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ORIGINAL ARTICLE

Development and storage study of reduced sugar soy containing compound chocolate

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Abstract Possibility of using full fat soy flour (FFSF) for replacer for whole milk powder (WMP), stevia-mannitol blend as replacer for sugar and soybean oil (SBO) as replacer for cocoa butter in chocolate manufacture without impairing the sensory quality characteristics of chocolate was explored. Data on the sensory evaluation of WMP, sugar and cocoa butter substituted chocolates revealed that 40% (w/w) of WMP, sugar and cocoa butter can be successfully substituted by FFSF, stevia-mannitol blend and SBO, respectively in the preparation of high protein and low sugar chocolate without impairing the sensory attributes. Lecithin was found to be optimum at 0.32% (w/w) level of chocolate mix. Protein content of optimized formulation increased by 21.8% over control. Storage study of the product indicated an increase in hardness, free fatty acid content, peroxide value, total plate count, yeast and mold count, whereas a decrease in moisture content, pH value and sensory scores. The optimized chocolate was found acceptable (score ≥7.0) after 90 days of storage at 16±1°C and ~65% RH.

Keywords Chocolate · Full fat soy flour · Stevia · Cocoa butter · Soybean oil

Introduction

Chocolate is a suspension of cocoa solids and sugar in a continuous fat phase (Stewart and Timms 2002). It is a general observation that consumption of chocolate leads to dental caries, constipation, diabetes, obesity and increase in cholesterol level. Besides, chocolates in general are found to be low in protein and have a high fat value (Anon 2007). All these factors may eventually lead to low consumer acceptance of the product. Soy flour has great potential for replacing milk powder in chocolate (Akinwale 2000) due to its high protein and isoflavones content. Refined soy flour, a natural antioxidant, is added to confectionery to prevent spoilage (Riedel 1990). Problem of high sucrose content in chocolate can be solved by incorporating alternative sweeteners or their blends. Stevia (*Stevia rebaudiana*) is a natural and calorie free sweetener (Gardana et al. 2003). Replacement of sugar by artificial sweeteners often necessitates addition of filler, to increase the bulk of the product (Glicksman and Farkas 1966). Polyols, like mannitol as bulk sweeteners, are of interest in the design of both reduced calorie foods as well as sugar-reduced or sugar-free foods (Dias 1999). Replacement of cocoa butter in chocolate with vegetable fat helps to decrease the cholesterol level (Chaveron et al. 1992). Lecithin, a phospholipid, is used as an emulsifier to improve fluidity of chocolate mix (Anon 1984). Chocolate can be prepared with much lower cocoa butter content if lecithin is present, and since cocoa butter is an expensive ingredient, the economic value of lecithin is obvious (Minifie 1989). Lees (1990) reported that major causes of deterioration of chocolate and confectionery products are fermentation, rancidity and molds. Keeping these factors in view, the present study was undertaken to develop protein rich and low sugar chocolate and study its storage stability.

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Materials and methods

Soybean (*Glycine max*) variety 'PS-1042' was procured from Crop Research Centre of G.B. Pant University of

Agriculture and Technology. Cocoa butter and cocoa liquor were obtained from M M Cocoa Product Pvt. Ltd., Baddi, Himachal Pradesh, India. Soya lecithin was obtained from High Media Laboratories Pvt. Ltd., Mumbai, whereas stevia-mannitol blend was from Stevia Biotech Pvt. Ltd., New Delhi. Whole milk powder (WMP) was obtained from Quality Dairy (India) Ltd., Faridabad, India. Vanillin was procured from Central Drug House (P) Ltd., New Delhi. All other ingredients used were purchased from local market.

Preparation of full fat soy flour (FFSF) FFSF was prepared from dehulled soybean as described by Loh (2006). Whole soy bean was cleaned and dehulled using a dehuller designed and fabricated by Post Harvest Process and Food Engineering Department of the University. The dehulled beans were boiled in 0.35% $CaCl_2$ solution for 45 min using salt solution to soybean ratio of 5:1. After draining the liquid, the sample was washed with hot water (~80°C) for 1 h followed by air drying (~45°C, 10 h). The treated soy cotyledons were ground to fine powder in a domestic grinder (Khera Instruments Pvt Ltd., Azadpur, Delhi) so as to pass through 60 mesh sieve. The FFSF was packed in air tight container and stored at 8°C until use.

Preparation of chocolates All the chocolate samples were manufactured as per the formulation given by Selamat et al. (1998) with minor modifications. For preparation of 100 g of chocolate-mix, cocoa liquor (28 g) and cocoa butter (14.3 g) were melted. To melted cocoa liquor and half of the cocoa butter, ground sugar (30.7 g) and whole milk powder (WMP) (26.5 g) were added and mixed manually. The mix was held at 75°C for 4 h, meanwhile rest of the cocoa-butter and lecithin (0.16 g) were added drop wise to the mix. Thereafter, mix was tempered by cooling to 28°C and then reheating to 32°C. Vanillin (0.16 g) and salt (0.16 g) were thoroughly mixed into it. The mix was cooled to 10°C for 1 h and poured into already tempered (~32°C) mould with gentle tapping to avoid air bubble formation. After setting the mix in refrigerator (10°C), the chocolates were demolded and wrapped in aluminium foil.

Optimization of ingredients FFSF was added at 20, 40, 60, 80 and 100% (w/w) level of WMP in the chocolate formulation. The selected recipe was used to prepare low sugar chocolates by replacing 20–100% of sugar with stevia-mannitol blends. Same levels of cocoa butter were replaced by soybean oil (SBO) in chocolates containing optimized levels of WMP replacer (FFSF) and sugar replacer (stevia-mannitol blend). Optimization was done on the basis of sensory evaluation. Lecithin as an emulsifier was optimized in the formulation after trying 0.16, 0.32, 0.48, 0.64 and 0.80% (w/w) levels in the chocolate mix. Storage study of optimized products was conducted to assess the stability at 16±1°C and 65% RH for up to 90 days.

Physico-chemical characteristics Proximate composition, titratable acidity (expressed as % lactic acid), free fatty acids (% oleic acid), peroxide value (meq peroxide/kg) of chocolate samples were determined according to AOAC (1984) procedures. Carbohydrate content in the samples was determined by difference. In vitro protein digestibility was estimated by modified procedure of Akeson and Stahman (1964). Minerals (Ca, Fe, Mn and Zn) were estimated by atomic absorption spectrophotometer (AAS 4141, Electronics Corporation of India Ltd., Hyderabad, India) using the wet ashing procedure as described by Raghuramulu et al. (2003). Phosphorus was determined as per the method given by Ranganna (1986), while Na and K were estimated by flame photometry. Water activity (a_w) of chocolate samples was determined by dew point hygrometer (FA-st/1 Model, GBX, France), whereas pH was measured using a digital pH meter (ECI Ltd, India). Total plate counts and coliform counts (APHA 1992) and yeast and mold count (IS 1980) in compound chocolate were also enumerated. The calorific value of chocolate was calculated by summing up the products of multiplication of carbohydrate, fat and protein content by factors 4, 9 and 4, respectively (Swaminathan 2003). Samples were evaluated sensorily by 10 panelists, on 9-point Hedonic scale, where 9 and 1 represented liked extremely and disliked extremely, respectively (Larmond 1977). Hardness of chocolate samples was measured on Texture analyzer (Stable Microsystem Model TA-XTZi, UK) using 2 mm dia cylindrical stainless steel probe (Probe code-P/2). All the samples were taken in size of 2.5×2.0×1.0 cm for analyses. Force calibration was carried out by using 25 kg load cell and the texture analyzer settings were fixed (return to start option, pre test speed - 2 mm/sec, test speed - 0.5 mm/s, post test speed - 5 mm/s, rupture test distance - 1 mm, distance - 10 mm, force - 0.98 N, time - 5 s, count - 5).

Statistical analysis Statistical analysis of data was carried out using analysis of variance technique as per Snedecor and Cochran (1968), while the effect of single treatment on different parameters was measured by paired *t*-test as described by Raghuramulu et al. (2003). Mean of three replicates was used in calculations.

Results and discussion

Optimization of FFSF Table 1 shows that as the level of FFSF was increased from 20 to 100% (w/w) of WMP in chocolate mix, hardness values increased ($p \leq 0.05$) from 36.7 N to 50.1 N. Increase in hardness of chocolates on

Table 1 Effect of incorporation of different ingredients on instrumental hardness and sensory scores of chocolate

	Hardness, N	Colour and appearance	Flavour	Body and texture	Overall acceptability
WMP: FFSF					
100:0 (control)	36.7 f	8.7 a	8.6 a	8.2 a	8.6 a
80:20	38.6 e	8.6 b	8.2 b	8.0 b	8.4 b
60:40	41.1 d	8.6 c	8.0 c	7.8 c	8.2 c
40:60	44.8 c	8.5 d	7.4 d	6.9 d	6.9 d
20:80	47.4 b	8.4 e	6.4 e	6.2 e	6.1 e
0:100	50.1 a	8.4 e	6.2 f	5.4 f	6.0 f
F-value	**	**	**	**	**
CD at 5%	1.12	0.076	0.069	0.074	0.051
Levels of stevia, %					
0	41.1 f	8.6	8.0 a	7.8 b	8.2 a
20	42.0 e	8.6	7.9 b	8.0 a	8.0 b
40	43.2 d	8.6	7.6 c	8.0 a	7.8 c
60	44.8 c	8.6	6.7 d	7.9 a,b	6.8 d
80	46.7 b	8.7	6.5 e	7.8 b	6.4 e
100	47.2 a	8.7	6.2 f	7.6 c	6.2 f
F-value	**	ns	**	**	**
CD at 5%	0.52	–	0.10	0.15	0.14
Cocoa butter: Soybean oil					
100:0	43.2 a	8.6 b	7.6 a	8.0 a	7.8 a
80:20	41.0 b	8.6 b	7.4 b	7.7 b	7.7 b
60:40	38.1 c	8.7 a	7.3 c	7.5 c	7.6 c
40:60	34.3 d	8.7 a	6.8 d	7.3 d	6.9 d
F-value	**	**	**	**	**
CD at 5%	0.42	0.043	0.025	0.069	0.046
Lecithin, %					
0.16	38.10	8.7	7.3	7.5	7.6
0.32	35.14	8.7	8.0	8.0	8.0
t-value	17.47	0.802	38.04	12.37	20.47
Interpretation	s	ns	s	s	s

WMP: FFSF: Ratio (w/w) of WMP, Means followed by different letters differ significantly at 5%, **Significant at 1%, Levels of stevia: Per cent replacement of sugar with stevia, Cocoa butter: soybean oil: Ratio (w/w) of Cocoa butter, Levels of lecithin: Per cent lecithin (w/w) of chocolate mix, s: significant, ns: non-significant, n=3 for hardness and n=10 panelists for sensory evaluation

incorporation of soy protein isolate was reported by Hutton and Campbell (1981) due to its ability to absorb cocoa butter during conching, which reduces the mix viscosity. The increase in hardness could prevent the soy flour chocolates from melting easily (Selamat et al. 1998). The colour and appearance scores for control preparation were 8.7, which decreased to 8.4 on incorporation of FFSF. The colour of FFSF containing chocolates was darker as compared to the control chocolate. This may be due to incorporation of high protein sources (soy protein), which can interact with sucrose to produce caramel-like or dark colour during conching process. Such dark coloured chocolates were not liked by the panelists. On incorporation of FFSF in chocolate mix, the flavour scores decreased from 8.6 to 6.2, but the products were acceptable (score≥ 7.0) up to 60% (w/w) level of WMP. The body and texture scores decreased from 8.2 to 5.4. This decrease at higher level of incorporation (beyond 40% (w/w) of WMP) of FFSF may be due to grittiness of FFSF. Overall acceptability scores of chocolates decreased from 8.6 to 6.0 during complete replacement of WMP. On the basis of overall acceptability scores, 40% replacement of WMP by FFSF in chocolate mix was found optimum.

Optimization of stevia Overall increase in instrumental hardness from 41.1 N to 47.2 N was observed when level of stevia was increased from 20 to 100% (w/w) of sugar (Table 1) which may be due to melting resistance property of stevia (Salem and Massoud 2003). Slight increase in colour and appearance scores (from 8.6 to 8.7) may be due to finer particles of stevia (as compared to ground sugar), over which colour of cocoa solids could easily be dispersed. The flavour scores decreased ($p \leq 0.05$) from 8.0 to 6.2 possibly due to slight after taste associated with stevia, which was also reported earlier (Phillips 1989, Tanaka 1997; Savita et al. 2004). Such after taste hindered the use of stevia beyond 40% level of sugar. The body and texture scores initially increased from 7.8 to 8.0 and then decreased, to 7.6 which may be due to presence of mannitol in stevia powder, which provided sufficient bulk to the product at lower level of replacement but at higher level of replacement of sugar with stevia it could not mimic attributes of sugar as bulk sweetener. Inability of stevia-mannitol blend for providing sufficient bulk to fruit juice RTS was also noticed by Sharma et al. (2009). On the basis of overall acceptability score, it was found that stevia could replace 40% of sugar in FFSF containing chocolate. Mohler et al. (1981) stated that sugar content in chocolate formulation could be reduced to 46% of sugar without affecting adversely the overall acceptability scores. Lower level of optimization of sugar replacer in the present investigation may be due to presence of FFSF, which was not used as an ingredient in supporting study.

Optimization of soybean oil Table 1 indicates that as the level of SBO in the formulation was increased, hardness of chocolates decreased from 43.2 to 34.3 N, which may be due to low melting point or low level of saturated fatty acids in SBO as compared to cocoa butter (Stewart and Timms 2002). Selamat et al. (1998) reviewed the fact that cocoa butter, which is used to maintain the total fat of the chocolates, also contributes to increase the hardness of chocolate. The colour and appearance scores of products increased due to gloss provided by SBO to chocolate surface. However, the flavour scores decreased ($p \leq 0.05$) and were found to be satisfactory up to 40% level of replacement of cocoa butter. These observations are in agreement with the findings of Johnston (1972). Body and texture scores of chocolate decreased ($p \leq 0.05$) from 8.0 to 7.3, which may be due to differences in physical properties of SBO and cocoa butter. Overall acceptability scores also decreased. Thus 40% replacement of cocoa butter with SBO was found optimum. Total replacement of cocoa butter with equivalent fats in a chocolate coating formulation was optimized by Nesaretnam and Razak (1992). However, lower level of optimization in present study may be due to differences in the properties of cocoa butter replacer and cocoa butter equivalent fats. It was also found that as the level of SBO increased, viscosity of chocolate mix decreased which led to difficulty in demoulding of chocolates after setting. At higher level of replacement (>60% of cocoa butter), demoulding of chocolate became impossible. This finding is in accordance with Barnett (1978).

Optimization of soy lecithin As the level of lecithin increased, overall acceptability scores also increased but at higher level of incorporation (>0.32% w/w of chocolate mix), chocolates could not be demoulded because of decrease in viscosity of chocolate mix. As the level of lecithin increased from 0.16 to 0.32% (w/w) of chocolate mix, hardness values decreased (from 38.1 to 35.1 N), colour and appearance scores (8.7) remained unaffected whereas, body and texture, flavour and overall acceptability scores increased from 7.3 to 8.0, 7.5 to 8.0 and 7.6 to 8.0, respectively. Based on these observations, 0.32% level (w/w) of lecithin in chocolate mix was found optimum.

Proximate composition and calorific value Table 2 shows composition of control and FFSF chocolates. Akinwale (2000) also found that moisture content of chocolates decreased when defatted soy flour was added in the milk chocolate formulation at 25% (w/w) level of milk powder. Increase in protein content in C_2 over control (C_1) was 21.8%. Higher value of protein than their control counterparts by incorporation of soy flour was reported by Thakur et al. (2008) in various food preparations. Calorific content of optimized preparation (583.1 kcal/100 g) was higher than control (574.5 kcal/100 g). This was apparently due to the fact that FFSFcontaining chocolates (C_2) had higher fat content than control (C_1).

In vitro protein digestibility In vitro protein digestibility of optimized chocolates (C_2) was lower compared to control

Table 2 Quality characteristics of chocolates

	Control (C_1)	Optimized (C_2)
Composition, %		
Moisture	5.0	4.9
Protein	8.4	10.3
Crude fat	41.0	42.6
Ash	2.6	2.5
Carbohydrate (by difference)	43.0	39.7
Calorific value, kcal/100 g	574.5	583.1
In vitro protein digestibility, %	92.6	86.9
Minerals, mg/100 g		
Ca	173.2	170.9
P	185.0	276.3
Fe	0.83	0.75
Na	124.0	101.4
K	265.7	256.5
Mn	0.078	0.140
Zn	3.2	3.3
Water activity	0.495	0.417

(n=3)

(Table 2). Slightly lower protein digestibility of optimized chocolates in comparison with control preparation may be due to presence of anti nutritional factors in soy flour, which inhibited the action of proteolytic enzymes.

Mineral content The difference in mineral content of chocolates in the present study (Table 2) and earlier study (Hui 1983) may be due to differences in recipe, ingredients and their levels and different types of replacers used in the formulation. This study showed that optimized preparation had higher contents of P, Mn and Zn, whereas lower content of Ca, Fe, Na and K as compared to control.

Water activity, pH and titratable acidity After incorporation of various ingredients pH values were slightly affected (Table 2). This showed almost neutral effect of various ingredients. The water activity and titratable acidity of C_1 and C_2 were 0.495 and 0.217 and 0.417 and 0.257%, respectively.

Fat stability Lower values of FFA and PV for C_1 may be attributed to presence of higher amount of cocoa butter, which was less prone to oxidative and hydrolytic rancidity. Resistance of cocoa butter against hydrolytic and oxidative rancidity was also reported by Johnston (1972) and Young (1983).

Microbial count Total plate count and yeast and mold count of C_1 were higher ($p \leq 0.05$) than the microbial load of C_2. This may be due to higher moisture content and water activity of control chocolate (Table 2). Coliforms could not be detected in freshly prepared chocolate samples.

Changes during storage Moisture content decreased ($p \leq 0.05$) throughout the storage period.One of the possible reasons for increasing hardness during storage of confections is decrease in moisture content (Fox and McSweeney 1998). Similar observation was seen in the present investigation. Ali

Table 3 Changes in physico-chemical, microbial and sensory characteristics of chocolates during storage at 16±1°C

Chemical	Product	Storage period, days								
		0	15	30	45	60	75	90	F-value	CD
Moisture, %	C_1	5.0	4.8	4.7	4.3	4.1	4.0	3.9	**	0.079
	C_2	4.9	4.5	4.1	3.8	3.7	3.6	3.4	**	0.058
Hardness, N	C_1	36.7	36.1	36.2	37.3	37.3	38.1	38.4	**	0.76
	C_2	35.1	35.7	36.3	37.0	37.7	38.3	39.0	**	1.48
pH	C_1	7.0	7.0	6.9	6.8	6.7	6.5	6.5	**	0.118
	C_2	7.1	7.1	7.0	7.0	6.9	6.8	6.8	*	0.16
Titratable acidity, % lactic acid	C_1	0.27	0.34	0.46	0.50	0.55	0.58	0.59	**	0.0084
	C_2	0.26	0.30	0.39	0.40	0.45	0.47	0.50	**	0.011
Free fatty acid, % oleic acid	C_1	0.21	0.26	0.31	0.40	0.45	0.53	0.59	**	0.014
	C_2	0.58	0.64	0.88	1.20	1.39	1.55	2.08	**	0.083
Peroxide value, m eq/ Kg of sample	C_1	3.0	3.3	3.9	4.3	4.7	5.2	5.4	**	0.110
	C_2	3.2	4.6	5.2	6.4	7.2	8.6	9.0	**	0.150
Microbial count (log_{10}cfu/g)										
Total plate count	C_1	3.4	3.8	3.9	4.1	4.2	4.4	4.5	**	0.042
	C_2	3.3	3.7	3.9	4.0	4.1	4.3	4.4	**	0.026
Yeast and mold count	C_1	2.4	2.6	2.7	2.9	2.9	2.9	3.1	**	0.014
	C_2	2.3	2.4	2.5	2.8	2.9	2.9	3.0	**	0.029
Sensory score										
Colour and appearance	C_1	8.7	8.7	8.7	8.6	8.6	8.6	8.6	**	0.035
	C_2	8.7	8.7	8.7	8.7	8.7	8.6	8.5	**	0.031
Flavour	C_1	8.6	8.6	8.5	8.5	8.5	8.4	8.4	**	0.037
	C_2	8.0	8.0	8.0	7.9	7.9	7.9	7.9	**	0.073
Body and texture	C_1	8.2	8.2	8.1	8.1	8.1	8.0	8.0	**	0.086
	C_2	8.0	8.0	8.0	8.0	7.9	7.9	7.9	**	0.079
Overall acceptability	C_1	8.6	8.6	8.5	8.5	8.5	8.5	8.4	**	0.056
	C_2	8.0	8.0	8.0	8.0	7.9	7.9	7.9	ns	–

*,** & ns: As in Table 1, C_1, C_2: As in Table 2

$n=10$ panelists for sensory evaluation and $n=3$ for other parameters et al. (2001) also reported post hardening in chocolates during storage at 18°C An increase in titratable acidity as an indicator of glycolytic changes in milk based confectionery during storage was also reported by Sarkar et al. (2002).

An increase ($p \leq 0.05$) in FFA content was observed during storage. Increase in FFA content during storage of butter paper packaged chocolate was observed by Yadav et al. (2009) and Ali et al. (2001). Values of FFA content in cocoa butter containing chocolates (control) were lower than the SBO containing chocolates (C_2). This was apparently due to the fact that cocoa butter is sufficiently saturated to exhibit excellent resistance to hydrolytic rancidity as a result of the activity of fat splitting enzymes (Johnston 1972).

PV increased ($p \leq 0.05$) during storage (Table 3). Lower content of peroxides in control samples throughout storage period may be due to presence of higher content of cocoa butter in its formulation which was reported as very resistant fat against oxidative deterioration. Retardation of oxidative rancidity of chocolates due to presence of natural antioxidants of cocoa butter was also reported by Becker (1951).

Microbial characteristics Normally, chocolate and other confectionery products are regarded as microbiologically stable and safe for consumption. Owing to the inherent low water activity, it cannot support the growth and proliferation of bacterial pathogens but high nutrient content, almost neutral pH (6–7) and long storage duration of chocolates make them perfect medium for microbial proliferation. Potential of chocolate mix as perfect medium for microbial growth was also reported by Baylis et al. (2004). Hence, microbiological changes in chocolate samples during storage were also determined. Optimized chocolate showed lesser standard plate count as compared to control chocolate throughout the storage period. It may be due to non fermentative behaviour of stevia (Sasaki 1983) as it is a non-nutritive sweetener and cannot support the growth of microflora. Similar trend was also observed in yeast and mold count. No coliform count could be detected in any chocolate samples throughout the storage study indicating that the chocolates were prepared and stored in hygienic conditions.

Sensory characteristics Generally colour and appearance scores for chocolates were not affected by the addition of any ingredient, as dark colour of cocoa liquor masked the colour of any added ingredients in the investigation. Colour and appearance marginally changed during storage. Flavour of control was superior. This may be due to presence of higher amount of cocoa butter, which has been reported to be a protective coating or package for chocolate flavour (Keeney 1972). Generally sensory scores decreased during storage. The control as well as the optimized chocolates were sensory acceptable (≥7.0) even after 90 days of storage at 16±1°C and RH ~65%.

Conclusion

Acceptable quality chocolates could be prepared using FFSF, stevia-mannitol blend and SBO at 40% replacement level of whole milk powder, sugar and cocoa butter, respectively. Lecithin at 0.32% (w/w) level of chocolate mix could be used to improve the overall acceptability of the product. The developed chocolate stored well at 16±1°C and ~65% RH for 90 days.

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