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Storage stability of processed flaxseed powder

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Abstract
Flaxseeds are well known source of alpha-linolenic acid, an essential fatty acid. Owing to the presence of this fatty acid makes the flaxseed highly susceptible to oxidation resulting in decreased shelf stability. In the present investigation flaxseeds were subjected to different processing treatments and packed. Processed flaxseeds were ground and stored at two different temperatures (refrigerated temperature and elevated temperature) in low density polythene zip lock pouches. These powders were assessed for peroxide value and free fatty acid values. It was found that samples stored at refrigerated temperature showed maximum shelf-stability for about 120 days in terms of lower peroxide and free fatty acid values.

Keywords: Flaxseeds, processing treatments, peroxides, free fatty acid, temperature, storage

Introduction
The use of milled flaxseed in various types of food products is getting popularized day by day. Milled flaxseed up to 15% by weight is mostly used in whole wheat or multigrain bread and other bakery products formulations.

It is generally believed that milled flaxseeds have limited shelf life owing to the high content of alpha-linolenic acid; therefore milled flaxseeds are stored at ambient temperatures in triple-wrapped paper bags with plastic liners until required for use (Malcolmson *et al.*, 2000) [18]. Usually whole flaxseeds are not advised to be consumed as these seeds are not completely digested in the digestive tract, and also the bioavailability of its bioactive compounds is also impaired (Austria *et al.*, 2008) [5]. Therefore, it is assumed that the milling of flaxseed is an excellent strategy to increase the bioavailability of the bioactive compounds and incorporation into various food products. The heat treatments and other processing treatments viz., germination, steaming, soaking may increase product shelf life and also helpful in eliminating/reducing antinutritional compounds particularly cyanogenic glycosides and phytic acid (Kajla et al., 2015) [15].

However, due to presence of high concentration of polyunsaturated fatty acids, flaxseeds are susceptible to oxidation by exposure to heat and oxygen. The oxidation of lipids in flaxseed is undesirable as it decreases the functional potential of seeds by destroying the functional fatty acids and might produce secondary oxidation products which may be harmful to human health (Burenjargal and Totani, 2009) [9]. The functional component, alpha-linolenic acid (ALA) is prone to rapid and/or extensive oxidation by exposure to air, light, or temperature resulting in potential alteration in the nutritional composition and quality of food. The production of oxidized compounds, such as lipid peroxides, leads to a reduced shelf life. In the literature, few studies are available on the stability of alpha-linolenic acid in milled flaxseeds during processing and storage. The food industry is interested in knowing the processing conditions that preserve the alpha-linolenic acid from flaxseed so that milled flaxseeds may become attractive technological strategy for the utilization for the enrichment of the products nutritionally as a source of naturally bioactive compounds. Therefore, the present study was planned with the objective to evaluate the stability of variously processed flaxseeds by measuring peroxide value and free fatty acid value at a regular interval of time.

Materials and Methods
Flaxseeds varieties *viz*., JLS-6, JLS-9, JL-23 & JL-27, were procured from Jawaharlal Nehru Krishi Vishawvidyalaya, Jabalpur, Madhya Pradesh, India. The whole seeds were cleaned and stored in tin cans at refrigerated temperature. Flaxseeds were subjected to different processing treatments viz., germination, roasting, autoclaving and soaking. All the methods were adapted from Kajla and Sharma, (2016) [16].

For germination sterilised flaxseeds were spread in petri plates having filter paper saturated with demineralised water underneath. Seeds were allowed to germinate at room temperature. All viable seeds germinated within 48 h.

The flaxseeds were roasted in the household microwave oven for 2.5 minutes. Weighed amount (100 g) of flaxseed samples were kept in wire mesh sieve and placed in autoclave. Autoclaving was done at 121 ^{0}C for 15 min. One hundred gram flaxseeds were added to 250 ml water and kept for 2 hours.

Variously treated flaxseeds powder were ground and packed in low density polyethylene (LDPE) pouches. The plastic pouches containing flaxseed powder were kept for storage at two different temperatures:

1. Elevated temperature (35±2 ^{0}C) 2. Refrigerated temperature (05±2 ^{0}C)

Samples were taken out after a regular interval of seven days for analysis. The study of powder was conducted till the powders became unacceptable on basis of peroxide value and free fatty acid levels.

Peroxide Value (PV)

Peroxide value of fresh and stored processed flaxseed powder was assessed by using standard method of AOAC, (2005) [1]. Fat content of the sample was extracted by keeping the sample overnight in chloroform: methanol (2:1 v/v) mixture. Solvents were vaporated and lipid/fat phase was left behind. To each fat containing flasks 30 ml acetic and chloroform was added and swirled to dissolve. After that, 0.5 ml of saturated potassium iodide solution was added, kept for exact one minute with occasional shaking and 30 ml of distilled water was added. Then 0.5 ml of 1% starch solution was added and titrated with 0.01 N sodium thiosulphate with vigorous shaking until all iodine from chloroform layer was released and blue colour disappeared. The blank was also prepared in the similar manner. Peroxide value was reported as meq/kg.

Free fatty acid value (FFA)

Free fat acid value was expressed as mg of potassium hydroxide required to neutralize free fatty acids of 100 g flour and was estimated as described by AOAC, (2005) [1].

Ten gram sample was extracted with petroleum ether on soxhlet apparatus. The residue was dissolved in extraction flask with 50 ml benzene-alcohol- phenolphthalein solution and titrated with potassium hydroxide till orangish-pink appeared. Blank titration was prepared as above and its value was subtracted from titration value of sample.

Results and Discussion

Peroxide value (PV)

The PV is a measure of rancidity at the early stages and it shows good correlation with organoleptic flavour scores. Use of PV as flavour quality indicator is reliable during the initial stages of lipid oxidation as the peroxide value increases to a maximum and then decreases with increase in storage time. According to the Codex Alimentarius Commission, (2006) [10] standard for virgin oils and cold pressed fats and oils, edible oil must have peroxide value less than 10 meq O_2/kg oil.

The data pertaining to peroxide values of variously processed flaxseed varieties is presented in Table 1. The peroxide values control flaxseed varieties at refrigerated temperature ranged from 0.47 (JLS-9), 0.52 (JL-27), 0.63 (JLS-6) 0.78 meq O_2/kg (JL-23). While among the processed flaxseed varieties slight variations though non- significant ($p<0.05$) were observed as compared to control at zero day of storage. Malcolmson *et al.*, (2000) [18] reported peroxide values in the range of 0.2 to 0.4 meq O_2/kg of oil in various Canadian flaxseed varieties. Roasted and germinated varieties showed slight lower values at 0 day of storage in comparison to other treatments. Peroxide values for roasted flaxseed varieties varied from 0.35 (JLS-9) to 0.70 (JL-23) meq O_2/kg while germinated varieties exhibited peroxide values in range of 0.35 (JLS-9) to 0.65 (JL-23) meq O_2/kg. Scott *et al.*, (2004) [22] flaxseed samples indicated lower peroxide values in roasted flaxseeds. On the other hand, peroxide values of non-roasted sesame seeds were higher than those of roasted samples which might be due to destruction of the endogenous antioxidants (Hassan, 2013) [13].

On the contrary, wet heat treatments including autoclaving, soaking and steaming combination of these showed a slight increment in peroxide values of all the four flaxseed varieties. In the autoclaved samples peroxide values ranged from 0.58 (JLS-9) to 0.85 (JL-23) meq O_2/kg. In steamed samples also peroxide values showed a slight increment as compared to control and other processed flaxseed varieties. The peroxide values for the steamed samples varied from 0.66 (JL-23) to 0.86 (JL-27). The significantly higher level of peroxide values of these treatments as compared to control or unprocessed flaxseed varieties are in agreement with the observations of Bankole *et al.*, (2005) [6] who reported higher peroxide values for steamed melon seeds. This variance could might be attributed to the faster decomposition rates of the hydroperoxy fatty acids (primary oxidation products, unstable) into secondary oxidation products such as ketones, aldehydes, epoxides which are more stable and largely contribute to off flavours and objectionable odours develpoment in deteriorated oilseeds, thereby, resulting in decreased peroxide values. This is further supported by several researchers (Amoo and Asoore, 2006 [3]; Mohamadzadeh *et al.*, 2009) [19], they too reported the faster decomposition rates of hydroperoxy fatty acids into stable secondary products. On the contrary, studies of Negedu *et al.*, (2013) [20] showed that peroxide values of autoclaved castor seeds were decreased as compared to unautoclaved samples.

Peroxide values for control/unprocessed flaxseed varieties increased significantly ($p<0.05$) with increase in time period of storage. Similar trend was followed among the variously processed flaxseed varieties. In the control flaxseed varieties, the peroxide values of increased from 0.52,0.78, 0.63 and 0.47 (0 day) to 5.04, 5.24, 5.34 and 5.15 on 120th day of storage for varieties JL-27, JL-23, JLS-6 and JLS-9 respectively. On the other hand, the mixed variety and Linott, Canadian flaxseed varieties showed a slight increase in peroxide values throughout the 128 days of storage (Malcolmson *et al.*, 2000) [18]. In sesame seeds peroxide values increased over one year period of storage (Bhattacharya *et al.*, 2013) [7]. Increase in peroxide values with increase in storage period was due to absorption of moisture, exposure to heat, light and oxidation (Abulude *et al.*, 2007) [2]. Peroxide values increased during storage at varied relative humidity, thus confirming the development of rancidity.

Similar pattern of increase in peroxide value during storage period was observed for variously processed flaxseed powder. All the processed flaxseed powder of the four varieties showed similar trend in increase of peroxide values with increase in storage time. Herchi *et al.*, (2015) [14] peroxide values increased slightly with increase in germination period but the increase in peroxide value was in safer limit as prescribed by Codex Alimentarius Commission, (2006) [10]. Lower acid and peroxide values have indicated that germinated flaxseed oil was more suitable as edible oil (Herchi *et al.*, 2015) [14]. Similar to the present findings, Scoot *et al.*, (2004) [22] and Hassan, (2013) [13] also observed increase in peroxide value with storage time in roasted flaxseeds and sesame seeds respectively. Higher peroxide value of wet heat treated flaxseed samples followed by oven drying might be due to the prolonged exposure of the seeds to heat which promoted faster formation of hydroperoxides as compared to the control samples (Mohamadzadeh *et al.* 2009) [19].

Peroxide values of the control and processed flaxseed varieties were also evaluated at elevated (35 ^{0}C) temperature. Data regarding the peroxide values at elevated (35 ^{0}C) temperature is presented in Table 2. The peroxide values at elevated temperature varied from 0.52 to 10.26 meq O_2/kg from 0 day to 45th day of storage. These increased values of peroxides demonstrated that flaxseed samples deteriorated rapidly at elevated temperatures. Similar trend of drastic increase in peroxide values was observed among all the processed flaxseed varieties. Peroxide value is an index of early rancidity; therefore, the high peroxide value pertains to poor resistance of the oil due to peroxidation during storage (Hassan, 2013) [13]. Similarly findings were also quoted by Mariod *et al.*, (2012) [17] in case of raw, roasted and boiled safflower oil after 3 days of storage at 70 ^{0}C, who noticed drastic increase in the peroxide values at elevated storage temperature.

Statistical analysis at different storage conditions revealed that storage period and storage temperature significantly affected the shelf stability of flaxseed powders irrespective of variety and processing treatment. It can be concluded from the present study that the quality in terms of decreased peroxide values was maintained in all flaxseed samples stored at refrigerated temperature. Higher storage temperature rapidly spoiled the flaxseed samples by aggravating the rate of oxidation, thus the flaxseed samples became rancid and of inferior quality within a period of 30 days irrespective of the processing treatment.

Table 1: Peroxide values (meqO_2/kg) and free fatty acid (%) values of processed flaxseed powder at refrigerated temperature

Variety		JL-27					JL-23				
STORAGE DAYS											
Treatments	Parameter	0	30	60	90	120	0	30	60	90	120
C	PV	0.52±0.02	2.30±0.05	6.79±0.08	4.66±0.01	5.04±0.04	0.78±0.03	2.6±0.04	6.89±0.06	4.60±0.09	5.24±0.03
C	FFA	0.05±0.03	0.10±0.07	0.36±0.04	0.94±0.06	0.94±0.09	0.04±0.01	0.11±0.12	0.39±0.13	0.94±0.19.	0.98±0.12
R	PV	0.46±0.12	2.16±0.17	5.72±0.11	4.14±0.29	4.72±0.17	0.7±0.02	2.56±0.05	5.83±0.11	4.26±0.14	4.84±0.14
R	FFA	0.12±0.14	0.24±0.15	0.47±0.09	1.13±0.15	1.14±0.08	0.11±0.08	0.26±0.15	0.53±0.26	1.11±0.06	1.13±0.23
G	PV	0.45±0.05	1.99±0.08	4.51±0.27	4.53±0.31	4.08±0.19	0.65±0.16	1.75±0.02	4.65±0.29	5.04±0.22	5.03±0.11
G	FFA	0.18±0.04	0.3±0.07	0.57±0.14	1.21±0.15	1.22±0.07	0.13±0.12	0.28±0.06	0.59±0.19	1.16±0.13	1.15±0.08
A	PV	0.62±0.12	2.36±0.05	5.94±0.30	4.71±0.28	4.95±0.22	0.85±0.09	2.81±0.17	5.83±0.22	4.74±0.07	5.85±0.15
A	FFA	0.19±0.9	0.30±0.17	0.59±0.16	1.38±0.13	1.37±.0.12	0.15±0.05	0.30±0.08	0.59±0.17	1.27±0.14	1.27±0.17
So	PV	0.55±0.8	2.34±0.07	5.95±0.25	4.67±0.22	4.85±0.13	0.83±0.13	2.85±0.19	5.15±0.14	4.25±0.17	4.87±0.06
So	FFA	0.21±0.04	0.32±0.02	0.62±0.11	1.39±0.11	1.37±0.09	0.17±0.03	0.32±0.09	0.6±0.06	1.38±0.11	1.38±0.03

Variety		JLS-6					JLS-9				
STORAGE DAYS											
Treatments	Parameter	0	30	60	90	120	0	30	60	90	120
C	PV	0.63±0.02	2.70±0.06	6.5±0.12	4.90±0.04	5.34±0.09	0.47±0.03	2.17±0.05	6.62±0.03	4.54±0.05	5.15±0.03
C	FFA	0.07±0.04	0.12±0.09	0.54±0.08	1.12±0.06	1.12±0.06	0.03±0.04	0.11±0.06	0.42±0.04	1.05±0.06	1.11±0.01
R	PV	0.58±0.06	2.43±0.11	5.81±0.09	4.67±0.05	4.87±0.03	0.35±0.06	2.01±0.09	5.15±0.05	4.09±0.07	4.55±0.04
R	FFA	0.14±012	0.19±0.09	0.41±0.14	1.25±0.03	1.25±0.05	0.17±0.09	0.28±0.13	0.46±0.06	1.12±0.05	1.14±0.06
G	PV	0.55±0.16	2.23±0.18	4.65±0.06	5.105±0.01	5.09±0.07	0.35±0.04	1.77±0.09	3.49±0.07	4.92±0.12	4.64±0.09
G	FFA	0.18±0.17	0.26±0.12	0.51±0.17	1.3±0.16	1.31±0.02	0.19±0.15	0.29±0.15	0.55±0.08	1.29±0.14	1.34±0.08
A	PV	0.75±0.14	2.74±0.19	5.04±0.01	4.845±0.14	5.04±0.04	0.58±0.03	2.38±0.21	5.86±0.08	4.78±0.23	4.85±0.05
A	FFA	0.20±0.09	0.29±0.18	0.56±0.21	1.32±0.09	1.35±0.09	0.21±0.07	0.33±0.05	0.57±0.06	1.35±0.04	1.37±0.06
So	PV	0.70±0.07	2.75±0.16	5.11±0.11	4.84±0.07	5.19±0.04	0.58±0.08	2.36±0.03	4.32±0.07	3.65±0.11	4.45±0.03
So	FFA	0.20±0.02	0.33±0.12	0.57±0.09	1.34±0.02	1.36±0.09	0.21±0.09	0.34±0.02	0.58±0.05	1.38±0.01	1.37±0.04

Table 2: Peroxide values and free fatty acid values of processed flaxseed powder at elevated (35 ^{0}C) temperature

Variety		JL-27			JL-23		
Treatments	Parameter	0	15	30	0	15	30
C	PV	0.52±0.02	4.64±0.11	10.26±0.02	0.78±0.05	4.95±0.02	11.73±0.12
C	FFA	0.05±0.04	1.17±0.04	2.01±0.06	0.04±0.01	1.40±0.02	2.25±0.04
R	PV	0.46±0.02	4.51±0.07	12.45±0.11	0.7±0.02	4.85±0.03	12.88±0.04
R	FFA	0.12±0.03	1.2±0.08	2.07±0.02	0.11±0.04	1.46±0.06	2.3±0.23
G	PV	0.45±0.12	5.15±0.05	13.24±0.21	0.65±0.06	5.34±0.07	13.55±0.45
G	FFA	0.18±0.05	2.145±0.04	2.17±0.06	0.13±0.07	2.42±0.08	2.47±0.03
A	PV	0.62±0.06	3.22±0.03	12.43±0.09	0.85±0.04	3.55±0.04	12.51±0.04
A	FFA	0.19±0.09	1.68±0.06	1.65±0.10	0.15±0.03	1.35±0.02	1.37±0.05
So	PV	0.55±0.03	3.56±0.07	11.35±0.11	0.83±0.06	3.85±0.05	11.27±0.07
So	FFA	0.21±0.14	1.34±0.03	1.36±0.07	0.17±0.07	1.57±0.07	1.54±0.02

Variety		JLS-6			JLS-9		
Treatments	Parameter	0	15	30	0	15	30
C	PV	0.63±0.02	4.17±0.05	10.35±0.21	0.47±0.09	4.03±0.03	10.09±0.02
C	FFA	0.07±0.05	1.53±0.03	2.15±0.02	0.03±0.03	1.45±0.01	2.27±0.06
R	PV	0.58±0.09	4.23±0.04	12.34±0.03	0.35±0.04	4.36±0.05	12.54±0.07
R	FFA	0.14±0.04	1.31±0.06	2.45±0.05	0.17±0.05	1.50±0.02	2.34±0.09

G	PV	0.55±0.02	5.26±0.07	13.44±0.06	0.35±0.04	5.32±0.21	13.33±0.04
G	FFA	0.18±0.03	2.55±0.05	2.56±0.04	0.19±0.07	2.33±0.04	2.38±0.02
A	PV	0.75±0.07	3.46±0.04	12.09±0.07	0.58±0.06	3.82±0.06	12.8±0.01
A	FFA	0.21±0.02	1.55±0.02	1.55±0.05	0.21±0.09	1.44±0.03	1.44±0.04
So	PV	0.70±0.08	3.77±0.03	11.43±0.06	0.585±0.11	3.35±0.04	11.50±0.05
So	FFA	0.20±0.09	1.42±0.07	1.45±0.03	0.21±0.15	1.5±0.02	1.47±0.03

Free fatty acid value

Free fatty acids formation in fats and oils takes place as a result of enzymatic hydrolysis by lipases, metal ions acting as free radicals or at elevated temperature storage conditions.

The trend in free fatty acid levels in control and processed flaxseed powder at refrigerated temperature is presented in Table 1. The free fatty acid values ranged from 0.03% (JLS-9) to 0.07% (JLS-6). Free fatty acid values are similar to those reported by Malcolmson *et al.*, (2000) [18] in two different varieties of flaxseed. Among the processed varieties slight variations were noticed in free fatty acid values. In roasted flaxseed varieties, free fatty acid values increased to a small extent viz., 0.05% to 0.12%(JL-27),0.04% to 0.11% (JL-23) 0.07% to 0.14% (JLS-6) and 0.03% to 0.17% (JLS-9) ; however the increase was not significant ($p<0.05$). Contrary to present findings, Scott *et al.*, (2004) [22] who reported that control samples had statistically higher free fatty acid contents in comparison to roasted samples. The free acidity which is an index free fatty acid content due to enzymatic activity in the was found to be very low and equal to minimum acceptable value of 0.21% as oleic acid as recommended by the Codex Alimentarius Commission for oil seeds. However, free fatty acid values for germinated samples varied from 0.13% (JL-23) to 0.19% (JLS-9). Herchi *et al.*, (2015) [14] found that free acid value varied significantly ($p<0.05$) during the germination period. The lower free fatty acid value of germinated flaxseed oils infers better quality and good shelf stability. Free fatty acid values increased slightly upon all the wet heat treatments including soaking. Free fatty acid values at 0 day followed slight increasing trend being highest in soaked + autoclaved samples followed by soaking + steaming, steaming and then soaking followed by autoclaving.

As the storage period increases, free fatty acid values increased for control as well as processed flaxseed varieties. Free fatty acid values in control samples increased from 0.05% to 0.94% (JL-27); 0.04% to 0.98% (JL-23); 0.07% to 1.12% (JLS-6); and 0.03% to 1.11% (JLS-9) from 0 day to 120th day of storage. Results of the present findings are well in accordance with the studies of Malcolmson *et al.*, (2000) [18], who also observed significant increase in free fatty acid values for various flaxseed samples with increase in storage time, though the increase was well in acceptance range of edible oil (2% oleic acid). Nytker *et al.*, (2006) [21] also reported increase in free fatty acid with increase in storage time, temperature and moisture content. Among the processed flaxseed varieties similar trend was observed for free fatty acid values. All the processed flaxseed powder exhibited higher free fatty acid values as compared to control, however, among the processing treatments, no significant difference existed between treatments for free fatty acid value. Wet heat treatments followed by soaking slightly increased the free fatty acid values in flaxseeds in comparison to control. This increase upon processing could be due to greater liberation of free fatty acids by the heating or by the conversion of the oil into its constituent fatty acids. It has been reported in the literature that increasing the moisture content and temperature of seeds, the levels of free fatty acids (FFA) increased due to the hydrolysis of triglycerides (Mohamadzadeh, 2009) [19]. Similar results were also reported by increased free fatty acid content in stored vegetable oils (Atinafu and Bedemo, 2011) [4]. On the other hand, germinated and roasted flaxseed varieties showed little increment in free fatty acid values in comparison to control as well as processing treatments. In roasted samples free fatty acid values varied from 0.14% to 1.25% (JL-23), the other varieties also followed similar trend, however not significant differences were observed among the roasted varieties. Similar results were also presented by Hassan, (2013) [13] for increase in free fatty acid values of roasted sesame seeds as compared to unroasted sesame seeds.

Data presented in Table 2, revealed that free fatty acid content increased with increase in storage time and storage temperature of all the processed flaxseed varieties. This might be due to activation lipases in milled samples and quickly hydrolysed the triglycerides into free fatty acids, diglycerides and mono glycerides and eventually these enzymes decomposed all the triglycerides present in the samples over a storage period. Similar changes were reported by Gulla and Waghrey, (2011) [12]. The hydrolytic changes though not predominant, the formation of free fatty acids was found to increase with increase in time of storage. Though initial levels of free fatty acids were found to be different in the blends, rate of formation was found to be almost parallel, recording a drastic increase after 12 months of storage. Data observed clearly depicted that at refrigerated temperature free fatty acid were well in acceptable range as per Codex Alimentarious, while at elevated temperature the powder remained stable only for 15 days as indicated by higher values of free fatty acids. Therefore, it can be concluded that milled/ground flaxseed can be suitably stored at refrigerated temperature for a long period of time without significant alterations in its edible quality.

Conclusion

From the present investigation it was concluded that milled flaxseeds can be safely stored up to 120 days at refrigerated temperature as indicated by the peroxide values and free fatty acid values which were well in acceptable limits as per standards. As a consequence of its shelf stability processed flaxseed may be stored and can be utilized as feasible alternative to food product enrichment and value addition.

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