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Effect of thermal treatments on the storage life of pearl millet (*Pennisetum glaucum*) flour

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ABSTRACT

Effect of pre-milling treatments, viz. roasting and hydrothermal treatment was evaluated on pearl millet [*Pennisetum glaucum* (L) R Br] flour stored at ambient condition (average maximum and minimum temperatures 33.1±4°C and 21.4±2°C, respectively, and 84.4±2% RH) and controlled condition (30±1°C at 50±2% RH) packed in zip-lip low density polyethylene (LDPE) pouches of 10 µm thickness having permeability 23.13g/m^2 24h. Roasting and hydro thermal treatment significantly reduced fat acidity by 8% and 12%, phytic acidity by 10% and 4% and trypsin inhibitor activity by 9% and 5%, respectively, of pearl millet flour. It was observed that pearl millet flour can be stored at controlled condition in superior form than ambient condition. Storage period had significant ($P<0.05$) effect on moisture content, fat acidity, phytic acid and trypsin inhibitors activity of pearl millet flour. It was found that untreated, roasted and hydrothermally treated pearl millet flour can be stored up to 2, 2 and 30 days at ambient conditions and 3, 3 and 45 day, respectively, at controlled condition without undue deterioration in quality.

Key words: Fat acidity, Moisture content, Pearl millet flour, Phytic acid, Storage, Trypsin inhibitors activity

Biochemical composition of pearl millet [*Pennisetum glaucum* (L) R Br] indicates that it is a good source of energy, protein, vitamins and minerals (Osman 2009). However, bioavailability of the nutrients is restricted due to the presence of anti-nutritional factors such as phytic acid, tannins, goitrogens, oxalic acid and trypsin inhibitors. These compounds interfere with mineral bioavailability, carbohydrates and protein digestibility through inhibition of proteolytic and amylolytic enzymes.

The phytic acid is present in the germ whereas, polyphenols are in peripheral areas of the pearl millet grain (Simwemba *et al*. 1984). Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium, iron and as with protein residue. The binding can result in insoluble salts with poor bioavailability of minerals (Coulibaly *et al.* 2011). Hence, it is important to reduce the phytic acid and polyphenols to avail the nutritional benefits of this grain.

Pearl millet grain is light in weight (3-15mg) but has a proportionally larger germ (17.4%) than all other cereal grains, except maize (Taylor 2004). It contains a higher content of triglycerides, which are rich in unsaturated fatty acids. Pearl millet flour used for food preparation like *roti* (flat bread), *bhakri* (stiff *roti*) and porridge or gruel is produced by milling, either through traditional or mechanical processes. However, pearl millet flour turns bitter and rancid within a few days of storage period, due to lipolysis and subsequent oxidation of the resulting de-esterified unsaturated fatty acids (Lai and Varriano- Marston 1980). This is caused by an active lipase enzyme which is responsible for the breakdown of glycerides and consequent increase of free fatty acids (Pruthi 1981 and Arora *et al.* 2002). These chemical changes are usually manifested as off-flavours during storage, especially under conditions of moderately high moisture and oxygen exposure (Nantanga *et al*. 2008).

Various treatments were applied to pearl millet to prevent the development of rancidity in the stored flour, including decorticating the grains (Abdebrahman *et al*. 1983), the use of antioxidants (Kapoor and Kapoor 1990), application of dry heat treatment to grain (Chavan and Kachare 1994) or flour (Kapoor and Kapoor 1990), acid soaking and hot water blanching (Chavan and Kachare 1994, Archana *et al.* 1998), use of malting (Archana *et al.* 1998), preservation of pearl millet flour by refrigeration (Mohamed *et al.* 2011), use of different containers for storage like gunny sacks, earthen pots, tin cans and polythene bags (Chaudhary and Kapoor 1984); application of thermal treatment for producing shelf- stable and also pre-cooked flour (Nantanga *et al*. 2008), use of pre-milling treatments, viz. pearling, heat treatment and fermentation

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which increased storage life of pearl millet flour up to 6 days without undue deterioration in quality at ambient condition (Tiwari *et al.* 2014). The available literatures clearly indicate that shelf-life of pearl millet flour was very limited. Therefore, the objective of this study was to evaluate the effects of dry and wet heat treatments of storage life to inactivate anti-nutritional content of pearl millet grains through some pre-milling treatments like roasting and hydrothermal treatment and thus evaluate the storage stability of flour at different storage conditions.

MATERIALS AND METHODS

The pearl millet (cv. Pusa Composite 443) was procured from the Experimental Farm of the Indian Agricultural Research Institute, New Delhi (India). The grain were carefully cleaned and freed from foreign materials.

After cleaning, the treatments like roasting and boiling were applied to the grain. Heat treatment was carried out using a twin screw extruder (BTPL, Kolkata) at 110°C temperature for duration 60 sec as beyond these limits the grain sample started showing either puffing or burning/ over-heating (Tiwari *et al.* 2014).

For hydro-thermal treatment, 2 kg of sample was held in a pan of boiling water (1:1 grain to water, w/v) for 15 min. Thereafter, the water was drained using a colander. The wet grain was spread on trays in thin layer of less than 1 cm. The trays were placed in a tray dryer at 60°C for 2 hr (Nantanga *et al*. 2008) to bring the moisture content up to 5.63±0.02%.

The untreated, roasted and hydro-thermally treated pearl millet grain was ground into flour using a hammer mill (Sanco, India) and packed in zip-lip low density polyethylene (LDPE) pouches of 10 µm thickness having permeability 23.13g/m^2/24 hr. The pouches containing 20 g sample were stored at two different conditions i.e., ambient condition (average maximum and minimum temperatures 33.1 ± 4°C and 21.4 ± 2°C, respectively, and 84.4 ± 2% RH) and controlled condition (30 ± 1°C at 50 ± 2% RH). Samples were withdrawn at the intervals of 15 days and analysed for moisture content, phytic acid, trypsin inhibitors activity and fat acidity. The untreated, roasted and hydro-thermally treated flours stored at ambient condition were represented as T_1, T_2, T_3, respectively, whereas T_4, T_5 and T_6 represents untreated, roasted and hydro-thermally treated samples stored at controlled condition (30 ± 1.5°C at 50 ± 2% RH).

Moisture content of a flour sample was determined by drying at 105±2°C for 24 hr in an oven to constant mass (AOAC, 1990). Fat acidity was determined by AOAC (1990) method 14.067, rapid method for corn. Phytic acid was estimated by the method described by Wheeler and Ferrel (1971). Trypsin inhibitor activity (TIA) was assayed according to the Hamerstrand *et al.* (1981) using BAPA (Nbenzoyl-DL-arginine-P-nitroanilide hydrochloride) and trypsin. TIA, expressed as TUI/mg of sample was calculated from absorbance read at 410 nm against sample blank in a spectrophotometer.

Statistical analysis of data was done using SPSS 16.0. Univariate analysis in general linear model was done for analysis of variance at 5% level of significance.

RESULTS AND DISCUSSION

Effect of pre-milling treatments on moisture content of flour during storage period

Moisture content has important role in determining storage life of flour. Lower the moisture content, longer is storage stability. The moisture content of the samples varied from 5.58-5.66% (wb). The moisture content of all the samples increased significantly during storage period (Fig. 1). At the end of the storage period (60 days), the moisture contents of the flour from untreated, roasted and hydro-thermally treated grains were found to be 11.61, 12.09, and 12.44% (wb), respectively, at ambient storage. The corresponding values of moisture contents at controlled storage conditions (50% RH and 30°C) were found to be 11.50%, 11.82% and 11.93% (wb), respectively. The gain in moisture content was due to hygroscopic nature of the flour, storage environment (temperature, relative humidity) as well as nature of the packaging material. Similarly, Nantanga *et al.* (2008) reported that moisture content of heat treated flours were increased significantly ($P<0.05$) during storage. Linear relationships between moisture content and storage period were found for all the samples with coefficient of determination >0.9 (Table 2).

Analysis of Variance (Table 1) indicated significant effects of treatments, storage period and their interaction n moisture content of flour.

The lowest increase in moisture content during storage

Table 1 ANOVA for moisture content, fat acidity, phytic acid and trypsin inhibitors activity of pearl millet flour during storage

Source	df	Moisture content		Fat acidity		Phytic acid		TIA	
		MS	F	MS	F	MS	F	MS	F
SD	4	82.4	619.4**	167182.8	1.3E3**	259561.2	6.5E3**	2.12	1.5E3**
Treatment	5	2.11	15.82**	179424.5	1.4E3**	11252.2	281.8**	0.55	375.3**
SD × Treatment	20	0.86	6.49**	16598.6	125.5**	1228.9	30.78**	0.02	10.9**
Replications	1	0.16	1.17	138	1.04	161	2.03	0.00	1.09
Error	29	0.13		132		39.93		0.10	

SD refers to storage days, TIA refers to trypsin inhibitors activity, df refers to degrees of freedom, MS refers to mean sum of square, F refers to F value, ** refers to significance at (P=0.05).

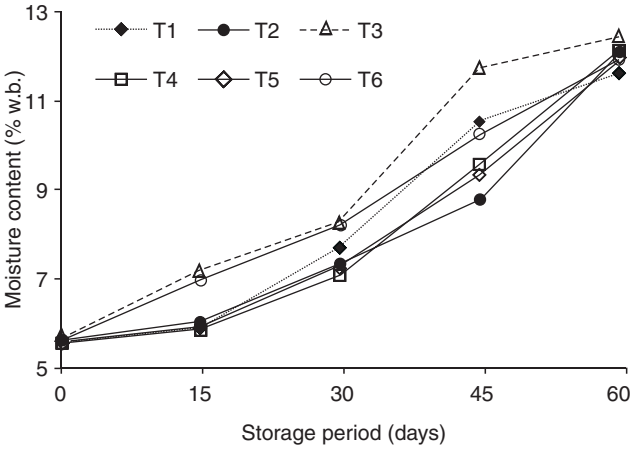

Fig 1 Effect of pre-milling treatment on moisture content of flour during storage period.

period was observed in flour obtained from untreated grain, whereas highest increase was in hydro-thermally treated flour. This indicated hydro-thermally treated grain produced flour with maximum hygroscopicity. The increase in moisture content was more rapid in ambient storage due to higher ambient relative humidity.

Though moisture content of flour increased during storage period, it remained below the maximum moisture content limit (13%) suggested by FAO/ WHO (FAO/ WHO, 1995 and Nantanga *et al.* 2008) for pearl millet flour intended for human consumption.

Effect of pre-milling treatment on fat acidity of flour during storage

Increase in fat acidity is one of the indicators of deterioration of quality of flour during storage. Analysis of Variance (ANOVA) (Table 1) revealed significant effect of treatments, storage period and their interaction on fat acidity of pearl millet flour. Fat acidity of untreated, roasted and hydro-thermally treated flour was determined as 26, 24, and 23 mg KOH/100g, respectively. Decrease in fat acidity on heat treatment has also been reported by Chavan and Kachare (1994) and Tiwari *et al*. (2014). The lower fat acidity value of hydrothermally treated samples could be the result of thermal treatment which had inhibited lipase before the grains were milled (Nantanga *et al.* 2008).

The fat acidity was found to be less at controlled than ambient condition at any particular storage period. With increasing storage period, fat acidity of the samples increased. The increase in fat acidity during storage indicated an increase of de-esterified fatty acids due to lipolysis (Nantanga *et al.* 2008). At the end of 60 days of storage, the fat acidity of untreated, roasted and hydro-thermally treated flour increased from 26 to 493 mg, 24 to 485 mg, 23 to 36.5 mg KOH/100g, respectively, at ambient storage and 26 to 474 mg, 24 to 443.5 mg and 23 to 32.5 mg KOH/100g, respectively. Also, the rate of increase in fat acidity was maximum in untreated and roasted sample compared to hydrothermally treated samples. This indicated that the hydrothermal treatment had better inhibited lipase, due to the higher specific enthalpy of wet heat than dry-thermal treatment of roasting of grain (Nantanga *et al.,* 2008).

Arora *et al.* (2002) found that fat acidity of untreated and heat treated pearl millet flour increased from 30.3 to 123.7 mg KOH/100g and 28 to 50.5 mg KOH/100g, respectively, during 28 days of storage period. They also suggested that heat treatment of pearl millet grain before milling to flour did not show any adverse effect on its quality in terms of colour, appearance, aroma, texture, taste and overall acceptability during 28 days of storage period at room temperature. Similarly, Nantanga *et al.* (2008) showed that fat acidity of flour from the untreated grain increased from 0.11 to 3.73 g KOH/kg during three months storage, whereas the wet thermally treated samples showed no significant increase (P>0.05). Also, Tiwari *et al.* (2014) found that fat acidity was above 30 mg KOH/100g in the untreated pearl millet flour even at the initial day, whereas heat treated flour exceeded this limit on 6th day. Increase in the fat acidity from 36.8 to 67.1 mg, and 22.1 to 37.8 mg KOH/100 g of untreated and heat treated pearl millet flour, respectively occurred during 8 days of storage. Chavan and Kachare (1994) found that fat acidity increased by over

Table 2 Relationship of moisture content, phytic acid, fat acidity and TIA of flour with storage period

Moisture content	Phytic acid	Fat acidity	TIA
$M_{T1} = 0.111\times + 4.93$	$P_{T1} = 99.03\times + 105.2$	$F_{T1} = 111.4\times - 10.25$	$T_{T1} = 0.014\times + 7.91$
$R^2 = 0.946$	$R^2 = 0.925$	$R^2 = 0.886$	$R^2 = 0.949$
$M_{T2} = 0.104\times + 4.874$	$P_{T2} = 84.13\times + 103.8$	$F_{T2} = 108.8\times - 23.75$	$T_{T2} = 0.018\times + 7.228$
$R^2 = 0.900$	$R^2 = 0.958$	$R^2 = 0.925$	$R^2 = 0.9444$
$M^{T3} = 0.121\times + 5.429$	$P_{T3} = 94.60\times + 159.0$	$F^{T3} = 3.2\times + 21.3$	$T_{T3} = 0.019\times + 7.566$
$R^2 = 0.956$	$R^2 = 0.965$	$R^2 = 0.946$	$R^2 = 0.919$
$M_{T4} = 0.111\times + 4.70$	$P_{T4} = 93.37\times + 108.8$	$F_{T4} = 110.9\times - 73.05$	$T_{T4} = 0.013\times + 7.834$
$R^2 = 0.918$	$R^2 = 0.897$	$R^2 = 0.990$	$R^2 = 0.992$
$M_{T4} = 0.106\times + 4.83$	$P_{T5} = 82.44\times + 111.3$	$F_{T5} = 103.4\times - 65.8$	$T_{T5} = 0.020\times + 7.172$
$R^2 = 0.923$	$R^2 = 0.936$	$R^2 = 0.991$	$R^2 = 0.929$
$M_{T6} = 0.105\times + 5.436$	$P_{T6} = 93.52\times + 136.0$	$F_{T6} = 2.3\times + 21$	$T_{T6} = 0.016\times + 7.516$
$R^2 = 0.991$	$R^2 = 0.984$	$R^2 = 0.994$	$R^2 = 0.918$

TIA refers to trypsin inhibitors activity.

five fold both in the flour of the control and of hot-air heated grain, whereas it remained almost unchanged in the flour of the boiled grain during storage under ambient conditions for a month. Kaced *et al*. (1984) reported that the fat acidity in pearl millet increased steadily from 20 to 60 mg/100g KOH during 10 days of storage period, indicating hydrolytic decomposition as well as oxidative degradation.

Tiwari *et al.* (2014) suggested the critical limit of fat acidity in pearl millet flour as 30 mg/100g. Also, Lai and Marston (1980) reported that in pearl millet flour, under all storage conditions the change in odour corresponded to a fat acidity level of 30 mg KOH/100 g of meal. In the present study, untreated, roasted and hydro-thermally treated pearl millet flour exceeded the fat acidity value after 2, 2 and 30 days of storage at ambient condition, whereas after 3, 3 and 45 days of storage at controlled condition, respectively.

Effect of pre-milling treatment on phytic acid content of pearl millet flour during storage

Phytic acid in cereals chelates mineral cations and interacts with proteins forming insoluble complexes, which leads to reduced bio-availability of minerals and reduced digestibility of protein (Reyden and Selvendran 1993, Mohamed *et al.* 2010). When the phytate level is reduced, there is more available phosphorus than phytate in the grain, the food then becomes more nutritious. The suggested average phytate intake in the U S and the U K ranged between 631 and 746 mg/day; in Finland 370 mg; in Italy 219 mg; and in Sweden a mere 180 mg/day (Coulibaly *et al*. 2011). Changes in phytic acid content during storage of pearl millet flour are depicted in Fig 2. Phytic acid content was found to be 238 mg/100g in untreated flour which reduced to 213 mg/100g in roasted flour and 228 mg/100g in hydro-thermally treated flour. The apparent decrease in phytate content during thermal processing may be partly due to the formation of insoluble complexes between phytate and other components, such as phytate protein and phytate-protein-mineral complexes or to the inositol hexaphosphate hydrolyzed to pentakis- and tetraphosphate (Siddhuraju and Becker, 2001).

Tiwari *et al.* (2014) reported that roasting of pearl millet grain at 110°C for 60 s duration reduced phytic acid content of flour from 728 to 410 mg/100 g (43.68%). Similarly, Chauhan *et al*. (2015) found that popping of pearl millet grain significantly reduced phytic acid content from 516.37 to 373.82 mg/100g, which may be ascribed to the heat treatment. Daniel and Egbuonu (2011) too reported that roasting and boiling significantly (P≤0.05) reduced phytate from 1.54±0.04 mg/g to 0.13±0.03 mg/g and from 1.54±0.04 mg/g to 0.18±0.11 mg/g, respectively, in case of asparagus bean flour.

It was further observed that increase in storage period led to significant (P≤0.05) increase in phytic acid content of the flour (Table 1). During storage for 60 days, the phytic acid content increased to 579, 527 and 600 mg/100g, respectively, in untreated, roasted and hydrothermally treated

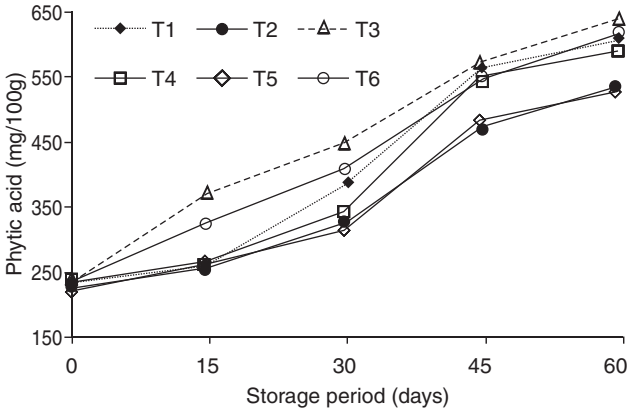

Fig 2 Effect of pre-milling treatment on phytic acid of flour during storage period.

flour at ambient storage. At controlled storage, the corresponding values of phytic acid were found to be 560, 519, 587 mg/100g, respectively. It was also observed that at any particular day of storage, phytic acid content was less at controlled condition than at ambient condition in all the flour samples. The increase in phytic acid might be due to increase in moisture content of the flour. The enzyme phytase is sensitive to temperature and humidity and hence susceptible to degradation during storage (Jacela *et al.* 2010). Due to degradation of phytase, there is increase in phytic acid. Mobilizations of myoinositol pool from various organelle's membrane during lipid peroxidation to cytoplasmic pool might be resulted in increase in phytic acid during storage.

A linear relationship was observed between the phytic acid and the storage days with high coefficient of determination (Table 2).

Effect of pre-milling treatment on trypsin inhibitors activity of flour during storage

The presence of protease inhibitors in the diet leads to the formation of the irreversible trypsin enzyme-trypsin inhibitor complex, causing a trypsin drop in the intestine and a decrease in the diet protein digestibility, leading to slower growth. Therefore, the reduction of trypsin inhibitors is useful in improving nutritional quality of grain with respect to protein digestibility.

Trypsin inhibitors activity in untreated, roasted and hydro-thermally treated flour was found to be 7.81 TUI/mg, 7.10 TUI/mg and 7.41 TUI/mg, respectively (Fig 3). Better inhibition of trypsin inhibitors activity was observed in roasted and hydrothermally treated pearl millet flour than the untreated flour. It could be due to the effect of heat treatments which cause the destruction of protease inhibitors, which interfere in protein digestibility.

Analysis of Variance (ANOVA) indicated highly significant effect of pre-milling treatments, storage period and their interaction trypsin inhibitors activity on the flour (Table 1). Trypsin inhibitor activity of the samples that were subjected to different pre-milling treatment increased

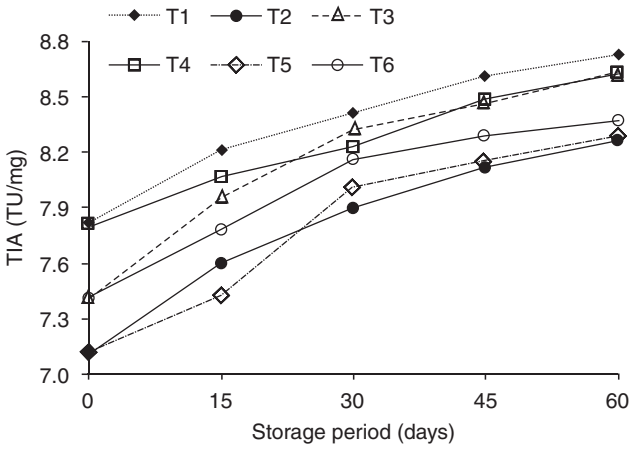

Fig 3 Effect of pre-milling treatment on trypsin inhibitors activity during storage period.

significantly with increasing storage period (Fig 3). The increase in trypsin inhibitors activity at the end of 60 days of storage at ambient condition was observed as 8.73 TUI/mg, 8.26 TUI/mg and 8.62 TUI/mg, respectively, in untreated, roasted and hydrothermally treated flour. At controlled storage, the corresponding values of trypsin inhibitors activity were found to be 8.63 TUI/mg, 8.29 TUI/mg and 8.37 TUI/mg, respectively. The activation in trypsin inhibitors activity during storage may be due to gain in moisture content and other storage conditions. Also, the linear nature of the plots obtained indicated that increased of trypsin inhibitor activity conforms to increase in storage period (Table 2).

Daniel and Egbuonu (2011) reported that roasting and boiling treatment significantly ($P<0.05$) reduced trypsin inhibitor activity from 5.58 ± 0.89 TUI/g to 0.16 ± 0.06 TUI/g or 96% and from 5.58 ± 0.89 TUI/g to 0.74 ± 0.04 TUI/g or 87%, respectively, in a time dependent manner in case of asparagus bean flour. The decrease of TIA activity may be attributed to the utilization of trypsin inhibitor as energy source, or degradation by peptic and pancreatic hydrolytic enzymes during germination.

From the present study it could be concluded that roasting and hydrothermal treatment caused significant reduction in phytic acid of 10% and 4%, respectively, in pearl millet flour. These two pretreatments also reduced fat acidity and trypsin inhibitors activity significantly. Pearl millet flour stored at controlled condition was better in form than ambient condition. The moisture content, fat acidity, phytic acid and trypsin inhibitors activity increased with increase in storage period. Based on the critical levels of fat acidity (30 mg KOH/100g), untreated, roasted and hydrothermally treated pearl millet flour could be stored upto 2, 2 and 30 days at ambient conditions whereas the samples kept at controlled condition could be stored upto 3, 3 and 45 days, respectively. This investigation will be beneficial in encouraging the utilization of pearl millet, which is still unexploited despite its numerous nutritious and therapeutic benefits.

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