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Proximate, Physicochemical and Functional Analysis of Herbal Frozen Dessert

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ABSTRACT: The aim of the study was to estimate the physiochemical, proximate and sensory attributes of functional frozen dessert prepared with *Plectranthus ambionicus* herb and rice bran oil. Inclusion of these compounds results in variety of health benefits like antioxidant properties, antimicrobial properties etc which naturally absent in normal frozen dessert. The inclusion level of herb was standardized based on sensory evaluation of panelists. The development of frozen dessert includes the addition of herb in different concentrations and replacement of saturated fat by rice bran oil to obtain a product with functional attributes. Among the various concentrations, 30% herb obtained highest overall acceptability value of 8.76 was chosen as a finalized combination. The increase of the herbal extract leads to decrease in viscosity, overrun, pH, total solids, carbohydrates whereas increases the titratable acidity, moisture, ash, protein content of the frozen dessert. There was a significant increase in the antioxidant activity, total phenolic content and total flavonoid content. Thus, by using fat substitute and herb in frozen dessert resulted in antioxidant rich functional dairy product.

Keywords: Functional, rice bran oil, herbal, frozen dessert, antioxidant.

INTRODUCTION

According to World Health Organization survey, 70–80% of the world's population relies on modern medicine, primarily herbal sources, for their primary treatment (Chan, 2003). The herbs are not only used in home medicine but also added as an ingredient in food systems like flavouring agents, preservatives and health substance. *Plectranthus amboinicus* (Indian Borage) is a perennial herb belonging to the family Lamiaceae which occurs naturally throughout the tropics and warm regions of Africa, Asia, and Australia. Studies revealed that there are 76 volatile and 30 non-volatile compounds belonging to different classes of phytochemicals such as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols, aldehydes. *Plectranthus amboinicus* (Indian borage) has a wide range of anti-microbial, anti-inflammatory, anti-tumor, wound healing, anti-epileptic, larvicidal, antioxidant, and analgesic activities. Also, it is effective against respiratory, cardiovascular, oral, skin, digestive, and urinary diseases (Greetha *et al*., 2016). In order to substitute synthetic chemicals in food and boost the amount of antioxidant substances, especially in high-fat diets, plant extracts are being used more and more. Antioxidants work as cell protectors in people and protect DNA (deoxyribonucleic acid) from damage and splicing, which lowers the risk of several cancers. (Amarowicz and Shahidi 2017).

Consumers have become more health conscious and growing interest of consumers towards the functional foods has led the food industry to increase the functional value of dairy products (Chandan *et al*., 2007). Fat substitutes are compounds that help replace the mouth feel of fats by contributing a greater viscosity to the liquid phase in the mouth (Thomas and Nora 2016) Omega-3 fatty acids are polyunsaturated fatty acids. Directions of the American Heart Association (AHA) also recommends that consumers increase their intake of omega-3 fatty acids for healthy lifestyle. Omega 3 fatty acids play an important role in the development of the infant's brain, eyes, and cardiovascular system and also aids in immunomodulatory functions, lowering HDL value, mood disorders, age-related macular degeneration, etc. (Goyal *et al*., 2014). Rice bran oil (RBO) helps to boost the immune system and prevent the process of premature aging and age-related neurodegenerative diseases. Because of its cardiac-friendly phytochemicals and antioxidant potentials, RBO is one of the healthiest

edible oils due to its balanced source of saturated, monounsaturated, and polyunsaturated fatty acids with a ratio of 0.6:1.1:1. RBO has been categorized as healthy edible oil for human consumption and has attained the status of "heart-healthy oil" (Lai *et al*., 2019).Gamma oryzanol is considered as a potent antioxidant in rice bran oil. A test tube study found that, when comparing to vitamin E, gamma oryzanol effectively stopped tissue oxidation (Hiramitisu and Armstrong 1991). Recent research indicates that RBO has 2.5–5 times better oxidative stability than groundnut oil (Nayik *et al*., 2015). RBO has been shown to effectively lower the Low density Lipoprotein (LDL) by 7–10% and is an excellent source of mono-unsaturated fats and cholesterol. The substance's antioxidant action is maintained by the high smoking point (213^{o}C), which inhibits fatty acid breakdown at high temperatures. Ice cream is a promising carrier for the stabilization and in vivo administration of bioactive chemicals because of its structure, colloidal form, and low-temperature storage (Soukoulis *et al*., 2014). The structure, colloidal makeup, and low-temperature storage of ice cream make it a particularly attractive carrier for the stabilization and in vivo administration of bioactive substances. Incorporation of Indian borage into frozen desserts will make the herb organoleptically acceptable and reduce the effect of the consumption of frozen desserts such as colds, cough and the shelf life will be increased due to the antioxidant activity of the herb. Hence this project is aimed to develop Indian borage and Fat substitute incorporated frozen dessert.

MATERIALS AND METHODS

Fresh milk was procured from cattle dairy farm, college of food and dairy technology. The herb was collected from the CFDT campus. Butter, sugar, Rice bran oil, skim milk powder, stabilizer was procured from local market.

Methods. The frozen dessert was prepared with varying the proportion of herbal extract. The formulation was presented in Table 1. Flow chart for the preparation of herbal extract was shown in Fig. 1. Flow chart for the preparation of herbal frozen dessert was shown in Fig. 2. The proportion of milk, skim milk powder, sugar, fat in each sample was kept constant.

Table 1: Formulation of herbal frozen dessert.

Treatments	Milk (ml)	Fat (%)	Sugar (gm)	Stabilizer (%)	Herb (%)
C	750	10	250	0.5	10
T_1	750	10	250	0.5	20
T_2	750	10	250	0.5	30

Preparation of herbal extract. The fresh leaves were washed in running water for three times. Herb and distilled water in a ratio of 1:2 was extracted 90ºC for 15 mins. Supernatant after filtration was stored at -4ºC for further usage.

Preparation of frozen dessert. The frozen dessert was prepared as per (Sukumar De, 2008). The milk was preheated to 40ºC. Then the mix ingredients like skim milk powder, sugar, RBO, stabilizer were added at 60ºC. Then the mix were homogenized and kept for aging at 4ºC overnight. Herbal extract (10%, 20%, 30%) was added before freezing. After freezing the frozen dessert was packed at -20ºC.

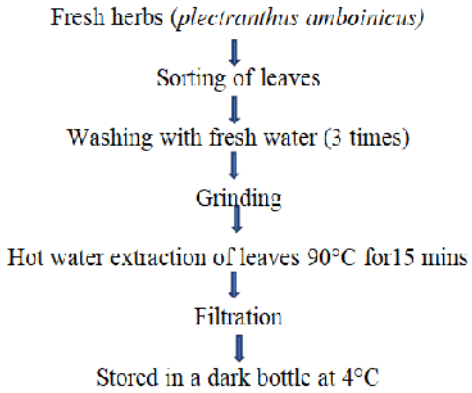

Fig. 1. Flow chart for the preparation of herbal extract.

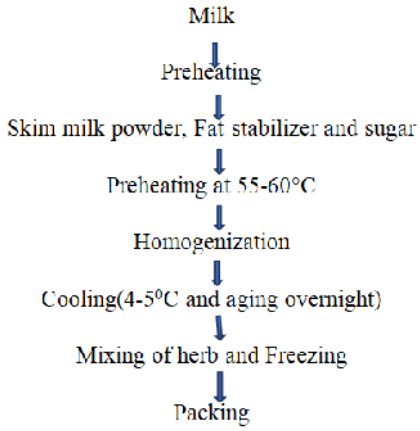

Fig. 2. Flow chart for the preparation of frozen dessert.

Sensory evaluation. The organoleptical evaluation of frozen dessert was done by panel member, who belongs to the staffs and students. The panelists received a standard score card (with a "9" point hedonic scale) to record their ratings for the product's general acceptability as well as sensory qualities colour and appearance, flavour, texture, taste and overall acceptability (Maximo *et al*., 1984).

Proximate composition. The proximate composition includes moisture, protein, fat, total ash, fibre and total carbohydrate according to their respective standard methods as described in (AOAC, 2000).

Total Solids: Total Solids were calculated by using FSSAI Laboratory Manual 1.

Physiochemical properties. pH: The pH was determined using a pH meter (AOAC, 2006).

Acidity: Acidity was determined in the frozen dessert as prescribed in Arbuckle, (1986).

Overrun: Overrun were calculated by the method given in (Sukumar De, 2008). A known volume of mixture was weighed accurately (W1) and then the same volume of frozen dessert was weighed (W2) and the overrun was determined as follows:

$$\% \text{ Overrun} = [(W1 - W_2)/W_2] \times 100$$

Melting resistance: Melting quality of ice cream was observed by placing a scoopful of the sample on a dish and noting its response to melting from time to time as the other qualities of ice-cream was examined. FSSAI Part:1 (2012)

Viscosity: The Viscosity of samples was determined as prescribed in (Muse and Hartel 2004).

Specific gravity: Specific gravity was determined as prescribed in Arbuckle, (1986).

Antioxidant activity. The DPPH (0.1 mM) reagent was prepared with methanol .3.8 ml of reagent was added to 100 µL of the sample, the mixture was kept in vortex for 15 s, followed by incubation of the tubes in the dark for 24 h at room temperature. By using spectrophotometer, the absorbance value was estimated at 515nm. The Results given as a inhibition percentage of DPPH radical (Akca and Akpinar 2021).

Total polyphenolic activity. The Total polyphenolic activity of frozen dessert samples was determined as prescribed by Beskow *et al*. (2015). 0.2g of sample were mixed with 1ml of folin- ciocalteu reagent and kept of 5 mins. 0.8 ml of 7.5% sodium carbonate were added to the test tube and incubated for 1 hr. the absorbance value was measured by spectrophotometer at 725nm using gallic acid as a standard. The total phenolic content of sample was estimated by using a calibration curve (R2 =0.999) and denoted as mg of gallic acid equivalents (GAE)/mL.

Total flavonoids content. The Total flavonoids of frozen dessert samples was determined as prescribed by Zhishen *et al*. (1999). A 12.5 ml of distilled water was mixed with 0.5 g of sample. Then 0.75 ml of 5% $NaNO_2$ solution was added. Followed by 6 mins incubation, 10% sodium carbonate (1.5 ml) was added. and following a 5-min incubation, 5 ml of 1 M NaOH was added. Then the absorbance value was measured by spectrophotometer at 510nm. The total flavonoid content is reported as catechin equivalents (mg/g) using a standard curve. (R2 = 0.997).

Statistical analysis. The SPSS software v20.00 (SPSS Inc. Chicago, IL, USA) was used. One-way ANOVA and Duncan's Multiple Range Test were utilized to analyses significant differences between the results. The experiments were assayed in duplicates and the analyses were performed with three parallels. The results were expressed as mean ± SE and the least significant difference at $P < 0.05$ was calculated using Duncan's multiple range tests as per Snedecor and Cochran, (1994).

RESULTS AND DISCUSSION

Sensory evaluation. *Plectrantus ambionicus* is a rich source of phenols and flavonoids which produce various functional benefits. The herb is commonly used as flavoring agent in food products. RBO contains 37% of polyunsaturated fatty acid and 42% of monounsaturated fatty acids. The main of this study is aimed to replace the saturated fatty acid which present in butter into unsaturated fatty acids. The effect of herb and fat level on the sensory attributes of frozen dessert were presented in Table 2. The average values of color and appearance, taste, texture, flavor and overall acceptability showed highly significant difference within the treatments. The highest overall acceptability obtained by T_3.so it had been finalized for further analysis. The study was comparable with (Brito-oliveira and Pinho 2016) who developed β carotene ice cream.

Table 2: Effect of herb level on the sensory acceptance of herbal frozen dessert.

Treatments	Colour and Appearance	Flavor	Texture	Taste	Overall acceptability
C	7.67±0.12^{b}	8.00±0.00^{c}	8.13±0.09^{c}	7.93±0.20^{d}	8.13±0.09^{d}
T_1	7.13±0.09^{a}	6.53±0.13^{a}	7.53±0.13^{b}	6.67±0.23^{ab}	7.13±0.09^{b}
T_2	7.73±0.11^{b}	7.40±0.13^{b}	7.47±0.13^{b}	7.07±0.26^{b}	7.60±0.13^{c}
T_3	7.73±0.11^{b}	8.73±0.11^{d}	8.73±0.11^{d}	8.20±0.22^{d}	8.20±0.22^{d}
T_4	6.80±0.14^{a}	6.47±0.13^{a}	6.20±0.10^{a}	6.33±0.12^{a}	6.47±0.13^{a}
T_5	7.07±0.24^{a}	7.07±0.22^{b}	6.47±0.13^{a}	6.47±0.13^{ab}	6.47±0.13^{a}
F value	7.343**	39.058**	64.093**	14.744**	30.366**

Proximate composition. Incorporation of herbal extract significantly affected the values of moisture, protein, ash, fibre, carbohydrate and energy value, pH and acidity in the frozen dessert which is presented in Table 3. There was no significant difference for fat percentage. The addition of herbal extract increased the moisture because the herb itself contains 95.3% of moisture content (Gupta *et al*., 2005). The ash and protein content were increased when compared to the control. Fibre is not detected in control, but there was a increasing trend in herbal frozen dessert. This study was in line with Reddy *et al*. (2013). Gupta *et al.* (2005) stated that the edible portion of herb contains 0.6% protein, 1.06 % ash and 1.87 % dietary fibre respectively. The carbohydrate and energy value were reduced due to the replacement of total solids by water which were added in the preparation of frozen dessert. The similar findings were observed by Giri and Rao (2014).

Table 3: proximate composition of herbal frozen dessert.

Parameters	Treatments		T value
	Control	HFD	
Moisture (%)	63.32± 0.15	67.13± 0.07	21.827**
Fat (g/100g)	10.45± 0.13	10.16± 0.05	1.958 ^{NS}
Protein (g/100g)	3.52± 0.01	3.92± 0.02	14.142**
Carbohydrate (g/100g)	22.33± 0.08	17.85± 0.08	37.068**
Energy value (Kcal)	195.16± 0.13	179.73± 0.08	95.935**
Fibre (g/100g)	0	0.02± 0.01	15.834**
Ash (g/100g)	0.63± 0.01	0.81± 0.00	10.941**

Physiochemical properties. The physiochemical properties of herbal frozen dessert were presented in Table 4. Addition of herbal extract increased the titratable acidity where in turns drops the pH. This might due to the presence of rosemaric acid, chlorogenic acid, caffeic acid, coumaric acid and phenolic compounds present in herb. Goraya and Bajwa (2015) reported the acidity of ice cream was enhanced by the addition of amla containing ascorbic acid. Total solids and viscosity were decreased due to the addition of herbal extract which contains higher moisture content and less total solids. The milk protein's whipping ability was decreased by hindering the incorporation of air results in decreased overrun. Similar findings were reported by Patil *et al*. (2018) who performed comparative studies in herbal and crystal menthol ice cream. This is also because of the modified oil in water emulsion or other physical state conversion. There was no significant difference in specific gravity. The melting time of the herbal frozen dessert increased when compared to ice cream. This might be due to the increased concentration of herbal extract which was incorporated in the preparation. The findings of this study were on par with Ali *et al*. (2015). They evaluated that the difference in the melting time is due to the effect of higher concentration of herb addition. Siddhu and Singh (2011) also noticed the enhancement of melting time attributed by the addition of dates in ice cream.

Table 4: Physiochemical properties of herbal frozen dessert.

Parameters	Treatments		T value
	Control	Herbal frozen dessert	
pH	6.35± 0.05	6.26± 0.12	0.605*
Titratable acidity (%)	0.181± 0.01	0.271± 0.01	9.798**
Overrun (%)	39.12± 0.06	33.24± 0.53	9.937**
Viscosity (cp)	27.01± 0.04	24.54± 0.02	44.694**
Specific gravity	1.05± 0.05	1.01 ± 0.00	0.834 ^{NS}
Melting resistance (ml/min)	0.54± 0.01	0.62± 0.01	3.664**
Total solids (%)	36.73± 0 .58	32.89± 0 .73	4.069**

Functional properties. Frozen dessert incorporated with *Plectranthus ambionicus* herb and rice bran oil showed significant difference in the functional properties like antioxidant activity, total phenolic content and total flavonoid content (Table 5). The antioxidant activity of the herbal frozen dessert was increased. This might be due to the considerable amount of polyphenols and phytosterols naturally present in *Plectranthus ambionicus* herb and rice bran oil respectively. The inclusion of herbs powder in the ice cream significantly altered the antioxidant properties of the ice cream samples. The total polyphenolic content of herbal frozen dessert was increased than the control. On account of the inherent polyphenols present in herb (thymol, carvacrol, caffeic acid, gallic acid, rosemaric acid, coumaric acid etc,) induced the phenolic content in frozen dessert. Akca and Akpinar (2021), studied the effects of Grape, pomegranate, Sesame Seed Powder

and Their Oils on Probiotic Ice Cream. The results concluded that among the seed pulp powders, the grape seed had the highest phenolic content and antioxidant activity. Subsequently, the total flavonoid content of herbal frozen dessert was also increased because of the natural supply of flavonoid compounds in the herb. The findings were in close resemblance with (Vital *et al.*, 2017), disclosed that the significant load of polyphenols displays functional characteristics in grape juice residue ice cream.

Table 5: Functional properties of herbal frozen dessert.

Parameters	Treatments		T value
	Control	Herbal frozen dessert	
Antioxidant activity (%)	4.73± 0.09	77.87± 0.16	161.268**
Total Polyphenolic activity (mgGAE/mL)	0	12.43± 0.01	103.694**
Total Flavonoid content (mg/g)	0	3.88± 0.02	175.20**

CONCLUSION

The functional properties produced by the secondary metabolites which are naturally present in the herb includes alkaloids, polyphenols make these herb as a health substance. People in the recent times have retrieved the knowledge of herbal usage because of the scenario with the COVID epidemic. *Plectranthus ambionicus* herb and rice bran oil can be used to formulate functional products because it contains significant amounts of polyphenols and phytosterols which displays antioxidant activity. Addition of herbal extract significantly altered the proximate composition and physiochemical characteristics. Ice creams formulated with functional ingredients showed higher functional properties than the control. However, the amount of herbal extract in the frozen dessert above 30 percent reduced the sensory scores significantly ($P<0.01$). It can be concluded that *Plectranthus ambionicus* herb and rice bran oil can be used as a natural ingredient to formulate a novel dairy product with enriched functional properties. Future studies can be made to improve the textural stability of frozen dessert with increased herbal extract concentrations.

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Conflict of Interest. None.

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