Table 1: carrot juice (5, 10 and 15 per cent), mushroom juice(3, 6 and 9 per cent), moringa leaves juice (5, 10, 15 per cent), dates juice (1, 2 and 3 per cent) and encapsulated DHA oil (1, 2 and 3 per cent) with sugar (8, 10 and 12per cent), flavourant (0.1, 0.15 and 0.2 per cent) and colourant (0.05, 0.06 and 0.07 per cent) respectively.

D. Experimental Designs

The ingredients levels were optimized through conjoint analysis, the number of cards shown in Table 2. Statistical conjoint analysis was carried out and 16 treatments were established, to which sensory analysis conducted for all the treatments. The sensory score obtained for all the 16 treatments were statistically analyzed to find out the best attributes. The conjoint analysis for selecting the best ingredient levels of ideal final products in 100ml was based on the percentage of utility estimate and relative importance and the treatment number 10 was selected as best one with the following attributes combination: Carrot – 15ml, Mushroom – 6ml, Moringa – 5ml, Dates – 2ml and DHA algal oil – 1ml.

Table 1: Preliminary trials for selecting ingredients level of fortified milk beverage.

Sr. No.	Ingredients	Levels (%)		
1.	Carrot juice	5	10	15
2.	Moringa leaves juice	5	10	15
3.	Irradiated Mushroom juice	3	6	9
4.	Dates juice	1	2	3
5.	Encapsulated DHA algal oil	1	2	3
6.	Sugar	8	10	12
7.	Flavourant	0.1	0.15	0.2
8.	Colourant	0.05	0.06	0.07

Table 2: Design of experiment for standardizing ingredient level of fortified milk beverage using conjoint analysis.

Card ID	Carrot juice (ml)	Irradiated mushroom juice (ml)	Moringa leaves juice (ml)	Dates juice (ml)	DHA juice (ml)
T1	15	3	10	3	1
T2	5	6	15	1	1
T3	5	3	5	2	1
T4	5	6	5	3	3
T5	5	9	10	1	1
T6	5	3	5	1	1
T7	10	3	15	3	1
T8	5	3	10	2	3
T9	15	9	15	1	3
T10	**15**	**6**	**5**	**2**	**1**
T11	10	9	5	2	1
T12	15	3	5	1	2
T13	10	6	10	1	2
T14	10	3	5	1	3
T15	5	3	15	2	2
T16	5	9	5	3	2
DHA – Docosahexaenoic acid					

E. Proximate Analysis

Total carbohydrates. Total carbohydrates were determined by the method of Lane and Eynon as reported by Ranganna (2005).

Crude protein. The protein content of fortified milk beverage was estimated by Kjeldahl method using Kjeltron protein analyser as described in AOAC (1990).

Fat. The fat content of fortified milk beverage was determined by solvent extraction method using Soxhlet apparatus as described in AOAC (2006).

Moisture. The moisture content of fortified milk beverage was determined by AOAC (2006) method.

Total Ash. The ash content of fortified milk beverage which represents the inorganic residue remaining after distraction of organic matter was estimated using standard method of AOAC (2006).

Fibre. The fibre content of fortified milk beverage was determined by the method described in AOAC (2000).

Total solids. The total solid of fortified milk beverage sample was determined as described in AOAC (2006).

Total Calorie. The total calorie content of fortified milk beverage was estimated using standard method of AOAC (1990) which gives total energy value obtained from beverage.

RESULT AND DISCUSSION

A. Conjoint analysis for quality attributes of Fortified milk beverage

The result of conjoint analysis for ingredient levels of final product is presented in Table 3 where Pearson's R and Kendall's tau values were 0.613 and 0.572 respectively indicating a better fit to the data. Accordingly, the card ID number 10 that contain carrot juice (15 per cent), irradiated mushroom juice (6 per cent), moringa leaves juice (5 per cent), dates juice (2 per cent) and encapsulated DHA algal oil (1 per cent) was selected as the best combination of fortificants to be incorporated in 100ml of the beverage. The conjoint analysis was useful method for judging the acceptance level of novel foods and could be applicable in a number of studies to evaluate their attributes was suggested by Annunziata and Vecchio (2013).

B. Level of ingredients added in preparation of 200 ml Fortified milk beverage

The level of ingredients added for the preparation of 200 ml Fortified milk beverage was shown in Table 4. The ingredients added in fortified milk beverage were skim milk, carrot juice, irradiated mushroom juice, moringa juice, dates juice, encapsulated DHA algal oil and sugar which were added in the quantity of 121.58 ml, 30ml, 12ml, 10ml, 4ml, 2ml and 20g respectively. Finally, the natural identical orange flavour and colour were added in the quantity of 0.3ml and 0.12ml respectively before in-bottle sterilization at 121º C for 15mins and stored at ambient temperature.

C. Proximate analysis of Fortified milk beverage

The results of proximate composition viz. moisture, carbohydrate, protein, fat, total ash content, fiber, total solids and total calories of the control (skim milk) and fortified milk beverage is shown in Table 5.

The fortified milk beverage had the mean moisture content of 84.64 per cent and the corresponding value for control was 90.50 per cent. The result was on par with Mittal and Bajwa (2012), who worked on the quality characteristics of low calorie milk drinks contain moisture content of 87.3 per cent respectively.

The mean fat content of fortified milk beverage was 0.75 per cent and the corresponding value for control was 0.41 per cent. The increase in fat content of product may be due to the addition of fruits and vegetables which were all rich in fat soluble vitamins. The result was similar with Hussein *et al*. (2020) developed a fortified almond milk with carrot juice and quinoa seed powder contain fat content of 0.5 per cent respectively.

Table 3: Conjoint analysis for quality attributes of Fortified milk beverage.

Attributes	Levels	Utility Estimate	Relative Importance (%)
Carrot Juice (%)	5	-1.303	**29.95**
	10	-.583	
	15	**1.886**	
Mushroom Juice (%)	3	-.615	**20.14**
	6	**1.268**	
	9	-.653	
Moringa Juice (%)	5	**.972**	**15.43**
	10	-.551	
	15	-.421	
Dates Juice (%)	1	-.543	**21.52**
	2	**1.355**	
	3	-.812	
Dha alagal oil (%)	1	**.816**	**12.96**
	2	-.442	
	3	-.374	
(Constant)		11.912	
*Pearson's R value = 0.613**; Kendall's tau value = 0.572**; n= 30*			

Table 4: Level of ingredients added in the preparation of Fortified milk beverage.

Sr. No.	Ingredients	Quantity
1.	Skim milk	121.58 ml
2.	Carrot juice	30 ml
3.	Moringa leaves juice	10 ml
4.	Irradiated mushroom juice	12 ml
5.	Dates juice	4 ml
6.	Encapsulated DHA algal oil	2 ml
7.	Sugar	20 g
8.	Flavourant	0.3 ml
9.	Colourant	0.12 ml

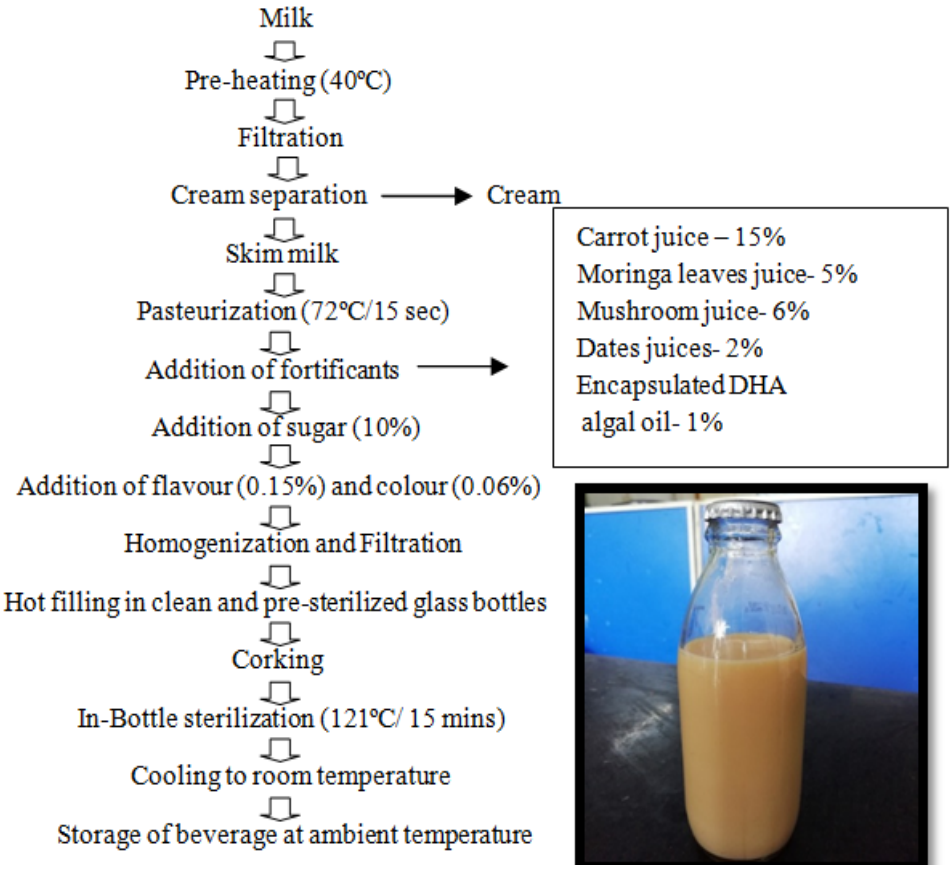


Fig. 3. Flow chart for preparation of Fortified milk beverage.

The mean protein content of fortified milk beverage was 5.49 per cent and the corresponding value for control was 3.45 per cent and the result was concurrence with the findings by Jothylingam and Pugazhenthi (2013) developed dietetic herbal flavoured milk were the protein content ranged from 3.02 to 4.03 per cent. The addition of moringa leaf juice, carrot juice and dates juice might be the reason for the increase in protein content of fortified milk beverage.

The fibre content of fortified milk beverage was 0.97 per cent and the corresponding value for control was below detectable level and the result was similar with Quarcoo (2008) developed the *Moringa oleifera* leaf beverage with mixture of carrot juice, pineapple juice and ginger distillate contain fibre content of 1.8 g. The increase in the fibre content of developed beverage might be due to addition of carrot, moringa leaves, mushroom and dates in the developed beverage.

The fortified milk beverage had the total ash content with mean value of 0.89 per cent and corresponding value for control with mean value of 0.66 per cent. Addition of various nutrient rich ingredients like carrot, moringa leaves, mushroom, dates increased the ash content of fortified milk beverage. Mittal and Bajwa (2012) observed the same results in the quality characteristics of low calorie milk drinks that the total ash content of product were 0.67 per cent respectively.

The mean carbohydrate content of fortified milk beverage was 7.24 per cent and for control was 4.88 per cent and the result is in concurrence with the findings of Hussein *et al*. (2020) developed the fortified almond milk with carrot juice and quinoa seed powder contains carbohydrate content of 5.22 per cent. The addition of fruits and vegetables in fortified milk beverage leads to increase in carbohydrate content.

The mean total solids of control were 9.49 per cent, while for fortified milk beverage the range was 15.36 per cent. The observed values are in concurrence with the findings by Shukla *et al*. (2018) developed the sterilized mango based dairy beverage were total solids content was 15.46 per cent. The mean total calories value of fortified milk beverage was 57.80 Kcal/100ml and corresponding value for control with mean value of 37.90Kcal/100ml. The reason for increase in calories must be due to addition of sugar, fat and other ingredients present in the developed beverage. The result was similar with the findings by Mittal and Bajwa (2012) observed the quality characteristics of low calorie milk drinks were calorific value of 40.0 kcal/100ml respectively.

Table 5: Proximate analysis (Mean ±SE) [@] of Fortified milk beverage.

Parameters	Control	Product	t value
Moisture (%)	90.50±0.050	84.64±0.015	110.193**
Carbohydrates (%)	4.88± 0.020	7.24±0.009	102.582**
Protein (%)	3.45±0.024	5.49±0.007	80.950**
Fat (%)	0.41±0.082	0.75±0.011	4.103*
Ash (%)	0.66±0.012	0.89±0.015	11.233*
Fiber (%)	00±0.00	0.97±0.006	161.695**
Total solids (%)	9.49±0.050	15.36±0.015	110.193**
Total calories (Kcal)	37.90±0.075	57.80±0.101	157.296**

@- Average of six trials: ** - Highly Significant (P≤0.01); *- significant (P≤0.05)
Control – Unfortified milk beverage; Product – Fortified milk beverage

CONCLUSION

One of the trendiest processing techniques to overcome nutritional deficiencies is food fortification, which is considered to be more cost effective in improving the health. For complete well-being, our body requires vitamins and minerals as nurturing agent that can curb the micronutrient deficiencies. Such micronutrient deficiencies can be alleviated by supplementing the fortified milk beverage prepared in this study. Fortified milk beverage is a drink that is designed to help children's health by contributing to their growth performance, brain development and in preventing from malnutrition. The major function of fortified milk beverage is to overcome micronutrient deficiencies but at the same time, it also energy for vital activities of the body.

The above mentioned optimal level of each ingredient was standardized by using conjoint analysis as statistical tool. Since milk lacks DHA, which is essential for the growth and development of brain for children, this beverage was fortified with DHA by incorporating encapsulated DHA algal oil. The proximate composition such as moisture, protein, fat, carbohydrate, total ash contents, total solids and total calories of the developed beverage were analyzed using standard procedures. All these nutritional composition of fortified milk beverage eventually had higher values than the control. Statistical analysis revealed that there was a highly significant (P≤0.01) for the moisture, carbohydrate, protein, fiber, total solids and total calories between the control and fortified milk beverage while significant (P≤0.05) difference was found in the fat and total ash content of control and DHA fortified milk beverage.

Though a variety of beverages like flavoured milks, soft drinks are available in the commercial market, the fortified milk beverage has a unique place because it comes under the multi-nutrient beverage derived from natural resources which will never impart any negative impact on the health of the children's. Furthermore, blending of natural resources in the form of fortified milk beverage that can also function as a health drink could be considered for a new product development.

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Conflict of Interest: None.

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