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Standardization and nutrient analysis of β-carotene enhanced chocolate

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Abstract
Micronutrient deficiency is raising concern worldwide, especially among children and pregnant women in developing countries, posing a significant risk to the nutritional status. This study aimed to develop β-carotene enhanced chocolates with the addition of processed green leaves (*Moringa oleifera* and *Basella alba*) and evaluated for sensory characteristics and nutritional composition. Researcher found that among the moringa chocolate with incorporation level of 8 per cent was best accepted by panel member. Proximate analysis results showed that addition of processed moringa leaf powder significantly increased the protein, ash and fiber content from 5.73 to 7.68, 4.12 to 4.89 and 5.36 to 7.50 g/100g respectively, However, the fat, total carbohydrates, available carbohydrates and energy content decreased from 30.67 to 28.57, 57.99 to 56.73, 54.60 to 49.23 g/100g respectively and 531 to 514 Kcal respectively. The mineral content (iron, zinc, calcium and phosphorus) increased significantly in β-carotene enhanced chocolates compared to control. The β-carotene content (9345 µg/100g) of developed chocolates was found significantly ($p<0.05$) higher than the control chocolate (887 µg/100g). Although moringa leaf substitution raised most of the nutritional contents, the maximum consumer acceptability was recorded in control chocolate followed by moringa chocolate, which was statistically similar to moringa chocolate. The results indicated that β-carotene enhanced chocolates supplemented with 8 per cent processed moringa leaf powder provide the enriched nutritional quality and can potentially contribute to the improvement of food and nutritional security of the vulnerable populations. Furthermore, *Moringa olefera* as super green can help to encourage functional food preparation, commercialization and acquaintance with society.

Keywords: β-carotene enhanced chocolates, *Moringa oleifera,* Nutritional composition, β-carotene

Introduction

Chocolate is a luxury food that evokes a range of stimuli which activates pleasure during its consumption. It is not age specific and consumed by children, youth and old age people. Chocolate is not only a common confectionery product but also has an important social role being an inherent part of many celebrations. Some of them are even crossing the confectionery industry line by aiming at consumer interest in health. The chocolates benefit from the positive effects of cocoa as well as from various additional ingredients such as dried berries, herbs, seeds, nuts, dietary fibre or probiotics. Another group of products focuses on the ethical and ecological aspects of production (Abhishek *et al*., 2010) [1].

Green leafy vegetables are the cheapest of all the vegetables within the reach of poor men, being the richest in their nutritional value. Green leafy vegetables occupy an important place among the food crops as these provide adequate amounts of many vitamins and minerals for humans. They are rich sources of β-carotene, ascorbic acid, riboflavin, folic acid and minerals such as calcium, iron and phosphorous (Aryal *et al.*, 2018) [5].

In nature, there are many underutilized green leafy vegetables (UGLVs) with promising nutritive value, which can feed an ever-growing population (Gupta and Yadav, 2016)[8]. Now-a-days, underutilized green leafy vegetables are becoming important as a means of nutritional alternatives to value addition and noble product development. Thus, in the present study author aimed to develop β-carotene enhanced chocolate using underutilized moringa and basella leaves.

Methodology

The study was conducted in the Department of Food Science and Nutrition, UAS, GKVK Bengaluru. The material used in the standardization of β-carotene enhanced chocolates is procured from the local wholesale market and after primary processing stored in an airtight container at a suitable temperature.

β-carotene enhanced chocolate formulation and production
Formulation

Basella chocolates were standardized using 1, 2, 3, 4 and 5 per cent processed basella leaves powder and moringa chocolates were standardized using 2, 4, 6, 8 and 10 per cent of processed moringa leaf powder and with appropriate combinations of the dark chocolate compound, milk compound, almond, cashew nut, resins and sugar. Chocolate prepared without the addition of UGLVs was kept as control chocolates. The trial samples were nominated as B_1, B_2, B_3, B_4, B_5, M_1, M_2, M_3, M_4, and M_5, whose formulations are depicted in Table 1.

Production
The dry ingredients (dark chocolate compound, white chocolate compound, dry fruits and sugar) were weighed in the required ratio. Almonds, cashew nuts and resins were cut into small pieces and dried in a hot air oven at 55ºC±2 for 1 hour to achieve a crisp texture. The double boiler method was used to melt the chocolate compound till it reaches 45 to 50ºC. After melting of chocolate compound was kept for cooling till the temp reaches 30ºC. Then processed moringa leaves and dried dry fruits were added to the required ratio and mixed nicely. Then mixture was poured into a silicone mold and tap the mold to remove air inside the chocolates and refrigerated at 4ºC for 15 minutes, demoulded the chocolate and packed in suitable packaging material (Fig.1).

Table 1: Standardization of β-carotene enhanced chocolates

Ingredients	Control	Basella chocolate					Moringa chocolate				
		Treatment									
		B_1	B_2	B_3	B_4	B_5	M_1	M_2	M_3	*M_4	M_5
Dark chocolate compound	57	56	55	54	53	52	55	53	52	49	47
Milk chocolate compound	20	20	20	20	20	20	20	20	20	20	20
Processed leaves	0	1	2	3	4	5	2	4	6	8	10
Dry fruits	15	15	15	15	15	15	15	15	15	15	15
Sugar	8	8	8	8	8	8	8	8	8	8	8
		Rejected by panel members					*M_4 was the best accepted				

Sensory evaluation of β-carotene enhanced chocolates
Basella and moringa chocolates along with control were evaluated for sensory characteristics by a panel of semi-trained judges on a nine-point hedonic scale. The panel members for sensory evaluation consisted of staff and students of the Department of Food Science and Nutrition, UAS, Bengaluru (Plate 1). The coded samples were presented along with a glass of water at room temperature. Panellists were advised to rinse their mouth with water after tasting each sample. All sensory attributes were explained to panellists before sensory analysis.

Nutritional composition of β-carotene enhanced products
Best accepted β-carotene enhanced products were analysed for proximate composition (AOAC, 2005) [2], mineral content (AAS method), vitamin C (AOAC, 2005) [2] and β carotene as per the methods quoted by AOAC, 2005 [2].

Statistical analysis
All data were analysed using Statistical Package for Social Sciences (SPSS) IBM SPSS Statistics software. Analysis of variance (ANOVA) was used to compare any significant differences between the samples. Values were expressed as means ± standard deviations while differences were considered significant at $p<0.05$. All analyses were carried out in triplicate.

Fig 1: Flow chart of formulation and production of β-carotene enhanced chocolates

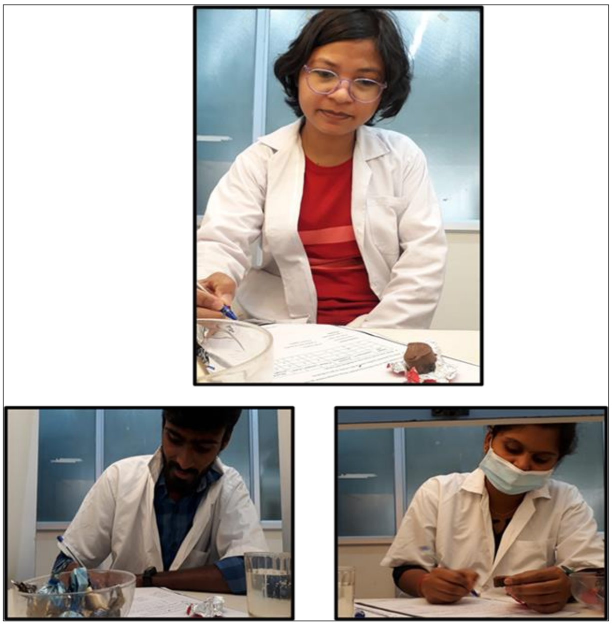

Plate 1: Sensory evaluation of β-carotene enhanced chocolate

Results and Discussion

Results and discussion deal with the tabulation of the data based on a statistical analysis of the experiment and presentation of the results with relevant discussions. The results of the study are presented under the following headings:

β-carotene enhanced chocolates

Basella and moringa chocolates were standardized by adding different levels of processed basella and moringa leaves powder with appropriate combinations. Similarly, Zaid and Nadir (2014) [12] prepared chocolates by using different levels of moringa leaves powder (MLP) and recorded that the incorporation of MLP in chocolates up to 20 per cent did not cause any significant effect on the organoleptic quality attributes of chocolate. A higher proportion of leaf powder led to a reduction in palatability and creamy texture of chocolates which makes the chocolate unacceptable. Aroyeun *et al.* (2019) [4] developed innovative spicy chocolate by partial replacement of cocoa nibs with different spice powders and reported a significant difference in proximate composition.

Subjective evaluation of β-carotene enhanced chocolates

In order to select the best acceptable combination of ingredients which results in an acceptable product was evaluated by sensory panellists based on subjective parameters. The sensory evaluation showed very acceptable scores for the control and moringa chocolates as comparable to the basella chocolate.

As depicted in Table 2 there was significantly higher ($p<0.05$) overall acceptability score was reported for control chocolate (8.85) followed by moringa chocolate M_4 (8.82). It was found that moringa chocolate (M_4) with 8 per cent of leaf powder was most accepted among the treated chocolate with respect to appearance (9.00), texture (8.67), colour (8.95), flavour (8.57), taste (8.86), aroma (8.88) and overall acceptability (8.82). However, basella chocolates were found unacceptable and did not include in the further analysis as they scored below 5 score (overall acceptability 5.10 to 3.82) on the nine-point hedonic scale by panel members due to bitter taste, rough texture and lack of palatability.

Lee *et al.* (2018) [9] standardized oat chocolate by using dark chocolate, white chocolate, fresh cream, antioxidants (green tea and lavender) and cactus in different proportions. Similarly, Chandegara *et al.*, (2018) [6] approved that milk chocolate prepared using dairy milk (control) was the most acceptable whereas chocolates treated with vegetable milk were significantly different in acceptability from the control. Muhammad *et al*. (2021) [10] developed chocolate with the addition of powdered mung bean, fenugreek seed and moringa leaf, which were added in different ratios and analysed for sensory attributes. The results showed that the addition of mung bean, fenugreek seed and moringa leaf significantly decreased the acceptance for all the parameters and got a score range of 3.8-4.7, 2.1-2.9 and 4.5–4.8 respectively.

Table 2: Sensory evaluation of β-carotene enhanced chocolates

Chocolates		Appearance	Texture	Colour	Flavour	Taste	Aroma	OAA
Control		8.86	8.86	8.98	8.74	8.88	8.80	8.85
Basella chocolate	B_1	6.26	4.86	5.76	4.14	4.86	4.71	5.10
	B_2	6.48	4.52	4.07	4.29	4.71	4.52	4.77
	B_3	6.14	3.43	4.02	3.24	4.52	4.19	4.26
	B_4	6.17	3.05	4.05	2.81	3.33	3.52	3.82
	B5	6.00	3.01	3.92	2.53	2.99	2.00	3.40
Moringa chocolate	M_1	9.00	8.36	8.79	8.41	8.86	8.60	8.67
	M_2	8.24	7.62	8.07	7.00	6.81	7.95	7.62
	M_3	8.76	8.05	8.76	7.74	7.64	7.76	8.12
	M_4	9.00	8.67	8.95	8.57	8.86	8.88	8.82
	M_5	8.00	7.11	7.33	6.98	6.32	6.91	7.11
F test		*	*	*	*	*	*	*
S.Em±		0.16	0.14	0.15	0.19	0.14	0.15	0.07
CD at 5% Level		0.43	0.40	0.42	0.54	0.40	0.42	0.20

OAA: overall acceptability
* Significant ($p<0.05$)

Nutritional composition of β-carotene enhanced chocolate

The nutritional parameters estimated for the best accepted β-carotene enhanced chocolate (moringa chocolate) include proximate composition, mineral content and β-carotene content. The values are projected here;

Proximate composition Proximate analysis refers to the quantitative analysis of macromolecules in food. A combination of different techniques was used to determine nutrient composition. The proximate composition of β-carotene enhanced chocolate is explained with the help of Table 3.

Moisture (%)

Moisture content was found to be significantly higher ($p<0.05$) in moringa chocolate (2.13±0.06%) as compared to control chocolate (1.50±0.30%) and higher moisture content in moringa chocolates may be due to the addition of sugar in the form of caramels. Similarly, Fatmawati *et al*. (2020) [7] also reported the moisture content of the moringa chocolate bar ranged between 1.89 to 2.91 per cent which is quite similar to the present study. The moisture of chocolate moisture content must be between 0.5 and 2.50 per cent and higher moisture affects rheological parameters and consequently caused difficulty in chocolate flow properties This is because of sugar agglomeration leading to grittiness in the chocolate and also causing an increase in its viscosity and yield value [4].

Protein (g/100g)

Protein helps the body to fight against infections in the form of antibodies. Proteins are crucial to the living course and carry out wide functions essential for life. Hence, protein is one of the vital nutrients required for the body and should be provided in sufficient amounts through diet (Arora *et al*., 2003) [3]. The protein content of β-carotene enhanced chocolates increased from 5.73±0.10 to 7.68±0.47 g/100g with a significant difference. The per cent protein obtained in this study was higher than the work of Aroyeun *et al.* (2019) [4] who reported a value of 6.34 to 7.44 in innovative spicy chocolate. Fatmawati *et al.* (2020) [7] reported 5.32 to 8.09 per cent of protein in different variations of the moringa chocolate energy bar.

Fat (g/100g)

Fat is an important component of the diet and performs different functions in the body. It imparts palatability and retards stomach emptying time and also the presence of fat in the diet is required for absorption and utilization of fat-soluble vitamins (Murthy *et al.*, 1996) [11]. The higher fat content was recorded in control chocolates (30.67±0.72 g/100g) as compared to moringa chocolate (28.57±0.71). It shows that addition of moringa leaf powder replaced the significant amount of fat in chocolate. These results are in accordance with the findings of Aroyeun *et al.* (2019) [4] who reported 31.53 to 34.43 per cent of fat in chocolate.

Ash (g/100g)

Ash refers to inorganic material and residue that remains after heating, excludes water and other organic material such as fat, protein, *etc.* Ash content represents the total mineral content of food. Moringa chocolate had a significantly higher ash content (4.89±0.21 g/100g**)** than control chocolate (4.12±0.04g/100g) which was higher than the values reported by Aroyeun *et al.* (2019) [4]. This signifies the good mineral content in moringa incorporated chocolates.

Crude fibre (g/100g)

Crude fibre is the residue obtained by subsequent washing with acid and alkali. The higher crude fibre was found in moringa chocolate (7.50±0.46 g/100g). This shows that addition of moringa leaf had increased the crude fibre content of chocolates which makes these chocolates functional in nature. These findings are similar to the findings of Aroyeun *et al.* (2019) [4], who reported values for crude fibre between 2.35 to 0 2.68 per cent. The values obtained were far higher than the one reported by Fatmawati *et al.* (2020) [7] who reported a range of values of 0.33 to 0.48 g/100g. It is obvious that the chocolate containing moringa leaves can have health benefits and can serve as a snack that can enhance the health of the gastrointestinal tract. It could contribute to easy bowel movement also. However, the values were within the regulated levels for standard chocolate.

Table 3: Proximate composition of β-carotene enhanced chocolates (g/100g)

Chocolates Nutrients	Control dark chocolate	Moringa Dark chocolate	F-test	S.Em±	C.D. at 5% level
Moisture (%)	1.50±0.30	2.13±0.06	*	0.09	0.3
Protein	5.73±0.10	7.68±0.47	*	0.23	0.75
Fat	30.67±0.72	28.57±0.71	*	0.53	1.74
Ash	4.12±0.04	4.89±0.21	*	0.07	0.24
Fiber	5.36±0.43	7.50±0.46	*	0.3	0.99
Total CHO	57.99±0.99	56.73±0.34	*	0.54	1.21
Available CHO	52.60±1.35	49.23±0.69	*	0.66	2.19
Energy (Kcal)	531±2.75	514±3.84	*	2.58	8.53

CHO: Carbohydrates

* Significant ($p<0.05$)

Total and available carbohydrates (g/100g)

Carbohydrates are energy-yielding substances. Although starch and other digestible carbohydrates are needed by the body for energy, non-digestible carbohydrates add bulk to the diet, which is equally important. Total and available carbohydrate was found to be highest in control chocolate (57.99±0.99 and 52.60±1.35 g/100g respectively) followed by moringa chocolate (56.73±0.34 and 49.23±0.69 g/100g) which is in tandem with the reports of Aroyeun *et al.* (2019) [4] and Fatmawati *et al.* (2020) [7], who reported the carbohydrate contents of chocolate between 47.83 to 59.63 per cent. As total and available carbohydrates are computed. The sample which has lower amounts of other nutrients was found to have a higher content of carbohydrates and vice versa.

Energy (Kcal)

The higher energy value was recorded in control chocolate (531±2.75 Kcal), followed by moringa chocolate (514±3.84Kcal). the higher energy value due to higher fat content in control chocolates. As energy is computed using the formula, the sample which had higher amounts of protein, fat and carbohydrate, was found to have higher amounts of energy. However, Fatmawati *et al.* (2020) [7] reported a lesser energy value (406.16 Kcal) than the present study. The difference may be due to the variation of the other ingredients used, where all the proximate parameters except moisture contributes to overall calorific value.

Mineral estimation (mg/100g)

Table 4 depicts that significantly higher iron (9.76±0.11 mg/100g), zinc (3.89±0.05 mg/100g), calcium (425.09±5.35mg/100g) and phosphorus (340.99±2.83 mg/100g) contents were reported in moringa chocolates as compared to control chocolate. These findings are in line with the findings of Fatmawati *et al.* (2020) [7] who observed that the addition of processed moringa leaf powder increased the mineral contents.

Table 4: Mineral composition of β-carotene enhanced chocolates (mg/100g)

Chocolates	Iron	Zinc	Calcium	Phosphorus
Control dark chocolate	4.85±0.32	3.41±0.07	163.56±11.29	271.59±3.97
Moringa dark chocolate	9.76±0.11	3.89±0.05	425.09±5.35	340.99±2.83
F-test	*	*	*	*
S.Em±	0.11	0.04	3.81	3.10
C.D. at a 5% level	0.37	0.12	12.60	10.26

* Significant (p<0.05)

β-carotene content (µg/100g)

The observation of Table 5 shows, the β-carotene content of chocolates. The β-carotene content in the chocolate increased from 887±80 µg100/g in control chocolates to 9345±160 µg/100g in moringa chocolate. A significantly (p<0.05) higher amount was recorded in moringa chocolates as compared to control chocolates which indicate the addition of moringa leaf powder increased β-carotene content significantly. β-carotene is one of the antioxidant that needs to be provided through foods. Hence confectionary made with moringa incorporation may fulfil the needs of children and college students.

Table 5: β-carotene content in β-carotene enhanced chocolates (µg/100g)

Chocolate	β-carotene
Control dark chocolate	887±80
Moringa dark chocolate	9345±160
F-test	*
S.Em±	0.132
CD at 5%	0.202

* Significant (p<0.05)
NS: Non-significant

Conclusion

β-carotene enhanced chocolate is a noble confectionary product with the goodness of super green *Moringa oleifera*. The product will help in uplifting the processing scenario of *Moringa oleifera* in the community. In terms of nutrition, it is a collected source of nutrients with higher protein, fibre, calcium, β-carotene and lower fat content which makes it a premium product.

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