



Formulation and Quality Evaluation of Antioxidant Rich Bars Enriched with Chia seed, Whole Mango, Apple and Guava

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Abstract

An insalubrious diet regime and lack of physical exercise are the root causes for the causation of metabolic diseases. Thus, need for food choices that are nutritious, rich in antioxidants and are ready to eat has grown to a great extent. Accordingly, the present study was planned to develop an antioxidant rich bar with puffed wheat, Chia seed, mango (Leather+peel powder), apple (whole fruit Leather), guava (whole fruit Leather), peanut and honey in different proportions using standard bar moulds. Therefore, three types of bars were prepared, namely mango Chia seed bar, apple Chia seed bar and guava Chia seed bar. Formulated mango Chiaseed bar of 55g showed the highest overall acceptability with 2g mango peel powder and 8g mango leather. Apple Chiaseed bar and guava Chia seed bar were found acceptable on incorporation of 5g leather of apple and guava respectively. The fatty acid profile of the selected bars showed significant increase ($p < 0.01$) in PUFA content. A significant increase ($p < 0.01$) was found in vitamin C and β -carotene content, both of which act as a good natural antioxidants. The bioactive component and antioxidants such as total phenols, flavonoids, tannins showing DPPH, FRAP, ABTS and RPA activity elevated to significant ($p < 0.01$) levels with the highest content in mango chia seed bar. The bar prepared was acceptable and nutrient-dense, therefore can be consumed by the general population to prevent and cure lifestyle diseases by increasing antioxidant content in their diet.



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Introduction

In India over the last two decades, over nutrition and obesity have emerged as huge public health problems, increasing the prevalence of non communicable diseases such as diabetes, cardiovascular disease (CVD), stroke, cancer etc especially in urban areas. Major risk factors for occurrence of these diseases are heredity, sedentary lifestyle, unhealthy dietary habits such as high energy intake, skipping of meals, high fat intake especially harmful fats like trans-fats, proteins of animal origin and high sugar intake. Another important factor for the causation of metabolic and lifestyle diseases is an increased level of oxidative stress. To put simply, an imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage is termed as 'oxidative stress'.¹ Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Antioxidants are said to be "compounds that protects biological systems against the potentially harmful effects of processes and reactions that can cause excessive oxidation".² Therefore, recommended amounts of cereals, pulses, oilseeds, nuts, milk and milk products, fruits and vegetables are important constituents of balanced diet for normal as well as therapeutic diet providing an adequate amount of nutrients and antioxidants needed by the body for a healthy living.

Chia seed (*Salvia hispanica* L.) belongs to the Lamiaceae (Mint) family and is an annual herb that is recently being recognized as a novel food and is receiving considerable scientific attention worldwide. It is a rich source of omega-3, omega-6 fatty acids, soluble dietary fibers, proteins and phytochemicals. Many *in-vitro* studies have proven the antioxidant activity of chia seed components. Therefore it has nutritional benefits in the prevention of several non-communicable diseases such as obesity, hypertension, cardio-vascular disease (CVD), cancer and diabetes.³ Many products have been formulated incorporating chia seed, flour and gel. In one such study conducted, Chia and quinoa seed flours were successfully incorporated in cookies by partially substituting wheat flour. Substitution of 10% chia and 15% quinoa seed flour resulted in the cookies with best sensory acceptability and marked

increase in protein, dietary fiber, polyunsaturated fatty acids, minerals.

Fruits being rich in many bioactive components and antioxidants are crucial to be included in daily diet. It is reported that low consumption of fruits in diet is the third major risk factor of CVDs following the high blood pressure and cigarette smoking, which accounts for more than 5 million deaths worldwide in 2010.⁴ Fruits contain many compounds including flavonoids, phenolics, thiols, carotenoids, tocopherol, and glucosinolates which may exert health benefits.⁵

Among the fruits, Mango (*Mangifera indica* L.) is a good source of dietary antioxidants, such as ascorbic acid, carotenoids and phenolic compounds. Several studies have indicated that a leucocyanidin, catechin, epicatechin, chlorogenic acid and quercetin are the main phenolics found in mango.⁶ Mango peel is also found to be a rich source of dietary fibers, starch, pectin and antioxidants including ascorbic acid, carotenoids and phenolic compounds.⁷ Apple (*Malus × domestica* Borkh.) consumption have shown to reduce the risk of cancer, cardiovascular diseases, asthma and diabetes. Apple contains a variety of phytochemicals such as quercetin, catechin, phloridzin and chlorogenic acid as strong antioxidants. The peel of apple fruit also possesses all these phytochemical compounds including additional flavonoids that is not present in flesh such as quercetin glycosides so the fruit should be utilized as a whole.⁸ Guava (*Psidium guajava* L.) contain high level of ascorbic acid, phytofluene, β -carotene, lycopene, rubixanthin, cryptoflavin, lutein, and neochrome makes it a highly nutritious food.⁹ Peel of guava is also found to have a good reducing power according to several studies.

Many studies showing the substitution of Chia seed as a functional ingredient and its effect on nutritional and sensory qualities have been conducted. The cookies were prepared substituting chia and quinoa seed flour in place of refined wheat flour at 5%, 10% and 15%. Significant differences in physical, physicochemical and nutritional properties were observed.¹⁰ The 10% (Chia cookie) gave best sensorial results with increased levels of omega 3 fatty acid, phenols, flavanoids and antioxidants. In another similar study, 10%, 15 % and 20% of a

pregelatinized low amylose ready to-eat rice flour was substituted with a 1:1 mixture of chia and quinoa seed flours.¹¹ Substitution significantly enhanced levels of proteins, dietary fibers, iron, calcium, potassium, sodium and zinc. Palmitic, stearic and oleic acids were proportionately higher. Beside this relative rise in ferric and cupric ion reducing antioxidant potentials was reported.¹¹

Formulation of ready to eat bars using different nutrient dense ingredients has been conducted by many researchers. One such product formulated was cereal bars utilizing guava pulp at 10% and 15 % concentration and the results showed higher protein, moisture and lower fat content in the cereal bars.¹² In another study, development of energy bar was done using different levels of flaxseed (0–20%) in addition to cereals and pulses with varying levels of sweeteners (45, 50, and 55%) to deliver a nutritious food to the consumer.¹³ The total calories obtained from the energy bar showed significant increase with the increasing levels of flaxseed. This energy bar sample also showed the maximum protein (12.41%), crude fat (11.86%), ash (1.65%), iron (3.77 mg/100 g), crude fiber (2.18%) and omega-3 as alpha-linolenic acid (22.50%, fatty acid basis) content. The sensory score for overall acceptability for samples with 10% flaxseed and 55% sweeteners and 15% flaxseed and 45% sweeteners were at par. Beside this in one such kind of study four different oat bar formulas (F1, F2, F3 and F4) from quick oat flakes, sunflower, chickpea and pumpkin seeds were prepared. The results indicated that the substitution of oat with sunflower, chickpea and pumpkin seeds significantly enhanced the nutritional value of the resultant bars. Protein, fat, total phenols, total flavonoids, tannins and antioxidant activity significantly increased. Sunflower formula was the most valuable addition with the highest acceptability followed by pumpkin formula.¹⁴

For the development of nutritious product with potential health benefits a thorough analysis of different parameters is needed. In accordance to the ongoing discussion, the present study was planned to prepare ready to eat bars using ingredients rich in nutrients and antioxidants and was further analyzed for the nutritional and sensory attributes of the developed bars.

Material and Methods

Procurement of Raw Material

Chia seeds (Black Variety) were procured from Herbo Foods, Vadodara, Gujarat. Peanuts (SG 99) were procured from oilseeds section of department of Plant Breeding and Genetics, Mango (var. chausa) and Guava (var. Punjab pink) was procured from fruit research substation of Gangian and Patiala respectively of Punjab Agricultural University, Ludhiana (Punjab), India. Apple and honey were purchased from the local market of Ludhiana.

Processing of Pulp and Peel

The procured fresh mango fruit were sorted, washed thoroughly and cut into small pieces. The mango pulp was then heated at 50–55°C for 5 minutes adding 5% sugar and 0.2% citric acid. While heating the pulp, sugar and citric acid were mixed properly, and the mixture was further grinded and spread into a thin layer (0.8cm) in aluminium trays. The drying of mango pulp in a tray-drier at 55°C for 4–5 hours was done and the leather prepared was cut, rolled, and stored in laminated pouches under refrigeration temperature (2 to 4°C). The mango peels were dehydrated at 60°C for 6–8 hours after the proper washing and cutting of the peels. The dehydrated peels (5% moisture) were then grinded to the fine powder using oster, 500 watt grinder and were stored in laminated aluminium pouches at temperature of -18°C.

Processing of Other Raw Materials

The peanut and Chia seed were checked for any infestation or damage and were roasted at 180°C for 20 minutes separately. Puffed wheat, roasted Chia seed and peanuts were then grounded finely using electronic grinder (Make: Oster, 500 watt, Model: 5011, India) which were then stored in sealed laminated aluminium pouches in deep refrigerator (-18°C) till further use.

Preparation of Bars

Three distinct types of antioxidant rich bars were developed namely Mango chia seed bar, guava chia seed bar and apple chia seed bar. The control bar was prepared using puffed wheat, peanut and honey i.e. 30g, 10g and 15g respectively. The control bar was further enriched with addition of Chia seed in 3, 4, 5, 6 g levels in the first trial, and in the highly

acceptable level (5g) of first trial three different whole fruits (mango, apple and guava) was incorporated. The incorporation of mango fruit leather was done in 4, 8, 12 g level while the mango peel powder was added in 1, 2, 3g level of incorporation. Moreover incorporation of apple and guava fruit leather was done in 5, 10, 15g level of incorporation. The ingredient mixes of the antioxidant rich bars were developed to provide nutrient and antioxidant rich wholesome snacks. The ingredient mix was binded with the addition of honey and bars of 55g were developed pouring the mixture to the moulds of size 11cm (L), 3cm (B) and 1.5cm (H). The bars were then kept for setting at refrigeration temperature for 1 hour, further were packed in aluminium sheets. The prepared bars were stored under refrigeration temperature till analysis.

Sensory Evaluation of Bars

Sensory evaluation of formulated antioxidant rich bar was conducted for both the trials i.e. incorporation of Chia seed followed by three different whole fruits. Sensory evaluation by 10 semi trained penalists (were all professors and Ph.D scholars of age 30-60 yrs) of Department of Food and Nutrition, Punjab Agricultural University, Ludhiana was done using 9- point hedonic scale for different parameters such as appearance, colour, texture, flavour, taste and overall acceptability.¹⁵

Quality Evaluation of the Bars

Fatty Acid Composition

Using the dried sample, fatty acid composition was determined by conversion of oil to fatty acid methyl esters (FAMES), which was analysed with a gas chromatograph (Varian CP 3800, USA), attached with a flame ionization detector and a capillary column of 50 m length and 0.25 mm internal diameter having CP-SIL 88 stationary phase.¹⁶ The temperature for programming was kept at 200°C for 13 minutes while keeping the injector and FID at temperature of 250°C. Reference FAME mix was also analysed under similar operation conditions. The samples were analysed for saturated fatty acids (SFAs) (palmitic acid – C16:0 and stearic acid – C18:0), mono unsaturated fatty acid (MUFA) (oleic acid – C18:1) and poly unsaturated fatty acid (PUFAs) (linoleic acid – C18:2, linolenic acid – C18:3, arachidonic acid – C20:4), FAMES and were expressed as relative area percentage.

Mineral and Vitamin Composition

For the estimation of selenium content, the sample was wet-digested in a mixture of nitric acid and perchloric acid mixture in 5:1 ratio (v/v) using a hot plate and the content in the extract was determined by atomic absorption spectrophotometry (AAS, Varian model).¹⁷ Ascorbic acid content was determined by extracting ascorbic acid in xylene layer and reading it at 500nm.¹⁸ Beta carotene was determined by column chromatography method.¹⁹

Bioactive Components Determination

The bioactive components namely total phenols, total flavonoids and tannins were analyzed. For the estimation of total phenols and flavonoids extraction of sample components in the 80% methanolic extract (acidified at pH 2) was done and then was determined further using the standard procedures. Total phenols were determined by colorimetric methods using Folin-Ciocalteu reagent.²⁰ The total flavonoids content was analysed by aluminium chloride colorimetric method.²¹ The extract of tannins prepared in distilled water was treated with Folin-Denis reagent and sodium carbonate according to AOAC (1985) and the absorbance was read at 700 nm after 30 min.²²

Antioxidant Activities Analysis

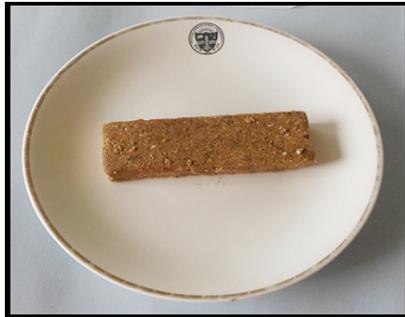
The antioxidant activity was determined by various means such as DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity, Ferric Reducing Antioxidant Power (FRAP) assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Activity and Reducing Power Assay (RPA). For estimation of antioxidant activities, the sample extract was prepared with acidified 80 % methanol which was further analyzed using the standard protocols. The free radical scavenging activity of the different sample aliquots taken was determined by addition of DPPH reagent and reading was taken using spectrophotometer at 517 nm.²³ The protocol of Benzie and Strain (1999) was used to determine the reduction of ferric tripyridyltriazine complex (III) to ferrous (II) compound due to the electron donating capacity of antioxidant.²⁴ In RPA, the ability of sample to reduce potassium ferricyanide to ferrocyanide and then reacting to ferric chloride to form ferric ferrous complex was determined) taking the readings at 700nm using spectrophotometer.²⁵ The capacity

of sample extract to reduce the ABTS cation radical generated due to the reaction of ABTS with potassium persulphate was measured at 734nm.²⁶

Statistical Analysis

All the determinations were carried out in triplicate and the results are given as mean ± standard

deviation. Data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) Version 16.0. To compare the significant differences in mean values of control and antioxidant rich bars, independent T- test was employed. Level of significance was expressed at 5% and 1%.



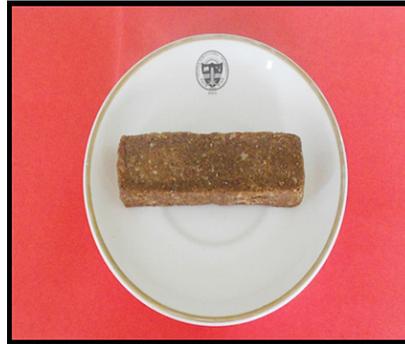
Control (Puffed wheat + peanuts + honey)



Puffed wheat+peanuts+honey+5g Chiasseeds



Puffed wheat+peanuts+honey+5g Chiasseeds+ 2g mango peel powder+8g mango fruit leather



Puffed wheat+peanuts+honey +5g Chiasseeds+ 5g apple fruit leather



Puffed wheat+peanuts+honey +5g Chiasseeds+ 5g apple fruit leather

Fig. 1: Developed bars with incorporation of chiasseed and mango, apple and guava fruit

Table 1: Sensory evaluation of the bars

Proportions	Sensory parameters					
	Appearance	Colour	Texture	Flavour	Taste	Overall Acceptability
Chiaseed bar						
T1(control) (30g PW+10g P+15g H)	8.20±0.35	8.25±0.64	8.35±0.53	8.10±0.85	8.00±0.38	8.18±0.35
T2(control + 3g CS)	7.45±0.25	7.4±0.26	7.5±0.36	7.45±0.36	7.35±0.46	7.43±0.18
T3(control + 4g CS)	7.45±0.17	7.50±0.13	7.65±0.28	7.55±0.48	7.6±0.24	7.55±0.46
T4 (control + 5g CS)	7.7±0.62	7.65±0.55	7.75±0.56	7.65±0.36	7.8±0.36	7.71±0.3
T5 (control + 6g CS)	7.2±0.49	7.4±0.84	7.35±0.19	7.25±0.29	7.3±0.35	7.3±0.29
χ^2 value#	19.18**	21.59**	18.85**	13.318*	11.54*	30.36**
Mango chiaseed bar						
T1 (Control)	8.00±0.26	8.00±0.94	7.50±0.27	7.25±0.47	7.55±0.33	7.69±0.64
B1 (control + 5g CS+1g MPP+4g MFL)	7.85±1.03	7.85±0.84	8.00±0.69	7.65±0.29	7.70±0.46	7.81±0.25
B2 (control + 5g CS+2g MPP+8g MFL)	7.90±0.57	8.00±0.38	8.05±0.37	8.30±0.57	8.25±0.94	8.10±0.4
B3 (control + 5g CS+3g MPP+12g MFL)	7.10±0.37	7.10±0.36	7.40±1.20	7.40±0.93	7.40±0.74	7.28±0.26
χ^2 value#	8.95*	12.32**	10.18*	13.39**	8.58*	15.07**
Apple chiaseed bar						
T1(Control)	8.00±0.36	7.70±0.57	7.60±0.56	7.50±0.37	7.60±1.08	7.68±0.36
B1 (control + 5g CS+5g AFL)	8.00±0.70	7.90±0.46	7.90±0.28	7.90±1.03	8.05±0.48	7.95±0.47
B2 (control + 5g CS+10g AFL)	7.80±0.31	7.80±0.26	7.80±0.85	7.70±0.19	7.55±0.37	7.73±0.29
B3 (control + 5g CS+15g AFL)	7.10±0.47	7.00±0.73	7.20±0.93	7.00±0.46	7.10±0.29	7.08±0.47
χ^2 value#	8.37*	8.60*	3.99 ^{NS}	7.08 ^{NS}	7.58 ^{NS}	9.29*
Guava chiaseed bar						
T1 (Control)	7.80±0.73	8.00±0.57	7.70±0.26	7.80±0.18	7.00±0.38	7.66±0.84
B1 (control + 5g CS+5g GFL)	8.00±0.26	8.10±0.28	7.80±0.47	7.85±0.46	8.10±0.66	7.97±0.48
B2(control + 5g CS+10g GFL)	7.80±0.45	7.80±0.75	7.75±0.26	7.35±0.36	7.30±0.75	7.60±0.63
B3(control + 5g CS+15g GFL)	8.00±0.29	7.90±0.47	7.60±0.47	7.10±0.19	6.70±0.83	7.46±0.58
χ^2 value#	0.50 ^{NS}	1.57 ^{NS}	0.392 ^{NS}	5.18*	12.23**	4.82*

Results and Discussion

Sensory Evaluation of Bars

The developed bars have been shown in Fig 1. In the first standardization, the chiaseed were incorporated in the control bar. The scores for chia seeds bar statistically revealed that all the treatments were significantly different with regards to parameters like appearance, colour, texture, flavour, taste and overall acceptability as presented in Table 1. Among the treatment, the bar incorporated with 5 g of chia seeds (T4) had the highest score for overall acceptability of 7.71. Hence, T4 was selected for further incorporation of three different fruits i.e. mango, apple and guava individually in the second standardization.

In the second standardization, mango, apple and guava were incorporated in bar prepared with 5g of chiaseed. In the first trial, incorporation of mango fruit leather and mango peel powder together was done. The mango pulp to peel ratio in fresh fruit was found to be in 1:4. But after dehydration, the yield of both mango leather and mango peel powder was concentrated to approximately 4.5 times. Therefore, the incorporation of mango peel powder and mango leather was done in a similar ratio to that of fresh fruit. The prepared mango chiaseed bar with the incorporation of chiaseed, mango fruit leather and mango peel powder was found to have greater acceptability than the control bar. The data shows

that the highest overall acceptability score of 8.10 was obtained for bar B2 (with 5g chiaseed, 2g mango peel powder and 8g mango fruit leather) among the other bars with different level of incorporation. In one such study, a sponge cake was prepared with partial substitution of wheat flour with mango pulp and mango peel flour at the concentration of 5, 10, 20 and 30 % respectively and sensory evaluation of the developed sponge cake showed formulation with 10 % mango pulp and mango peel flour respectively to be most acceptable.²⁷

The apple fruit was incorporated after mango, in the form of apple fruit leather prepared from whole fruit. The sensory evaluation revealed that the control bar scored 7.68 in overall acceptability whereas the B1 treatment with 5g apple fruit leather obtained the highest scores for overall acceptability i.e. 7.95. A significant difference was found between appearance, colour and overall acceptability of all treatments. A study was conducted to incorporate apple pomace powder in refined flour as a source of dietary fibre for the preparation of bread.²⁸ The level of incorporation was standardized by using refined flour and pomace powder in the ratio of 80:20, 70:30 and 60:40. Sensory analysis for getting highly acceptable ratio was carried out and it was found that ratio of 70:30 to be best in overall acceptability among all the other treatments.²⁸

The third fruit incorporated was guava fruit, in the form of guava fruit leather prepared from the whole fruit. Likewise, mango and apple, incorporation of whole guava fruit leather was done in 5g, 10g and 15g level of incorporation in the acceptable chia seed bar. The sensory evaluation revealed that the control bar scored 7.66 in overall acceptability whereas the B1 treatment with 5g guava fruit leather obtained the highest scores for overall acceptability i.e. 7.97 (Table 4.8). A significant difference was found between sensory parameters such as flavour, taste and overall acceptability of all treatments. One such study report the development of cereal-based bars using different varieties of guava pulp was done at different concentrations of 10 and 15 %. The sensory evaluation showed satisfactory acceptance of the bars up to 10% guava pulp regarding most of the sensory parameters.²⁹

Fatty Acid Composition

Six fatty acids namely, palmitic (C16:0), stearic (C18:0), oleic (18:1), linoleic (18:2), linolenic (C18:3) and arachidonic (C20:4) acids were identified in selected antioxidant-rich bars (Tables 3). With these fatty acids the determination of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) was also done. It was depicted through the results that there was a significant increase ($p < 0.05$) in palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) in the selected antioxidant-rich bars when compared to control bar.

The results of saturated fatty acids (SFA) show that there is a significant increase in saturated fatty acids among all the selected bars. The control bar was reported to have an SFA of 10.30 %. The saturated fatty acid was found to be varied from 12.66 to 12.80 % and was found to be highest in guava chia seed bar. The monounsaturated fatty acids (MUFA) was found to be significantly ($p < 0.05$, $p < 0.01$) increased in the selected antioxidant-rich bars except for apple chia seed bar where non-significant increase occurred. In the control bar the MUFA content was found to be 45.18 %. The MUFA content among selected bars was found to be highest in guava chia seed bar i.e. 46.05 % and lowest was observed in apple chia seed bar 46.05 %. Among all the bars significant ($p < 0.01$) increase was found in the polyunsaturated fatty acid (PUFA) content ranged from 38.28 % (mango chia seed bar) to 39.81 % (guava chia seed bar). The control bar was observed to have PUFA content of 33.85 %.

The content of unsaturated fatty acid increased significantly due to the incorporation of chia seed rich in omega 3- fatty acids. The results were consistent with developed flax oats nutty granola bars results show the SFA, MUFA and PUFA content to be 17.79, 51.34 and 28.70 respectively which is varying from the results of the present study because of difference in ingredients used.³⁰ The study reported the development of soup powder incorporating microencapsulated flaxseed oil to enrich the omega-3 fatty acid, consumption of which plays a major role in preventing cardiovascular diseases.³¹

Mineral and Vitamin Composition

The mineral and vitamins such as selenium, ascorbic acid and β-carotene which exhibit the antioxidant properties were determined in the control and selected antioxidant rich bars so as see the effect of incorporation and the results are presented in Table 2. The incorporation of chiaseed and different whole fruits to the control bars was found to have significant increase in β-carotene and vitamin C content of the bars while a non-significant increase in the selenium content occurred.

The selenium content of selected apple chiaseed and guava chiaseed bar was observed to be significantly increased (p<0.01) while a non-significant increase in the selenium content of mango chiaseed was found. The selenium content of the selected antioxidant rich bar ranged from 14.18 to 14.76 µg/ 55g. However, vegetables and fruits contains trace amounts of selenium (0.001–0.067 µg/g).³²

Table 2: Fatty acid composition of bars

Products	Fatty Acids (%)									
	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4	SFA	MUFA	PUFA	
Control Bar	8.75±0.10	1.55±0.12	45.18±0.17	22.52±0.17	11.32±0.11	10.30±0.53	nd	45.18±0.22	33.85±0.60	
MCB (Acceptable)	9.97±0.12	2.74±0.11	46.56±0.11	25.34±0.10	12.93±0.09	nd	nd	46.56±0.25	38.28±0.15	
t-value	13.099**	13.096**	11.936**	33.183**	5.206**	-	-	13.173**	11.936**	12.393**
Control Bar	8.75±0.10	1.55±0.12	45.18±0.17	22.52±0.17	11.32±0.11	10.30±0.53	nd	45.18±0.22	33.85±0.60	
ACB (Acceptable)	10.08±0.18	2.58±0.11	46.05±0.80	25.52±0.17	13.66±0.21	nd	nd	46.05±0.22	39.18±0.37	
t-value	11.113**	10.436**	1.853NS	25.407**	7.080**	-	-	12.748**	1.853NS	13.060**
Control Bar	8.75±0.10	1.55±0.12	45.18±0.17	22.52±0.17	11.32±0.11	10.30±0.53	nd	45.18±0.22	33.85±0.60	
GCB (Acceptable)	10.12±0.22	2.68±0.32	47.33±0.40	25.80±0.72	14.01±0.86	nd	nd	47.33±0.53	39.81±0.13	
t-value	9.788**	5.773*	8.465**	7.763*	5.226**	-	-	7.482**	8.465**	16.775**

Values are given as Mean±SD * (p<0.05) ** (p<0.01) NS- Non significant MCB-mango chiaseed bar ACB- apple chiaseed bar GCB- guava chiaseed bar C16:0- palmitic acid C18:0- stearic acid C18:1- oleic acid C18:2- linoleic acid C18:3 - linolenic acid C20:4- arachidonic acid SFA- saturated fatty acid MUFA- monounsaturated fatty acid PUFA- polyunsaturated fatty acid

Ascorbic acid content of all selected bars increased significantly ($p < 0.01$) as presented in Table 2. Ascorbic acid recorded among all the selected antioxidant rich bars was found to be highest in guava chiaseed bar (10.29mg/55g) and lowest in apple chiaseed bar (1.27mg/55g) due to highest and lowest content of ascorbic acid in guava and apple respectively. Incorporation of whole mango, apple and guava found to increase the ascorbic acid content of all selected bars. observed ascorbic acid content of 0.07 to 1.10 mg/100g was in The cereal bars enriched with flours of acerola residues was found to increase the ascorbic acid content of 0.07 to 1.10 mg/100g.³³ In a study, ascorbic acid content of 50.6mg/100g in developed anti-oxidant rich nutrient bar was observed.³⁴

The results presented in (Table 2) shows that there was a significant ($p < 0.01$) increase in the β - carotene content of all the bars except in bars incorporated with apple fruit where a non-significant decrease in β -carotene content was observed. The β -carotene content in all the selected antioxidant rich bars was found to be ranged from 18.41 to 325.24 μ g/55g. The highest ascorbic acid content was found to be present in mango chiaseed bar while the lowest ascorbic acid content was observed in apple chiaseed bar. The significant increase in mango and guava incorporated bars is due to their high content of β -carotene. The study found β -carotene content of 0.384 % to be present in cereal bars incorporated with quinoa.³⁵

Table 3: Mineral and vitamin composition of bars

Products	Selenium (μ g/ 55g)	Vitamin C (mg/ 55g)	β -carotene (μ g/ 55g)
Control Bar	13.51 \pm 0.10	0.85 \pm 0.01	24.55 \pm 5.31
MCB (Acceptable)	14.18 \pm 0.10 ^{abc}	4.95 \pm 0.03 ^c	325.24 \pm 5.31 ^a
t-value	1.959 ^{NS}	1658.28 ^{**}	69.296 ^{**}
Control Bar	13.51 \pm 0.10	0.85 \pm 0.00	24.55 \pm 5.31
ACB (Acceptable)	14.40 \pm 0.08 ^{ab}	1.27 \pm 0.01 ^e	18.41 \pm 9.20 ^c
t-value	11.75 ^{**}	211.289 ^{**}	1.000 ^{NS}
Control Bar	13.51 \pm 0.10	0.85 \pm 0.00	24.55 \pm 5.31
GCB (Acceptable)	14.76 \pm 0.07 ^a	10.29 \pm 0.02 ^a	76.71 \pm 4.58 ^b
t-value	17.254 ^{**}	3512.9 ^{**}	25.21 ^{**}

Values are given as Mean \pm SD * ($p < 0.05$) ** ($p < 0.01$) NS- Non significant Figures with different superscripts –a, b, c, d, e, f are significantly different ($p < 0.05$) MCB- mango chiaseed bar ACB- apple chiaseed bar GCB- guava chiaseed bar

Bioactive Components

Total phenols, total flavonoids and tannin content were determined in the selected antioxidant rich bars formulated with the incorporation of chiaseed, whole mango, apple and guava in different combination and proportion. The results observed for selected and control bar has been presented in Table 4.

The results for total phenols reveals the significant increase ($p < 0.01$) in the selected antioxidant rich bars as compared to the control bar. The control bar was seen to have a total phenols content of 64.50mgGAE/55g. A highest total phenol among the selected bars was found to be in guava

chiaseed bar (97.01 mgGAE/55g) while lowest was observed in apple chiaseed bar (69.93 mgGAE/55g). The increase in total phenol content was due to the incorporation of chiaseed possessing good phenolic content. The phenolic content of 79.00mgGAE/100g in dry soup mix containing 46.296 % whole barley flour and 23.148 % roasted flaxseeds powder was reported.³⁶

The total flavonoid content of selected antioxidant rich bars ranged from 53.55 to 56.68 mgQE/55g. The results revealed that development of selected bars resulted in significant increase ($p < 0.01$, $p < 0.05$) in total flavonoid content of all the bars. Mango

chiaseed bar was found to have higher flavonoid content than the other two types of bar. A significant increase was reported in total flavonoid content from 170.36 to 252.60mgRE/100g in beetroot enriched nutribars with the ingredients like flaxseeds, Bengal gram powder, coconut chips and roasted sesame seeds.³⁷ A significant increase was observed in Total Phenolic Content and Total Flavanoid Content values after substitution and with increasing levels of chia and quinoa seed flours in the cookies.¹⁰

The control bar was observed to have a tannin content of 31.20mg/55g. The content of tannin in the selected antioxidant rich bars was found to lie in a range of 36.88 to 55.32mg/55g. The highest content of tannin was observed in guava chiaseed bar. A significant increase in the condensed tannin content was observed ranging from 725.00 to 893.70µgCE/100g in snack bars added with common bean flour as a source of bioactive compounds.³⁸

Table 4: Bioactive components of the bars

Products	Total Phenols (mgGAE/ 55g)	Total Flavonoids (mgQE/ 55g)	Tannins (mg/ 55g)
Control Bar	64.50±0.43	48.48±0.39	31.20±6.50
MCB (Acceptable)	71.22± 0.24 ^c	56.68±0.39 ^a	48.23±2.46 ^a
t-value	23.500**	25.720**	63.43**
Control Bar	64.50±0.43	48.48±0.39	31.20±6.50
ACB (Acceptable)	69.93±0.25 ^c	55.50±0.68 ^a	36.88±2.45 ^{bc}
t-value	19.000**	35.588**	1.414NS
Control Bar	64.50±0.43	48.48±0.39	31.20±6.50
GCB (Acceptable)	97.01±0.25 ^b	53.55±0.39 ^b	55.32±4.25 ^a
t-value	113.500**	35.922**	75.76**

Values are given as Mean±SD * (p<0.05) ** (p<0.01) NS- Non significant Figures with different superscripts –a, b, c, d, e, f are significantly different (p < 0.05) MCB-mango chiaseed bar ACB- apple chiaseed bar GCB- guava chiaseed bar

Antioxidant Activities

2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity, Ferric Reducing Antioxidant Power (FRAP), 2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and reducing power assay (RPA) were determined analyzing the antioxidant activities in the selected bars. DPPH and ABTS measure the free- radical scavenging activity of the sample while FRAP and RPA measure the reducing power of the product. The results observed for all the formulated and control bar have been presented in Table 5.

The results for DPPH scavenging activity reveals the significant increase ((p<0.01) in the selected antioxidant rich bars as compared to the control bar. The control bar was seen to have a DPPH scavenging activity of 76.38 % inhibition. . A highest DPPH scavenging activity among the selected bars was found to be in mango chiaseed bar i.e. 80.09 %

inhibition. In one such study, the lower antioxidant activity of 33.87% was observed in cereal bar developed using quinoa.³⁵

The ABTS scavenging activity, measured as mmol Trolox equivalent per 55g of sample was significantly (p<0.01) increased in the selected bars ranging from 39.60 to 45.49 mmolTE/55g, highest to be found in mango chiaseed bar. The ABTS activity of control bar was found to be 38.17 mmolTE/55g. The ABTS scavenging activity of oat bars was found by to be varying from 21.87 to 29.18 % in four different formulations, showing the increase in scavenging activity of bars with the incorporation of cereals, nuts and fruits.³⁹

Ferric reducing power assay (FRAP) activity actually measures only the reducing capability based upon the ferric ion. The results revealed that FRAP activity

of selected antioxidant rich bars significantly ($p < 0.01$) increased as compared to control bar. FRAP activity of control bar was found to be $81.55 \mu\text{molFe}^{2+}/\text{g}$. in the selected antioxidant rich bars the FRAP activity was observed to be highest in mango chiaseed bar i.e. $480.40 \mu\text{molFe}^{2+}/\text{g}$ followed by apple and guava chiaseed bar i.e. $396.48 \mu\text{molFe}^{2+}/\text{g}$ and $325.91 \mu\text{molFe}^{2+}/\text{g}$ respectively. Increase in FRAP content of rolled oat-based snack bars was reported within various levels (0, 5, 10, 15, 20 %) of green banana flour to be ranged from 24.83 to $30.50 \mu\text{mol Fe(II)}/100 \text{ g}$.⁴⁰ The FRAP activity in barley flaxseed based dry soup mix was found to be $121.33 \mu\text{mol Fe (II)}/\text{g}$ which is less than the results reported in present study may be due to the higher FRAP content in the ingredients used.³⁶

The reducing power of an antioxidant compound reveals the presence of reductones which is based on the breaking of the free-radical chain reaction by donating a hydrogen atom, therefore preventing peroxide formation. Reducing Power Assay (RPA) expressed as mg Trolox Equivalent/55g showed non-significant increase among all the selected bars except mango chiaseeds bar in which significant ($p < 0.05$) increase was observed. The increased RPA activity among the selected bar was found to be in a range of 94.17 to $222.92 \text{ mg TE}/55\text{g}$. The RPA activity in the control bar was observed to be $63.33 \text{ mg TE}/55\text{g}$. It has been observed that the thermal processing alters the antioxidant profile and generate more antioxidants that contribute in increased antioxidant activity.⁴¹

Table 5: Antioxidant activities of bars

Products	DPPH (% Inhibition)	ABTS (mmolTE/55g)	FRAP ($\mu\text{molFe}^{2+}/\text{g}$)	RPA (mg TE/55 g)
Control Bar	76.38±0.30	38.17±0.33	81.55±0.56	63.33±0.42
MCB (Acceptable)	80.09±0.50	45.49±0.11	480.40±0.35	222.92±0.42
t-value	11.031**	36.108**	922.17**	254.68*
Control Bar	76.38±0.30	38.17±0.33	81.55±0.56	63.33±0.42
ACB (Acceptable)	78.43±0.38	42.30±0.57	396.48±0.30	94.17±0.42
t-value	7.347**	10.783**	841.59**	23.75**
Control Bar	76.38±0.30	38.17±0.33	81.55±0.56	63.33±0.42
GCB (Acceptable)	78.14±0.86	39.6±0.25	325.91±0.13	126.94±0.24
t-value	3.364 NS	5.965**	816.76**	83.75**

Values are given as Mean±SD * ($p < 0.05$) ** ($p < 0.01$) NS- Non significant Figures with different superscripts –a, b, c, d, e, f are significantly different ($p < 0.05$) MCB-mango chiaseed bar ACB- apple chiaseed bar GCB- guava chiaseed bar

Conclusion

A bar composed of puffed wheat (20g), peanut (10g) and honey (10g) was enriched with chiaseed, whole mango, guava and apple fruit to improve the nutritional quality. The highest acceptability for incorporation of chiaseed (5g), whole mango fruit (10g), whole apple fruit (5g) and whole guava fruit (5g) was observed. The mineral and vitamin content such as calcium, ascorbic acid and β -carotene was observed to be increased. Besides, the PUFA content of the bars was increased to a great extent. Bioactive and antioxidant content was improved to

a greater extent in the developed antioxidant rich bar and thus can be consumed by the population suffering from lifestyle diseases so as to increase the antioxidant content in their diet. The diet rich in antioxidants and PUFA will eventually contribute to the prevention of metabolic diseases.

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Conflict of Interest

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