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ANALYZING THE EFFECTS OF FORTIFYING MILK BEVERAGES WITH *DOCOSAHEXANOIC ACID* ON THEIR SENSORY EXPERIENCE, FUNCTIONAL ATTRIBUTES AND MICROBIOLOGICAL QUALITY

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ABSTRACT

Food fortification involves deliberately increasing the content of essential micronutrients in a food product to enhance its nutritional quality and provide health benefits while minimizing potential health risks. Fortification with essential nutrients like vitamins and minerals, sourced from fruits and vegetables. This research aimed to standardize a fortified milk beverage using natural resources rich in micronutrients and fatty acids. Additionally, the study focused on assessing the functional and microbiological parameters of the developed beverage. The selection of ingredient levels was determined through conjoint analysis, and in-bottle sterilization was chosen as the heat treatment method to extend the shelf life of the fortified milk beverage. The processing involved bottle sterilization at 121˚C for 15 minutes. The functional composition of the developed beverage revealed, it has an antioxidant activity (%) of 31.81±0.89 and 17.63±1.57. Similarly, the TPC (mg GAE/g) of 22.18±0.84 and 18.41±0.548 on the 0th and 90th days, respectively. The microbial activity of the fortified milk beverage was not detected in the total bacterial, coliform, spore, yeast and mould count in both unfortified and fortified beverages after the retort process.

Keywords: antioxidant activity, micronutrients, microbial activity and fortification.

INTRODUCTION

Micronutrients, which encompass vitamins and minerals play an important role as they are essential in minute quantities for promoting growth, ensuring overall health and well-being, preventing diseases and facilitating fundamental physiological processes within the human body. Micronutrient deficiencies can have severe repercussions, including premature mortality, compromised health, visual impairments, hindered physical growth and impediments to cognitive development. To address these deficiencies, diverse strategies such as fortifying foods, broadening dietary options, providing nutritional education, and administering micronutrient supplementation have been employed by (Manoharan *et al.* 2021). The WHO and UNICEF computed a worldwide assessment that nearly 190 million children are deficient in vitamin A with more than 2 million people deficient in other key micronutrients like iron, iodine and zinc (Venkatesh *et al.* 2021; Randhawa *et al.* 2013). Food fortification of essential micronutrients, including vitamin B12, vitamin A, vitamin D, iron, iodine and folic acid in various food products. One such fortified product is milk beverages designed to enhance the health of children and adolescents, promoting their growth, and brain development, and safeguarding against malnutrition. These deficiencies not only have a significant impact on health, but also associated with economic costs, estimated to range between 0.8% and 2.5% of the gross domestic product as estimated by (Stein and Qaim, 2007 and Subhashini *et al.* 2018). Skim milk, a nutrient-rich by-product resulting from the separation of cream, contains essential elements such as lactose, proteins, minerals and vitamins.

These components hold significant value as a dietary sources for human consumption. The primary source of skim milk production is associated with the cream industry, owing to its low fat content, making it an ideal supporting material in various food production processes. Additionally, carrots are recognized as a valuable source of β-carotene, a precursor to vitamin A, which plays a pivotal role in addressing vitamin A deficiency (VAD), as previously documented by (Fratianni *et al.* 2010 and Ramachandran *et al.* 2022). Mushrooms exposed to sunlight were a good source of vitamin D2 fortification with foods is a safe and effective way to increase 25(OH) D levels in children and adults as studied by (Keegan *et al.* 2013 & cardwell *et al.* 2018). (Gopalakrishnan *et al.* 2016) studied the nutritive importance and medicinal application of Moringa oleifera and the leaves were rich in minerals, vitamins and other essential phytochemicals also used as a potential anti-oxidant, anti-cancer, anti-inflammatory, anti-diabetic and anti-microbial agent. Omega-3 DHA fatty acids have shown potential benefits in managing mental health conditions such as bipolar depression and major depressive disorder. (Charles et al. 2019) highlighted fish oil as an excellent natural source of preformed omega-3 DHA, along with other crucial nutrients such as iron, zinc, magnesium, and vitamins B3, B6, C, and E. Conjoint analysis, often referred to as trade-off analysis, is a multivariate method employed to gain insights based on consumers and their preferences for products or services. It aids in making predictions regarding the ideal ingredient levels for product concepts. (Navarasam et al. 2021 & Sowdambika et al. 2022) proposed the use of conjoint analysis, specifically in the context of food products, to select and define quality attributes related to ingredient levels in multi-attribute product concepts. When presented with a substantial array of combinations for evaluation by a sensory panel, the risk of encountering a high non-response rate becomes apparent (Junaid et al. 2023 & Adhithya et al. 2022). To mitigate this issue, one effective approach is to employ the creation of a set of cards using the orthogonal array method, a process facilitated through SPSS statistical software. This method streamlines the evaluation process and enhances panel responsiveness. Furthermore, it is noteworthy that food fortification is recognized as a prominent strategy for addressing malnutrition. This approach is considered to be more efficient in developing nutrient-enriched food products, delivering nutritional benefits, and achieving results in a shorter time compared to other forms of food assistance (Manoharan and Ramasamy, 2013). Ottaway (2009) suggested that the development of a fortified milk beverage had a dual purpose. It was not only intended to address or prevent specific nutritional deficiencies but also aimed to enhance the overall well-being of individuals across different age groups. This multi-micronutrient fortified beverage was enriched with a range of components, including carrot juice, mushroom juice, moringa leaves juice, seaweed and Fish oil. These ingredients served as valuable sources of essential micronutrients such as vitamin A, and vitamin D, minerals like iron and calcium, and DHA fatty acids. This composition was designed to effectively combat nutritional deficiencies.

MATERIALS AND METHODS

Raw Material Collection

Fresh cow milk sourced from the Community Cattle Center (CCC) at the College of Food and Dairy Technology in Koduvalli was utilized to produce skim milk by separating cream. Sugar was procured from the local market in Alamathi Village, Chennai, for the preparation of the DHA fortified milk beverage. Harvested Carrots (Daucus carota), purchased from the local market in Redhills, Chennai were used in the beverage's preparation. Additionally, fresh Oyster mushrooms (Pleurotus ostreatus) obtained from the local market in Redhills, Chennai, and Moringa leaves (Moringa olifera) purchased from Alamathi local market were included in the DHA fortified milk beverage. Fish oil sourced from M/s. Geltec Private Limited in Mumbai was also used in its preparation. Red seaweed (Kappaphycus alvarezii) collected from the Seaweed Research and Utilisation Association in Mandabam, Rameshwaram district, Tamil Nadu, was another component included in the DHA fortified milk beverage. For flavour and colour enhancement, natural identical mango super alphonso flavour and orange colour were purchased from M/s. CEC Flavour and Fragrances Pvt. Ltd in Chennai. Finally, glass bottles of 200 ml capacity were purchased from Bharat Bottles, a local market in Chennai, to package and store the developed DHA-fortified milk beverage.

Optimized Level of Ingredients

The optimization process was performed using conjoint analysis software for the different level of ingredients and the optimized levels are carrot juices (20 %), moringa leaves juice (4 %), mushroom juice (2.5%), Seaweed (1 %) and fish oil (1 %) were added along with sugar (14%), Mango Super Alphonso flavour (0.15 %) and orange colour (0.02 %) and then filtered after homogenization.

Filling and Sealing

The 200 ml fortified milk beverage was carefully poured into glass bottles and sealed using a manual crown corking machine. To ensure safety during the retort processing, the bottles were filled up to three-fourths of their capacity, leaving adequate space in the neck to prevent breakage. The hot filling process was employed to minimize microbial contamination from the surrounding environment, securing the quality of the product.

Sensory Analysis

The sensory analysis of the DHA-fortified milk beverage sample was performed using 9 point hedonic scale test (Maximo et al., 1984).

Antioxidant Activity

According to Brand-Williams et al., (1995), the DPPH method was performed to measure the total antioxidant activity of the DHA-fortified milk beverage. The DPPH radical scavenging activity assay measured the extract's capability to donate hydrogen or scavenge free radicals. The DPPH radical is a stable form of free radical that is reduced to diphenyl picryl hydrazine when it interacts with an antioxidant compound that can donate hydrogen.

Three millilitres of 60-M DPPH were added to a 250-liter aliquot of the sample solution, which was then vortexed. After 20 minutes of darkness, the reaction liquid was stirred, and the absorbance was determined at 517 nm. As a blank, DPPH with ethanol was utilized. The colour of the solution was shifted from deep violet to bright yellow over time. The antioxidant chemicals in the milk caused the DPPH to be reduced, which caused the colour shift. The reduction was assessed by measuring the absorbance decrease at 517 nm.

Total Phenolic Content

The total phenolic content of DHA-fortified milk beverages was determined according to the Follin - Ciocalteu method adopted by (Singleton and Rossi, 1965). The Gallic acid standards that have been prepared were measured by using UV-vis spectrophotometer (Perkin-Elmer Lambda 35,Perkin Elmer Inc., USA) at 765 nm wavelength. The outcomes were expressed as milligrams of gallic acid equivalents (GAE). All experiments were performed in triplicate.

Microbial Analysis

Microbiological analysis viz. standard plate count, coliform count and yeast and mould counts were also performed for the DHA-fortified milk beverage as per the standard protocol described in BIS: 1981, SP: 18 (Part XI).

Statistical Analysis

The statistical analysis was performed using the Software of Statistical Package for Social Sciences (SPSS) 16.0 as per the standard procedure adopted by (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSIONS

In this present research work, efforts were made to analyse their effects on sensory, functional and microbial characteristics in retort processed DHA-fortified milk beverages.

Sensory Evaluation Score for DHA-Fortified Milk Beverage using 9 Point Hedonic Scale

The sensory evaluation score for DHA fortified milk beverage using 9- point hedonic is represented in Table 1. The sensory attributes of DHA-fortified milk beverage such as colour and appearance, taste, flavour, mouth feel and overall acceptability had a mean value of 8.63, 7.85, 8.38, 8.60 and 8.50 while the control had a mean value of 5.35, 4.33, 4.11, 5.43 and 5.25 respectively.

The statistical analysis revealed a highly significant ($P \leq 0.01$) difference between the control and various treatments concerning colour and appearance, taste, flavour, and overall acceptability. Across all sensory attributes, the product consistently exhibited the highest mean value for colour and appearance compared to the control. Figure 1 illustrates the mean sensory evaluation scores between the control and the DHA-fortified milk beverage. From the scores, it is clear that the sensory evaluation score is highly significant between control and DHA-fortified milk beverage and the developed product is more pleasing with a higher mean overall acceptability. The result obtained was similar with the findings of Anandh *et al.* (2014) who studied the retort processed rose flavoured milk and Jayalalitha *et al.* (2022) who explained the iron fortified yoghurts reported better sensory scores than stored yoghurt after 3 days and 7 days of storage study.

Table 1. Sensory score (Mean ±SE)@for DHA fortified milk beverage using 9- point hedonic scale

Beverage	Sensory attributes (9-point hedonic scale)					
	Colour and appearance	**Taste**	**Flavour**	**Mouth feel**	**Overall acceptability**	**F value**
Control	$5.35^{b}\pm0.076$	$4.33^{a}\pm0.088$	$4.11^{a}\pm0.144$	$5.43^{b}\pm0.088$	$5.25^{b}\pm0.042$	**43.701****
Product	$8.63^{b}\pm0.111$	$7.85^{a}\pm0.076$	$8.38^{b}\pm0.079$	$8.60^{b}\pm0.093$	$8.50^{b}\pm0.057$	**13.917****
t value	**24.286****	**30.143****	**25.860****	**24.694****	**45.215****	

@- Average of six trials (Different superscripts in a same row differs significantly)
**-Highly Significant (P≤0.01); Control – Unfortified milk beverage; Product – DHA fortified milk beverage

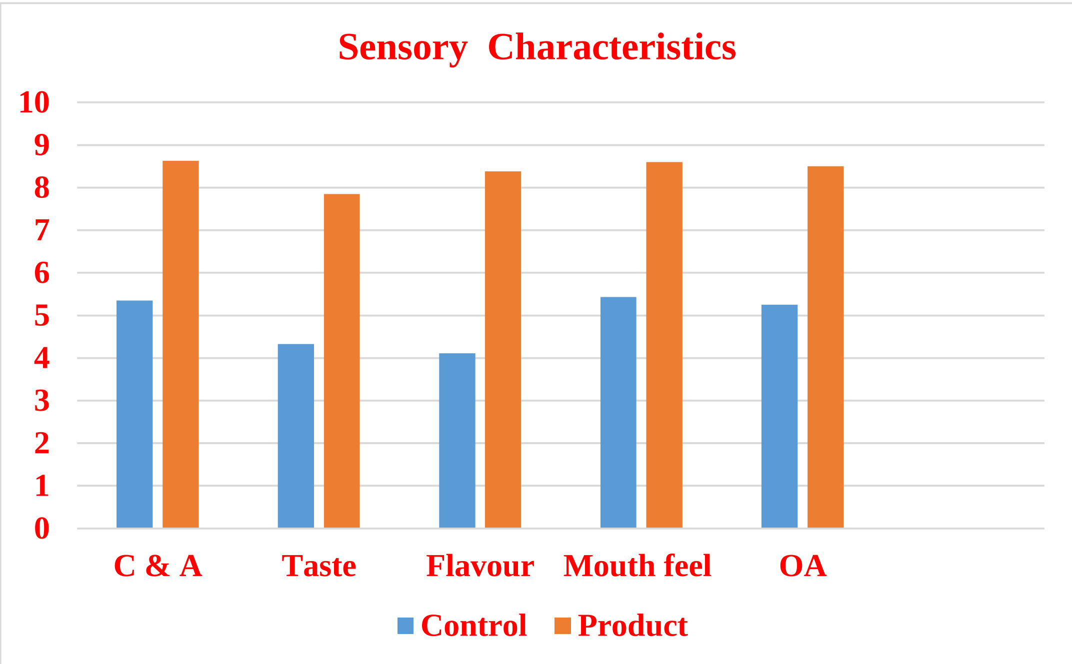

Fig. 1 : Sensory Analysis of DHA fortified milk beverage during storage at ambient temperature

Antioxidant Activity and Total Phenolic Content for DHA-Fortified Milk Beverage during the storage period.

The AOA and TPC storage period was shown in Figure 2. The mean ± SE value of the Antioxidant activity of DHA-fortified milk beverage was 31.81±0.891 and 17.63±1.573 (%) on 0th and 90th days was shown in table 2 and total phenolic content of DHA-fortified milk beverage was 22.18±0.85 to 18.41±0.55 (mg GAE/g) on 0^{th} and 90^{th} days as shown in Table 3. These results were almost similar to the findings of (Ashoush and Gadallah, 2011). The observations were by the finding of (El-Said *et al.* 2014) who revealed that the TPC of aqueous and methanolic extract of PPE for stirred yoghurt was in the range of 13.98 to 14.81 mg and 14.83 to 15.80 mg gallic acid/g respectively. Regarding the antioxidant activity, the obtained results closely mirrored the findings of Singh et al. (2002). Concerning the storage study, both the phenolic content and antioxidant activity in DHA fortified milk beverages decreased day by day which might be due to the loss of these activities with the advancement of the storage period. The outcomes of Hala et al. (2010) obtained were nearly akin to the discoveries previously documented and who reported that the decrease in total phenolic content and AOA % might be due to highly unstable compounds that undergo numerous enzymatic and chemical reactions during food storage.

Table 2. Antioxidant activity (Mean ±SE)[@] of DHA fortified milk beverage during storage at ambient temperature

Beverage	Storage days				F value
	0	30	60	90	
Control	0	0	0	0	-
Product	31.81^{b}±0.891	28.81^{a}±3.451	21.71^{b}±1.032	17.63^{a}±1.573	**21.269****
t value	**98.727****	**20.466****	**78.611****	**59.183****	

@- Average of six trials (Different superscripts in a same row differs significantly)
**-Highly Significant (P≤0.01); Control- Unfortified milk beverage; Product – DHA fortified milk beverage.

Table 3. Total phenolic content (Mean ±SE)[@] of DHA fortified milk beverage during storage at ambient temperature

Beverage	Storage days				F value
	0	30	60	90	
Control	0	0	0	0	-
Product	22.18^{a}±0.845	21.59^{a}±0.578	19.55^{b}±0.572	18.41^{b}±0.548	**43.139****
t value	**64.533****	**78.290****	**93.240****	**89.884****	

@- Average of six trials (Different superscripts in a same row differs significantly)
**-Highly Significant (P≤0.01); Control- Unfortified milk beverage; Product – DHA fortified milk beverage.

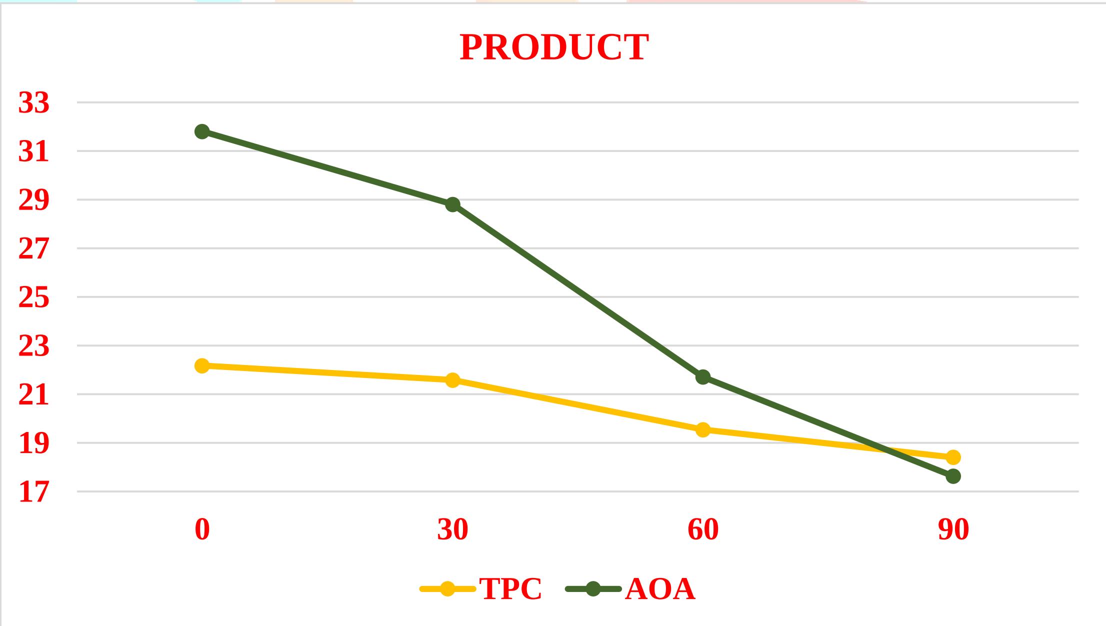

Fig. 2 : Antioxidant activity and Total phenolic content for DHA fortified milk beverage during the storage period.

Microbial Growth for DHA-Fortified Milk Beverage during the storage period

The colonies were calculated according to the procedure given by (Pugazhenthi *et al.* 2020) Sample was serially diluted before plating. Plates were incubated in replicates of various dilutions under study. Retort processing provides a highly sterile product with an extended shelf- life. So, it can be understood that at 121℃ with 15 minutes in processing temperature and time. The occurrence of bacterial and fungi colonies is reduced to a much greater extent. The examination of microbial content in the beverages was carried out throughout the storage period at room temperature.

Analysis conducted on the 0, 30, 60, and 90th day revealed that the total plate count, coliform count, and yeast and molds count were undetected throughout the 90 day storage duration at room temperature, both in the control and the DHA-fortified milk beverage. Similar findings were reported by (Anandh *et al.* 2014) in their storage studies of retort processed rose flavoured milk and by (Deka *et al.* 2001) in their studies of the spice mixed fruit juice RTS beverage. In bottle sterilization of these products results in complete destruction of micro flora and indicates a proper sterilization with no post sterilization contamination.

The result is in accordance with the findings by (Shukla *et al.* 2018) that microbial analysis of sterilized mango based dairy beverage during storage up to 75 days was not detected with standard plate count, spore formers, yeast and molds respectively. In the present study, the spore count, yeast and moulds were not detected in both the control and treated throughout the storage period and it indicates the complete sterility of product and no post contamination. The observed result is in concurrence with the findings by Mittal and Bajwa (2014) reported that the low calorie milk drinks contain no spore counts, yeast and molds until 150 days storage at room temperature.

Cost of Production of DHA Fortified Milk Beverage

Table 4 presents the production costs of DHA-fortified milk beverage. The market price of ingredients was considered to calculate the cost of the DHA-fortified milk beverage. Considering the levels of inclusion, the estimated cost of producing a 200 ml beverage was determined. The production cost for developing a 200 ml DHA-fortified milk beverage amounted to Rs.24.50, while for the control, it was Rs.17.10.

Table 4. Cost of production of DHA fortified milk beverage

Ingredients	Cost (Rs/ Kg or L)	Inclusion level 200 ml (of ingredients in ml or gm)		Cost of ingredients per 200 ml (Rs)	
		Control	Fortified	Control	Fortified
Skim milk	30.00	200.00	142.66	6.00	4.30
Sugar	40.00	28.00	28.00	1.10	1.10
Carrot	60.00	-	40.00	-	2.40
Moringa Leaves	50.00	-	8.00	-	0.40
Mushroom	370.00	-	5.00	-	1.85
Fish oil	1000.00	-	2.00	-	2.00
Seaweed	750.00	-	2.00	-	1.50
Flavourant	2300.00	-	0.30	-	0.70
Colourant	6000.00	-	0.04	-	0.25
Processing and bottling costs	10.00	-	-	10.00	10.00
Total				**17.10**	**24.50**

The cost for the development of DHA-fortified milk beverages is presented in Table 4. The increased cost incurred in preparation of DHA-fortified milk beverage was due to the cost of fish oil, a natural identical flavouring and colouring agent. The production cost for preparation of DHA-fortified milk beverage is Rs. 24.50 which can be commercialized and affordable. The cost of commercially available fortified beverages was higher than the cost of developed DHA-fortified milk beverages.

The developed beverage was prepared with micronutrients rich ingredients from natural resource, which helps in nutritional deficiency disorder, growth and development, brain development, improves physical and mental health of adolescent.

CONCLUSION

Food fortification is a contemporary processing technique aimed at mitigating nutritional deficiencies, and it is regarded as a cost-effective means of enhancing overall health. Our bodies require essential vitamins and minerals as nurturing agents to combat micronutrient deficiencies. The fortified milk beverage developed in this study serves as a solution to address such deficiencies, contributing to adolescent growth, brain development, and malnutrition prevention. Besides its primary function of overcoming micronutrient deficiencies, this beverage also provides energy for essential bodily functions. To determine the optimal levels of each ingredient, conjoint analysis was employed as a statistical tool. This beverage was fortified with fish oil because milk lacks DHA, a critical nutrient for adolescent brain growth and development.

In this work, AOA, TPC, microbial studies and cost analysis were analyzed using standard procedures. The fortified milk beverage exhibited higher values for AOA and TPC components compared to the control. The statistical analysis indicated notably significant differences ($P \leq 0.01$) and significant differences ($P \leq 0.05$) in sensory attributes between the fortified milk beverages and the control. The absence of microbial growth during storage suggests the efficacy of the retort process. Further analysis using standard methods is required to assess the proximate composition, which includes moisture, protein, fat, carbohydrates, total ash contents, total solids, total calories, and nutritional components of the fortified milk beverage.

Despite the availability of various beverages like flavoured milk and soft drinks in the commercial market, the fortified milk beverage holds a unique position as a multi-nutrient beverage derived from natural resources, promoting the well-being of children without imposing any negative health impacts.

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