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Storage Stability of Bio Fortified Pearl Millet Flour

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Abstract **– Pearl millet is an important food for millions of people and is a major source of calories and the vital component of food security in the developing world. It is important to know the keeping quality of the processed pearl millet for consumption, changes and the level of pathogenic bacteria that would make the processed flour unfit for consumption in longer storage periods.** *Aim* **: To assess the effect of packaging and temperature on the shelf life of the millet flour**. *Methods***: Pearl millet cultivars "Dhanshakthi" and "Proagro" were procured from ICRISAT, Telengana, Andhrapradesh. The samples were cleaned, roasted and milled into fine free flowing flour. Three layered packaging(two HDPE covers and one Zip-loc sandwich bags) was adopted coded (PA–PROAGRO, DS- DHANSHAKTHI) and dated. A control sample was kept unpacked in a cloth bag at room temperature. The samples were analyzed every fortnight for the nutrients, free fatty acid content and peroxide value and microbial load.**

Results**: The free fatty acid (FFA) content gradually increased from 0.071±0.002 (Proagro (RT) to 0.826±0.010 (Proagro (RF). FFA increase was the least in refrigerated samples. The peroxide value (PV) of the pearl millet flour samples remained at zero (0) till 45 days of storage in Dhanshakthi-RT, Dhanshakthi-RF, Proagro-RT and Proagro-RF. The moisture gain was 53.8 (Dhanshakthi-RT), 49.2 (Dhanshakthi-RF), 31.9 (Proagro-RT) and 22.5 (Proagro-RF), 70.2(Dhanshakthi-UP) and 44.3 (Proagro- UP) percent on storage. The fat content of Dhanshakthi and Proagro flour declined steadily from 0 to 60 days. The unpacked samples had the highest microbial count. The refrigerated sample (Dhanshakthi RF) had the least Total bacterial count (02 x 10^5 to 04 x10^5 CFU/g).**

Conclusion: **Rancidity can be reduced in pearl millet flour when packed in HDPE covers and stored at refrigerated temperature.**

Keywords **– Free Fatty Acid, Pearl Millet, Peroxide Value, Rancidity.**

I. INTRODUCTION

Millets are small seeded grasses having substantial benefits such as less water intensive, pest and drought resistance, high yield in the areas with water scarcity. It also possesses remarkable edible and nutritive values and health benefits. It lends itself to various processing and food manufacturing techniques. Realizing the nutrient richness of these grains they are now considered as "nutria-cereals".

.Pearlmillet (*Pennisetumglaucum)* also known as "*Kambu"* in Tamil, "*kambam*" in Malayalam and "*Bajra*" in Hindi was originally used by Indians since ancient times. Pearl millet flour has a severe problem during storage and it was observed to produce off-flavor and bitter taste [1]. The high fat content of pearl millet plays a major role in its shelf life because hydrolysis and oxidation of triglycerides release non esterified fatty acids. Good storage conditions can prevent these problems.

Flour packed and stored at right conditions can prevent the loss or gain of moisture, entry of microorganisms, changes in fatty acid profile Good packaging serves two purposes which are essentially technical and presentational. Technical aspects in packaging aim to extend the shelf life by better protection from all the hazards during storage. The temperature variation on flour products from millet grain could result in either hydrolytic or oxidative rancidity, triggering free – radical reaction, destabilizing flour quality and shelf stay. The high–density polyethylene package under sealed condition could prevent free radical build up, prolong its shelf stay and prevent microbial proliferations [2]

This study entitled **"Storage stability of bio fortified pearl millet flour"** was taken up to assess the effect of packaging and storage temperature on the shelf life of bio fortified pearl millet flour.

II. MATERIALS AND METHODS

II.1.Grain Procurement

The biofortified pearl millet cultivars *viz* **Proagro** and **Dhanshakthi** developed as biofortified varieties by **ICRISAT, HYDREBAD** were selected for this study**.** Freshly harvested samples were procured from the above mentioned institution to carry out the study.

II.2.Processing of Pearl Millet

II.2.1. Cleaning

The procured pearl millet was cleaned manually free of stones, sand and other extraneous materials as this may affect the quality of the millet and its products.

II.2.2. Roasting and Milling

Roasting improves colour, texture and flavor of the grains [3]. Hence the pearl millet samples were dry roasted on medium flame for 10minutes till the emergence of roasted aroma. The relative humidity on the day of roasting was 60 percent and the room temperature was 23°C. The roasted pearl millet samples were milled into flour in a hammer mill and sieved with a mesh size of 60. Sieving and milling were repeated twice to get a very fine flour.

II.2.3 Packaging and Storage

The two (Proagro and Dhanshakthi) milled pearl millet flours were packed in HDPE covers which have excellent chemical resistance, good water vapour barrier and great impact resistance. For the purpose of packing and subsequent analysis 300g of the flour were separately

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portioned and placed in HDPE covers and this was placed in a second HDPE cover and sealed well.

The double sealed covers were packed in standard household Zip-loc brand sandwich bags of 1.5 Mil. Totally three covers were there to prevent the entry of moisture. The samples packed were then coded (PA–PROAGRO, DS-DHANSHAKTHI) and dated to facilitate easy identification and retrieval on 0,15,30,45 and 60th day for analysis. A control sample was kept unpacked in a cloth bag at room temperature. The packaged samples were stored under two different temperatures namely room temperature and refrigerated temperature for 60 days. The average room temperature was 27.5 (range 23-32 ˚C) and the refrigerated temperature was 4˚C. It was ensured that the refrigerator's power supply was continuous without interruption.

II.2.4. Estimation of Quality Parameters

The biofortified pearl millet samples were analyzed every fortnight for Acid insoluble ash, Free Fatty acid, Peroxide value, Moisture, Protein, Fat, Ash, Fibre, Carbohydrates, Energy, β Carotene, Iron, Zinc, Phytate. The total plate count was done on the pearl millet samples at an interval of 15 days till 60 days.

II.3. Statistical Analysis

Results of the nutrient and quality analysis were subjected to statistical analysis of t-test and ANOVA using SPSS version 16.0.

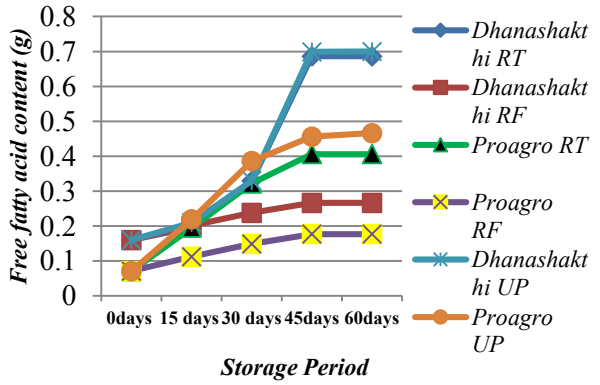
III. Results and Discussion

III.1. Quality Analysis of Pearl Millet Flours

III.1.1 Acid Insoluble Ash Content (AIA)

The acid insoluble ash content of Dhanshakthi and Proagro flours was more or less same from day 0 to day 60. However Proagro contained a slightly higher amount of AIA than that of Dhanshakthi. The unpacked samples' AIA increased slightly from day 15 onwards, whereas in the packed samples AIA showed an increase only on day 60. This indicates the presence of silica in Proagro sample and further cleaning is required for the same.

III.1.2. Free Fatty Acid Content



RT-Room temperature RF- Refrigerated temperature UP – Unpacked. All values are means of triplicates

Fig. 1. Free fatty acid content of biofortified pearl millet flours

The level of **free fatty acid** (FFA) is a good measure of the storage conditions of either the grain or the flour. The present pearl millet flour samples' free fatty acid content gradually increased from 0.071±0.002 (Proagro (RT) to 0.826±0.010 (Proagro (RF). All the samples showed an increase in FFA value irrespective of number of days as well as temperature of storage. However the increase in FFA value was the highest (6.5 times) in Proagro (UP). It was followed by Proagro- RT (5.7 times) and Dhanshakthi –UP (4.3 times).

It could be very well understood from the above discussion that FFA increase was the least in refrigerated samples. A high FFA value is mainly due to hydrolytic changes associated with the action of lipolytic enzymes [4]The increase in lipase activity during storage may have lead to a significantly higher FFA value in stored bio fortified pearl millet flour samples. The above results indicate that level of rancidity could be diminished with concurrent increase in shelf life if pearl millet flours are packed in HDPE covers and refrigerated.

III.1.3 Peroxide Value

The **peroxide value** (PV) of the pearl millet flour samples remained at zero (0) till 45 days of storage in Dhanshakthi-RT, Dhanshakthi-RF, Proagro-RT and Proagro-RF. The dry roasting that was done before milling the pearl millet into flour could have partially inactivated the lipolytic enzymes that are responsible for degradation of fat triglycerides. This could be cited as a reason for nil peroxide value till 45 days in packed samples and 30 days of storage in unpacked samples.

Table I Peroxide value of biofortified pearl millet flour

Sample	Peroxide value (%)				
	0	15	30	45	60
Dhanashakthi (RT)	0.0	0.0	0.0	0.0	0.20± 0.03 *
Dhanashakthi (RF)	0.0	0.0	0.0	0.0	0.22±0.01 *
Proagro (RT)	0.0	0.0	0.0	0.0	0.25±0.02*
Proagro (RF)	0.0	0.0	0.0	0.0	0.20±0.03*
Dhanashakthi (UP)	0.0	0.0	0.0	0.10± 0.01	0.20 ±0.02*
Proagro unpacked (UP)	0.0	0.0	0.0	0.12± 0 .01	0.25 ±0.03*

RT-Room temperature **RF**- Refrigerated temperature
UP – Unpacked *P value (<0.05)All values are means of triplicates

After one and half months of storage the PV had increased up to 0.25±0.03 percent (Proagro RT) with a concurrent increase in moisture content and this could have triggered the hydrolytic rancidity. [4] had also reported an increase in hydrolytic action with increase in moisture content. The unpacked samples' peroxide value started increasing by day 45 and at 60 days it was double that of 45th day value, owing to the exposure to atmosphere that accelerates aerobic degradation of fats. The increasing trend of PV during storage is in agreement with the observation of [5] and [6] but [7] reported that initially peroxide values increased and thereafter it decreased contrary to the present finding. [8] had also observed an increase in peroxide value of up to 18.292meq/kg in breakfast cereals packed in HDPE covers. There was a positive correlation between the peroxide and free fatty

acid values of the six different pearl millet samples with the "r" value ranging from 0.48 to 0.69. This indicates that as the free fatty acid increases the peroxide value also increases indicating deterioration in the quality of pearl millet flour.

III.2 Nutrient Content of Pearl Millet flours

III.2.1 Moisture Content

Table II Moisture content of biofortified pearl millet flour

Sample	Moisture content (g percent)				
	Days of storage				
	0	15	30	45	60
Dhanashakthi (RT)	6.11±0.01	6.88±0.02	7.89±0.025	8.12±0.02	9.40 ± .025
Dhanashakthi (RF)	6.11±0.01	7.89±0.015	7.50±0.025	7.98±0.01	9.12±0.025
Proagro (RT)	8.08±0.02	8.70±0.025	9.56±0.026	9.89±0.01	10.66±0.03
Proagro (RF)	8.08±0.02	8.96±0.03	9.11±0.01	9.15±0.01	9.90±0.011
Dhanashakthi (UP)	6.11± 0.01	6.98±0.02	8.99±0.025	9.12 ± 0.02	10.40±0.025
Proagro (UP)	8.08±0.02	9.70±0.025	10.56±0.026	10.89±0.01	11.66±0.03

RT-Room temperature **RF**- Refrigerated temperature **UP** – Unpacked. All values are means of triplicates

The **moisture** content of the pearl millet flour samples gradually increased from 0 day to 60th day of storage. The moisture gain was observed even in samples packed in HDPE covers. The gain in moisture was 53.8 (Dhanshakthi-RT), 49.2 (Dhanshakthi-RF), 31.9 (Proagro-RT) and 22.5 Proagro-RF),70. Dhanshakthi-UP) and 44.3 (Proagro-UP) percent. This indicates that the packaging needs to be further improved to prevent moisture entry. Compared to room temperature storage, the packed and refrigerated samples had lower moisture gain. The upward movement in moisture content during storage noticed in the present study is in concurrence with the observation of [9], [10], [11] and [12] who did shelf life studies on maize flour, *sev*, cowpea fortified maize weaning mix and vermicelli.

III.2.2 Protein Content

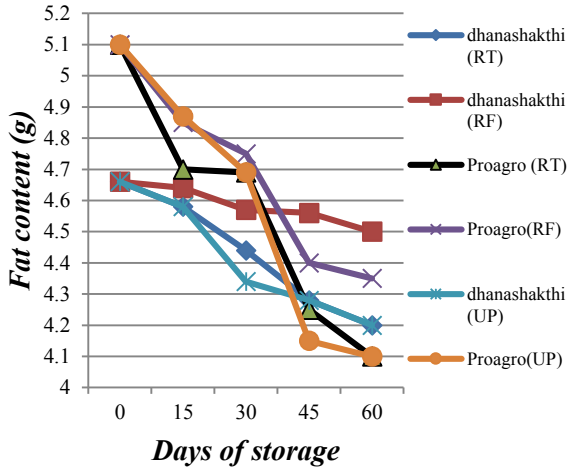
Not withstanding the temperature of storage and packing, both the cultivars' protein got decreased significantly after 60 days. The percent reduction of protein in Proagro was greater (22.9 percent; Proagro-UP), compared to that of Dhanshakthi (18 percent; Dhanshakthi–UP). [13] and [14]had also observed significant reduction in protein content throughout the storage period. This is in accordance with the current observation and the above authors attributed the reduction to the interaction between reducing sugars and amino acids (Maillard reaction). [15] had reported that refrigeration process of the whole raw flour slightly decreased the protein content. The present finding corroborates the same.

III.2.3 Fat Content

The fat content of Dhanshakthi as well as Proagro flour samples declined steadily from 0 to 60 days of storage. At the start of the study the fat content was 5.1 (Proagro –RT, RF and UP) grams percent and 4.66 (Dhanshakthi- RT, RF, UP) grams percent. On the last day (60th) of the analysis the fat content was found to be 4.20 (Dhanshakthi RT), 4.50 (Dhanshakthi RF), 4.10 (Proagro RT), 4.35 (Proagro RF), 4.15 (Dhanshakthi UP), 4.10 (Proagro UP) grams percent. This is given in Fig 2.

The present finding is in accordance with [16], [17] had also found a significant decline in crude fat during storage and the former attributed the same to the activities of lipolytic enzymes and that of the fermenting micro flora during storage. [13] had opined that reduction in fat was due to the oxidation of unsaturated fatty acids with atmospheric oxygen and moisture uptake. In addition, oxidative reactions can reduce the nutritional quality of food.

The 60th day values of fat and free fatty acid were found to be highly negatively correlated (r = -0.90 to -0.97).



RT-Room temperature RF- Refrigerated temperature UP- Unpacked, All values are means of triplicates

Fig. 2. Fat content of biofortified pearl millet flour

Likewise the fat and peroxide value were also negatively correlated and the values ranged from -0.60 to -0.87 among the six pearl millet samples packed and stored at different temperatures. It could be inferred that as the FFA and PV which are markers for flour quality increases with a corresponding decrease in the fat content.

The coefficient of variation (CV) is a measure of spread that describes the amount of variability relative to the mean. The coefficient of variation can be used to compare distributions obtained with different units. The lesser values of CV indicate greater stability. In the present study the coefficient variation was lesser for the refrigerated samples (Dhanshakthi RF and Proagro RF) indicating that they are more stable than the unpacked as well as samples stored at room temperature. Analysis of variance indicated a significant difference in the fat content of the pearl millet samples on the 60th day of storage.

III.2.4 Ash Content

The quantity of ash in Dhanshakthi and Proagro flours at the beginning of the study was 4.26±0.02 and 3.78±0.02 grams percent. Both Dhanshakthi and Proagro stored at dissimilar temperatures with and without packing showed a significantly enhanced ash content on day 60. [18] reported that the enzymatic activities of the

microorganisms during storage could lead to the changes in the ash content of food. While [19] reported a decrease in ash content in fermented millet, which is divergent to the present finding, [20]detected an increase in ash content in fermented maize cowpea blends similar to the current study.

III.2.5 Fibre Content

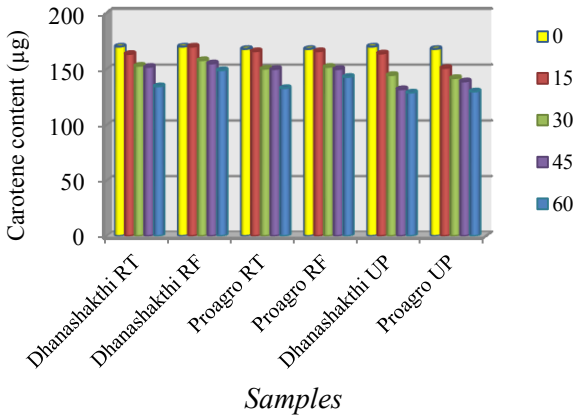
Dhanshakthi flour had the highest soluble fibre (1.60±0.1) on day "0", the lowest soluble fibre content was observed in Proagro 60th day. The changes in total fiber content may be due to processing involving heat, such as roasting as indicated by [13] in a study carried out with wheat flour. Students "t" test indicated a significant difference in the soluble fibre as well as the insoluble fibre content

III.2.6 Carbohydrate Content

Both Dhanshakthi and Proagro stored at different temperature with and without packing showed a non significant decline in carbohydrate content on day 60.This may be due to the maillard reaction between the generated reducing sugar and amino acids during storage. [13] and the utilization of carbohydrate particularly sugars by the growing microorganisms as energy sources [21].

III.2.7 Carotene Content

The carotene content of the pearl millet flours got reduced considerably on storage. The reduction was regular and consistent. While dhanshakthi flour had the highest carotene (170.12±0.54 µg) on day "0", the lowest carotene content was observed in proagro–up (130±0.02µg) 60th day sample. The reduction ranged between 14.17 (Dhanshakthi–UP) and 31 (Dhanshakthi-UP) percent. This was in agreement with the observations of [22] and [6] that degradation of carotenoids also increases with increase in storage temperature and moisture content. This is given in the figure3



RT-Room temperature **RF**- Refrigerated temperature, **UP** – Unpacked *P value (<0.05)All values are means of triplicates

Fig. 3. Carotene Content of the biofortified pearl millet flour

III.2.8 Iron Content

The iron content of Dhanshakthi (RT) and Proagro on day "0" was 9.6±0..6 and 8.40±0.6mg percent. After 60 days of storage all the samples exhibited a loss in iron content which was between 48 (Proagro-RF) and 77 (Dhanshakthi-UP) percent. However the reduction in iron content was significantly higher in Dhanshakthi than Proagro- samples. The reason for this reduction could be because mineral content especially iron, were reduced with the increase in storage period of pearl millet due to leaching or obliteration of minerals on heating as per the observation of[23]

III.2.9 Zinc Content

There was not much difference in the zinc content of Dhanshakthi (3.68mg) and Proagro (3.40 mg). Zinc also registered a mild non significant reduction on storage which was 17.24 (Dhanshakthi UP) to 42(Proagro RF) percent.

III.3 Anti nutrient Content of Pearl Millet Flours

Dhanshakthi and Proagro was found to be 0.602±0.008 and 0.2633±0.020 grams percent. According to [24], phytate content in pearl millet ranges from 172 to 327mg/100g whereas [25] reported that phytate content ranged from 969.3mg to 1101 mg per 100 g in pearl millet. The present finding is between the above two reported ranges.

III.4 Microbial count of Pearl Millet Flours

The total bacterial plate count of pearl millet during the storage period is given in the Table 3.The unpacked samples had the highest microbial count, followed by Dhanshakthi-RT.

Table III Microbial count of the bio fortified pearl millet flour

Sample	Microbial count (cfu/g)				
	Days of storage				
	0	15	30	45	60
Dhanshakthi (RT)	$0x10^5$	$0x10^5$	$0x10^5$	$0x10^5$	$03x10^5$
Dhanshakthi (RF)	$0x10^5$	$0x10^5$	$0x10^5$	$0x10^5$	$02x10^5$
Proagro (RT)	$0x10^5$	$0x10^5$	$0x10^5$	$0x10^5$	$03x10^5$
Proagro (RF)	$0x10^5$	$0x10^5$	$0x10^5$	$0x10^5$	$03x10^5$
Dhanshakthi (UP)	$0x10^5$	$0x10^5$	$0x10^5$	$2x10^5$	$04x10^5$
Proagro (UP)	$0x10^5$	$0x10^5$	$0x10^5$	$2x10^5$	$04x10^5$

RT-Room temperature **RF**- Refrigerated temperature **UP** – Unpacked All values are means of triplicates

The refrigerated sample Dhanshakthi RF had the least Total bacterial count, which ranged from 02 x 10^5 to 04 x10^5 CFU/g [26] enlightens the standard microbial count of flour as $5x10^4$.

Results of present investigation is below the codex standard are comparable with those found by [27] who had also seen similar trend during storage of wheat flour. The increase in microbial load during storage in ordinary packaging may be due to the influence of increased moisture content [28].

These results suggests that the pearl millet flour can be stored for 45 days under packed conditions and 30 days in unpacked conditions without any appreciable descent in quality.

IV. Conclusion

This study revealed that the pearl millet flour packed in HDPE pouches is stable to 60 days at refrigerated conditions. This study's results will encourage the

researcher, miller, retail seller, as well as purchaser, as it would help to store flour usable for a longer time without considerable changes in overall quality. It would also encourage utilization of pearl millet grains, which is still unexploited even with its numerous nutritious and medicinal benefits.

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