EFFECT OF LINSEED OIL SUBSTITUTION ON PHYSICO-CHEMICAL PROPERTIES OF COOKIES

Sharoon Masih*, Zafar Iqbal**, Atta Muhammad Arif, Muhammad Rafiq**, Ghulam Rasool**** and Anjum Rashid*****

ABSTRACT

In a study conducted at National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan during 2012, linseed oil was used to replace shortening in standard cookie formula to check its stability and suitability in baked products. Cookies were prepared with five levels of linseed oil (0, 25, 50, 75 and 100%). Treatments effects were significant while storage effects were found non significant on physical characteristics i.e. width, diameter and spread ratio of cookies. The biscuits prepared from 100 percent linseed oil showed the highest width (279mm) and spread ratio (46.39), while maximum thickness (62.16mm) was noted in standard shortening. The increasing levels of substitution of linseed oil increased proportionately cookie width (275.00 mm in control to 279mm in 100% linseed oil) and spread factor (44.24 in control to 46.39 in 100% linseed oil). There was a linear decrease in cookies thickness from 62.16mm (control) to 60.14mm (100% linseed oil) with increasing levels of substitution. Different levels of linseed oil substitution for cookies preparation and their storage depicted significant differences for moisture, fat and nitrogen free extract (NFE) contents while non significant differences were recorded for protein, fiber and ash contents. Maximum mean values for protein (7.81%), ash (0.63%) and fibre (0.22%) were recorded in cookies prepared from 50 percent substitution of linseed oil. The cookies prepared with standard shortening depicted maximum moisture (3.06%) whereas, maximum fat contents (25.18%) were recorded for cookies prepared from 25 percent replacement of shortening with linseed oil. However, the highest NFE values (65.57%) were recorded for cookies made from 75 percent substitution of shortening with linseed oil. The data further showed that moisture content increased while protein, ash, fat, fibre and NFE contents decreased with increased storage duration.

KEYWORDS: *Linum usitatissimum;* vegetable oil; shortening; cookies; physicochemical characteristics; Pakistan.

INTRODUCTION

Linseed (*Linum usitatissimum*) belongs to family Linaceae. Linseed oil is yellow in color. Flaxseed as well as its extractions has been in the use of

***Food Technology Section, Post-harvest Research Centre, **Oilseeds Research Institute, AARI, Faisalabad ***Department of Dairy Technology, UVAS, Lahore, ****National Institute of Food Science and Technology, UAF, Pakistan.**

people as diet from beginning of civilization in different continents (8). Knowledge about the health benefits of linseed promoted its requirement and utilization in foodstuff and drinks, value added foods and dietetic supplements. Its seed contains 40 percent oil on weight basis. However, α-linolenic acid (ALA) constitutes 55 percent of its oil (26). The linseeds and its oil contain an abundance of micronutrients and phyto-chemicals which are helpful for better health. A balance between ratios of omega-3 to omega-6 fatty acids in the diet is desirable for a better health. Ideal ratio is 1:2, while linseed oil has increased ratio (4:1) which is similar to fish oil. Hence it is a good source of omega-3 (ω3) fatty acids. Vegetable oils except linseed and canola are low in α-linolenic acid. Linseed oil is readily oxidized if not stored properly due to high level of linolenic acid (29). It has quality to reduce the cholesterol level and low density lipids in mammals especially in women (23).

Wheat is main cereal grain providing highest protein and energy as compared to any other cereal. Biscuits are concentrated source of energy and supply two times more energy than bread. These are best in dietetic significance, deliciousness, compactness and convenience (21). Whole as well as ground linseed is included into a variety of bakery products. It contributes specific nut like aroma to different bakery products like bread, waffles, pancakes when used at a level of six to eight percent of the total ingredients in formulation (8).

Fats and oils are important ingredients in baked items depending upon the type of bakery products. Specific properties of pasteries and biscuits are dependent upon fat as these contain high amount of fat. The characteristics of dough like visco-elasticity are affected by the nature and quantity of fat. Fat quantity is critical for proper development of structure and decreasing amount of fat or replacing liquefied oil for hard fat resulted in a discernible reduction in dough stiffness. Higher fat content results in soft textured dough in short doughs.

Today the linseed enjoys increasing popularity throughout the world as a functional food. But unfortunately in Pakistan its use is not much popular. Present study was planned to evaluate the suitability of linseed oil in cookies and to check its effect on the physical and chemical characteristics of cookies.

MATERIALS AND METHODS

A study was conducted at National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan during the year 2012 in completely randomized design (factorial) and repeated quadruplicately. Linseed and other materials for preparation of cookies were purchased from local market. Oil was extracted from linseed by using screw type mechanical press from the local market. The extracted linseed oil was refined and purified by using degumming, neutralization and bleaching processes (19). The oil was heated at 60-80°C and then a small amount of phosphoric acid (0.25%) (50-500 mg/kg citric acids solution) was added alongwith water for hydration. The gums were separated from the oil by centrifugation. Neutralization was done by adding 18% NaOH solution to linseed oil at 65°C to convert free fatty acids into sodium soaps which was removed by centrifugation. Linseed oil was double refined to ensure removal of free fatty acid. Acid activated bleaching clay was added to the oil @1-3%. The mixture was agitated, heated and clay was removed by filtration.

Physico-chemical analysis of oil: The color of oil was measured following AOCS Method Cc 13e-92 in a Lovibond Tintometer (Model F, BS 684, The Tintometer Limited) using 5.25-inch cell. The cell of Lovibond Tintometer was first rinsed with linseed oil and reading was observed. The results were expressed as number of glass standards (R and Y). The refractive index and specific gravity was determined by AOAC Official Method 921.08 and 9201.212 through Abbe's refractometer and Pycnometer, respectively at 25°C (4). Acid value and saponification value were determined by AOCS official method No. Cd 3d-63 and Cd 3-25, repectively (2).

Proximate analysis: Cookies prepared from different levels of linseed oil were stored at room temperature. Wheat flour and cookies were chemically analyzed for moisture content, crude protein, crude fat, crude fiber, ash and nitrogen free extract (3).

Preparation of cookies: Cookies were prepared according to the method as described by AACC (3). Shortening was used according to the ratios as mentioned in Table 1.

Table 1. Different levels of substitution of linseed oil in cookie preparation.

Treatments	Normal shortening (%)	Linseed oil (%)
T_0	100	-
T_1	75	25
T_2	50	50
T_3	25	75
T_4	-	100

Physical analysis of cookies: The width, thickness and spread factor for cookies was determined fortnightly upto 45 days according to the method described by AACC (3).

Statistical analysis: The data obtained for each parameter were subjected to statistical analysis using Fisher's analysis of variance technique and mean values obtained were compared according to Duncans multiple range test (36).

RESULTS AND DISCUSSION

Physical characteristics of linseed oil

The data regarding physico-chemical characteristics of linseed oil revealed that color of refined linseed oil was 3R and 30Y (Table 2). The color of oil is a legitimate factor in determining its value and is not desirable for a particular use of oil. It is affected by the method of extraction, storage conditions and other factors (27). The specific gravity of refined linseed oil was 0.921 at 25°C (Table 2). Specific gravity is a diagnostic criterion for assessing the purity of oil. Pure linseed oil has specific gravity ranging from 0.925 to 0.935 which is higher than other vegetable oils due to high content of linolenic acid (27). Refractive index is useful for identification, establishing purity and recording progress of reaction (isomerization and hydrogenation). It is higher in oil with more saturation and reduces with declining molecular weight of fatty acids and increasing temperature. The refractive index of refined linseed oil was 1.47 at 40°C (Table 2) which is in line with previous findings by Popa *et al.* (26) and Przybylski (27) who reported 1.469 and 1.475 refractive index of linseed oil, respectively.

Table 2. Physico-chemical characteristics of linseed oil

(a) Physical characteristics	
Color of oil	3R & 30Y
Specific gravity	0.921±0.05
Refractive index	1.47±.07
(b) Chemical characteristics	
Acid value	1.15±0.06
Saponification value	190±9.5

Chemical characteristics

Saponification value is the content of NaOH required to saponify a certain amount of sample and is inversely related to average molecular weights of fats. It depicts the actual molecular weight of fatty acids. The saponification value of refined linseed oil was 190 (Table 2) similar to the results reported by Popa *et al.* (26). Przybylski (27) also observed saponification value of linseed oil ranged from 187-195 mg KOH/g. Acid value measures free fatty acid content of oil and depicts deterioration in sensory properties of oil (27). Oils with higher value result in greater refining losses and more susceptible to rancidity. Higher concentration of free fatty acids in edible oils make it unfit for dietary use. Free fatty acids develop strident and disagreeable aroma in dietary fats and oils and their occurrence is not desirable in edible oils and fats (40). According to the standards ISO 150, ASTM D234 (7) and British Pharmacopoeia (5) linseed oil should have acid value less than 4.0 mg KOH/ g oil. The acid value of refined linseed oil was 1.15 (Table 2) which is higher than previously reported acid value (0.80 mg KOH/ g oil) for linseed oil by Popa *et al.* (26). A little variation in results could be due to difference in the handling of experimental materials and environmental conditions.

Chemical composition of wheat flour

Analysis of wheat flour is given in Table 3. Mebpa *et al.* (22) agree to the results who reported 11.31 percent moisture, 12.86% protein, 1.40% fat, 0.82% crude fiber and 0.46% ash contents in wheat flour. Saeed *et al.* (30) have also reported similar results (moisture content 11.90%, 11.70% protein, 1.68% fat, 0.07% crude fiber, 2.10% ash and NFE 76.39%) for wheat flour. Chemical composition of wheat flour varies under different storage conditions (3).

Table 3. Proximate composition of wheat flour.

Characters	Values
Moisture (%)	12.48±0.62
Crude protein(%)	10.08±0.50
Crude fat(%)	1.09±0.05
Crude fiber(%)	0.31±0.02
Ash(%)	0.55±0.03
Nitrogen free extract (NFE)(%)	75.49±3.77

Physical characteristics

Cookie width (W): The data (Table 4) depict that different treatments significantly affected the width of cookies made by substituting standard fat with linseed oil. A slight increase in cookies width recorded with an increase of linseed oil. Highest mean value for cookie width was observed in T_4 (279.00mm) while, lowest in T_0 (275.00mm) (Table 4). However, other treatments differed non significantly from each other. Jacob and Leelavathi (15) reported that cookies made from sunflower oil showed higher value for width (8.8cm) than non-emulsified shortening ‘dalda’ (8.1cm). Cookie width increases linearly by increasing level of rice bran oil (34). The findings of present studies are in contradiction with the results reported by Sharif *et al.* (32) (width decreased from 44.15 to 36.53mm) and Sharif *et al.* (33) also reported a decrease in width of cookies as a result of replacement of normal shortening with rice bran oil. Use of different levels of emulsifiers and their combinations increased the width of cookies (20, 25).

Table 4. Effect of different treatments on physical characteristics of cookies.

Treatments	Width (mm)	Thickness (mm)	Spread factor
T_0	275.00±1.38 c	62.16±0.31 a	44.24±0.22 c
T_1	276.30±1.38 bc	61.69±0.31 ab	44.77±0.22 bc
T_2	277.00±1.39 b	61.12±0.31 b	45.32±0.23 b
T_3	276.80±1.38 bc	60.85±0.30 bc	45.49±0.23 b
T_4	279.00±1.40 a	60.14±0.30 c	46.39±0.23 a

The effect of storage was non-significant on the width of cookies. The results showed that at 0 day, T_4 exhibited maximum width 280.00 mm while, T_0 (0 linseed) exhibit minimum width (275.00 mm). Cookie width showed declining trend during storage. It decreased from 277.20 mm at 0 day to 276.00 mm after 45 days (Table 5). These results are in concordance with the findings of Sharif (31) and Sharif *et al.* (33). Treatments had significant impact on cookie width while, non-significant effect was observed in case of storage period (14).

Table 5. Effect of storage period on physical characteristics of cookies.

Storage duration (Days)	Width (mm)	Thickness (mm)	Spread Factor
0 days	277.20±1.39	61.20±0.31	45.30±0.23
15 days	277.20±1.39	61.20±0.31	45.30±0.23
30 days	276.60±1.38	61.18±0.31	45.22±0.23
45 days	276.00±1.38	61.19±0.31	45.14±0.23

Cookies thickness: The mean values for cookies thickness revealed a significant difference among linseed oil treatments. The highest value (62.16 mm) was noted in cookies prepared from zero linseed oil while 100 percent linseed oil got minimum thickness (60.14 mm) (Table 4). A decrease in cookies thickness with an increased level of lineed oil was due to quick drying of linseed oil as reported earlier (8) due to higher concentration of linolenic acid. Sharif *et al.* (34) reported that cookies thickness decreased with increasing level of rice bran oil. Present results are in contradiction to those reported by Sharif (31) and Sharif *et al.* (33) who found increasing trend with progressive increase in rice bran oil. The differences might be due to different composition of linseed and rice bran oils and processing conditions.

Cookie thickness was non significantly affected by storage. The results show that thickness decreased from 61.20 mm at 0 day to 61.19 mm after 45 days. The findings of current studies about storage have also been reported by Sharif *et al.* (32, 33) and Saeed *et al.* (30). Different treatments showed substantial influence while trivial or insignificant effect of storage is reported on the thickness of cookies prepared from different shortenings (14).

Spread factor: Particle size index of flour, moistness and type of fat used are major factors determining spread factor of cookies in baking (35). Cookie diameter and thickness was also affected by spread ratio which in turn are affected by relative competency of different ingredients for water availability and of flour or any other constituent for absorption of moisture during dough preparation (11). The results (Table 5) show significant effect of treatments on spread ratio of cookies due to substitution of varying levels of linseed oil with normal shortening. The increasing trend was observed for cookie spread ratio with the proportional rise of linseed oil. Linseed oil dries quickly resulting in a decrease in cookie thickness with increasing level of linseed oil which ultimately resulted in a slight increase in spread factor. The spread factor of cookies, prepared from different treatments ranged from 44.24 (T_0) to 46.39 (T_4) (Table 4). Cookies made from special shortening, (Dalda) which is non-emulsified hydrogenated fat exhibited lower values for spread. Biscuits prepared from liquid fat i.e. oil showed early spread and hence spread lasted for more time than solid fats. (15). Also Cookies prepared from sunflower oil showed more spread ratio (8.38) as compared to hydrogenated fat (Dalda) (7.58) (15).

Patel and Rao (25) studied effects of sugar, fat and emulsifiers on cookies made from four blends of wheat and black gram and reported increase in spread factor with increasing addition of sugar and fat. Use of different levels and combinations of emulsifiers resulted in significant increase in spread ratio of cookies (16, 20). However, present findings are in contradiction with findings of Adair *et al.* (1) who reported decrease in cookie spread by reducing the dose and substitution of butter with mungbean paste in peanut butter cookies. Sharif *et al.* (32) and Sharif *et al.* (35) found that spread ratio decreased with increasing rice bran oil, Saeed *et al.* (30) reported decrease in cookie spread from 42.35 to 40.00mm with increasing sweet potato flour while, Hussain *et al.* (13) found reduction in spread ratio from 48.49 to 47.71mm with 30 percent incorporation of linseed flour. Differences may be due to different composition of linseed and rice bran oil or may be due different nature of ingredients used by different research scholars.

Storage insignificantly affected the cookie spread ratio. Spread factor varied from 45.14 to 45.30 during 45 days storage. The data showed that at 0 day, T_4 exhibited maximum spread factor (46.55 mm) while T_0 minimum spread factor (44.23 mm). Decreasing trend was observed in spread factor of cookies during storage. Similar results have also been rported earlier (14, 30, 34).

Proximate analysis of cookies

Moisture content: Both substitution levels of linseed oil and storage showed substantial influence on moisture content in all cookie samples. On an average, T_0 got the highest value (3.06%) for moisture content where as, lowest (2.85%) in T_2. The moisture content showed considerable increment in all cookies with increasing storage duration. Variation in moisture content among treatments seemed to be due to differences in affinities of oil and shortening blends towards water as the cookies made from standard shortening attained maximum moisture content.

The mean values for moisture content of cookies varied from 2.61 to 3.25 percent for different storage periods. Highest moisture content (3.25%) was noted after 45 days while, lowest (2.61%) on zero day storage (Table 7). This increase was primarily due to packaging material (polythene bags). The packaging was not airtight and lack of temperature control resulted in an increase in moisture contents of cookies. Moreover, cookies absorbed moisture from surrounding atmosphere due to hygroscopic behavior of wheat flour. An increase in moisture contents of cookie samples during storage has also been reported by Wade (38), Leelavathi and Rao (16), Rao et al. (28), Pasha et al. (24), Iqbal (14), Butt *et al.* (9) and Sharif *et al.* (35) either due to atmosphere or packaging materials. Higher values for moisture contents of cookies differed to those reported by Iqbal (14) and Sharif *et al.* (35). It might be due to different nature and composition of shortening and oils used and also differences in storage environment.

Crude protein: Analysis of variance depicted that storage had highly significant effect on crude protein content whereas treatment and interaction had non-significant effect. Protein content was maximum (7.81%) in T_2 while, the lowest in T_3 (7.78%) (Table 6). Protein content decreased progressively at all intervals during 15 days storage. Statistical analysis showed non-significant variance between different substitution levels but substantial change for storage days. Mean protein content decreased from 7.85 at 0 day to 7.73 percent after 45 days (Table 7).

Pasha *et al.* (24), Butt *et al.* (9) and Sharif *et al.* (35) also reported decreasing trend in protein content of cookies during storage. Treatments showed non-significant while, storage period significantly affected the protein content in cookies prepared from rice bran oil (34). Protein content decreased during storage (6.56 to 6.41%) in biscuits prepared from composite flour (10) and 7.99 to 7.80 percent during storage period of 45 days (35). Reduction in protein content of cookies throughout storage might be due to increasing level of moisture which enhances proteolytic activity.

Crude fat: Different substitution levels of linseed oil and storage period revealed substantial effects on fat contents of cookies. The highest value for fat contents (25.18%) was noted in T_1 against the lowest value (22.99%) in T_0 (Table 6). These values are slightly higher than those presented by Waheed *et al.* (39). Mean fat contents of cookies at 0 day were 24.18 percent which decreased thereafter to 24.00 percent after 45 days of storage (Table 7). Waheed *et al.* (39) reported that fat content of cookies decreased from 22.87 to 21.69 percent throughout storage period (45 days). This decline in fat content throughout storage might be due to moisture uptake by cookies from the surrounding air and break down of fats to different compounds as reported earlier (10, 13, 18, 32, 34, 35).

Table 6. Effect of different treatments on chemical characteristics of cookies.

Treatments	Moisture content (%)	Protein content (%)	Ash content (%)	Fiber content (%)	Fat content (%)	NFE (%)
T_0	3.06±0.02a	7.80±0.04a	0.59±0.03c	0.20b	22.99±0.11 e	65.37±0.33a
T_1	2.88±0.01bc	7.79±0.04ab	0.57±0.03d	0.20b	25.18±0.13 a	63.35±0.32e
T_2	2.85±0.01c	7.81±0.04a	0.63±0.04a	0.22a	24.99±0.13 b	63.51±0.32d
T_3	2.92±0.02ab	7.78±0.04ab	0.60±0.03b	0.20b	23.94±0.12 c	64.57±0.32c
T_4	2.88±0.01bc	7.79±0.04ab	0.60±0.03b	0.20b	23.41±0.12 d	65.14±0.33b

Table 7. Effect of storage period on chemical characteristics of cookies.

Days	Moisture content (%)	Protein content (%)	Ash content (%)	Fiber content (%)	Fat content (%)	NFE (%)
0 days	2.61±0.01 d	7.85±0.04 a	0.64±0.04a	0.26a	24.18±0.12 a	64.45±0.32 a
15 days	2.80±0.01 c	7.82±0.04 ab	0.61±0.03b	0.22ab	24.15±0.12 ab	64.40±0.32 ab
30 days	3.00±0.02 b	7.77±0.04 bc	0.58±0.03c	0.18b	24.08±0.12 bc	64.37±0.32 bc
45 days	3.25±0.02 a	7.73±0.04 c	0.55±0.03d	0.16b	24.00±0.12 c	64.31±0.32 c

Crude fiber: There were non-significant differences for crude fiber content in cookies due to different levels of substitution of linseed oil and storage duration (Table 6, 7). However, cookies prepared from 50 percent replacement of normal shortening showed the highest value (0.22%) for fiber contents. The fiber contents for all other treatments were 0.20 percent (Table

6). Waheed *et al.* (39) reported fiber content of cookies varied from 0.270 to 0.279 percent. Lower values in present studies might be due to variation in composition of flour used for cookie preparation. Although there were non-significant differences for fiber contents during storage, yet freshly prepared cookies depicted maximum fiber contents (0.26%) which decreased to 0.16 percent on 45^{th} day of storage (Table 7). These confirm the findings of Waheed *et al.* (39) who reported decrease in fiber content of cookies during storage from 0.320 to 0.231 percent. Decrease in crude fiber during storage might be due to increase in moisture content which was engrossed from air as reported by Pasha *et al.* (24), Iqbal (14), Sharif *et al.* (33) and Butt *et al.* (9).

Total ash: Analysis of variance showed non-significant variation for ash content of cookies among treatments and storage. Maximum ash content (0.63%) was recorded in T_2 (% linseed oil) while, minimum (0.57%) in T_1 (25% lineed oil) (Table 6) which is in line with previous findings (12) while 0.57-0.58 percent ash contents were observed in cookies made from interesterified palm and cottonseed oil blends. At start of storage (day 0) average value recorded for ash content was 0.64 percent for all cookie samples which declined to 0.55 percent at 45^{th} day (Table 7). Storage had non-insignificant effect on ash content of biscuits (9, 24, 33). Ash content decreased from 0.74 to 0.64 percent (14), 0.74- 0.64 percent (33) and 0.64-0.52 percent after 45 days storage (12). This might be owing to uptake of moistness from air.

Nitrogen free extract (NFE): Both substitution levels and storage gave most important impact on NFE. NFE content was higher (65.37%) in T_0 while, the lowest (63.35%) in T_1 (Table 6). Findings of current studies are well braced by the results of Waheed *et al.* (39) with 67.53 to 67.66 percent NFE. The findings of current studies are also in concordance with the results reported by Butt *et al.* (9) and Sharif (31) who determined non-significant variations in NFE content of cookies.

Mean NFE content for all treatments showed a significant decrease after 15 days interval. NFE was 64.45 percent at 0 day which decreased to 64.31 percent after 45 days of storage (Table 7). These results are in close proximity with those reported by Sharif *et al.* (33) who found that NFE content ranged from 68.23 to 68.56 percent which reduced from 68.06 to 68.29 percent after 45 days of storage.

CONCLUSION

Demand and consumption of linseed in food and beverages, functional foods and dietetic supplements has augmented because of more awareness in people about health. Being carrier of omeg-2 fatty acids, if it is used in proper proportions in cookies preparation, it could improve the health status of masses. It can be safely used up to 50 percent level of substitution with good consumer acceptance, physical characteristics and shelf stability. Cookies made from 50 percent inclusion of linseed oil can be stored up to 30 days without any significant differences in their quality if stored under appropriate conditions.

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