International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 14-19

Received: 09-11-2018
Accepted: 13-12-2018

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Mineral characteristics and shelf life studies of cereal and legume based value added *Ladoos*

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Abstract
Due to increase in population, deficiency diseases and rapid urbanization in recent years, people have shifted towards consumption of coarse cereals. The study was therefore, done to assess the nutritional characteristics and shelf life study of value added *ladoos* prepared from sorghum, soybean and newly released wheat varieties (WH-1129 and HD-2967) flour blends. Sorghum (HJ-541) and wheat (WH-1112 and HD-2967) grains were processed into flour and wheat flour used to supplement sorghum and soybean flours at different proportions. Results showed that the values of WH-1129 wheat flour supplemented *ladoos* were 63.42, 302.08, 4.71, 4.68 and 84.60 mg/100g of calcium, phosphorus, iron, zinc and magnesium, respectively, whereas, HD -2967 wheat flour supplemented *ladoo* also contained significantly ($P \leq 0.05$) higher contents of calcium, phosphorus, iron, zinc and magnesium i.e. 62.75, 355.05, 4.44, 4.41 and 81.84mg/100g, respectively compared to control. The *in vitro* availability of calcium, iron and zinc of control *ladoos* were 28.19, 2.20 and 1.78mg/100g, respectively. Availability of calcium and zinc increased significantly ($P \leq 0.05$) to 34.38 and 2.99mg/100g, respectively in WH-1129 supplemented *ladoos* whereas in HD-2967 supplemented *ladoos*, it increased significantly ($P \leq 0.05$) to 33.58 and 2.79mg/100g, respectively. The availability of iron decreased significantly ($P \leq 0.05$) to 2.00mg/100g in HD-2967 supplemented *ladoo* compared to control. Shelf life studies showed that there was a significant ($P \leq 0.05$) increase in the fat acidity content of all types of *ladoos* from zero to 90 days of storage. The use of sorghum in *ladoos* making would greatly enhance the utilization of this crop in many sorghum cultivating developing countries where the crop has not been optimally utilized, for commercialization and promotion for use to prevent deficiency diseases.

Keywords: *Ladoos*, Value added, Supplementation, *In vitro* availability, Shelf life

Introduction
Wheat (*Triticum aestivum*) is the most common cereal available all over the world and today is even more in demand for its abundant health benefits. Through decades, wheat has been one of the major cereals crops in the world. The protein content of wheat is 11.8 g per 100 g and the energy content is 346 kcal per 100g. Wheat is rich in minerals (calcium, magnesium, potassium, sulphur, chlorine, manganese, zinc, copper and vitamins (vitamin B and E). The global trend towards urbanization has resulted in diets markedly deficient in dietary fibre, minerals and phytochemicals, which are essential in preventing various health ailments of the affluent society. Therefore, there is need to change diet from refined wheat and rice diets to coarse cereals and millets based diets for prevention of diabetes and other degenerative diseases.

Millets have an important place in human diet. In recent years, there has been increasing recognition of the importance of millets as the substitute for major cereal crop considering their low cost, nutritive value than cereal. In developing countries, with the current rate of increase in population and with less than adequate irrigation facilities, millets can adequately meet the demand for additional food supply, so it's considered as 'the way of life', 'food of the people' and 'food for hungry'.(Asma *et al*., 2006) [2]. Besides they are rich source of dietary fibre, phytochemical and micronutrient and hence they are rightly termed as 'nutri-cereals' (Seghal and Kawatra, 2003) [18]. Millets are considered as crop of food security because of their sustainability in adverse agro-climatic conditions (Singh *et al*., 2004) [21]. India contributes about 16% of the world's sorghum production. It is the fourth most important cereal crop in the country. Although millets are nutritionally superior to cereals, yet their utilization is not wide spread. One possible way of extending their utilization could be by blending them with wheat flour after suitable processing (Singh *et al.*, 2005) [20]. In order to overcome the nutritional deficiency, coarse grains are blended with legumes and other

ingredients in appropriate proportion of development of composite flour which will have excellent nutritional qualities with low cost (Devaraju, 2003) [5].

The soybean (*Glycine max*) is a species of legume native to East Asia and is classed as an oilseed rather than a pulse by the UN Food and Agricultural Organization (FAO). In India, soybean is mainly grown in the States of Madhya Pradesh, Maharashtra, Rajasthan, Karnataka and Uttar Pradesh. Agro climate conditions of Haryana are also suitable for cultivation of soybean. Soy is truly seems to be a wonder food. Soy is an excellent source of dietary fibre and protein (43%). As a high quality, complete protein, soy protein is comparable in protein quality to the protein found in animal sources, such as meat, milk and eggs. Soy protein products can be good substitutes for animal products because, unlike some other beans, soy offers a 'complete' protein profile (Henkel, 2000). Soybean also contain Omega-3 fatty acids, which are special fat components that benefit many body functions.

There is a great potential for utilization of wheat, sorghum and soybean flours for development of different types of value added products (Sangwan, 2002) [17]. Value addition to the existing diet is an effective way of improving nutrition security. Value can be added to a commodity by upgrading its quality, reducing perishability, extending shelf life, ensuring off season availability, changing their form and purity as required in the market. To get high nutritional value product, coarse grain and millets can be used instead of refined wheat and rice, which are comparatively cheaper and provide more nutrients (Prakash, 2004) [12]. Various types of traditional *ladoos* are prepared from multigrain flours. Multi-grain *ladoo* is galactogouge and nutritionally rich product. The product provides good amounts of biological proteins, functional property, vitamins, minerals and adequate fibre and has a good satiety value (Naidu *et al*., 2013) [10]. With a surging prevalence of various health ailments attributed to consumption of refined cereals, the focus is now on fibre rich millet based value added products which serve as good vehicle for carrying the added proteins and dietary fibre.

Therefore, the present study was undertaken to develop *ladoos* from wheat, sorghum and soybean flour and analyze their nutritional quality and shelf life studies.

Material & Methods

The present study was carried out in the Department of Foods and Nutrition, I.C. College of Home Science, CCS Harayana Agricultural University, Hisar, Haryana.

Procurement of raw materials

Seeds of wheat (*Triticum aestivum*, WH-1129, HD-2967 and C-306), and (Sorghum vulgare, HJ-541) used in this study were procured in a single lot from the breeders, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Soybean (*Glycine max*) flour along with other ingredients required for the development of value added products were purchased from the local market of Hisar.

Preparation of wheat and sorghum flour

The wheat and sorghum grains were cleaned to remove dirt, dust and admixture of other food grains and ground in an electric grinder (Cyclotec, M/s Tecator, Hoganas, Sweden) to make flour thus obtained flour were sieved through a 60 mesh sieve and packed in airtight plastic containers for further analysis.

Preparation of wheat-sorghum-soybean composite flours

Wheat flour (WHF) of both the wheat varieties, sorghum flour (SGF) and soybean flour (SBF) were mixed in the ratio of 60:30:10 and 40:40:20 for development of composite flour.

Development and Organoleptic characteristics of value added *ladoos*

Development of value added *ladoos* from wheat, sorghum and soybean flour blends

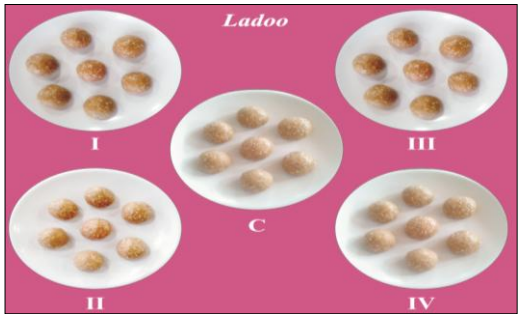
Table 1: Ingredients and preparation method for development of *ladoos*

Supplementation level (%)	Wheat flour (g)	Sorghum flour (g)	Soybean flour (g)	Ghee (g)	Sugar (g)	Gingelly seeds (g)
Control(100%WF)	100	-	-	55	50	30
WF : SGF : SBF						
60 : 30 : 10	60	30	10	55	50	30
40 : 40 : 20	40	40	20	55	50	30

Method

- Roasted wheat, sorghum and soybean flours separately in skillet and mixed together.
- Then add roasted and grounded gingelly seeds and ghee.
- Allowed the mixture to cool.
- Added sugar and mixed well and made the *ladoos*.

The preparation method of value added *ladoos* is presented in Table 1. Using two ratios (60:30:10 and 40:40:20) of each wheat variety flour (WF), sorghum flour (SGF) and soybean flour (SBF), four types of *ladoos* were developed. 100% wheat flour *ladoos* prepared from C-306 were kept as control.



Wheat-Sorghum-Soybean flour blend *ladoos*

C = Control (C-306)
I = Wheat flour (WH-1129) + Sorghum flour+ Soybean flour (60:30:10)
II = Wheat flour (WH-1129) + Sorghum flour+ Soybean flour (40:40:20)
III = Wheat flour (HD-2967) + Sorghum flour+ Soybean flour (60:30:10)
IV = Wheat flour (HD-2967) + Sorghum flour+ Soybean flour (40:40:20)

Organoleptic Characteristics

The *ladoos* were organoleptically evaluated by a panel of ten judges for sensory parameters like colour, appearance, flavour, texture, taste and overall acceptability using 9 point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9 to like extremely). Between tasting different samples, participants rinsed their mouth with warm water. On the basis of organoleptic acceptability, from each category the ladoos rated higher for organoleptic characteristics were selected for further study.

Analysis of total and available mineral contents

Total minerals

Acid digestion

To one g ground sample in a 150 ml conical flask, 25-30 ml of diacid mixture (HNO_3 : $HClO_4$ :: 5:1, v/v) was added and kept overnight. The contents were digested by heating until clear white precipitates settled down at the bottom. The volume was made to 50 ml with double distilled water. The crystals were filtered through Whatman No. 42 filter paper and used for the determination of total calcium, iron, zinc, magnesium and phosphorus.

Calcium, iron, zinc and magnesium in acid digested samples were determined by Atomic Absorption Spectrophotometer according to the method of Lindsey and Norwell (1969) [9].

$$\text{Minerals (mg/100g)} = \frac{\text{Reading (conc. µg/ml)} \times \text{Volume made}}{\text{Weight of sample (g)} \times 1000} \times 100$$

Estimation of phosphorus

Phosphorus was determined colorimetrically by using the method of Chen *et al.* (1956) [4].

Reagents

i. Ascorbic acid (10%)
ii. Ammonium molybdate (2.5%)
iii. Reagent C: 6N H_2SO_4, water, 2.5 per cent ammonium molybdate and 10 per cent ascorbic acid were mixed in the ratio of 1:2:1:1 (v/v), respectively. This reagent was prepared fresh.
iv. Standard phosphorus solution: 0.351 g pure and dry anhydrous monopotassium dihydrogen orthophosphate was dissolved in a few ml of water and 10 ml 10N H_2SO_4. The volume was made to one litre with water. This stock contained 80 µg P/ml.
v. Working standard phosphorus solution: Twenty five ml stock solution was diluted to one litre, which served as working standard solution and contained 2 µg P/ml. Two or three drops of chloroform were added to preserve the solution.

Procedure

Mineral extract (0.1ml, obtained from acid digestion) was pipetted in a test tube and volume was made to four ml with water. Four ml reagent C was added and mixed well. The contents were incubated at 37ºC in water bath for 90 minutes. It was removed and allowed to cool to room temperature and absorbance was read at 720 nm against a suitable blank. Standard curve was plotted using one to eight µg P.

Available calcium, zinc and iron (*in vitro*)

Calcium and Zinc availability (*in vitro*)

Available calcium and zinc in the sample were extracted by method of Kim and Zemel (1986) [8]

Reagents

i) 0.1% pepsin in 0.1 N HCl.
ii) HCl
iii) NaHCO3
iv) 0.5% pancreatin
v) 5% bile

Procedure

Two g finely ground sample was taken in a conical flask and 3 ml distilled water was added to rehydrate it. To this, 20 ml of pepsin solution (0.1% pepsin in 0.1 N HCl) was added. The pH was adjusted to 1.5 with dilute HCl. The contents were incubated at 37°C in a shaker cum water bath for an hour. After incubation the pH of the contents was raised to 6.8 with sodium bicarbonate solution. Then 2.5 ml of suspension containing 0.5% pancreatin and 5% bile were added and the contents were again incubated at 37°C for an hour in an environmental shaker. Then the contents were taken out and total volume was made to 50 ml with distilled water. Contents were then immediately centrifuged at 5000 rpm for 45 min at 5°C. Supernatant were collected and recentrifuged at 2500 rpm for 45 min at 5°C. The supernatant was filtered through an ashless filter paper (Whatman No. 42) and the filterate was oven dried, digested in the diacid mixture and proceeded for the estimation of calcium and zinc by Atomic Absorption Spectrophotometer method (Lindsey and Norwell, 1969) [9].

Iron availability (*in vitro*)

Extraction: Ionizable iron in the samples was extracted according to the procedure of Rao and Prabhavati (1978). Two g sample was mixed with 25 ml pepsin HCl (0.5% pepsin in 0.1 N HCl) in a conical flask. The pH of the mixture was adjusted to 1.35 with HCl and incubated at 37°C for 90 min in an environmental shaker. After incubation, pH was adjusted to 7.5 with NaOH and again incubated at 37°C in an environmental shaker for 90 min. Contents of the flask were centrifuged at 9000 rpm for 30 min and the supernatant was filtered through Whatman No. 42 filter paper. The filtrate was used for determination of ionizable iron.

Ionizable iron

Free form of iron in the filtrate which reacts with α', α'-dipyridyl was determined as described by AOAC (2000) [1].

Reagents

i) α', α'-dipyridyl solution: Dissolved 0.1 g dipyridyl in water and made the volume to 100 ml.
ii) Hydroxylamine hydrochloric acid (10%)
iii) Acetate buffer solution: Dissolved 8.3 g anhydrous sodium acetate (dried at 100°C) in water, added 12 ml acetic acid and made the volume to 100 ml with water.
iv) HCl
v) Iron standard solution (0.01 mg iron/ml): Dissolved 3.512 g $Fe(NH_4)_2H_2SO_4.6H_2O$ in water, added two drops of

HCl and made to 500 ml with water. Ten ml of the solution was further diluted with water and made to 500 ml. This solution contained 0.01 mg iron per ml.

Estimation: Ten ml filtrate was taken in 25 ml volumetric flask and one ml 10% hydroxylamine hydrochloride solution was added. Then five ml acetate buffer solution was added, the contents were mixed and then one ml dipyridyl solution was added. The volume was made to 25 ml with water and the contents were mixed well. The colour intensity was read at 510 nm.

For plotting a standard curve 10 to 15 ml of iron standard were taken in 100 ml volumetric flask, added 2.0 ml of HCl to each and made the volume to 100 ml with water. Blank was also prepared in similar manner. Ten ml of each of these solutions were taken in 25 ml volumetric flask and proceeded as mentioned above. 0.135 OD corresponded to 25 µg iron.

Shelf -life study of value added *ladoos*

For the shelf –life study, the most acceptable *ladoos* were stored for the period of 3 months in air tight plastic containers at room temperature. The *ladoos* were evaluated for fat acidity and peroxide value at regular intervals of 0, 15, 30, 45, 60, 75 and 90 days.

Fat acidity

The fat acidity was determined by the standard method of analysis (AOAC, 2000) [1].

Reagents

(i) Benzene-alcohol-phenolpthalein solution (0.02%): To one litre benzene, one litre alcohol and 0.4g phenolphthalein was added and mixed.
(ii) Potassium hydroxide solution (0.0178 N).

Procedure

Ten gram sample was extracted with petroleum ether on Soxhelet apparatus. The solvent of the extract was completely evaporated on steam bath. The residue was dissolved in extraction flask with 50ml benzene-alcohol-phenolpthalein solution and titrated with standard potassium hydroxide (1g/lt) to orange pink colour. Blank titration was made on 50ml benzene-alcohol-phenolpthalein and this value was substracted from titration value of the sample. Fat acidity was calculated as mg of potassium hydroxide required to neutralize free fatty acids of 100g of flour.

Fat acidity = 10 × (T-B)

Where,
T = ml of KOH required to titrate sample extract
B = ml KOH required to titrate blank

Peroxide value

Peroxide value of stored products at 0, 15, 30, 45, 60, 75 and 90 days was determined by the method of AOAC (2000) [1].

Reagents

(i) Acetic acid: chloroform solution (3 : 2, v/v)
(ii) Saturated potassium iodide solution
(iii) 0.01 N sodium thiosulphate solution
(iv) Starch solution: One gram soluble starch was dissolved in cold distilled water to make thick paste. Then boiled distilled water was added and boiled for one minute while stirring. When completely dissolved, the volume was made to 100 ml.
(v) Potassium hydroxide solution (0.0178 N)

Procedure

Five gram sample was taken in conical flask. Thirty ml acetic acid-chloroform mixture was added to the flask and swirled to dissolve. Then 0.5 ml saturated potassium iodide solution was added. Kept for one minute with occasional shaking and 30 ml distilled water was added. This was slowly titrated against 0.01 N sodium thiosulphate with vigorous shaking until yellow colour almost disappeared. Then 0.05 ml starch solution was added and titration continued with shaking vigorously to release all iodine from chloroform layer until blue colour just disappeared. The blank was run in the similar way. Peroxide value was calculated as:

$$\text{Peroxide value (meq peroxide/100g)} = \frac{\text{(S-B) x N x 1000}}{\text{Weight of sample}}$$

Where,
B = Volume (ml) of $Na_2S_2O_3$ used for titration of blank
S = Volume (ml) of $Na_2S_2O_3$ used for titration of sample
N = Normality of $Na_2S_2O_3$ solution

Statistical analysis

Suitable standard statistical methods were used for analysis of data (Sheoran *et al*., 1998).

Result and Discussion

Total mineral contents of value added *ladoo*s

Table.2 showed that the control *ladoos* contained 46.73, 237.83, 3.88, 2.72 and 56.59 mg/100g, calcium, phosphorus, iron, zinc and magnesium, respectively. The WH-1129 wheat flour supplemented with sorghum and soybean flours had significantly ($P \leq 0.05$) higher contents of all minerals than control. The values were 63.42, 302.08, 4.71, 4.68 and 84.60 mg/100g of calcium, phosphorus, iron, zinc and magnesium, respectively. HD -2967 wheat flour supplemented *ladoo* also contained significantly ($P \leq 0.05$) higher contents of calcium, phosphorus, iron, zinc and magnesium i.e. 62.75, 355.05, 4.44, 4.41 and 81.84mg/100g, respectively compared to control. Value added *ladoos* developed from WH-1129 wheat flour had significantly ($P \leq 0.05$) higher contents of calcium, iron, zinc and magnesium significantly ($P \leq 0.05$) lower content of phosphorus than HD-2967 composite flour *ladoos*. Similar findings were also reported by other workers (Rani, 2008; Punia and Gupta, 2009; Chandel, 2014; Rana, 2015; Pandey, 2016) [15, 13, 3, 14, 11]. The increase in mineral contents of supplemented products might be due to high contents of calcium, phosphorus, magnesium and zinc in soybean flour as compared to wheat flour.

Table 2: Total mineral contents of cereal and millet based value added *ladoo*s (mg/100g, on dry matter basis)

Supplementation Level (%)	Calcium	Phosphorus	Iron	Zinc	Magnesium
Ladoo					
Control (100% WF)	46.73±0.04	237.83±5.18	3.88±0.06	2.72±0.04	56.59±0.16
WH-1129:SGF:SBF (40:40:20)	63.42±0.10	302.08±1.08	4.71±0.08	4.68±0.09	84.60±0.15

HD-2967:SGF:SBF (40:40:20)	62.75±0.07	355.05±2.93	4.44±0.08	4.41±0.05	81.84±4.44
CD(P≤0.05)	0.27	2.54	0.25	0.23	0.56

Values are mean ± SE of three independent determinations
WF = Wheat Flour, WH– 1129= Wheat Flour, HD-2967=Wheat Flour, SGF = Sorghum Flour,
SBF = Soybean Flour,

In vitro mineral availability of cereal and millet based value added _ladoos_

Table.3 indicated that the *in vitro* availability of calcium, iron and zinc of control *ladoos* were 28.19, 2.20 and 1.78mg/100g, respectively. Availability of calcium and zinc increased significantly (P≤0.05) to 34.38 and 2.99mg/100g, respectively in WH-1129 supplemented *ladoos* whereas in HD-2967 supplemented *ladoos*, it increased significantly (P≤0.05) to 33.58 and 2.79mg/100g, respectively. The availability of iron decreased significantly (P≤0.05) to 2.00mg/100g in HD-2967 supplemented *ladoo* compared to control. However it was observed that there was non-significant difference in the available calcium, iron and zinc contents of value added *ladoos* developed from WH-1129 and HD-2967 composite flours. The differences in mineral availability of control and supplemented products might be due to differences in the mineral content and antinutrient contents of raw flours used for product development. Similar result was also reported by Sangwan (2002) [17].

Table 3: *In vitro* mineral availability of cereal and millet based value added *ladoos* (%, on dry matter basis)

Supplementation level (%)	Available Calcium		Available Iron		Available Zinc	
	mg/100g	(%)	mg/100g	(%)	mg/100g	(%)
Ladoo						
Control (100% WF)	28.19±0.03	60.33±0.06	2.20±0.01	54.35±0.07	1.78±0.02	65.34±0.04
WH-1129:SGF:SBF (40:40:20)	34.38±0.02	54.20±0.08	2.16±0.03	45.77±0.06	2.99±0.02	63.85±0.12
HD-2967:SGF:SBF (40:40:20)	33.58±0.01	53.52±0.10	2.00±0.04	45.25±0.07	2.79±0.03	63.31±0.06
CD(P≤0.05)	0.80	0.26	0.16	0.22	0.20	0.25

Values are mean ± SE of three independent determinations
WF = Wheat Flour, WH– 1129= Wheat Flour, HD-2967=Wheat Flour, SGF = Sorghum Flour,
SBF = Soybean Flour,

Effect of storage period on fat acidity of value added _ladoos_

During storage, the fat acidity content of control *ladoos* increased significantly (P≤0.05) from 34.67 (0 day) to 62.00 (90 days) mg KOH/100g. The fat acidity of value added *ladoos* of WH-1129:SGF:SBF (60:30:10) and (40:40:20) flours increased from 39.67 to 70.67 and 43.67 to 71.33 mg KOH/100g, respectively during zero to 90 days of storage. Similarly, that of HD:SGF:SBF (60:30:10) and (40:40:20) increased significantly (P≤0.05) from 42.67 to 71.33 and 46.00 to 73.33 mg KOH/100gm, respectively during zero to 90 days of storage. There was a significant (P≤0.05) increase in the fat acidity content of all types of *ladoos* from zero to 90 days of storage. Though there was increase in the fat acidity of products during the storage but this increase was within the permissible limits. Results of the present study corroborated with those of other invsetigators (Hooda, 2002; Sangwan, 2002; Rana, 2015) [7, 17, 14]. Our findings also lend support to those of Supraja (2001) [22] and Chandel (2014) [3] who reported that fat acidity content of control and supplemented *ladoos* increased on increasing the storage period.

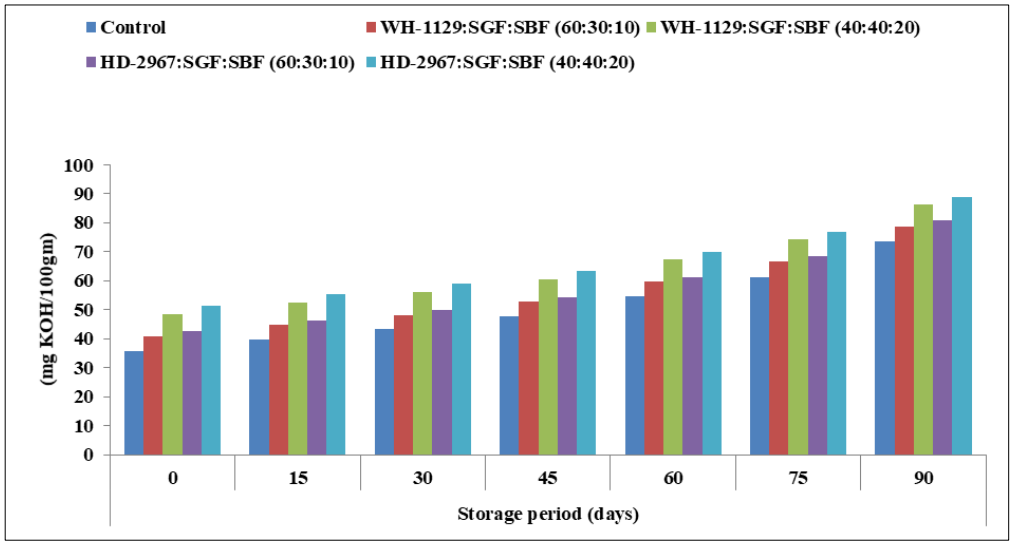


Fig 1: Effect of storage period on fat acidity (mg KOH/100gm) of cereal and millet based *ladoos* (on dry weight basis)

Effect of storage period on peroxide value of cereal and millet based value added _ladoos_

The peroxide value of control and supplemented storable *ladoos* were not detected up to 90 days of storage period at room temperature (in winter season). The peroxide value of control and supplemented storable *ladoos* were not detected up to 90 days of storage period at room temperature. These results clearly indicated the effectiveness of baking process in reducing the lipolytic activity in control and supplemented products and hence no rancidity was found in all value added

products during storage. The results of the present study are in close agreement with those of (Hooda, 2002 and Sangwan, 2002) [7, 17].

Conclusion

The study concluded that the cereal and legume based *ladoos* prepared by substituting wheat, sorghum and soybean flour at 40:40:20 level of substitution. There was a significant improvement in mineral content of value added *ladoos*. Hence, it is recommended that value added *ladoos* developed from wheat-sorghum-soybean flour blends which are rich in minerals (iron, zinc, calcium, magnesium and phosphorus) and have higher *in vitro* mineral availability (calcium and zinc) should be commercialized and promoted for use among population, suffering from malnutrition and deficiency diseases. In the face of increasing population and stagnant wheat and rice productions, coarse grain and millets can be a promising alternative in solving the problem of food insecurity and malnutrition. Therefore, if the production of the cereal and legume based value added products is taken on small scale or large scale as an entrepreneurial activity will help to raise the socio-economic status of not only farmer but also rural population at large.

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