Optimization and characterization of functional chocolate with addition of butter fruit milkshake powder as a source of phenolic, flavonoid and carotenoid

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Abstract: Butter fruit milkshake powder (BFMS) is a healthful mixture of avocado pulp and dairy components with high nutritional and antioxidant activity. In this study, an attempt has been made to enhance the nutraceutical and functional properties of the chocolate with BFMS powder at the 15-60% level based on the physical, nutritional, sensory attributes, and storage of study. Sensory studies of chocolate indicated that maximum sensory score was found at 30% incorporation of BFMS without adverse effects. BFMS powder's addition increased the contents of fat, dietary fibers, proteins, phenolic, flavonoid, and beta carotenoid content of chocolate. Mineral element (Ca, Mg, Fe, Zn, and Mn) were also increased with the addition of BFMS in chocolate. Storage studies of BFMS powder fortification in chocolate revealed that butter fruit milkshake chocolate (BFMSC) significantly more stable than control chocolate and accepted by consumers up to 60 days of storage. This study showed that BFMS powder fortified chocolate was nutritionally improved and economical with acceptable sensory properties. This may increase the market's revenue and enable chocolate companies to develop a functional new chocolate type.

Keywords: Avocado, Bioactive compound, Butter fruit milkshake powder, Chocolate; Shelf Life

Introduction

Chocolate is one of the most popular and consumed delicacies in worldwide. It is a complex multiphase system of particulate (cocoa, milk components, sugar) and continuous phases (milk fat, cocoa butter, and emulsifiers) and color, gloss, shape, surface texture, roughness, shininess, and translucency is its visual and appearance attributes (Briones et al. 2005). Chocolate represent a functional food product due to its high level of flavonoid content, which positively impacts human health (Wollgast and Anklam 2000). Latif (2013) also reviewed the impact of chocolate on the human health and illustrated that chocolate/cocoa is a rich source of antioxidants with radical scavenging activity due to large concentrations of flavonoids, epicatechin, catechin and, procyanidins. Cocoa mass also contains macro and micro minerals such as potassium (K), phosphorus (P), copper (Cu), iron (Fe), zinc (Zn), and magnesium (Mg).

In the last years, a marked change in food consumption patterns, consumers are getting more demanding in the food market for nutritious, palatable, affordable, and easily consumed food products (Mussatto and Mancilha 2007). Consumers would also like to have more alternative to choose food products than ever before. In view of the above, food technologist and researcher launch organic, prebiotic, probiotic, and high-cocoa polyphenol-rich chocolate that can be effectively found in the market (Erdem et al. 2014). Presently in COVID-19 pandemic situation, functional foods acquire a prominent place in the food market due to their capability to provide positive health effects beyond their conventional nutritional benefits. So, new food product development (NPD) has been increased using functional food ingredients. Also, government and companies invest in the food marketing field to develop new products.

Avocado (*Persea americana*) is a subtropical fruit, highly appreciated by consumers due to its nutritional values. Avocado (Butter fruit) a rich source of monounsaturated fatty acids that increase the level of HDL that is good for the heart; polyhydroxylated fatty alcohols (PFA) that suppress inflammatory response and protect against UV-induced damage in skin cells; lipophilic acetogenins exhibited the highest antioxidant capacity (Rosenblat et al. 2011; Souza et al. 2011;

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Bhuyan et al. 2019). Additionally avocado is rich in vitamins such as tocopherol, ascorbic acid, pyridoxine, beta-carotene, and minerals like K and Mg (Fulgoni et al. 2013). According to Ortiz-Avila et al. (2015), avocado consumption gives protection from hypersensitivity, coronary heart disease, oxidative damage, anti-mycobacterial activity, and improves immune systems.

However, an avocado grows only in a particular region, and its shelf life is also very short, so there is a need for researchers and food technologists to discover a method to preserve the avocado with its nutritional properties. So, developed butter fruit milkshake powder (BFMS) revealed that these products provide a healthful mixture of dairy and fruit components with high nutritional and longer shelf-life. With these contexts, the objective of present study was to optimize and characterize the functional chocolate using butter fruit milkshake powder as a source of phenolic, flavonoid and carotenoid as well as cell viability of chocolate and the effect of storage (two months) on the sensory attributes was also evaluated.

Materials and Methods

Raw materials (cocoa powder, milk powder, butter, sugar) for the preparation of chocolate were purchased from the local market. All the required chemicals used were of analytical grade.

Preparation of Butter fruit milkshake powder (BFMS)

Lyophilized butter fruit milkshake powder (BFMS) was prepared by the method described by Pandey et al. (2020) in which avocado pulp, pasteurized toned milk (Amul), sugar, and maltodextrin was mixed at 84, 28, 10, and 6 % respectively at -20°C under 0.035 mbar pressure.

Preparation of chocolate

According to the methodology reported by Mayank and Kumar (2012), the chocolate was prepared with minor modifications. Ingredients used were BFMS, cocoa powder, sugar, butter, whole milk powder (fat 26%). The quantity used to prepare control chocolate as follows: 100 g cocoa powder, 30 g milk powder, 62 g butter, 150 g sugar, and water 117 mL. Four blends were prepared with the replacement of cocoa powder by BFMS that is 100:0(control); 85:15, 70:30; 55:45; and 40:60 as shown in Table 1. Sugar syrup (one string) was made with a mixture of sugar and water in the oven for 4-5 min at 60°C. Then butter was mixed in sugar syrup well till melted and afterwards cocoa powder and milk powder mixed above mixture gradually till glossy. The mixer was then poured in a greased pan and refrigerated for 10-15 min until it became solid. Prepared control chocolate (CC) and BFMS chocolate (BFMSC) were packed and stored for further analysis.

Sensory admissibility of chocolate

Optimization of BFMS in chocolate was decided by organoleptic evaluation by 15 semi-trained panellists. The sensory evaluation

was planned in quiet with properly illuminated lights, humid free & ventilated room by maintaining hygienic condition. The sensory evaluation was done according to method explained by Żyżelewicz et al. (2018), where sensory attributes, i.e., colour and appearance, consistency (hardness, smoothness) and mouthfeel, flavour, and overall acceptability. The sensory analysis was done using 5-point scale, 5 meant extremely desired quality, 4 was desirable quality, 3 was tolerable quality, 2 represented dislike, and one was for a defective product.

Determination of nutritional composition and trace elements

A moisture analyzer was used to analyze moisture, which works on halogen heating technology. The protein content was estimated with Kjeldahl nitrogen analyzer (Kel Plus –DISIYL EMS), fat content with SOCS-PLUS model SCS8, Fibertec™ 1023 - semi-automatic crude fibre analyzer is used to determine crude fibre. All proximate composition of chocolate sample was analyzed according to AOAC (2000) method. The energy value was determined by bomb calorimeter (Sundy Science and Technology Co., Ltd).

Macro and microelements like calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and manganese (Mn) were detected by using AAS (Atomic Absorption Spectrophotometer) (Perkin Elmer) according to the method explained by Aparna et al. (2018).

Texture and color of chocolate

Customer adequacy is affected by the texture and color of chocolate. Color Flex EZ determined color value in term of L*, a*, and b*, where L* (lightness/ darkness), a* (redness/ greenness) and b* (blueness/ yellowness).

The texture of the chocolate was determined by using a TA.XT plus texture analyzer with a sharp cutting blade probe. The hardness and breaking strength of chocolate were recorded by force required to break, and the average value was recorded.

Analysis of Nutraceuticals for FGSE, CC, and FGSC

Estimation of polyphenolics and flavonoids contents

BFMS, CC, and BFMSC sample extracts were prepared for the estimation of polyphenolics and flavonoids compounds by the procedure described Tyagi et al. (2020) in which formic acid / water /methanol(0.3/29.7/70 v/v/v) used as extracted agent.

Folin-Ciocalteu phenol reagent is used to evaluate the total polyphenol content (TPC) of the sample by the method described by Stoilova et al. (2007). Standard curve was prepared by gallic acid (5-60µg /mL; $y = 0.00137x + 0.3059$; $r^2 = 0.989$; x is the concentration of solution; y is the absorbance). The results were expressed as GAEG-1 of dw.

Flavonoid content in sample extracts was determined by using the AlCl_3 colorimetric method explained by Tyagi et al. (2020). Rutoside trihydrate was used to plot standard curve ($5\text{--}120\mu\text{g/mL}$; $y = 0.0054x + 0.0821$; $r^2 = 0.9974$; x is the concentration of solution; y is the absorbance). The results were expressed in RTEg-1 of dw.

Carotenoid content

β -carotene was estimated by the method as suggested by Srivastava and Kumar (2003). 5g of a sample of butter fruit milkshake powder (BFMS) was grinded with few crystals of Na_2SO_4 and homogenized with 10 ml $(\text{CH}_3)_2\text{CO}$. It was decanted, and then the supernatant was collected and transferred supernatant to a separatory flask. 10 ml of petroleum ether was added in separating funnel and mixed. Two layers were found, the lower layer discarded and the upper layer collected and, OD was recorded at 452 nm. The result was expressed in $\mu\text{g}/100\text{mg}$.

Storage studies

Water activity

Water activity (a_w) of the chocolate samples was determined by water activity meter (Aqua-Lab, model 3TE, Decagon Devices Inc., Pullman, WA, USA) with 25°C temperature.

Peroxide value (PV)

Peroxide value was estimated by the American Oil Chemist's Society (AOCS) (1990) method. 3–5g of the sample was taken in an iodine flask then 20 mL of CHCl_3 followed by 30ml of CH_3COOH were added. 1 mL of KI solution was added to the sample and left to stand in the dark for 15 min at room temperature. After 15 min, 75mL distilled water was added to the sample, and the

content was titrated against $\text{Na}_2\text{S}_2\text{O}_3$ (0.01 N) using starch as an indicator of a colorless endpoint. The PV was expressed as meq O_2/kg of fat.

Thiobarbituric Acid (TBA) Value

The TBA value of the sample was estimated by the procedure given by Tarladgis et al. (1960). 5g of chocolate samples were placed in a flask, and 25 mL of C_6H_6 followed by 20 ml aqueous trichloroacetic acid solution (0.67 % w/v in water) was added. The obtained sample was shaken in a mechanical shaker for 2 h. The aqueous layer was separated using a separating funnel and heated over a water bath for 20 min. The absorbance was measured at 540 nm using a spectrophotometer. TBA reactive substances were calculated from a standard curve of malonaldehyde, a breakdown product of tetraethoxypropane.

Cost analysis

Cost calculation of butter fruit milkshake powder fortified chocolate was calculated using the standard methods by taking to account of the yield of butter fruit milkshake powder and all cost of raw material and processing (labor and electric) charges at the laboratory level.

Results and Discussion

Sensory acceptability of BFMS in chocolate

The data related to sensory evaluation was presented in Table 2. The sensory rating for color and appearance, flavor, consistency, and overall acceptability declined with the increase in BFMS powder concentration from 0–60%. The color scores significantly decreased as increased the level of butter fruit milkshake powder (15–60%) and ranged from 4.56 to 3.94, and the control chocolate

Table 1 Ingredients used for preparation of control chocolate (CC) and butter fruit milkshake chocolate (BFMSC)

Ingredient	CC (0%)	BFMS (15%)	BFMS (30%)	BFMS (45%)	BFMS (60%)
Cocoa powder (g)	100	85	70	55	40
Milk powder (g)	30	30	30	30	30
Butter (g)	62	62	62	62	62
BFMS (g)	-	15	30	45	60
Sugar (g)	150	150	150	150	150
Water (mL)	117	117	117	117	117

Table 2 Sensory score of chocolate formulation with varying of BFMS level Sensory acceptability of control chocolate (CC) and *Tinospora cordifolia* chocolate (TCC)

Formulation	Color and appearance	Flavour	Consistency	OAA
Cocoa powder: BFMS				
Control chocolate	4.87 ± 0.27	4.53 ± 0.24	4.20 ± 0.12	4.66 ± 0.23
85%:15%	4.56 ± 0.25	4.32 ± 0.22	4.14 ± 0.25	4.49 ± 0.22
70% : 30%	4.40 ± 0.25	4.13 ± 0.2	4.09 ± 0.28	4.33 ± 0.14
55% : 45%	4.09 ± 0.13	3.87 ± 0.24	3.53 ± 0.19	3.67 ± 0.17
40% : 60%	3.94 ± 0.27	3.07 ± 0.11	3.17 ± 0.15	3.23 ± 0.21

Fig.1 Optimized BFMS chocolate and control chocolate **Table 3** Nutritional composition of BFMS, CC, and BFMSC

Parameter	BFMS	CC	BFMSC
Moisture %	2.9±0.37	1.55±0.04	1.83±0.03
Protein %	17.2±0.14	5.92±0.07	11.98±0.01
Fat %	42.2±0.22	27.52±0.13	40.64±0.16
Ash%	2.78±0.09	1.54±0.04	2.14±0.02
Carbohydrate %	33.16	73.34	70.06
Crude Fiber %	0.84	0.47	0.822
Eneergy Value(kcal/100g)	277.11±0.29	522±0.29	531.05±0.11
Ca(mg/100g)	13.45±0.26	1432±5.03	1456±6.33
Mg(mg/100g)	29±0.38	497±3.13	504±2.83
Fe(mg/100g)	0.63±0.07	24.6±2.03	26.5±1.89
Zn(mg/100g)	0.67±0.04	11.4±1.81	11.6±1.57
Mn(mg/100g)	0.15±0.02	2.97±.51	3.18±.37
Colour	L*(65.83±0.69) a*(-4.5±0.57) b(22.83±0.17)	L*(29.03±0.77) a*(8.01±0.18) b*(6.48±0.35)	L*(31.45±0.21) a*(8.23±0.41) b*(6.59±0.61)
Texture (Hardness) in kg	—	8.13 kg±0.948	9.48±0.88

Values are Mean ± Standard deviation (SD) of triplicates

had a significantly higher score (4.87). The hardness of BFMSC was slightly hard and feeling gritty within the mouth due to the high fiber content of BFMS. The overall acceptability score of control chocolate was 4.66, 15 % BFMS (4.49), 30% BFMS (4.33), 45 % (3.67) and 60 % BFMS (3.23). These results showed that the addition of BFMS above 30 % adversely affected the sensory parameter of chocolate. Thus, it could be concluded that chocolate can be prepared by using 30 % butter fruit milkshake powder with acceptable sensory quality (Fig. 1). This result conforms with Al-Marazeeq (2018), who reported that sensory attributes decreased with increasing the addition of wheat germ (6-15%) and found that 10% supplementation of the wheat germ was more acceptable than other concentration.

Nutrient composition and trace elements of BFMS, CC, BFMSC

The nutritional analysis results of BFMS, CC, and BFMSC are depicted in Table 3. Butter fruit milk powder fortified chocolate possessed a good quantity of protein 11.98%, fiber 0.822%, lipid

40.64, and the gross energy value for BFMSC was 531.05 kcal/100g. The obtained result was higher than control chocolate because butter fruit milkshake powder processed a good quantity of protein, fat, fiber, and ash, as shown in the previous study. Fat content (40.64%) of optimized chocolate was partly in the form of monounsaturated fatty acid, which is good for the heart. Al-Marazeeq (2018) found similar results of improved mineral, and protein content when incorporating wheat germ in chocolate.

Minerals are essential for the maintenance of human health. Trace elements were determined by the atomic absorption spectrophotometer shown in Table 3 revealed that the calcium (1456mg/100gm), magnesium (504mg/100gm), iron (26.5mg/100gm), zinc (11.6mg/100gm), and Mn (3.18 mg/100g) of optimized chocolate was higher than the control chocolate. These result obtained because the avocado pulp has a good quality of macro and microelement (Fulgoni et al. 2013)

Color and texture analysis

Table 3 shows, color and texture value of control chocolate and optimized chocolate. The color values of control chocolate were L^* (29.03 ± 0.77), a^* (8.01 ± 0.18), and b^* (6.48 ± 0.35). Addition of BFMS powder to the chocolate, brightness values (L^*) was significantly increased, and the redness (a^*) and the yellowness (b^*) slightly increased when compared to the control sample. The addition of BFMS in chocolate affected textural. It was found the hardness proportionally increased with the addition of butter fruit milkshake powder in chocolate. The hardness of control chocolate is 8.13 kg, which increased with an increased level of BFMS. It happens because butter fruit milkshake powder has high moisture and fiber and makes a strong bond with cocoa protein. A previous study (Heo et al. 2019) also reported that muffin's hardness increased with the addition of enriched with dietary fiber from kimchi by product.

Phytochemical characteristics of CC, FGSF, and FGSC

Total phenolic and flavonoid content

Phenols are essential plant constituents. Various researches have confirmed that phenolic and flavonoid compounds are the most prominent antioxidative constituents in vegetables, fruits, and cereals (Choi et al. 2007). These constituents have a proportional relationship with total antioxidant activity. Table 4 shows the total phenolic and flavonoid content of BFMS, CC, and BFMS. The total phenolic content of butter fruit milkshake powder fortified chocolate was 932.14 mg/100 g of dry weight, which was high in comparison to control chocolate (758.14 mg/100g). TFC of the optimized chocolate extract against rutin was found to be 43.2 mg/100g of dry weight, respectively, which was more than the control chocolate (43.2 mg/100g dw). Butter fruit milkshake powder has higher phenolic and flavonoid content because of avocado rich in phenolic and flavonoid compounds (Vinha et al. 2013), which proportionally increased the TPC and TFC content in BFMS.

Beta carotene

BFMS is rich in carotenoid; hence the chocolate was prepared with BFMS Powder was rich in carotene content ($71.13 \mu\text{g}/100\text{g}$) shown in Table 4. Similar result was also reported by Hamdan et al. (2020) that carotenoid content of chocolate increases as incorporated with spirulina.

Shelf life study of chocolate

Effect on water activity

Several factors such as the raw materials used, the surface area of the materials, and the temperature and humidity of refining and conching may influence water activity of chocolate (Biquet and Labuza 1988). Water activity was increased from 0.391 to 0.532 in control chocolate (CC) and from 0.404 to 0.408 in butter fruit milk shake chocolate (BFMS) depicts in Table 5, during the storage of 0 to 60 days. The results depict that water activity of control chocolate and optimized chocolate increased during storage the 2 months of shelf life. Similar findings of increase in water activity in during storage were noticed in previous studies by Rossini et al. (2011) in white chocolate.

Effect on peroxide value

Quality loss and deteriorative problems in a food product are related to lipid degradation (Haak et al. 2006). In this regard, peroxide values increased for all chocolate samples during shelf life study for two months. The values presented in Table 5 depicts that the peroxide values of optimized chocolate 2.20 ± 0.21 to 3.88 ± 0.40 and control chocolate 2.24 ± 0.19 to 4.11 ± 0.13 increases with an increase in storage period from 0 to 60 days respectively. Moreover, the peroxide value for optimized chocolate was lower than CC during storage. This outcome is well supported by the observation of Rossini et al. (2011), who noticed that peroxide

Table 4 Total phenolic contents, total flavonoid contents and antioxidants potential of BFMS, CC, and BFMS

Parameter	BFMS	CC	BFMS
TPC(mg/100gdw)	416.2 ± 5.04	758.14 ± 7.15	932.14 ± 5.19
TFC(mg/100g dw)	24 ± 1.54	36.02 ± 1.50	43.2 ± 1.92
Beta carotenoid($\mu\text{g}/100\text{g}$)	502 ± 7.09	0.45 ± 0.001	71.13 ± 7.86

Values are Mean $\pm$ Standard deviation (SD) of triplicates

Table 5 Water activity, TBARS, and Peroxide value of butter fruit milkshake powder chocolate samples stored at 20°C for up to 2month

Days	Water activity			Peroxide value (mEq O ₂ /kg)						TBARS ($\mu\text{g}/\text{g}$)	
	0	30	60	0	30	60	0	30	60	0	60
Control chocolate(20°C)	0.391 $\pm$ 0.005	0.412 $\pm$ 0.010	0.532 $\pm$ 0.016	2.24 $\pm$ 0.19	2.65 $\pm$ 0.33	4.11 $\pm$ 0.13	5.84 $\pm$ 0.34	6.51 $\pm$ 0.52		8.1 $\pm$ 0.58	
BFMS(20°C)	0.404 $\pm$ 0.004	0.408 $\pm$ 0.007	0.530 $\pm$ 0.005	2.20 $\pm$ 0.21	2.36 $\pm$ 0.18	3.88 $\pm$ 0.40	5.60 $\pm$ 0.13	6.22 $\pm$ 0.42		7.55 $\pm$ 0.54	

Values are Mean $\pm$ Standard deviation (SD) of triplicates

Table 6 Cost analysis calculation for cost of butter fruit milk shake powder

Particulars	Amount
Total weight of pulp obtained in 1 kg of raw avocado	655 g
% yield of powder in raw avocado=(weight of powder/weight of raw avocado)*100	655/1000*100=65.5%
Total cost of 655 gram of avocado milk shake powder(Cost of raw avocado+ Processing charges+ miscellaneous)	Rs.278/-
Cost of raw avocado	Rs.178/kg
Processing charges(electricity used in drying +labor charges+ miscellaneous)	Rs.100/kg
Cost for 1 kg of Butter fruit milk shake powder	(278/655)1000= Rs. 424.43/Kg
Butter fruit milk shake powder cost	424.43/Kg or 42.44/100g

Table 7 Cost estimation of chocolate

Ingredient	Quantity	Rate(Rs/100g)	Total cost of control chocolate(Rs/-)	Total cost of BFMS(Rs/-)
Cocoa powder	100g for CC	120	120	84
	70g for BFMS			
Milk powder	30g	50	15	15
Butter	62g	48	29.76	29.76
BFMS	0 for CC	42.44	0	12.73
	30 for BFMS			
Sugar	150g	5	7.5	7.5
Water	117ml	2	2.34	2.34
Sub total			174.6	151.33

values of white chocolate prepared with antioxidants were lower than that of control.

Effect on TBA value

From the data represented in Table 5, it was noticed that increasing the storage period elevates the TBA value of both chocolate. The TBA value for optimized chocolate (BFMS) were 5.60 ± 0.13 , 6.22 ± 0.42 , and 7.55 ± 0.54 , and for control chocolate (CC) were 5.84 ± 0.34 , 6.51 ± 0.52 and 8.1 ± 0.58 at 0, 30th, and 60th days of storage, respectively. There was no significant change observed in BFMS and CC's TBA value from 0 to 60 days of storage. A similar finding was studied by Rossini et al. (2011) for white chocolate. The lower value of TBA delayed the onset of rancidity in chocolate.

Cost estimation

Cost analysis of BFMS powder

The cost analysis calculation of BFMS powder is shown in Table 6. The amount of total BFMS powder obtained from 1Kg of raw avocado after being processed (removal of moisture and wastage) was estimated 655 grams, and the yield of BFMS powder was found to be 65.5%, and the total cost for 1 Kg of BFMS powder was calculated as 424.43/Kg.

Cost estimation of chocolate

Cost of ingredient used and prepared chocolate shown in Table 7. The cost of optimized chocolate (Rs. 151.33/100g) in the laboratory was estimated to lower than control chocolate (Rs. 174.6/100g). The cost of cocoa powder used in control chocolate was higher than BFMS powder. Hence there was not much variation in the cost of control and optimized chocolate. So the use of butter fruit milkshake powder in preparation of optimized chocolate was found to be economical.

Conclusions

Butter fruit milkshake powder comprises protein, fiber, antioxidants, and phenolic compounds that are advantageous for health. Due to nutritional qualities and palatability of the butter fruit milkshake powder, the present study was carried out to make chocolate with BFMS that has acceptable sensory attributes and enhanced nutritional properties. The study findings based on sensory analysis suggested that 30% of butter fruit milkshake powder addition in chocolate in the place of cocoa powder is acceptable by consumers. BFMS powder's addition increased micro and macro minerals (Ca, Mg, Fe, Zn and Mn), proteins, dietary fibers, phenolic, flavonoids, and beta carotenoid contents of the chocolate. Shelf life investigation showed butter fruit milkshake powder fortified chocolate significantly more

stable than control chocolate and accepted by consumers up to 60 days of storage and presence of bioactive compounds in BFMS can be used to treat different irresistible illnesses. This kind of value-added chocolate may expand the food industry and the market with nutraceutical potential. Further, the histopathology and other compulsory examination are required before getting an endorsement from the administrative bodies. Different food items such as cookies, snack bars, nutritional bars, and candies can also be developed by supplementing the butter fruit milkshake powder.

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