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EFFECT OF UNGERMINATED AND GERMINATED FLAXSEED ADDITION ON THE RHEOLOGICAL PROPERTIES OF WHOLE WHEATMEAL AND WHEAT FLOUR

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ABSTRACT
Rheological properties of composite flour prepared by addition of ungerminated and germinated flaxseed flour in wheat flour and whole wheat meal were studied. The ungerminated and germinated flaxseed flour was at added at different levels 5%, 10%, 15% and 20%. It was observed that as the levels increased the development time increased significantly whereas stability time and mixing tolerance index decreased. A significant increase in paste temperature, peak viscosity, final viscosity, final viscosity upto 10% addition and later on started decreasing. Farinograph results showed that as the levels of incorporation of ungerminated and germinated flaxseed meal increased in wheat flour and whole wheat meal, the development time increased significantly whereas the stability time decreased.

1. Introduction

The globalization scenario in the new millennium has increased the demand for value-added bakery products due to change in perception, economic consideration, westernization, urbanization, busy lifestyle, increased women employment and increased per capita income. Due to health promoting properties and excellent nutrient profile of flaxseed, it has become a popular candidate for incorporation in human diet. The components of flaxseed, identified to exhibit the health benefits are fiber, lignans and linolenic acid (Omega-3 fatty acid). Moreover flaxseed is a good source of high quality protein, soluble fibers and phenolic compounds (Oomah and Mazza, 2008).

The process of germination fundamentally changes the nutrient composition of the seed. Nutrients such as enzymes, amino acids, and vitamins are substantially increased and become more bioavailable, allowing for better absorption. For example, sprouting doubles the antioxidant (ORAC) value of flaxseed. The "anti-nutrients" such as phytic acid, enzyme inhibitors and insoluble fibers are decreased; allowing for increased bioavailability and nutrient absorption. The dough rheological properties are influenced by the structure of the aggregates and their tendency to interact with each other. Quality and quantity of the proteins affect the water absorption capacity of the dough (Finney, 1984). The mechanical and rheological properties of the dough exert promising effect on the overall quality of baked products (Blokshma and Bushuk, 1988). The arrangement and interaction of constituents (especially proteins) and the structure of materials are the responsible factors affecting the rheological properties (Bushuk, 1985).

Flaxseed mucilage is composed of mainly polymeric carbohydrates while galacturonic acid, rhammose, galactose, fructose, glucose are also present in small quantities. It can help to improve the water absorption characteristics of the dough (Fedeniuk and Biliaderis, 1994).

There is more information on nutritional and physiological properties than on its use in food. However, the knowledge about effect of supplementation of flaxseed on the rheological properties of dough for bread making is scanty. This research was undertaken to examine effect of replacement of wheat flour and whole wheat meal with germinated and ungerminated flaxseed flour on the rheological properties of dough used for bread making.

2. Materials and methods

2.1. Raw materials

Commercial wheat flour and whole wheat meal were procured from the local market. Flaxseed variety LC 2063 was procured from Narayangarh farms (Khanna), Punjab Agricultural University, Ludhiana.

2.2. Germination of flaxseeds

Flax seeds were germinated using different time-temperature combination after soaking in water followed by drying.

2.3. Chemical analysis of raw material

2.3.1. Fatty acid profile analysis

Lipids were extracted from sample using methanol/chloroform (Christie, 1989). The lipid fraction was quantified gravimetrically. For the isolation of triglycerides (TAG), the lipid extract was dissolve with hexane and passed through a Pasteur pipette containing florisil retained by glass wool. The sample was eluted with hexane/diethyl ether 4:1 (v/v) (10 ml) and the solution was collected. The TAG was trans esterified into fatty acid methyl esters (FAME) with a sodium methoxide catalysis method. The FAME was analyzed using a Shimadzu GC-17A gas chromatography coupled with a flame ionization detector (FID) and equipped with a HP-Innowax column (30m X 0.32mm i.d., 0.25µm film thickness). Helium was used as the carrier gas with a spilt ratio of 15:1. The flow rate through column was 1.5ml/min. The injector temperature was 225°C and the detector temperature was 240°C. The initial column temperature 120°C was increased to 200°C at a rate of 4°C/min and then to 240°C at a rate of 10°C/min. Fatty acids were identified using a mixture of commercial methyl esters 68D. The proportion of each fatty acid was determined without correction factors. Fatty acids were analyzed in two replicates.

2.3.2. Total phenolics

Total phenolics were determined by colorimetric method. A known amount of sample was taken and extracted with 80 percent methanol in a volumetric flask and made to 100 ml with 80% methanol and filtered. 0.5 ml of filtrate was taken into a test tube containing 0.5 ml water. The Folin- Ciocalteau reagent (0.5 ml) then kept for 5 min, and saturated solution of sodium carbonate (1 ml) was mixed. Absorbance of the developed color after 60 minutes was measured at 760 nm using Spectronic-20 Spectrophotometer. A standard curve was plotted by taking known amount of Gallic acid as reference standard (Swain and Hillis, 1959).

2.3.3. Antioxidant activity

Free radical scavenging activity was determined by DPPH (diphenyl picrylhydrazyl) method. Five hundred micro litres of 0.5 mM DPPH solution and 2 ml of 80% methanol aqueous solution were mixed with 25 µL of methanolic extract of sample, and absorbance was determined under 517 nm (blank as 80 percent methanol and tris buffer) after maintaining at 20°C for 30 minutes. The free radical scavenging activity was evaluated by comparing the absorbance of the sample solution with control solution to which distilled water was added instead of sample (Koga et al., 2007).

Radical scavenging activity (%) =

$$\frac{\text{Control OD(0min)} - \text{Sample OD (30min)}}{\text{Control OD (0min)}} \text{x100} \quad (1)$$

2.3.4. Mineral analysis

Mineral analysis was done using Atomic Absorption Spectrophotometer by AOAC (2005) method.

2.3.5. Proximate composition of raw materials

Moisture, protein, ash and fat were determined by AACC methods 44.15 A, 46-11 A, 08-01 and 30-10 respectively.

2.3.6. Crude fibers

Crude fiber of raw grains and multigrain porridge was estimated using Fibertec (Foss Company). Switched on the instrument and preheated the hot plate. Dried capsules kept in hot air oven at 100^{o}C for 20 minutes. Cooled and weighed 1 g Formulation in capsules. Fix the capsules to the rotating stand. Defatting of breads was done if necessary. Added 250-275 ml of 1.25% H_2SO_4 to the large extraction cup and immersed the stand into the beaker. Acid extraction was done by boiling it for 30-40 minutes followed by its washing with hot water. Then alkali washing was done with 1.25% NaOH for the same time duration followed by hot water washing. Finally, acetone washing was given and the capsules were dried in oven for 2 hours at 130^{o}C. Cooled and weighed for crude fiber estimation.

2.3.7. Dietary fibers

The contents of total soluble and insoluble dietary fibers were determined using an enzymatic-gravimetric method (Lee et al. 1992).

2.3.8. Calorific value

Calorific value was determined by using Bomb Calorimeter, Parr Calorimeter assembly 6100 (Parr Instrument Company, Moline, Ilinois 61265, USA).

2.3.9. Rheological properties

Farinographic properties were determined using the Brabender farinograph (AACC 2000) and flour pasting properties were determined using a rapid viscoanalyser (RVA) starch master R & D pack V 3.0 (Newport Scientific Narrabeen, Australia).

3. Results and discussion

3.1. Standardization of germination time-temperature

Various trials of germination of flaxseed were conducted by varying the soaking and germination time. The different trials conducted for germination are given as follows:

A- Soaking- 4hrs; Germination-1 day; Drying-24 hrs.

B- Soaking-2 hrs; Germination-2 day; Drying-36 hrs.

C- Soaking- 2 hrs; Germination-1 day; Drying-36hrs.

On the basis of fatty acid analysis, best time-temperature combination i.e. G-C for germination was selected, which was soaking flaxseeds for 2 hours in water, germination for 1 day and at drying for 36 hours at a temperature of 40±5ºC. This combination gave the unique and healthy fatty acid profile from nutrition and stability point of view. It has low amounts of saturated fatty acids (approximately 6%); moderate amounts of monounsaturated fatty acids (approximately 41%) and beneficial omega-6 (linoleic acid) and omega-3(linolenic acid) fatty acid were 13% and 40%, respectively.

In the selected combination, on germination, palmittic acid, oleic acid, linoleic acid content were increased by 0.7%, 44% and 57% respectively as compared to ungerminated flaxseed, whereas, linolenic acid content decreased by 20% as compared to ungerminated flaxseed.

Increase in oleic acid content is desirable, as it has many health benefits associated with it. High concentration of oleic acid can lower the blood cholesterol level and lowers the risk of heart problems (Rickman, 2004). Decrease in linolenic acid content adds to stability of the product. As linolenic acid contains three double bonds in it, that is why, it is more prone to oxidation as compared to other unsaturated fatty acids and decreases the shelf life of the product. Thus, slight decrease in linolenic content is also beneficial. Although linolenic acid content decreased upon germination, but its beneficial effects didn't alter much. This is supported by increase in linoleic acid content, which makes the omega6 to omega 3 ratio unaltered.

3.2. Analysis of raw material

3.2.1. Proximate composition of raw material

The proximate composition of raw material was shown in Table 1. The germination of flaxseed resulted in an increase in protein, fat and fibers content.

3.2.2. Mineral analysis of raw materials

The mineral analysis of the raw materials like wheat flour, whole wheat meal, ungerminated and germinated flaxseed were carried out as shown in Table 2. The minerals like Zn, Ca and Mg were found to increase on germination of flaxseed whereas minerals like Cu, Fe and Mn decreased.

3.2.3. Farinographic properties of flour used for preparation of bread

A. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the farinographic properties

The effect of incorporation of germinated and ungerminated flaxseed meal at 0, 5, 10, 15, 20% levels in wheat flour on farinograph has been discussed in the Table 3, Figures 1 and 2. Water absorption increased significantly with the addition of the flaxseed meal. The stability time of dough decreased significantly with the addition of ungerminated and germinated flaxseed meal as compared to control. The change in stability due to the flaxseed meal might have been attributed to the dilution of gluten forming proteins that caused weakening of dough. This value gives some indication of the tolerance to mixing or strength of the flour and gluten breakdown the flour will have. The mixing tolerance index of the flaxseed containing breads was found to be less as compared to control breads. Flours that had a low mixing tolerance index tend to have a good tolerance to mixing; whereas, the higher the tolerance index, the weaker is the flour (Shuey et al., 1972). The results agreed with those obtained by Garden (1993) who reported that incorporating ground flaxseed into wheat flour significantly increased water absorption and dough development time but decreased stability. The longer dough development time could have resulted from the dilution of gluten and difficulty of mixing of flax seed flour and wheat flour homogenously.

B. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the farinographic properties

The water absorption of whole wheat-flaxseed meal was found to be more than that of wheat flour as represented by Table 4 and Figures 3 and 4.This might have been due to the high fibers content in the whole wheat meal.

Table 1. Proximate composition of raw material

Constituent	Wheat flour (Mean ± SD)	Whole wheat meal (Mean ± SD)	Ungerminated flaxseed (Mean ± SD)	Germinated flaxseed (Mean ± SD)
Moisture content (%)	14.93± 0.21	8.20±0.10	8.76±0.04	3.22±0.06
Ash content (%)	0.38±0.05	1.13±0.01	3.16±0.01	3.01±0.09
Protein (%)	11.47±0.24	12.03±0.14	20.33±0.25	27.69±0.27
Fat (%)	1.43±0.09	1.64±0.07	35.17±0.82	38.9±0.38
Crude fiber (%)	0.50±0.04	1.80±0.01	22.15±0.28	26.6±0.32
Dietary fiber (%)	0.50±0.07	1.40±0.03	19.70±0.19	24.90±0.24
Calorific Value (Kcal/g)	4.21±0.14	4.58±0.13	6.69±0.11	6.75±0.23
Antioxidant activity (%)	-	-	92.05±0.15	91.18±0.18
Total phenols (mg/ 100g)	-	-	307.36±0.29	238.56±0.31

Table 2. Mineral content (mg/Kg) of raw materials

Minerals (mg/Kg)	Wheat flour	Whole wheat meal	Ungerminated flaxseed	Germinated flaxseed
Cu	BDL*	0.90	9.85	9.40
Fe	16.92	25.64	88.02	82.36
Mn	4.26	14.81	31.47	30.42
Zn	5.55	13.91	34.03	42.53
Ca	183.33	281.05	1723.38	2532.86
Mg	917.31	1056.70	2737.97	3235.36

BDL*=Below Detection Limit

Table 3. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the farinographic properties

Formulation	Level (%)	Water Absorption (%)	Dough Development Time (minutes)	Stability Time (minutes)	Mixing Tolerance Index (BU)
Control	0	61.3	3.5	13.6	80
Ungerminated	5	62.9	6.9	6.2	32
	10	63.8	7.7	6.7	30
	15	62.4	8.0	6.4	45
	20	62.1	8.2	4.9	60
Germinated	5	62.1	70	7.7	26
	10	61.6	7.2	9.1	36
	15	61.6	8.9	5.6	30
	20	62.2	8.5	4.6	49
Critical Difference ($p \leq 0.05$)		0.30	0.27	0.39	3.76

Table 4. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the farinographic properties

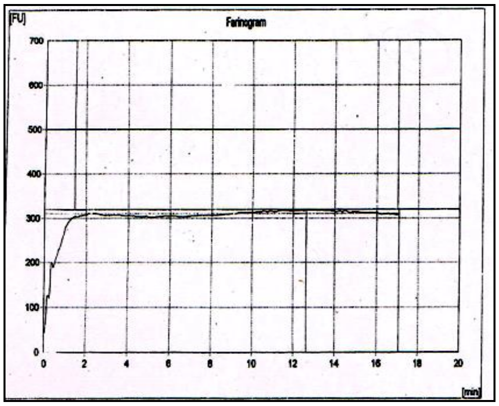
Formulation	Level (%)	Water Absorption (%)	Dough Development Time (minutes)	Stability Time (minutes)	Mixing Tolerance Index (BU)
Control	0	70.2	4.0	4.8	70
Ungerminated	5	72.9	3.9	4.9	59
	10	71.1	4.5	2.9	60
	15	70.9	4.8	4.9	55
	20	69.5	6.4	3.2	59
Germinated	5	70.2	4.2	5	68
	10	70.9	3.9	5.3	51
	15	69.7	4.7	3.3	70
	20	70.1	4.8	3.3	76
Critical Difference (p≤0.05)		0.46	0.19	0.24	1.71

Table 5. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the pasting properties

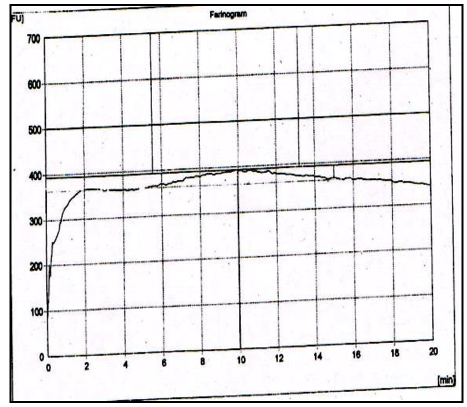
Breads	Level (%)	Parameters					
		Paste Temperature (ºC)	Peak Viscosity (cP)	Hold Viscosity (cP)	Final Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)
Control	0	92.80	1317	847	1658	470	811
Ungerminated	5	94.10	1555	1039	1869	470	759
	10	94.60	1436	961	1822	468	742
	15	94.85	1337	884	1758	454	738
	20	95.00	1156	826	1570	329	702
Germinated	5	94.75	1376	939	1714	438	775
	10	94.90	1386	938	1754	448	816
	15	94.90	1274	886	1662	388	775
	20	95.00	1132	806	1520	326	715
Critical Difference (p≤0.05)		0.32	4.35	2.28	3.95	2.42	4.16

Table 6. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the pasting properties

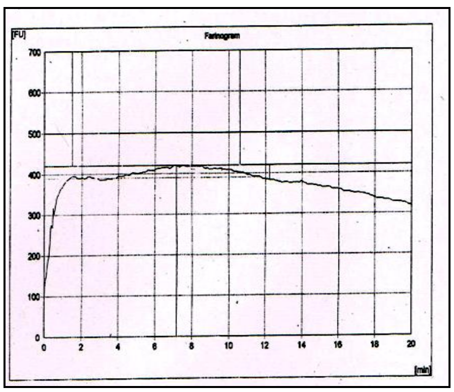
Breads	Level (%)	Parameters					
		Paste Temperature (ºC)	Peak Viscosity (cP)	Hold Viscosity (cP)	Final Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)
Control	0	91.9	756	538	1192	258	654
Ungerminated	5	93	856	598	1279	218	680
	10	95.6	800	588	1236	212	648
	15	95.2	718	543	1166	174	624
	20	0	648	500	1059	148	559
Germinated	5	92.85	752	536	1180	215	644
	10	94.55	737	538	1176	199	638
	15	95.6	734	552	1177	182	623
	20	0	686	537	1132	134	595
Critical Difference (p≤0.05)		0.44	3.07	9.89	2.28	3.83	3.87



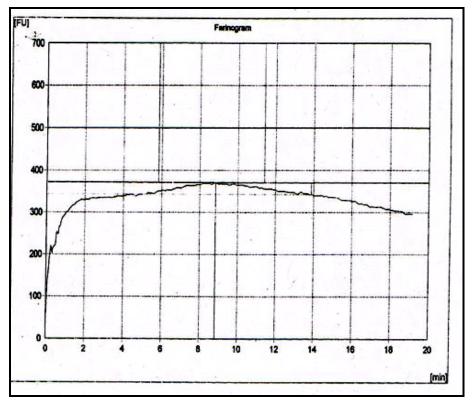
Control-whole wheat meal



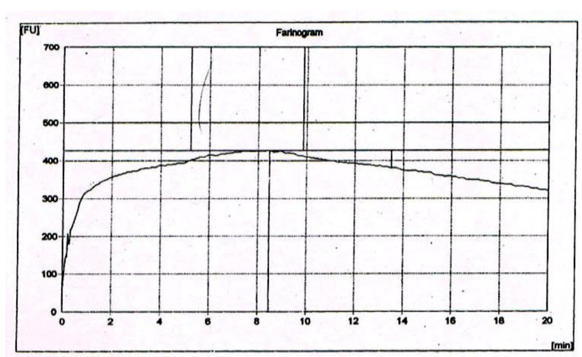
Whole wheat meal + 5 % germinated meal



Wheat flour +10 % Germinated meal

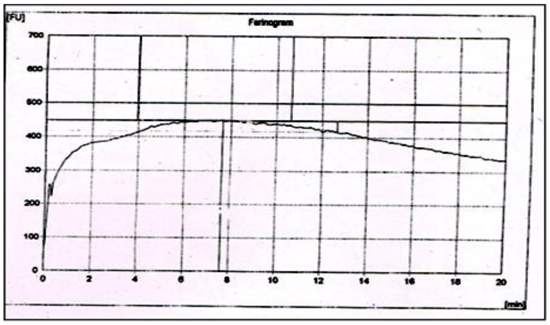


Wheat flour + 15 % Germinated meal

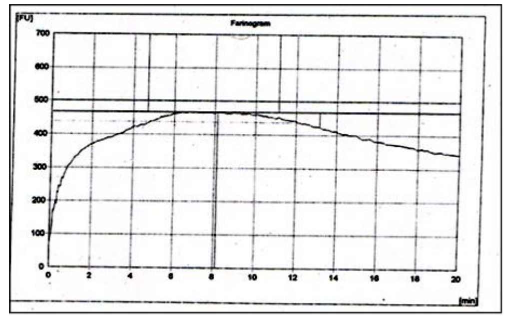


Wheat flour + 20 % germinated meal

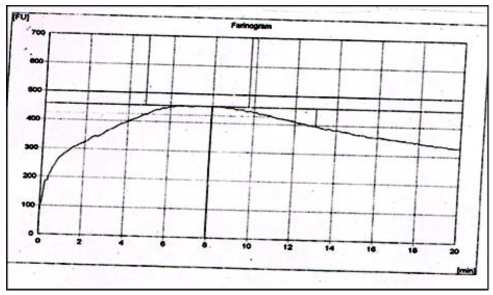
Figure 1. Farinographs showing effect of incorporation of different levels of germinated flaxeed meal



Wheat flour + 10 % ungerminated meal

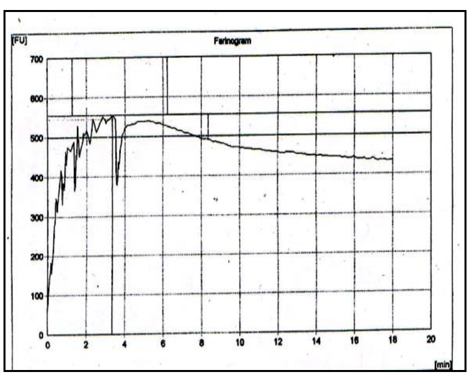


Wheat flour + 15 % ungerminated meal

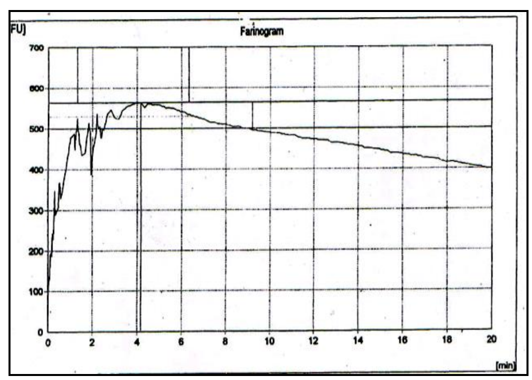


Wheat flour + 20 % ungerminated meal

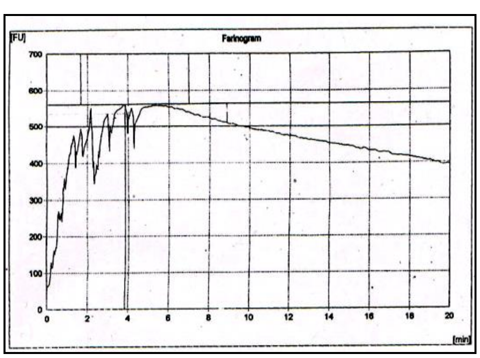
Figure 2. Farinographs showing effect of incorporation of different levels of ungerminated flaxeed meal



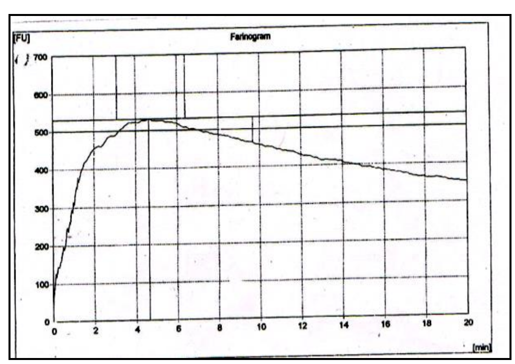
Control-whole wheat meal



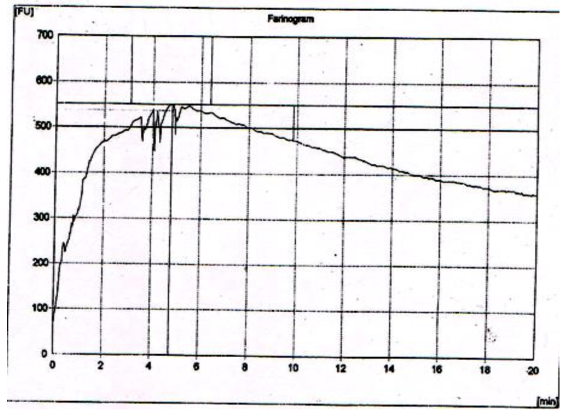
Whole wheat meal + 5 % germinated meal



Whole wheat meal+10 % germinated meal

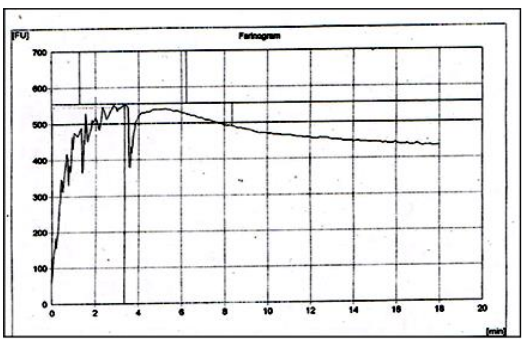


Whole wheat meal+15 % germinated meal

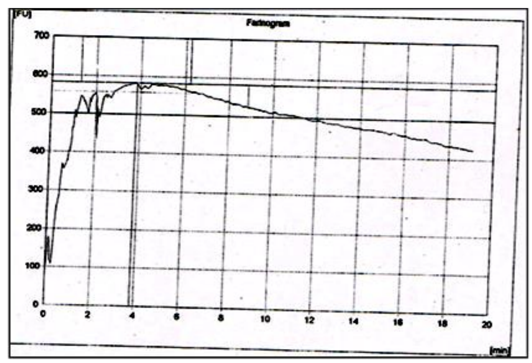


Whole wheat meal + 20 % germinated meal

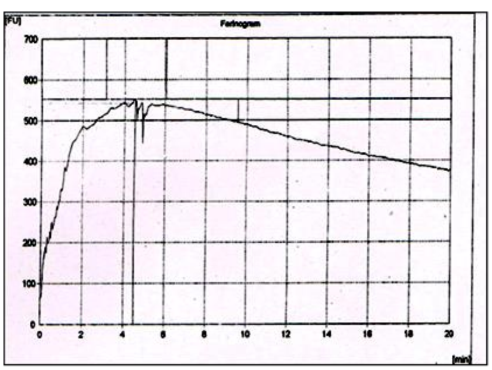
Figure 3. Farinographs showing effect of incorporation of different levels of germinated flaxseed meal



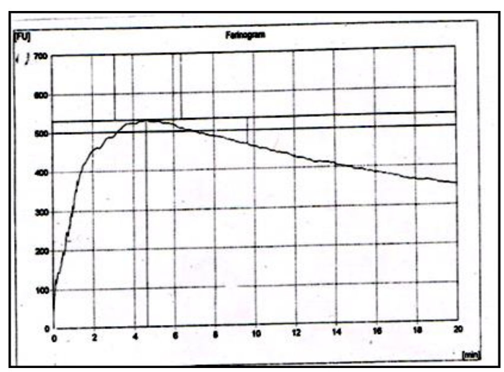
Control-whole wheat meal



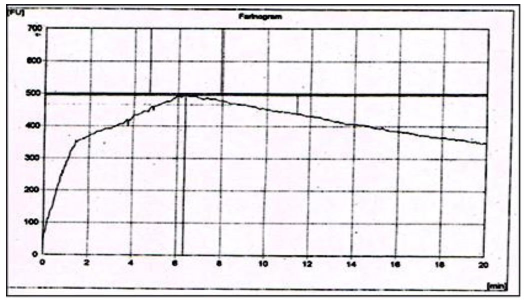
Whole wheat meal + 5 % ungerminated meal



whole wheat meal+10 % ungerminated meal



Whole wheat meal +15%ungerminated meal



whole wheat meal + 20 % ungerminated meal

Figure 4. Farinographs showing effect of incorporation of different levels of ungerminated flaxseed meal

The water absorption in the flaxseed breads was found to be significantly more than that of control breads at 5 and 10 percent level of substitution. The increased water absorption might have been due to the gum present in the flaxseed which possesses excellent water binding capacity (Konessni et al., 2005). Also, the flaxseed mucilage was found to be composed of polymeric carbohydrates that can help in improving the water absorption characteristics of the dough (Fedenuik and Biliaderis, 1994).

3.2.4. Pasting properties of flour used for preparation of bread

A. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in wheat flour on the pasting properties

Pasting is one of the most important properties of the starch, which often occurs in various types of flour during processing. The pasting properties of various blends prepared by the addition of ungerminated and germinated flaxseed meal were determined by use of rapid visco-analyser and are represented in the Table 5.

The pasting temperature of the flaxseed containing breads was found to increase significantly with the increasing level of addition of the ungerminated and germinated flaxseed meal. It increased to 95ºC at 20% level of incorporation of ungerminated flaxseed meal whereas in 10% germinated flaxseed meal incorporated breads, it increased to 95.1ºC as compared to the control which was 92.8ºC. Too much pasted starch will cause stickiness, small volume and prone to the stale of bread. On the other hand too little pasted starch cannot form a continuous phase to be involved in the gas wall of bread.

The peak viscosity for the ungerminated flaxseed meal added wheat flour was found between 1555 cP at 5% level of addition and 1436 cP at 10% level of addition after which it started to decline. Peak viscosities attained during the heating portions of tests indicate the water binding capacity of starch. Similar trend was observed in the hold viscosity and final viscosity which first increased significantly upto 10% level of addition of ungerminated and germinated flaxseed meal and then started decreasing.

Final viscosity was minimum at 20% level of addition (1570 cP) and maximum at 5% level of addition (1869 cP) in the flour incorporated with ungerminated flaxseed meal. In case of germinated flaxseed meal incorporated Formulation, it was found to be in the range of 1714 cP at 5% level of addition and 1520 at 20% level of addition.

Breakdown and setback viscosity were found to decrease with the increase in level of addition of flaxseed meal. The setback viscosity decreased to 702 cP at 20% cent level of addition of ungerminated flaxseed meal whereas it decreased to 715 cP at 20% cent level of addition of germinated flaxseed meal as compared to control which was 811 cP. Set back values have been reported to correlate with the ability of starch to gel into solid pastes.

The results are in accordance with Chetana et al. (2010) who found that on cooling to 50ºC, the viscosity decreased from 1147 to 680 BU and 499 for 40% incorporation of raw and roasted flaxseed powder whereas breakdown values decreased from 286 to 56 BU and 286 to 54 BU with an increase in level of incorporation of raw and roasted flaxseed powder. This indicated that the fiber fraction interacted with the wheat starch.

B. Effect of incorporation of different levels of ungerminated and germinated flaxseed meal in whole wheat meal on the pasting properties

The pasting temperature was found to increase significantly with the increase in level of addition of ungerminated and germinated flaxseed meal (Table 6). The peak viscosity was found to increase significantly upto 10% level of addition of both ungerminated and germinated flaxseed meal and then started decreasing whereas in case of germinated flaxseed meal addition, it significantly decreased with the increasing level of addition. The hold viscosity showed a significant decrease to 500cP after the addition of 20% of ungerminated flaxseed meal and 537 cP in germinated flaxseed meal added breads as compared to control which was 538 cP. Breakdown viscosity was also found to decrease significantly with the increase in level of addition of ungerminated and germinated flaxseed meal. It was found between 218 cP at 5% cent level of addition and 149 cP at 20% cent level of addition in case of ungerminated flaxseed meal incorporated breads.

Setback viscosity also showed a significant decrease with the increasing level of addition of flaxseed. The final viscosity is the most commonly used parameter to determine a particular starch based quality. It gives an idea of a material to gel after cooking. The decrease in pasting properties might have been due to the fibre present competing with the starch in sample for water. As viscosity of paste were directly related to the degree and extent of starch gelatinization and hence realignment during subsequent cooling. Any material that competes for water will restrict the amount of water available for the starch granules during starch gelatinization.

The pasting temperature of whole wheat meal was found to be less than that of wheat flour. Huang et al. (2007) also found lower pasting temperature and viscosities in whole wheat meal as compared to the commercial white flour. Breakdown is a measure of susceptibility of cooked starch granules to disintegration whereas setback is a measure of recrystallization of gelatinized starch during cooling (Beta and Corke, 2001). A low breakdown of wheat flour blends suggests that they are more stable under hot conditions than wheat flour. Moreover, wheat flour blends exhibit lower setback value indicating less amylose retrogradation as the system is cooled. In addition, the difference in pasting properties of wheat flour and wheat flour blends also could be due to other factors, such as particle size, enzyme activity or water-holding capacity.

4. Conclusions

It was observed that on germination of flaxseed, the fibre content and the protein content was found to increase. Farinograph results showed that as the levels of incorporation of ungerminated and germinated flaxseed meal increased in wheat flour and whole wheat meal, the development time increased significantly whereas the stability time decreased. Pasting properties like the breakdown viscosity and set back viscosity was found to decrease with increasing levels. However, stability time of germinated flaxseed added dough was more than that of ungerminated and can be further increased by adding improvers.

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