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;
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.

**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 FGSF, 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; r² = 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 AlCl₃ colorimetric method explained by Tyagi et al. (2020). Rutoside trihydrate was used to plot standard curve (5-120μg/mL; y = 0.0054x+0.0821; r² = 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 Na₂SO₄ and homogenized with 10 ml (CH₃₂CO. 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 µg/100mg.

Storage studies

Water activity
Water activity (aₜ) of the chocolate samples was determined by water activity meter (Aqua-Lab, model 3TE, Decagon Devices Inc., Pulluman, 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 CHCl₃ followed by 30ml of CH₃COOH 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 Na₂S₂O₃ (0.01 N) using starch as an indicator of a colorless endpoint. The PV was expressed as meq O₂/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 C₆H₆ 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

| 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 1 Ingrdients used for preparation of control chocolate (CC) and butter fruit milkshake chocolate (BFMSC)**

| Formulation | Color and appearance | Flavour | Consistency | OAA |
|-------------|----------------------|---------|-------------|-----|
| Control chocolate: BFMS | 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±0.20 | 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 |

**Table 2 Sensory score of chocolate formulation with varying of BFMS level Sensory acceptability of control chocolate (CC) and Tinospora cordifolia chocolate (TCC)**
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% BFMSC (4.49), 30% BFMSC (4.33), 45% (3.67) and 60% BFMSC (3.23). These results showed that the addition of BFMSC 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**

| 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            |
| Energy 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)   | L*(29.03±0.77)   | L*(31.45±0.21)   |
|                           | a*(-4.5±0.57)    | a*(8.01±0.18)    | a*(8.23±0.41)    |
|                           | b*(22.83±0.17)   | b*(6.48±0.35)    | b*(6.59±0.61)    |
| Texture (Hardness) in kg   | 8.13 kg±0.948    | 9.48 kg±0.88     |

Values are Mean ± Standard deviation (SD) of triplicates
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 BFMSC. 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/100 g). TPC of the optimized chocolate extract against rutin was found to be 43.2 mg/100 g of dry weight, respectively, which was more than the control chocolate (43.2 mg/100 g 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 BFMSC.

**Beta carotene**

BFMS is rich in carotenoid; hence the chocolate was prepared with BFMS Powder was rich in carotenote content (71.13 µg/100g) 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 (BFMSC) depicted 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

| Parameter                  | BFMS      | CC           | BFMSC       |
|----------------------------|-----------|--------------|-------------|
| TPC (mg/100gdw)            | 416.2±5.04| 758.14±7.15  | 932.14±5.19 |
| TFC (mg/100gdw)            | 24±1.54   | 36.02±1.50   | 43.2±1.92   |
| Beta carotenoid (µg/100g)  | 502±7.09  | 0.45±0.001   | 71.13±7.86  |

Values are Mean ± Standard deviation (SD) of triplicates

| Days | Water activity | Peroxide value (mEq O2/kg) | TBARS (µg/g) |
|------|----------------|-----------------------------|--------------|
|      | 0              | 30                          | 60           | 0            | 30           | 60           | 0            | 30           | 60           |
| 0    | 0.39±0.005     | 0.41±0.010                  | 0.53±0.016   | 2.24±0.19    | 2.65±0.33    | 4.11±0.13    | 5.84±0.34    | 6.51±0.52    | 8.1±0.58     |
| 30   | 0.40±0.004     | 0.40±0.007                  | 0.53±0.005   | 2.20±0.21    | 2.36±0.18    | 3.88±0.40    | 5.60±0.13    | 6.22±0.42    | 7.55±0.54    |

Values are Mean ± Standard deviation (SD) of triplicates
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 (BFMSC) 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 BFMSC 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 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 |

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

**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 BFMSC can use 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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