Development of Composite Meat Chocolate Fortified with Calcium and Plant Extracts

Composite Meat Chocolate- A Novel Meat Product

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Abstract

An attempt was made to improve the nutritional perception and textural stability of chocolate at higher tropical temperatures by developing a composite meat chocolate using meat protein powder and animal fat. The composite meat chocolate was fortified with calcium to reduce the cariogenicity (anticaries activity) and enriched with blueberry and raspberry extract (1%, 2% and 3%) as a preservative. The products were evaluated at 0, 2, 4, and 6 months of storage (25±1°C). Both, the protein and calcium content of the optimized meat chocolate increased ($p < 0.05$) by 93% and a significant ($p < 0.05$) decrease in fat, carbohydrate and total calories was recorded without any increase ($p > 0.05$) in cholesterol content. Addition of blueberry and raspberry extracts, particularly at 3% level, improved ($p < 0.05$) the lipid oxidative [TBARS (mg malonaldehyde/kg), DPPH (% inhibition), total phenolic content (mg/g)] and microbial [free fatty acids (% oleic acid), total plate count ($\log_{10}$ cfu/g)] stability while improving the sensory quality and functional value of the products.

Keywords: Composite chocolate, meat protein powder, calcium fortification, blueberry and raspberry, storage quality

1. Introduction

Liked by people of all ages due to their desirable sensory characteristics (Oracz et al., 2019), chocolates are cocoa-based products with complex multiphase systems of continuous (cocoa butter, milk fat and emulsifiers) and particulate (cocoa, sugar, milk components) phases (Li et al., 2021; Toker et al., 2020). While primary categories of chocolate (milk, dark, white and ruby) differ in their amount of cocoa solids, cocoa butter and milk fat (Tuenter et al., 2021), a product which contains more than 60 percent of chocolate by weight is known as a composite chocolate. It may also contain more than 10 percent by weight of the final product of some edible wholesome substances, such as fruits and nuts (FSSAI, 2011).

While chocolate is one of the most popular products throughout the world, chocolate-based products are often associated with a negative perception due to a high proportion of fat and sugar in their composition (Toker et al., 2018). Studies have suggested an association between consumption of diets rich in sugars and fats, such as chocolates, with the occurrence of several degenerative diseases such as obesity and cardio-vascular diseases (Li et al., 2021; Verde et al., 2021). Several authors have developed low calorie chocolates using noncarbohydrate nutritive sweeteners, such as polyols (Petković, 2019), however, their usage is not free from health risks and hazards (Tandel, 2011). This requires urgent scientific attention and there is a need for development of chocolates which contain greater proportion of proteins (replacing fats and carbohydrates) and other healthful compounds to improve the nutritional perception of the product (Kaur et al., 2017).

In the present study, a calcium-fortified and protein-rich composite chocolate was developed using chicken protein powder and calcium lactate and the cocoa butter was partly replaced with animal fat. Chicken protein powder was used due to its excellent nutritional characteristics such as high protein, low fat and low carbohydrate content, a high ratio of unsaturated fatty acids (polyunsaturated fatty acids and omega (ω)-3 fatty acids) and ease of digestion (Alagawany et al., 2019; Bhat et al., 2015a, b; Bhat and Bhat, 2011; Bhat and Pathak, 2009). Given the fact that calcium lactate is a tasteless and non-toxic compound that is commonly used in food industry, there is a possibility for its use in chocolate as an anticaries food additive for children. Calcium lactate has been reported to reduce the cariogenicity (enamel dissolution) of sugar candies significantly in both *in vitro* and *in vivo* studies (Turssi et al., 2014; Suda et al., 2006; Shrestha et al., 1982) and calcium has been reported to reduce the absorption of saturated fats in chocolate in human digestive system (Murata et al., 2001).

Replacing cocoa butter with some animal fats having comparatively higher melting point can be helpful in managing the textural and melting stability at higher tropical temperatures by increasing the heat-resistance of the milk chocolates (Kaur et al., 2017). The results of our study replacing cocoa butter with goat fat have been published in Kaur et al. (2017). Further, to improve the functional and sensory characteristics and storage quality of the developed products, three levels (1%, 2% and 3%) of blueberry and raspberry extract were used, separately, as a natural preservative. The developed products were stored at room temperature (25±1 ^{o}C) for six months and were evaluated for physicochemical parameters, lipid and microbial stability, oxidative stability, and sensory quality. The objective of the present study was to develop a composite meat chocolate using meat protein powder and animal fat to improve the nutritional perception and textural stability of chocolate at higher tropical temperatures. The composite meat chocolate was fortified with calcium to reduce the cariogenicity and enriched with blueberry and raspberry extract as a preservative.

2. Materials and methods

2.1. Meat protein powder

Commercially available dressed broiler chicken was procured from local market and deboned manually under strict hygienic conditions. The lean meat obtained after removal of all the tendons and separable body fat and connective tissues was minced in Sirman mincer (MOD-TC 32 R10 U.P. INOX, Marsango, Italy) using 6 mm plate. The minced chicken meat was evenly spread over aluminium trays and dehydrated at 75 ^{o}C in a hot air oven for 5 hours followed by grinding in a mixer to a powder form as per the method described by Ilansuriyan, Marimuthu, & Sasikala (2015). The coarse particles were removed using a sieve and the powder contained less than 5% moisture content.

2.2. Animal fat and other ingredients

Cocoa powder of brand "Cadbury" (Cadbury, Mumbai, India), cocoa butter of brand "Morde" (Morde, Mumbai, India) and milk powder of "Nestle" brand (Nestle, Haryana, India) were used in the preparation of the products. Soy lecithin (Product code RM637, Hi Media, Mumbai, India) and calcium lactate (Product code RM495, Hi Media, Mumbai, India) were purchased from Hi Media. Goat fat was used as a source of animal fat and was heated to 75 ^{o}C for 15 minutes in a hot air oven. All the chemicals used were of analytical grade and were purchased from standard firms (Hi Media and Sigma). Aluminium foil and low-density polyethylene (LDPE) film pouches (200 μm thickness) in natural colour were used for packaging of the product, pouches were sterilized by U.V. light.

2.3. Blueberry (*Vaccinium corymbosum*) and raspberry (*Rubus idaeus*) fruit extract

"Nature's Answer" blueberry fruit extract (Nature's Answer, Hauppauge, New York, USA) containing 155 mg/ml blue berry extract (water based, alcohol and gluten free) and "Herb Pharm" red raspberry extract (Herb Pharm, Oregon, USA) containing 628 mg/0.7 ml (organic cane alcohol based) were used.

2.4. Preparation of composite meat chocolate

The method described by Jeffery et al. (1977) was modified for the preparation of composite meat chocolate. A milk chocolate was prepared by thorough mixing of cocoa solids (10.5%), cocoa butter (36.0%), milk solids (11.0%), sugar (42.0%), emulsifier (lecithin 0.5%, added after half of conching time) and ethyl vanillin (0.1%). Said percentages were based on the total weight of the milk chocolate to produce an emulsion. The emulsion was subjected to conching process for minimum 6 hours using Prestige PWG 03 wet grinder (TTK Prestige Ltd, New Delhi, India). This was followed by tempering at 30-40 ^{o}C for 15-20 minutes. Emulsion was then put into moulds to obtain milk chocolate of desired shape. The product was wrapped in aluminium foil followed by LDPE packaging (outer packaging) and stored at room temperature (25±1°C) for six months. The developed product was assigned as control C1 (milk chocolate without animal fat and without meat protein powder). The product samples were drawn at 0-, 2-, 4- and 6-months intervals and assessed for storage quality and sensory parameters.

2.4.1. Optimization of animal fat

The cocoa butter was replaced with animal fat at 0, 10, 20 and 30% levels and rest of the ingredients were kept same. Based on sensory analysis and other quality parameters, the milk chocolate with 10% animal fat was chosen as optimum [cocoa solids (10.50%), cocoa butter (32.40%), milk solids (11.0%), animal fat (3.60%), sugar (42.0%), lecithin (0.50%) and ethyl vanillin (0.10%)]. The developed product was assigned as control C2 (milk chocolate containing optimized animal fat and no meat protein powder).

2.4.2. Optimization of meat protein powder

The composite meat chocolate was prepared by replacing milk chocolate emulsion containing animal fat (C2) with chicken meat protein powder at 0, 10, 20 and 30% levels and conching was done for at least 2 hours. Based on sensory analysis and other quality parameters, the milk chocolate with 10% meat protein powder was chosen as optimum [cocoa solids (9.45%), cocoa butter (29.16%), milk solids (9.90%), animal fat (3.24%), chicken meat powder (10.0%), sugar (37.80%), lecithin (0.45%) and ethyl vanillin (0.10%)]. The developed product was assigned as control C3 (milk chocolate containing optimized animal fat and meat protein powder).

2.4.3. Optimization of calcium lactate and addition of fruit extracts

Calcium fortified composite meat chocolate was developed by incorporation of calcium lactate at 0, 0.5, 1.0 and 1.5 % levels (by weight of composite meat chocolate) into the product. The product was assessed for acceptability based on quality parameters and sensory analysis and was optimized to 1% level [cocoa solids (9.45%), cocoa butter (29.16%), milk solids

(9.90%), animal fat (3.24%), chicken meat powder (10.0%), sugar (37.80%), lecithin (0.45%), ethyl vanillin (0.10%) and calcium lactate (1% of composite meat chocolate emulsion by weight)]. The developed product was assigned as control C4 (milk chocolate containing optimized animal fat, meat protein powder and calcium lactate). This was followed by addition of fruit extracts (1, 2 and 3% levels by weight) into the emulsion and was followed by conching for 30 minutes. The products developed were assigned as B1 (C4 containing 1% blueberry extract), B2 (C4 containing 2% blueberry extract), B3 (C4 containing 3% blueberry extract), R1 (C4 containing 1% raspberry extract), R2 (C4 containing 2% raspberry extract) and R3 (C4 containing 3% raspberry extract).

2.5. Calcium content (mg/g) and total cholesterol (mg/100g)

Calcium content of the samples was determined according to the method of AOAC (1999). The lipid extract of the samples was prepared using the method of Folch et al. (1957) and the total cholesterol in the lipid extract was estimated as per the method described by Hanel and Dam (1995).

2.6. pH

The pH of the samples was determined by the method of Bhat et al. (2018) as modified by Bhat et al. (2020a) using Ultra Turrex T10 tissue homogenizer (Janke and Kenkel, IKA labor Technik, Germany) and a digital pH meter (Product code 35613424, Oakton instruments, Singapore).

2.7. Proximate parameters (%)

The moisture (%), protein (%), fat (%) and carbohydrate content (%) of the samples were determined using standard methods (AOAC, 1999) as described by Singh et al. (2014).

2.8. Thiobarbituric acid reacting substances (TBARS) value

The TBARS value of composite meat chocolate was determined using the distillation method described by Bhat and Pathak (2012). The optical density was recorded at 538 nm and the value was expressed as mg malondialdehyde per kg of sample.

2.9. Free fatty acids (% oleic acid)

Free fatty acids (FFA) were determined by the method described by Noor et al. (2018) using the given formula.

$$\text{Free fatty acids (\% oleic acid)} = \frac{(0.1 \times \text{ml of KOH used} \times 0.282)}{\text{wt. of sample}} \times 100$$

2.10. DPPH radical scavenging activity

The ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (% inhibition) by the samples was determined by the method of Blois (1958) with minor modifications. The radical scavenging was determined by adding different concentrations of methanolic extract of samples (1 ml) and DPPH solution (0.1 mM, 2 ml) in test tubes which were stored in dark for 30 minutes and the absorbance was estimated at 517 nm using Systronics UV-VIS Spectrophotometer (Ahmedabad, Gujrat, India) against control (only vehicle solvent). Ascorbic acid was used as a standard.

$$\text{DPPH (\% inhibition)} = \frac{A_o - A_1}{A_o} \times 100$$

Where, A_o is absorbance of DPPH solution. A_1 is the absorbance of the reaction mixture (containing test compound and DPPH solution).

2.11. Total phenolics

Total soluble phenolics were determined with Folin-Ciocalteu (FC) reagent according to the method of Yu et al. (2002). The defatted sample (0.1 ml) was added to 0.5 ml of double distilled water and 0.5 ml of FC reagent was added to this mix. This was followed by the addition of 5 ml of 20% sodium carbonate after 5 min. The mixture was shaken thoroughly and allowed to stand for 2 h or more. The volume of the mixture was made to 6 ml using double distilled water and its absorbance was measured at 765 nm using Systronics UV-VIS Spectrophotometer. Gallic acid was used as a standard and the amount of total phenolic content was calculated as mg Gallic acid equivalents (GAE)/g of defatted sample.

2.12. Microbial counts

All the microbiological counts (total plate, psychrophilic, coliforms, yeast and mould) were determined according to the standard methods (APHA, 1984) as described by Bhat et al. (2010). Readymade media (Hi media, India) were used for the analysis. Stored samples of composite meat chocolate were opened in a laminar flow chamber sterilized by ultraviolet irradiation. Ten grams of the sample was taken aseptically and blended with 90 ml of 0.1 percent sterile peptone water with a pre-sterilized blend. Serial ten-fold dilution was made in pre-sterilized tubes containing 9 ml volume of 0.1 percent peptone water. The sample preparation was done near flame under laminar flow (Thermo Electron Corporation D-63505 Langenselbold, Robert Boschstr. 1, Germany).

2.12.1. Total plate count

Duplicate sets of sterilized Petri plates containing total plate agar were inoculated aseptically with 1 ml of aliquots from appropriate dilution. The plates were incubated at 35±2°C for 24 hours. Following incubation, plates showing 30-300 colonies were counted and expressed as $\log_{10}$ cfu/g of the sample.

2.12.2. Psychrophilic count

The same procedure was followed as used for total plate counts. The plates were incubated at 4±1 °C for 10-14 days.

2.12.3. Coliform count

The inoculated violet-red bile agar plates were incubated at 35±2 °C for 24 hrs. The number of red or purple colonies of about 0.5 mm diameter surrounded by a zone of precipitated bile were counted.

2.12.4. Yeast and mould count

The inoculated potato dextrose agar plates were incubated at 35±2°C for 5 days.

2.13. Sensory evaluation

Sensory evaluation of the product samples was carried out for appearance, taste, aroma, hardness, mouth feel and overall acceptability by a mixed gender panel of trained members composed of scientists and research scholars based on a 9-point hedonic scale, wherein 9 denoted "extremely liked" and 1 denoted "extremely disliked" (Singh et al., 2015a). The panellists were trained according to the guidelines of American Meat Science Association (1995) and were trained on product descriptions and terminology. Ten members of the panel replicated the experiment thrice. Samples were presented to the panellists and the serving order of the samples was randomized. Three-digit coded samples were served at room temperature (25 ^{o}C) and water was given for oral rinsing between the samples to avoid carry-over effect.

2.14. Statistical analysis

The experiments were repeated six times (n=6) and six independent batches of the products (control and treatments) were prepared. The data compiled were analysed by one-way ANOVA (calcium content and cholesterol) or two-way ANOVA (other parameters) except for sensory evaluation that was analysed using a repeated measurements ANOVA to investigate the effect of treatments and storage time by General Linear Model using Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). The measured variables were set as dependent variables and the results were presented as means ± standard errors. The fixed effects included in the model were treatments, storage time and their interactions. The random effects in the model were effects for batches and their interactions with fixed effects. For sensory analysis, treatment was considered as the main effect and panellists as random variable. The effect of storage time and treatments were analysed using Duncan's multiple range tests at 0.05 level of significance (Snedecor and Cochran, 1994).

3. Results and discussion

3.1. Physicochemical and proximate parameters

3.1.1. Cholesterol (mg/100g) and calcium (mg/g)

The mean values of cholesterol (mg/100g) of the products containing animal fat and meat protein powder are presented in figure 1. Both the replacement of cocoa butter with animal fat and the addition of meat protein powder (0, 10, 20 and 30%) resulted in a significant ($p < 0.05$) increase in cholesterol content of the products, however, no increase ($p > 0.05$) was observed at 10% level. Cholesterol is present in all animal cells as a structural component of cell membranes and is an important precursor for steroid hormones, bile acid and vitamin D. Increased dietary cholesterol has been reported to decrease the synthesis of endogenous cholesterol to maintain a constant cholesterol homeostasis (Hu et al., 2010; Lin et al., 1992) and its role in the development of cardiovascular diseases is not experimentally proven yet. Subsequently the recommendations of limiting the intake of cholesterol to 300 mg/day was removed from Dietary Guidelines for Americans during 2015-2020 (Soliman, 2018).

The mean values of calcium (mg/g) of the products containing different levels of calcium lactate are presented in figure 2. Calcium content, which reduces the cariogenicity (Shrestha et al., 1982) and absorption of saturated fats in chocolate (Murata et al., 2001), increased significantly ($p < 0.05$) at 0.5% level of incorporation and above. Dietary calcium has several health benefits such as reduced LDL- and increased HDL-cholesterol; blood pressure reduction; and prevention of osteoporosis, pregnancy induced hypertensive disorders and colorectal adenomas (Cormick and Belizán, 2019). Depending on the reference guidelines, dietary requirement of calcium may vary from 1000 to 1300 mg/day for human adults over 19 years of age (Cormick and Belizán, 2019).

3.1.2. Proximate parameters

The mean values of protein (%), fat (%) and carbohydrate (%) of the products containing meat protein powder are presented in figure 3. A significant ($p < 0.05$) increasing trend was observed in the protein (%) with increasing level of meat protein powder showing an increase of 93% at 10% level of incorporation (optimum level). Both fat (%) as well as carbohydrate (%) showed a significant ($p < 0.05$) decline with increasing level of meat protein powder. The total calories (fat + carbohydrate + protein) also decreased with addition of meat protein powder which can help in improving the nutritional perception of the product.

3.1.3. pH and moisture (%)

Table 1 presents the effect of storage and extracts (blueberry and raspberry) on physicochemical parameters (pH and moisture %) of composite meat chocolate. Both storage time as well as extracts showed a significant ($p < 0.05$) lowering effect on pH of the products. A significant ($p < 0.05$) decrease in pH of all the products with storage time may be explained by the Maillard's reaction which produces formic acid due to isomerization and degradation of lactose in milk and dairy products (van Boekel, 1998). Changes that occur during storage and induce protein cross-linking or dephosphorylation of caseins also result in a decrease in pH due to the release of protons (Karlsson et al., 2019). Decrease in the pH of the milk chocolate has been reported during storage (Pandey and Singh, 2011). The lower pH values of the extracts which contain high amounts of bioactive phytochemicals such as polyphenols including anthocyanins, proanthocyanidins and phenolic acids (Asakura and Kitahora, 2018) may be attributed to the lower pH values of the products containing blueberry and raspberry extract.

The decline in pH may also be attributed to lower pH of meat protein powder in comparison to milk chocolate (FDA, 2015).

Both storage time as well as extracts showed a significant ($p < 0.05$) effect on moisture (%) of the products. A significant ($p < 0.05$) decline was recorded in the moisture (%) of the products containing blueberry and raspberry extract with advancement of the storage time. Comparatively higher values of the products containing the extracts may be attributed to higher moisture content of these extracts. A significant ($p < 0.05$) effect of the storage time on moisture content of compound chocolate developed with reduced sugar soy has been reported (Pandey and Singh, 2011). Inclusion of other ingredients such as calcium lactate and meat protein powder might also be responsible for minor variations in the moisture content of the products.

3.2. Lipid stability

Table 2 presents the effect of storage and extracts (blueberry and raspberry) on lipid stability (TBARS value and free fatty acids) of composite meat chocolate. Lipid oxidation, which can affect nutritional quality, safety, and shelf-life, is a serious problem in chocolates due to a high percentage of fat from cocoa butter and milk (Rossini et al., 2011).

3.2.1. TBARS value (mg malonaldehyde/kg)

A significant effect of both storage time and extracts was recorded on the TBARS values of the products. All the products showed a significant ($p < 0.05$) increasing trend with advancement of storage time and significantly ($p < 0.05$) lower TBARS values were recorded for the products containing blueberry and raspberry extract at the end of the storage period, particularly at higher levels of incorporation (2% and 3%). Several studies have reported an increase in lipid oxidation of stored chocolate products (Kita et al., 2020; Rafsanjani et al., 2018; Rossini et al., 2011). A threshold value of up to 2.0 mg malondialdehyde/kg does not affect the sensory quality of the food products with moderate fat content (Bhat et al., 2020b). Raspberry and blueberry extracts, which are particularly rich in phenolic compounds such as ellagitannins, flavonoids, phenolic acids and flavan-3-ols, have strong antioxidant properties (Krauze-Baranowska et al., 2014; Giovanelli et al., 2013) and by furnishing hydroxyl groups can directly neutralize the radicals and inhibit the chain reactions of lipid oxidation. These free radical chain reactions consume molecular oxygen and unsaturated fatty acids and produce secondary oxidation products which leads to rancidity affecting the nutritive and sensory attributes of foods. Similar results have been reported for several products containing plant extracts during refrigerated storage (Singh et al., 2015b; Mahajan et al., 2015, 2016; Kumar et al., 2011).

3.2.2. Free fatty acids (% oleic acid)

The mean values of free fatty acids (FFA) increased significantly ($p < 0.05$) at the end of the storage for all the products, however, the values of all the products remained within acceptable limits which is expected to be less than 1.75% in cocoa butter and related products (Jonfia-Essien and Navarro, 2010) and 1.8% in meat products (Kelam et al., 2018). Several authors have reported a significant increase in FFA of chocolate products during storage (Pandey and Singh, 2011; Yadav et al., 2009). Free fatty acids are produced during microbial or enzymatic degradation of lipids and are highly vulnerable to oxidation and develop off-flavours and odours (Dilnawaz et al., 2017). Similar results have been reported for several products containing plant extracts during refrigerated storage (Dua et al., 2015; Jamwal et al., 2015; Pathak et al., 2009).

3.3. Oxidative stability

Table 3 presents the effect of storage and extracts (blueberry and raspberry) on oxidative stability (DPPH and total phenolic content) of composite meat chocolate.

3.3.1. DPPH (% inhibition)

A significant effect of both storage time and extracts was observed on the DPPH radical scavenging activity of the products. A significant ($p < 0.05$) decreasing trend was recorded in the DPPH values of all the products with advancement of storage time whereas significantly ($p < 0.05$) higher values were observed for the products containing blueberry and raspberry extract, particularly at higher levels of incorporation (2% and 3%). Due to their high levels of bioactive and phenolic compounds, chocolate and chocolate products are known to have high antioxidant properties (Oracz et al., 2019). Blueberry and raspberry extracts (20-100 µg/ml) have also been reported to have strong antioxidant properties with DPPH radical scavenging activities in the range of 38.50-87.90% and 37.60-87.0%, respectively (Basu and Camelia, 2016). Ingredients with low antioxidant capacity, such as meat protein powder and calcium lactate, caused a slight decrease in the antioxidant properties of the products.

3.3.2. Total phenolic content (mg gallic acid equivalents (GAE)/g)

A significant effect of storage time was recorded, and significant ($p < 0.05$) declining trend was observed with advancement of storage time for all the products. Arithmetically higher values ($p > 0.05$) were recorded for the products containing blueberry and raspberry extract at higher levels of incorporation (2% and 3%) which might be attributed to their antioxidant properties (Krauze-Baranowska et al., 2014; Giovanelli et al., 2013). Total phenolic content of blueberry and raspberry extracts have been reported to range from 250.1 ± 17.12 to 965.6 ± 2.9 mg GAE/g (Basu and Camelia, 2016).

3.4. Microbial quality

Table 4 presents the effect of storage and extracts (blueberry and raspberry) on microbial quality (total plate count, psychrophilic count, coliforms, yeast and mould) of composite meat chocolate.

3.4.1. Total plate count (TPC) and psychrophilic count ($\log_{10}$ cfu/g)

The mean values of TPC of all the products showed a significant ($p < 0.05$) increase with increasing storage time and similar increasing trend was also recorded for psychrophilic counts which were detected after 4 months of storage in all the products and increased significantly ($p < 0.05$) at the end of the storage. Both TPC and psychrophiles did not exceed the limit of acceptability of 5.33 log cfu/g and 4.6 log cfu/g, respectively, in all the products (Noor et al., 2017). Chocolate is generally regarded as microbiologically stable and allows slow microbial growth during long term storage. Increase in microbial counts has been recorded during storage for chocolate and cholate-based products (Orleans, 2011; Pandey and Singh, 2011). Significant ($p < 0.05$) effect of the extract was also recorded in the products containing blueberry extract (3%) and arithmetically ($p > 0.05$) lower values were recorded for the products containing blueberry and raspberry extracts, particularly at higher levels of incorporation. Both these extracts are rich in phenolic compounds with strong antimicrobial properties (Krauze-Baranowska et al., 2014; Giovanelli et al., 2013) and have experimentally proven activity against several bacteria such as *Clostridium sporogenes*, *Bacillus subtilis*, *Neisseria meningitidis*, *Staphylococcus epidermidis*, *Moraxella catarrhalis*, *Corynebacterium diphtheriae*, *Helicobacter pylori*, *E. faecalis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus and V. cholera* (Krauze-Baranowska et al., 2014; Jelena et al., 2014; Aleksandra et al., 2012).

3.4.2. Coliforms and yeast and mould count ($\log_{10}$ cfu/g)

No coliforms and yeast and mould counts were detected in any of the samples during six months of storage. Absence of coliforms means no contamination of the products has taken place indicating hygienic practices, clean handling, and sterilized packaging.

3.5. Sensory quality

Table 5 presents the effect of storage and extracts (blueberry and raspberry) on sensory quality of composite meat chocolate. A significant effect of storage time was observed on all the sensory parameters (appearance, aroma, taste, hardness, mouthfeel, and overall acceptability) whereas a significant effect of extracts was recorded on taste, aroma, mouthfeel, and overall acceptability. A decreasing ($p < 0.05$) trend was observed in the sensory scores of all the samples with storage time for all the sensory parameters and lowest scores were recorded at the end of the storage. A similar decrease in sensory quality and acceptability of chocolate and cocoa products has been reported (Bui and Coad, 2014; Popov-Raljić et al., 2013; Pandey and Singh, 2011) and has been attributed to various structural changes that occur during storage (Engeseth and Pangan, 2018). Fat bloom is one of the changes that can affect the appearance and sometimes the flavour and texture of the chocolate during storage (Bui and Coad, 2014). Significant ($p < 0.05$) decrease in the scores of overall acceptability with storage time might be attributed to the decline in the scores of other sensory attributes.

While a significant ($p < 0.05$) effect of extracts was recorded on taste, aroma, mouthfeel, and overall acceptability at the end of the storage, arithmetically higher ($p > 0.05$) values were recorded even for other sensory parameters for the products containing blueberry and raspberry extracts in comparison to the control. This might be attributed to aroma and polyphenolic compounds of blueberry and raspberry extracts having strong antioxidant and antimicrobial properties. These extracts have been reported to improve the sensory quality of other food products in several studies (Kryževicute et al., 2017; Ozarda et al., 2015; Gok and Bor, 2012). The higher sensory scores of the products containing these extracts might also be corelated with their lower values for other quality parameters and deteriorative processes, such as TBARS, FFA, and microbial counts, which have been reported to affect the lipid oxidation, lipolysis, proteolysis, production of bitter compounds and loss of volatile flavour components (Bhat et al., 2020b).

4. Conclusions

A composite meat chocolate was developed to improve the nutritional perception, anticaries properties and textural stability of the product using meat protein powder, animal fat and calcium lactate. Both the calcium as well as protein content of the optimized composite chocolate containing meat protein powder (10%), animal fat (10%) and calcium lactate (1.0%) increased significantly ($p < 0.05$) by 93%. A significant ($p < 0.05$) decrease in fat, carbohydrate and total calories was also recorded. Addition of blueberry and raspberry extracts, particularly at 3% level, improved the lipid oxidative and microbial stability and sensory quality of the products during storage.

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The authors declare that there is no conflict of interest. No outside funding was received.

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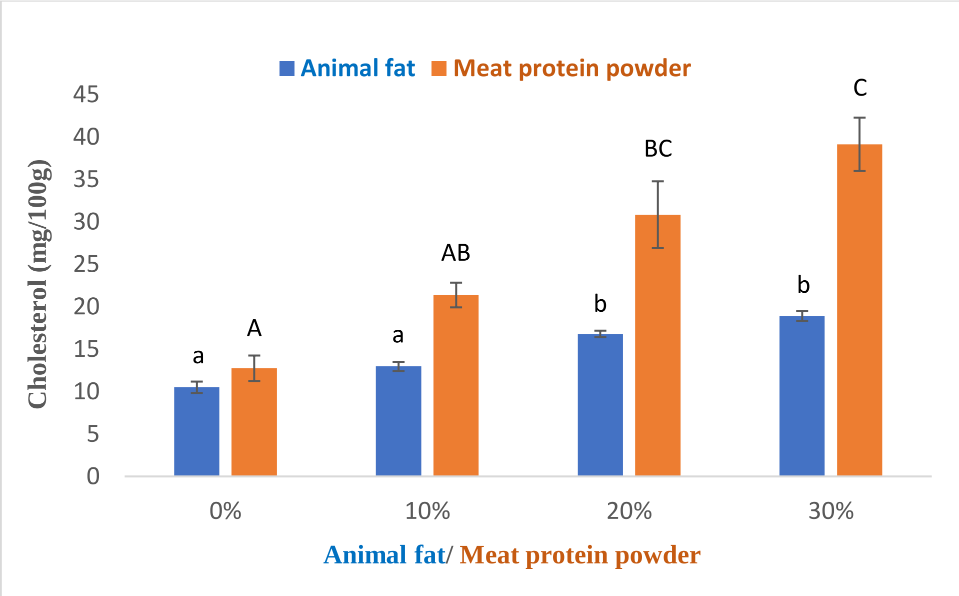
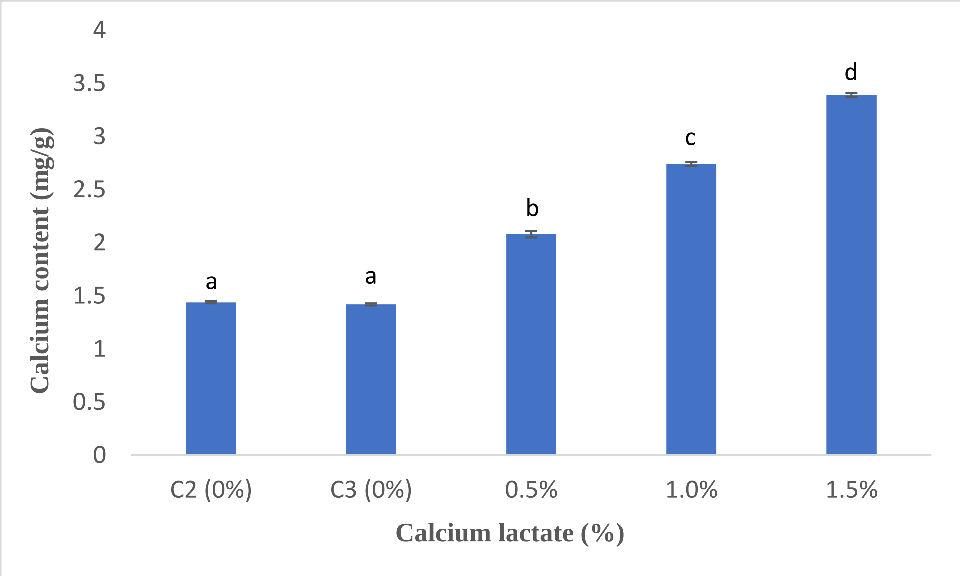


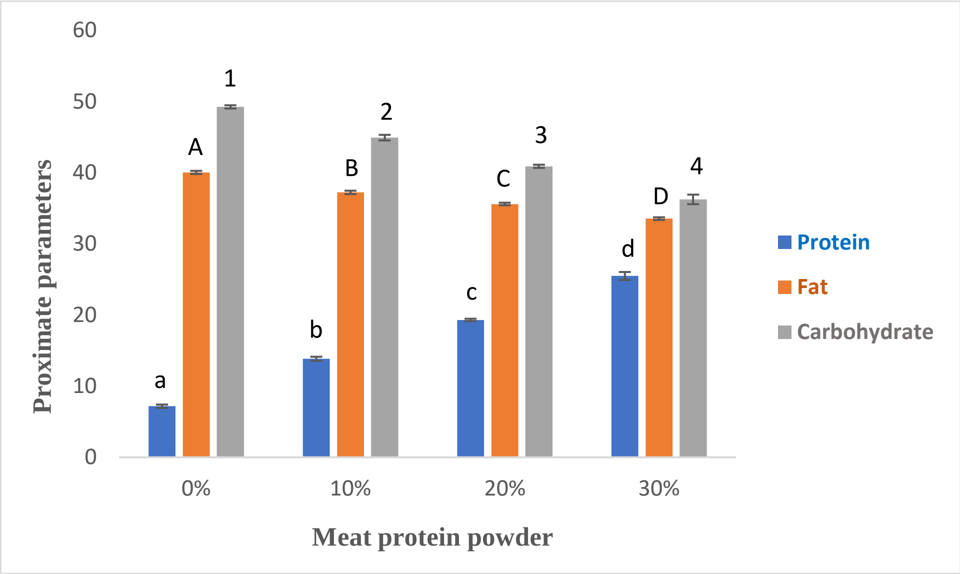
Different superscripts on blue (lower case alphabet) and brown (upper case alphabet) columns differ significantly (P<0.05)
Error bars represent the standard errors

Figure 1. Effect of animal fat and meat protein powder on the cholesterol content of composite meat chocolate



Different superscripts on columns differ significantly (P<0.05)
Error bars represent the standard errors

Figure 2. Effect of calcium fortification on the calcium content of composite meat chocolate [C2 (products containing animal fat only), C3 (products containing animal fat and meat protein powder)]



Different superscripts on blue (lower case alphabet), brown (upper case alphabet) and grey (numeric) columns differ significantly (P<0.05), Error bars represent the standard errors

Figure 3. Effect of meat protein powder on proximate parameters of composite meat chocolate

Table 1: Effect of blueberry and raspberry extract on physicochemical parameters of composite meat chocolate during storage (25±1ºC)

Sample	Storage period			
	0 months	**2 months**	**4 months**	**6 months**
pH				
C1	6.74 ±0.02^{Ce}	6.73 ± 0.04^{Cd}	6.49 ± 0.01^{Bc}	6.26 ± 0.02^{Ac}
C2	6.75 ±0.02^{Ce}	6.74 ± 0.02^{Cd}	6.51 ± 0.03^{Bc}	6.27 ± 0.02^{Ac}
C3	6.43 ±0.04^{Cd}	6.37 ± 0.02^{BCc}	6.28 ± 0.04^{ABb}	6.18 ± 0.02^{Ac}
C4	6.11 ±0.02^{c}	6.10 ± 0.02^{b}	6.07 ± 0.02^{a}	6.04 ± 0.03^{b}
B1	6.09 ±0.01^{bc}	6.09 ± 0.01^{b}	6.06 ± 0.04^{a}	6.03 ± 0.03^{b}
B2	6.06 ±0.02^{abc}	6.06 ± 0.02^{ab}	6.05 ± 0.03^{a}	6.01 ± 0.02^{ab}
B3	6.05 ±0.01^{abc}	6.05 ± 0.01^{ab}	6.04 ± 0.02^{a}	6.01 ± 0.03^{ab}
R1	6.07 ±0.01^{abc}	6.07 ± 0.01^{ab}	6.01 ± 0.04^{a}	5.97 ± 0.04^{ab}
R2	6.02 ±0.03^{ab}	6.02 ± 0.02^{ab}	6.00 ± 0.05^{a}	5.94 ± 0.01^{ab}
R3	6.01 ±0.01^{a}	6.01 ± 0.02^{a}	5.99 ± 0.02^{a}	5.93 ± 0.03^{a}
Moisture content (%)				
C1	1.51 ± 0.03^{a}	1.50 ± 0.03^{a}	1.49 ± 0.02^{a}	1.47 ± 0.02^{a}
C2	1.50 ± 0.01^{a}	1.50 ± 0.04^{a}	1.48 ± 0.02^{a}	1.47 ± 0.02^{a}
C3	2.11 ± 0.04^{c}	2.09 ± 0.04^{c}	2.08 ± 0.03^{c}	2.06 ± 0.05^{c}
C4	1.90 ± 0.06^{b}	1.89 ± 0.06^{b}	1.88 ± 0.05^{b}	1.85 ± 0.03^{b}
B1	3.13 ± 0.07^{Bd}	3.10 ± 0.06^{Bd}	3.10 ± 0.05^{Bd}	2.55 ± 0.06^{Ad}
B2	3.67 ± 0.04^{Be}	3.66 ± 0.03^{Be}	3.64 ± 0.03^{Be}	3.33 ± 0.04^{Ae}
B3	4.37 ± 0.03^{Bf}	4.36 ± 0.04^{Bf}	4.35 ± 0.08^{Bf}	3.79 ± 0.07^{Af}
R1	3.10 ± 0.04^{Bd}	3.09 ± 0.08^{Bd}	3.08 ± 0.04^{Bd}	2.43 ± 0.05^{Ad}
R2	3.60 ± 0.03^{Be}	3.55 ± 0.06^{Be}	3.52 ± 0.04^{Be}	3.08 ± 0.05^{Ae}
R3	4.18 ± 0.06^{Bf}	4.17 ± 0.03^{Bf}	4.13 ± 0.03^{Bf}	3.64 ± 0.06^{Af}

Mean ± SE with different superscripts in a row (upper case alphabet) and column (lower case alphabet) differ significantly ($P<0.05$), n = 6 for each treatment

C1 (milk chocolate without animal fat and meat), C2 (milk chocolate containing optimized animal fat), C3 (composite meat chocolate containing optimized animal fat and meat protein powder), C4 (C3 containing optimized calcium lactate), B1 (C4 containing 1% blueberry extract), B2 (C4 containing 2% blueberry extract), B3 (C4 containing 3% blueberry extract), R1 (C4 containing 1% raspberry extract), R2 (C4 containing 2% raspberry extract), R3 (C4 containing 3% raspberry extract)

Table 2: Effect of blueberry and raspberry extract on lipid stability of composite meat chocolate during storage (25±1ºC)

Sample	Storage period			
	0 months	2 months	4 months	6 months
TBARS value (mg malondialdehyde/kg)				
C1	1.54 ± 0.05^{A}	1.74 ± 0.17^{AB}	1.98 ± 0.17^{Ba}	3.52 ± 0.12^{Ccd}
C2	1.57 ± 0.10^{A}	1.77 ± 0.15^{AB}	2.11 ± 0.22^{Ba}	3.77 ± 0.13^{Cd}
C3	1.74 ± 0.09^{A}	2.04 ± 0.19^{A}	2.88 ± 0.14^{Bb}	4.73 ± 0.11^{Ce}
C4	1.56 ± 0.09^{A}	1.88 ± 0.15^{A}	2.26 ± 0.14^{Ba}	3.66 ± 0.08^{Cd}
B1	1.49 ± 0.08^{A}	1.76 ± 0.14^{AB}	2.12 ± 0.16^{Ba}	3.24 ± 0.08^{Cc}
B2	1.46 ± 0.15^{A}	1.70 ± 0.14^{AB}	2.07 ± 0.13^{Ba}	2.78 ± 0.11^{Cb}
B3	1.43 ± 0.11^{A}	1.63 ± 0.10^{AB}	1.91 ± 0.11^{Ba}	2.44 ± 0.12^{Ca}
R1	1.51 ± 0.09^{A}	1.80 ± 0.15^{AB}	2.18 ± 0.18^{Ba}	3.51 ± 0.13^{Ccd}
R2	1.49 ± 0.07^{A}	1.73 ± 0.10^{A}	2.13 ± 0.05^{Ba}	3.41 ± 0.15^{Ccd}
R3	1.45 ± 0.13^{A}	1.67 ± 0.19^{AB}	2.05 ± 0.11^{Ba}	3.23 ± 0.09^{Cc}
Free fatty acids (% oleic acid)				
C1	0.010 ± 0.003^{A}	0.014 ± 0.004^{A}	0.029 ± 0.005^{Aa}	0.076 ± 0.008^{Bab}
C2	0.012 ± 0.003^{A}	0.015 ± 0.004^{A}	0.032 ± 0.005^{ABa}	0.082 ± 0.007^{Bab}
C3	0.021 ± 0.004^{A}	0.027 ± 0.003^{A}	0.053 ± 0.008^{Bb}	0.096 ± 0.007^{Cb}
C4	0.018 ± 0.005^{A}	0.024 ± 0.005^{AB}	0.035 ± 0.006^{Aa}	0.080 ± 0.003^{Bab}
B1	0.017 ± 0.005^{A}	0.023 ± 0.005^{A}	0.032 ± 0.005^{Aa}	0.075 ± 0.006^{Bab}
B2	0.015 ± 0.005^{A}	0.020 ± 0.004^{A}	0.028 ± 0.006^{Aa}	0.071 ± 0.010^{Bab}
B3	0.013 ± 0.003^{A}	0.018 ± 0.004^{A}	0.025 ± 0.003^{Aa}	0.060 ± 0.004^{Ba}
R1	0.019 ± 0.004^{A}	0.024 ± 0.004^{A}	0.033 ± 0.005^{Aa}	0.077 ± 0.010^{Bab}
R2	0.017 ± 0.004^{A}	0.021 ± 0.006^{A}	0.030 ± 0.006^{Aa}	0.074 ± 0.006^{Bab}
R3	0.015 ± 0.003^{A}	0.198 ± 0.003^{A}	0.027 ± 0.005^{Aa}	0.065 ± 0.005^{Ba}

Mean ± SE with different superscripts in a row (upper case alphabet) and column (lower case alphabet) differ significantly ($P<0.05$), n = 6 for each treatment

C1 (milk chocolate without animal fat and meat), C2 (milk chocolate containing optimized animal fat), C3 (composite meat chocolate containing optimized animal fat and meat protein powder), C4 (C3 containing optimized calcium lactate), B1 (C4 containing 1% blueberry extract), B2 (C4 containing 2% blueberry extract), B3 (C4 containing 3% blueberry extract), R1 (C4 containing 1% raspberry extract), R2 (C4 containing 2% raspberry extract), R3 (C4 containing 3% raspberry extract)

Table 3: Effect of blueberry and raspberry extract on oxidative stability of composite meat chocolate during storage (25±1ºC)

Sample	Storage period			
	0 months	2 months	4 months	6 months
DPPH (% inhibition)				
C1	88.45 ± 1.06^{Cd}	88.10 ± 1.15^{Cd}	80.43 ± 2.5^{Bb}	67.14 ± 2.69^{Abc}
C2	88.22 ± 1.28^{Ccd}	$87. 91 \pm 1.44^{Ccd}$	78.06 ± 2.49^{Bab}	66.98 ± 2.77^{Abc}
C3	80.72 ± 1.89^{Ca}	80.39 ± 1.91^{Ca}	71.65 ± 2.55^{Ba}	53.68 ± 2.06^{Aa}
C4	81.26 ± 1.61^{Bab}	80.86 ± 1.32^{Bab}	76.93 ± 1.69^{Bab}	65.47 ± 2.40^{Ab}
B1	82.17 ± 3.00^{Babc}	81.39 ± 2.41^{Bab}	77.68 ± 2.78^{ABab}	70.46 ± 1.57^{Abc}
B2	83.25 ± 1.78^{Babcd}	82.42 ± 1.78^{Babcd}	78.78 ± 3.29^{ABab}	71.86 ± 2.42^{Abc}
B3	85.25 ± 2.10^{Babcd}	84.18 ± 1.70^{Babcd}	81.85 ± 1.90^{Bb}	72.25 ± 2.87^{Abc}
R1	84.35 ± 1.44^{Babcd}	82.89 ± 2.03^{Babcd}	79.96 ± 1.37^{Bb}	71.39 ± 1.50^{Abc}
R2	86.82 ± 2.23^{Bbcd}	85.54 ± 1.83^{Babcd}	80.82 ± 2.65^{ABb}	72.53 ± 4.17^{Abc}
R3	87.37 ± 1.51^{Bcd}	86.40 ± 1.17^{ABbcd}	81.65 ± 3.16^{Bb}	75.55 ± 3.08^{Ac}
Total phenolic content (mg/g)				
C1	5.49 ± 0.36^{B}	5.32 ± 0.38^{B}	4.94 ± 0.35^{AB}	3.88 ± 0.36^{A}
C2	5.47 ± 0.35^{B}	5.31 ± 0.37^{B}	4.92 ± 0.40^{AB}	3.84 ± 0.36^{A}
C3	4.88 ± 0.53^{B}	4.56 ± 0.60^{B}	4.20 ± 0.45^{AB}	2.91 ± 0.43^{A}
C4	5.04 ± 0.39^{B}	4.91 ± 0.48^{AB}	4.85 ± 0.60^{AB}	3.87 ± 0.48^{A}
B1	5.32 ± 0.56^{B}	4.97 ± 0.53^{AB}	4.90 ± 0.45^{AB}	3.99 ± 0.50^{A}
B2	5.43 ± 0.37^{B}	5.36 ± 0.54^{AB}	4.96 ± 0.51^{AB}	4.06 ± 0.52^{A}
B3	5.60 ± 0.25^{B}	5.41 ± 0.43^{AB}	5.14 ± 0.50^{AB}	4.12 ± 0.44^{A}
R1	5.17 ± 0.22^{B}	4.94 ± 0.22^{AB}	4.84 ± 0.38^{AB}	3.95 ± 0.41^{A}
R2	5.28 ± 0.33^{B}	5.17 ± 0.30^{AB}	4.95 ± 0.43^{AB}	4.00 ± 0.44^{A}
R3	5.42 ± 0.28^{B}	5.29 ± 0.40^{AB}	5.06 ± 0.39^{AB}	4.07 ± 0.54^{A}

Mean ± SE with different superscripts in a row (upper case alphabet) and column (lower case alphabet) differ significantly ($P<0.05$), n = 6 for each treatment

C1 (milk chocolate without animal fat and meat), C2 (milk chocolate containing optimized animal fat), C3 (composite meat chocolate containing optimized animal fat and meat protein powder), C4 (C3 containing optimized calcium lactate), B1 (C4 containing 1% blueberry extract), B2 (C4 containing 2% blueberry extract), B3 (C4 containing 3% blueberry extract), R1 (C4 containing 1% raspberry extract), R2 (C4 containing 2% raspberry extract), R3 (C4 containing 3% raspberry extract)

Table 4: Effect of blueberry and raspberry extract on microbial quality of composite meat chocolate during storage (25±1ºC)

Sample	Storage period			
	0 months	**2 months**	**4 months**	**6 months**
Total plate count (log_{10} cfu/g)				
C4	1.96 ± 0.04^{Ab}	2.12 ± 0.06^{ABb}	2.41 ± 0.04^{B}	3.76 ± 0.08^{C}
B1	1.84 ± 0.03^{Aab}	2.04 ± 0.06^{ABab}	2.33 ± 0.08^{B}	3.51 ± 0.05^{C}
B2	1.77 ± 0.05^{Aab}	2.01 ± 0.02^{ABab}	2.28 ± 0.05^{B}	3.47 ± 0.02^{C}
B3	1.72 ± 0.08^{Aa}	1.89 ± 0.07^{Aa}	2.14 ± 0.08^{B}	3.39 ± 0.06^{C}
R1	1.88 ± 0.06^{Aab}	2.07 ± 0.05^{ABab}	2.35 ± 0.03^{B}	3.56 ± 0.05^{C}
R2	1.80 ± 0.03^{Aab}	2.04 ± 0.04^{ABab}	2.3 ± 0.08^{B}	3.51 ± 0.07^{C}
R3	1.74 ± 0.07^{Aab}	1.93 ± 0.07^{Aab}	2.24 ± 0.09^{B}	3.44 ± 0.03^{C}
Psychrophilic count (log_{10} cfu/g)				
C4	ND	ND	2.05 ± 0.03^{A}	2.67 ± 0.07^{B}
B1	ND	ND	1.96 ± 0.03^{A}	2.60 ± 0.04^{B}
B2	ND	ND	1.91 ± 0.05^{A}	2.54 ± 0.05^{B}
B3	ND	ND	1.83 ± 0.04^{A}	2.46 ± 0.03^{B}
R1	ND	ND	2.01 ± 0.05^{A}	2.65 ± 0.06^{B}
R2	ND	ND	1.94 ± 0.06^{A}	2.57 ± 0.05^{B}
R3	ND	ND	1.87 ± 0.04^{A}	2.50 ± 0.03^{B}
Coliform count (log_{10} cfu/g)				

All samples	Not detected throughout the period of storage
Yeast and mold count (log_{10} cfu/g)	
All samples	Not detected throughout the period of storage

Mean ± SE with different superscripts in a row (upper case alphabet) and column (lower case alphabet) differ significantly (P<0.05), n = 6 for each treatment, ND = Not detected

C4 (product containing optimized meat protein powder, animal fat and calcium lactate), B1 (C4 containing 1% blueberry extract), B2 (C4 containing 2% blueberry extract), B3 (C4 containing 3% blueberry extract), R1 (C4 containing 1% raspberry extract), R2 (C4 containing 2% raspberry extract), R3 (C4 containing 3% raspberry extract)

Table 5: Effect of blueberry and raspberry extract on sensory quality of composite meat chocolate during storage (25±1°C)

Sample	0 months	2 months	4 months	6 months
Appearance				
C4	8.45 ± 0.26^{B}	8.26 ± 0.18^{B}	7.87 ± 0.30^{B}	6.24 ± 0.24^{A}
B1	8.49 ± 0.21^{B}	8.29 ± 0.24^{B}	7.90 ± 0.29^{B}	6.40 ± 0.21^{A}
B2	8.50 ± 0.28^{B}	8.31 ± 0.24^{B}	7.93 ± 0.18^{B}	6.48 ± 0.21^{A}
B3	8.51 ± 0.18^{B}	8.36 ± 0.12^{B}	7.97 ± 0.21^{B}	6.53 ± 0.19^{A}
R1	8.49 ± 0.25^{B}	8.30 ± 0.31^{B}	7.93 ± 0.37^{B}	6.35 ± 0.27^{A}
R2	8.50 ± 0.16^{B}	8.32 ± 0.25^{B}	7.96 ± 0.32^{B}	6.38 ± 0.25^{A}
R3	8.53 ± 0.14^{B}	8.39 ± 0.14^{B}	8.01 ± 0.21^{B}	6.41 ± 0.21^{A}
Taste				
C4	8.05 ± 0.24^{B}	7.89 ± 0.25^{B}	7.71 ± 0.45^{B}	5.82 ± 0.21^{Aa}
B1	8.12 ± 0.25^{B}	8.06 ± 0.38^{B}	7.76 ± 0.34^{B}	6.15 ± 0.24^{Ab}
B2	8.13 ± 0.23^{B}	8.11 ± 0.32^{B}	7.84 ± 0.30^{B}	6.19 ± 0.23^{Ab}
B3	8.20 ± 0.16^{B}	8.14 ± 0.45^{B}	7.93 ± 0.40^{B}	6.22 ± 0.28^{Ab}
R1	8.12 ± 0.22^{B}	8.09 ± 0.30^{B}	7.85 ± 0.20^{B}	6.13 ± 0.29^{Ab}
R2	8.17 ± 0.36^{B}	8.12 ± 0.16^{B}	7.89 ± 0.24^{B}	6.15 ± 0.21^{Ab}
R3	8.30 ± 0.27^{B}	8.17 ± 0.39^{B}	7.95 ± 0.48^{B}	6.19 ± 0.29^{Ab}
Aroma				
C4	8.20 ± 0.28^{B}	8.18 ± 0.24^{B}	7.87 ± 0.34^{B}	6.12 ± 0.36^{Aa}
B1	8.24 ± 0.25^{B}	8.20 ± 0.27^{B}	7.90 ± 0.37^{B}	6.29 ± 0.23^{Ab}
B2	8.26 ± 0.27^{B}	8.23 ± 0.24^{B}	7.92 ± 0.30^{B}	6.32 ± 0.31^{Ab}
B3	8.29 ± 0.26^{B}	8.26 ± 0.14^{B}	7.95 ± 0.36^{B}	6.41 ± 0.16^{Ab}
R1	8.25 ± 0.23^{B}	8.22 ± 0.27^{B}	8.01 ± 0.34^{B}	6.29 ± 0.11^{Ab}
R2	8.28 ± 0.29^{B}	8.24 ± 0.28^{B}	8.05 ± 0.28^{B}	6.31 ± 0.21^{Ab}
R3	8.36 ± 0.26^{B}	8.28 ± 0.19^{B}	8.10 ± 0.15^{B}	6.35 ± 0.15^{Ab}
Hardness				
C4	7.97 ± 0.18^{B}	7.92 ± 0.36^{B}	7.76 ± 0.37^{B}	6.15 ± 0.13^{A}
B1	$7. 96 \pm 0.18^{B}$	7.90 ± 0.22^{B}	7.76 ± 0.28^{B}	6.17 ± 0.16^{A}
B2	7.98 ± 0.30^{B}	7.92 ± 0.29^{B}	7.78 ± 0.37^{B}	6.24 ± 0.23^{A}
B3	7.99 ± 0.30^{B}	7.96 ± 0.28^{B}	7.80 ± 0.44^{B}	6.13 ± 0.22^{A}
R1	7.95 ± 0.28^{B}	7.89 ± 0.29^{B}	7.75 ± 0.39^{B}	6.15 ± 0.23^{A}
R2	7.96 ± 0.20^{B}	7.91 ± 0.21^{B}	7.75 ± 0.22^{B}	6.16 ± 0.16^{A}
R3	7.97 ± 0.32^{B}	7.94 ± 0.38^{B}	7.78 ± 0.36^{B}	6.21 ± 0.21^{A}
Mouthfeel				
C4	7.72 ± 0.33^{B}	7.64 ± 0.38^{B}	7.54 ± 0.35^{B}	5.88 ± 0.26^{Aa}
B1	7.74 ± 0.15^{B}	7.68 ± 0.32^{B}	7.58 ± 0.32^{B}	6.09 ± 0.15^{Ab}
B2	7.76 ± 0.26^{B}	7.71 ± 0.26^{B}	7.63 ± 0.33^{B}	6.11 ± 0.17^{Ab}
B3	7.82 ± 0.16^{B}	7.75 ± 0.24^{B}	7.68 ± 0.28^{B}	6.16 ± 0.24^{Ab}
R1	7.86 ± 0.30^{B}	7.70 ± 0.42^{B}	7.60 ± 0.33^{B}	6.05 ± 0.16^{Ab}
R2	7.89 ± 0.31^{B}	7.74 ± 0.21^{B}	7.65 ± 0.27^{B}	6.08 ± 0.17^{Ab}
R3	8.06 ± 0.29^{B}	7.80 ± 0.21^{B}	7.72 ± 0.33^{B}	6.13 ± 0.27^{Ab}
Overall acceptability				
C4	8.11 ± 0.28^{B}	7.93 ± 0.33^{B}	7.68 ± 0.38^{B}	5.84 ± 0.21^{Aa}
B1	8.12 ± 0.33^{B}	7.94 ± 0.33^{B}	7.73 ± 0.34^{B}	6.10 ± 0.33^{Ab}
B2	8.15 ± 0.43^{B}	7.96 ± 0.44^{B}	7.74 ± 0.45^{B}	6.13 ± 0.22^{Ab}
B3	8.21 ± 0.29^{B}	8.10 ± 0.31^{B}	7.82 ± 0.35^{B}	6.16 ± 0.16^{Ab}
R1	8.11 ± 0.36^{B}	7.94 ± 0.39^{B}	7.69 ± 0.44^{B}	6.09 ± 0.23^{Ab}
R2	8.14 ± 0.28^{B}	7.96 ± 0.27^{B}	7.72 ± 0.31^{B}	6.12 ± 0.27^{Ab}
R3	8.18 ± 0.27^{B}	8.05 ± 0.43^{B}	7.75 ± 0.42^{B}	6.13 ± 0.24^{Ab}

Mean ± SE with different superscripts in a row (upper case alphabet) and column (lower case alphabet) differ significantly (P<0.05)
Ten members of a trained panel replicated the experiment thrice based on a 9-point hedonic scale, wherein 9 denoted “extremely liked” and 1 denoted “extremely disliked”
C4 (composite meat chocolate containing optimized animal fat, meat protein powder and calcium lactate), B1 (C4 containing 1% blueberry extract), B2 (C4 containing 2% blueberry extract), B3 (C4 containing 3% blueberry extract), R1 (C4 containing 1% raspberry extract), R2 (C4 containing 2% raspberry extract), R3 (C4 containing 3% raspberry extract)