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Research Article

Effect of Antioxidant on the Shelf Life of Quality Protein Maize Flour

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ABSTRACT

Quality protein maize flour stored in two types of packaging material (LDPE covers and plastic box) along with antioxidant treatment was studied for changes like moisture peroxide value and microbial quality for a period of 6 months. In both the packages absorption of moisture was significantly more in control compared to treated sample. The antioxidant treatment had a role in onset and development of rancidity in treated samples which was evident from the more increase of moisture and peroxide value in control samples .Periodic assessment of microbial quality for bacteria, yeasts and moulds indicates that the flour samples were consistent with FAO specifications for consumption, up to three months in treated polythene covers; $0.50\pm0.6x10^5$ (bacteria) and $0.5\pm0.6x10^3$ (fungi) as well as in the box samples ($0.25\pm0.0x10^5$ and $0.5\pm0.0x10^3$) indicates that the antioxidant along with good manufacturing practices could extend the shelf life and packaging had very little effect on the growth of the microorganisms. The predominating organisms in both the packages and treatments at the end of six month storage period were *Bacillus subtilis*, *Saccharomyces cerevisiae*, proteus *mirabilis*, and *Klebsiella aeruginosa*.

Key words*:* Antioxidant, peroxide value, shelf life, rancidity.

INTRODUCTION

Maize is the third most important crop after wheat and rice, which serves as staple food for human beings, quality feed for animals as well as raw material for starch, food sweeteners, beverages and more recently ethanol. Maize also known as corn is a good source of starch (65-70%) protein (8-10%) fat (3-4%) and some of the important vitamins and minerals. However inspite of several important uses, maize has an inbuilt drawback of being deficient in two essential amino acids, viz lysine and tryptophan and the same has been overcome by developing quality protein maize (QPM) which contains twice the quantity of lysine and tryptophan, thus making it rich in quality of proteins.QPM can be a strong support to the mission of food and nutritional security of our country in various ways such as infant food, health mixes, convenience foods specialty foods and emergency ration (Jat *et al 2009*).

Maize can be processed into a number of ways; however the common method is dry milling. The dry milling of maize yields byproducts such as maize meal (whole flour) grits, suji (semolina) and bran (outer cover).Since whole flour along with fat content is a good source of micro and macro nutrients can be good replacer of fancied and highly processed foods. Supplementation with protein rich sources and preparation of acceptable products would not only improve nutritional value of maize but also adds variety to our daily diet.The QPM flour and suji (semolina) has found its place in one or the other way in some parts of our country like Uttar Pradesh, Punjab and Rajasthan for preparation of number of sweet and savoury dishes including thick and thin porridges, dry pancake, idli, dosa, vada, shev, chakkuli, laddu payasam and so on. The availability of flour which serves as basic raw material and its quality is at most important in order to popularize the QPM in other states among large masses.

During recent past there is a greater demand for high quality nutritious and safe food which is free of physical, chemical and microbial contamination. Farmers (growers) and consumers (users) must maintain and protect their produce (harvested grain) from insect and microbial damage (Sinha 1995).

Cereal and its products, particularly flour constitutes a major part of Indian daily diet and is generally considered to be microbiologically safe product as it has a low water activity(a_w 0.40-0.65) the growth of microorganisms is very less but contamination from various external sources cannot be ruled out. The level of contamination and composition of the micro flora are very important for risk assessment.The micro flora of flour is composed of a variety of microorganisms including yeasts, moulds, bacteria more specifically pathogenic organisms such as *Aspergillus brasiliensis*, *Bacillus subtilis*, *E coli*, *Enterococcus faecalis*, and *Salmonella spp*. Even though flour is not directly related to food born diseases, it is important to know the data on keeping quality of flour for consumption and level of pathogenic bacteria and other microorganisms that

would render the flour unfit for consumption in longer period storages. Grain and flour moisture level play an important role regarding its shelf life and growth of microorganisms. Lower the flour moisture better is storage stability. The deterioration of baking quality is also less at lower moisture content which is directly related to retarded respiration and activity of microorganisms (Staudt and Zeigler 1973). There are two process of degeneration in foods; hydrolysis and oxidation. Hydrolysis is the process by which triglycerides are broken down to release non-esterified fatty acids, though hydrolysis does not reduce the palatability of the flour, further oxidative breakdown of fatty acids is associated with bad taste and smell (Sauer 1992).

In order to encounter the above problems in storage, one needs to give greater importance to packaging. Food packaging has become very important not only in protecting the product from contamination by macro and microorganisms and their filth, but also preventing from loss or gain of moisture and shielding the product from oxygen to facilitate handling. 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. Presentational aspects increase sales by creating a brand image that the buyer instantly recognizes (Peter and Axtell, 1993).Hence flour should be stored in airtight containers or packaging material to prevent oxidative rancidity, mould attack, bacterial attack and insect infestation. So also the optimum moisture content of flour must be in relation to the length of storage envisaged and the prevailing ambient temperature and the relative humidity (Kent 1978). To prevent oxidative rancidity in flour, antioxidants are widely used as food additives to improve the oxidative stability of lipids and to prolong shelf life mainly for dried products and oxygen sensitive foods (Vermeiren *et al* 1999). Numbers of researches have been worked on use of antioxidants in relation to its shelf life for snack items (Waghray K and Gulla S 2010), breakfast cereals (Bult *et al* 2010) and studied the changes in moisture, FFA and peroxide levels in different food items. However the effect of antioxidant on shelf life and microbial quality of maize flour is still inadequate. Therefore the objective of this study is to determine the effect of antioxidant treatment and packaging on the shelf life of maize flour.

MATERIALS AND METHODS

The grains of quality protein maize (HQPM-7) were procured from AICRP (Maize) RRS, Uchani and were treated with 1% lime solution (Palacios-Fonseca *et al* 2009) drained washed and sundried until the moisture percentage reaches to around 9-10% (Flow chart 1).The grains were dry milled in a mini SS dry grinder mill (Sri Rajalakshmi Industrial Agency, Bangalore)and passed through 60BS sieve to get a fine powder (250 microns).The antioxidant Butylated hydroxyanisole(BHA)was obtained from Ajay Trading Industry, Bangalore. The QPM flour was divided into two parts; one part was treated with antioxidant BHA at the rate of 0.02%, calculated on the basis of fat content (Rule 59 of PFA act 1955), i.e. 0.99 mg/kg flour, since HQPM-7 contains 5.01% fat. Both the flours (treated; T and control; C) were packed in 200 gauge LDPE covers (P) and food grade plastic jars (B) followed by sealing of covers using sealing machine and stored for a period of 6 months under room temperature (25±5ºC).Every month samples were drawn and analyzed for moisture and peroxide value according to AOAC (1990).For microbial analysis, nutrient agar (Bacteria), Potato Dextrose Agar (Fungi) and Rose Bengal Agar (Moulds) were procured from Himedia, Bhuveshwar Plaza Mumbai. Freshly milled flours were subjected to microbial analysis immediately after milling and at an interval of one month up to six months. Serial dilution technique was followed where in 1 gram of each sample was dissolved in 9ml of distilled water then each diluents was plated out on a Nutrient agar for bacteria, Rose Bengal and potato Dextrose agars respectively for yeast and mould counts. The plates were incubated at 37ºC for 48 hours for bacterial growth and 27ºC for 72 hours for yeasts and moulds (Collins and Lyne, 1970).Colonies observed after incubation period were counted.

At the end of six month storage, samples were tested for pathogenic organisms on specific medium.Micro-organisms that are pathogenic and known to cause diseases *viz.*, bacteria, fungi and virus, which can cause illness through contaminated food, water or personal contact were studied.Common symptoms are gastrointestinal problems and vomiting .Samples were tested on Rose Bengal Chloramphenicol Agar (*Himedia*, MP640, 2010) for organisms such as *Aspergillus brasiliensis*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *E. coli* and *Enterococcus faecalis*. Hitouch HexaCrome Flexiplate (Himedia FL031, 2010) was used for differentiation of bacterial colonies such as *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. Fungal colonies of *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger* were differentiated using Hitouch Ogay Flexiplate (*Himedia*

FL032, 2010).The medium plates were kept in the incubator at 36ºc for 1 hour to evaporate the water droplets formed during storage in refrigerator, then the readymade plates were carefully opened with the lid on top. The plate with medium was inverted on the test surface and pressed for few seconds, and medium plate was lifted and put back into base plate. The lid was closed firmly and plates were incubated at 35-37ºc for 18-20 hours. As a result colony characters described in Hi media data sheet such as pink to red colonies (*E. coli*), blue to purple (*K. pneumoniae*), light brown (*P. mirabilis*), golden yellow (*S. aureus*), colorless (*P. aeruginosa*) blue colored colonies (*E. faecalis*) light blue with black spores (*Aspergillus niger*), green colored colonies (*Conidia albicans*), and colorless colonies (*Saccharomyces cerevisiae)* were observed due to the presence of indicator dye in the medium. Then the colonies were enumerated by counting the number of colonies in triplicates, and two-way Anova was used to test the significant difference between treated and control samples over a period of six months storage in two types of packaging material.

Maize
↓ After cleaning
Soak 500g grains in 1% lime water for 5 minutes
(10g of lime in 1 liter water)
↓
Give heat treatment at simmering temperature for 30 minutes
↓
Remove the vessel and leave it for overnight
Wash 3 – 4 times to remove lime
↓
Dry in sunlight
(Moisture level should be 9-10 %)
↓
Store in air tight container

Flow chart 1: Lime treatment of maize grain.

RESULTS AND DISCUSSION

The initial moisture content of the flour after milling was 8.11% which increased significantly in both the packaging material irrespective of the treatment (figure and table 1).In bottle as well as polythene covers the absorption of moisture was significantly more in control compared to treated sample. (9.68 % CB; 9.15 TB and 9.54CP; 9.03 TP). Similar study conducted by Waghray and Gulla 2010, reports that the moisture content of Sev increased from 2.2 to 3.7% for BHA treated and 2.8 to 4.6% for control over a period of 3.5 months. Afoakwa *et al* (2004) observed that moisture content of cowpea fortified maize weaning mix increased from 6.61 to 6.82% over a period of six month storage period. So also a study on storage of vermicelli indicates that moisture content increased from 7.7 to 8.0% for a period of two months (Midha and Mogra, 2007). Peroxide value usually used as an indicator of deterioration of fats, as peroxidation takes place the double bonds in the unsaturated fatty acid breakdown to produce secondary oxidation products which indicate rancidity (Ihekoronye and Ngoddy, 1985). A peroxide value of maize flour packed in two types of packages is presented in figure and table 2. The flour had an initial peroxide value of 0.63±0.03 which increased significantly to 1.46±0.05 and 1.39 ±0.05 meq/kg fat in box and cover (Treated) samples respectively. While the control flour for the same samples had a pv value of 1.63±0.06 and 1.52±0.07 respectively. Analysis of variance indicates a significant difference between the treatments, packaging material and months over six month storage period. Similar study conducted by Ugare (2008) indicates a PV of 0.20 for fresh barnyard millet flour which increased to 0.87 meq/kg fat at ambient storage conditions in LDPE covers over six month storage period .On the contrary the pv for fresh Khara Boondi was 8.5±0.67 which increased significantly to 33.2±0.09 in BHA treated sample and 47.2±0.38 for control was reported by Waghray and Gulla (2010).The antioxidant treatment had a role in onset and development of rancidity in treated samples which was evident from the more increase of moisture and peroxide value in control samples, however which was statistically non significant. Both the Samples (treated and control) had a PV of less than10 meq/kg of fat, the value where rancidity begins in fresh oils and fats. For rancid oils and fats the value should be above 20meq/kg of fat (Eagan 1981).

Microbial Analysis: The target microbes (fungi, bacteria and moulds) were not noticed up to two months in polyethylene cover (P) and plastic jars (B) irrespective of the treatment (Table 3 and 4). Since the grains were boiled in hot water during lime treatment, combined with low moisture content (8.11%) and low water activity along with hygienic handling of the product might have helped in inhibiting the growth of microorganisms and the same was supported by study conducted on quality evaluation of value added vermicelli by Midha and Mogra (2007) indicates that the total viable count reduced at low moisture content.

Malleshi *et al* (1989) observed that malted ragi products could be safely stored for 100 days at 11% moisture. For flour and maize meal, 12% water is a critical level below which no microbial growth will occur, above 12%, some xerophilic fungi can grow (Hesseltine and Graves, 1966). The fungal and bacterial population were noticed as the storage progressed ($0.75\pm0.5x10^{-3}$ C; $0.25\pm0.5x10^{-3}$T) and ($0.75\pm0.5x10^{-5}$ C; $0.25\pm0.5\ 10^{-5}$ T) cfu/gm of sample, respectively in polyethylene covers, While the counts were slightly more in box samples ($0.75\pm0.58x\ 10^{-3}$ C; $0.5\pm0.0\ x\ 10^{-3}$ T and $0.75\pm0.58\ x10^{-5}$ C; $0.25\pm0.0\ x\ 10^{-5}$ T). However there was no mould growth up to 3 months irrespective of the treatment and packaging material. At the end of 6 month storage period, the moisture and peroxide value as well as number of colony forming units/g increases irrespective of the treatment and packaging material.

However our study indicates that the microbial load was within the limit (up to 3 months in treated covers as well in box) prescribed by the International Microbiological Standards Recommended limit of bacteria contaminants for food of less than 10^6 cfu/g and a food product for consumption should have microbial count below $1x10^5$ cfu/ml, which also agrees with the Turkish legal limit of $1.0x10^5$ cfu/g (Turkish Food Codex 2001) and the mean mould counts should be around 10^3 cfu/g (France: Potus and Suchet 1989; Germany: Spicher 1986; Italy: Cicognani *et al* 1975; Ottogalli and Galli 1979). According to centre for food safety, the acceptance limit of bacteria should be 10^5 to $<10^6$ cfu/g. (Food and Environmental Hygiene Department) and the same value were also reported in guidelines of communicable diseases and public health by Gilbert *et al* 2000. According to Microbial Food safety- Indian Regulation (www.ilsi-india.org) solvent extracted soya flour should contain total bacterial count of less than 50,000/g, coli-form bacteria should be less than 10/ g, and salmonella bacteria should be absent in 25 gram of the sample for consumption .Hence the flour with treatment in both the packages met the above mentioned specifications up to 3 months and suitable for consumption.

The addition of BHA (antioxidant) along with airtight sealing might have inhibited the growth of microorganisms. Similar line of work carried out by Kapoor and Kapoor (1990) on keeping quality of pearl millet flour indicates that the Butylated Hydroxyanisole and Ascorbic acid (antioxidants) showed inhibitory effect against fungal and mould growth, contrary to this the flour stored in bottle with antioxidant had the fungal count $0.5\pm0.0\ x\ 10^{-3}$ and the Anova table indicates that statistically treatment had no significant effect on the microbial load but it could able to extend the shelf life slightly when stored in airtight containers.

At the end of 6 month storage period, 1ml of sample from 10^{-4} dilution was transferred to specific plates like Rose Bengal Chloramphenicol Agar using micropipette closed firmly and incubated at 25-30ºC for 48-72 hours. White spores of *Aspergillus brasiliensis* were found in control sample of both the packages (1.20±0.5in C and 1.5±0.58 in B) even the pearl millet flour was contaminated with mould *Aspergillus spp* at a level of 20-25 % (Badau, 2006) and colonies of cream colored *Bacilius subtilis* were found in cover ($1.25\pm0.5\ x10^4$) as well as in box sample ($1.0\pm0.0x10^{4,}$ table 5). *Bacillus subtilis* was isolated at a high frequency of 70% from unmalted pearl millet grains (Badau, 2006).These *Bacillus subtilis* and *Bacillus coagulans* are non pathogens, widely distributed and can spoil many foods held above refrigerator temperatures (Jay1987), so also this *Bacillus subtilis* is a spore bearer from soil and can easily come in contact with the grain (Collins and Lyne 1970). A study conducted by Lana *et al* (2002) on microbiology of wheat flour, indicates that the 81 percent of incoming wheat samples tested positive for *Bacillus spp* in general and *B cereus* in particular indicating significant degree of field contamination, or otherwise present in milling equipment but present in less than one spore per gram. Both the samples were free from *E coli* and *Enterococcus faecalis*. According to centre for food safety (Anonymous, 2007) the level of *E coli* and *Salmonella* should absent in 25 grams of sample, where as *S. aureas*, *C. perfringens*, *B cereus*, should be 20-<100 was acceptable limit. Hence our samples were within the limit described above. From the table 6 it is evident that Light brown colonies of *P mirabilis*(2.0±0.0CP, 1.0 ±0.0TP; 2.25±0.50CB,1.25±0.50TB) mucoid type blue to purple colony of *Klebsilla pneumoniae* (1.25±0.5CP;2.5±0.58CB,1±0.0TB) were did noticed in both the samples at very low levels. Pink to red coloured colonies describing *E coli*, golden yellow colonies of *S aureus* and blue dotted spots describing *Enterococcus faecalis* were absent in both treated and control samples of both the packages, indicating maintenance of hygiene at all the stages like lime treatment to the grain, drying, milling *etc*. study conducted by Lana *et al* (2002) on *wheat flour* indicated that *E coli* was detected sporadically in wheat flour samples at very low levels,(3.0 MPN/g) and majority of positive samples being at the minimum level of detection and usually in the early stages of milling or in the end products derived from outer grain layers, so contamination probably occurred during the conditioning process. *E coli*

Table 1: Effect of Antioxidant on the moisture content of QPM flour at room temperature

	M_0		M_1		M_2		M_3		M_4		M_5		M_6	
	C	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**
B	8.11 ± 0.10	8.19 ± 0.06	8.59 ± 0.3	8.38 ± 0.0	8.94 ± 0.04	8.56 ± 0.02	9.16 ± 0.03	8.78 ± 0.01	9.57 ± 0.03	8.85 ± 0.04	9.52 ± 0.01	8.83 ± 0.01	9.68 ± 0.01	9.15 ± 0.02
P	8.11 ± 0.10	8.19 ± 0.06	8.46 ± 0.04	8.26 ± 0.03	8.75 ± 0.04	8.48 ± 0.03	9.04 ± 0.05	8.76 ± 0.04	9.44 ± 0.03	8.78 ± 0.02	9.36 ± 0.03	8.74 ± 0.04	9.54 ± 0.04	9.03 ± 0.03
SEm±	0.021													
CD (0.05)	0.059													

***M_0-M_6** = Months Interval; **C**= Without Treatment/control; **T** = With Treatment; **P** = Polyethylene Cover; **B** = Box.*
**Values are means of three replications ±SD.*

Sl. No	**Sources**	**F Value**	**SEm±**	**CD (0.05)**
1	Treatments	150.83**	0.006	0.016
2	Months	1345.66**	0.010	0.030
3	Packaging material	21.97**	0.006	0.016
4	Treatments Vs Months	3.48**	0.015	0.042
5	Treatments Vs Packaging material	NS	0.008	NS
6	Months Vs Packaging	904.10**	0.015	0.042
7	Treatment Vs Month Vs Packaging	4.72**	0.021	0.059

***NS** = Non Significance; ** indicates the significance difference at 1%.*

Table 2: Effect of Antioxidant treatment on peroxide value of QPM flour at room temperature

	M_0		M_1		M_2		M_3		M_4		M_5		M_6	
	C	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**	**C**	**T**
B	0.63 ± 0.03	0.63 ± 0.03	0.66 ± 0.03	0.61 ± 0.02	0.96 ± 0.04	0.66 ± 0.03	0.93 ± 0.02	0.90 ± 0.04	1.22 ± 0.03	1.06 ± 0.03	1.35 ± 0.09	1.27 ± 0.05	1.63 ± 0.06	1.46 ± 0.05
P	0.63 ± 0.03	0.63 ± 0.03	0.72 ± 0.03	0.61 ± 0.05	0.72 ± 0	0.66 ± 0.05	0.78 ± 0.06	0.78 ± 0.06	0.96 ± 0.05	0.95 ± 0.02	1.14 ± 0.06	1.11 ± 0.02	1.52 ± 0.07	1.39 ± 0.05
SEm ±	0.026													
CD (0.05)	0.074													

***M_0-M_6** = Months Interval; **C**= Without Treatment/control; **T** = With Treatment; **P** = Polyethylene Cover; **B** = Plastic Box. *Values are means of three replications ±SD.*

Sl. No	Sources	F Value	SEm±	CD (0.05)
1	Treatments	120.45**	0.007	0.020
2	Months	378.38**	0.013	0.037
3	Packaging material	8.72**	0.07	0.020
4	Treatments Vs Months	13.80**	0.018	0.052
5	Treatments Vs Packaging material	15.00**	0.010	0.028
6	Months Vs Packaging	214.75**	0.018	0.052
7	Treatment Vs Month Vs Packaging	2.68*	0.026	0.074

*** indicates the significance difference at 1% and * indicates significant difference at 5%*

Table 3: Effect of antioxidants on the microbial load of qpm flour stored in polythene covers

MONTHS	TREATMENTS	Colony Counts in Polythene covers					
		Fungi		Bacteria		Moulds	
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}
M_0	C	0	0	0	0	0	0
	T	0	0	0	0	0	0
M_1	C	0	0	0	0	0	0
	T	0	0	0	0	0	0
M_2	C	0	0	0	0	0	0
	T	0	0	0	0	0	0
M_3	C	0.75±0.5	0	0.75±0.5	0	0	0
	T	0.25±0.5	0	0.25±0.5	0	0	0
M_4	C	1.0±0.0	0	1.25±1.0	0.25±0.5	0.25±0.5	0
	T	0.5±0.6	0	0.5±0.6	0	0	0
M_5	C	3±0.8	0	1.75±0.5	0.5±0.6	0.5±0.6	0
	T	1.75±1.0	0	0.75±0.5	0.25±0.5	0.5±0.6	0
M_6	C	4.5±1.3	0.75±1.0	2.75±1.0	1.0±0.0	1.25±1.0	0.25±0.5
	T	5.25±1.0	1.0±0.8	1.5±0.6	1.0±0.0	0.75±0.5	0
Correlation b/w moisture & microbial count		0.24	0.23	0.16	0.25	0.32	0.16
Correlation b/w peroxide value & microbial count		0.32	0.20	0.05	0.26	0.25	-0.12

The values are the means of colony forming units/gram with standard deviation.* ***LPC* *= LDPE polyethylene cover;* ***M_0-M_6*** *= Months Interval;* ***C****= Without Treatment/control;* ***T*** *= With BHA Treatment.*

Microbial Species	Dilution	Interaction effects between	SEm±	CD @ 0.05
Fungi	10^{-3}	Months	0.189	0.540**
		Treatments	0.101	NS
		Months & treatments	0.267	0.763**
	10^{-4}	Months	0.121	0.345**
		Treatments	0.065	NS
		Months & treatments	0.171	0.488**
Bacteria	10^{-5}	Months	0.171	0.489**
		Treatments	0.092	NS
		Months & treatments	0.242	0.692**
	10^{-6}	Months	0.081	0.233**
		Treatments	0.044	NS
		Months & treatments	0.115	0.329**
Moulds	10^{-4}	Months	0.129	0.369**
		Treatments	0.069	NS
		Months & treatments	0.183	0.521**
	10^{-5}	Months	NS	NS
		Treatments	NS	NS
		Months & treatments	NS	NS

*NS = Non significant; ** significance at 1%.*

Table 4: Effect of antioxidant on Microbial load of flour stored in Plastic box at room temperature

MONTHS	TREATMENTS	Colony Counts in BOX					
		Fungi		Bacteria		Moulds	
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}
M_0	C	0	0	0	0	0	0
	T	0	0	0	0	0	0
M_1	C	0	0	0	0	0	0
	T	0	0	0	0	0	0
M_2	C	0	0	0	0	0	0
	T	0	0	0	0	0	0
M_3	C	0.75±0.05	0	0.75±0.58	0.25±0.50	0	0
	T	0.5±0.0	0	0.25±0.0	0	0	0
M_4	C	1.75±0.50	0.25±0.5	1.25±0.96	0.75±0.5	0.75±0.5	0
	T	1.25±0.50	0.25±0.5	0.5±0.5	0.25±0.5	0	0
M_5	C	2.5±0.58	0.25±0.5	1.75±0.5	2.25±0.5	2.75±0.5	0.75±0.5
	T	2.25±0.50	0.5±0.58	0.75±0.58	1.5±0.58	1.0±0.0	0
M_6	C	4.5±1.0	1.0±0.0	2.75±0.5	4.0±0.82	5.0±0.82	1.75±0.5
	T	4.5±0.58	1.0±0.0	1.5±0.58	3.5±0.58	3.0±0.0	0.75±0.5
Correlation b/w moisture & microbial count		0.35	0.37	0.16	0.27	0.21	0.13
Correlation b/w peroxide value & microbial count		0.40	0.46	0.05	0.28	0.12	-0.01

The values are the means of colony forming units/gram with standard deviation; M_0-M_6 = Months Interval; **C= Without Treatment/control; **T** = With BHA Treatment.*

Microbial Species	Dilution	Interaction effects between	SEm±	CD @ 0.05
Fungi	10^{-3}	Months	0.157	0.447**
		Treatments	0.084	NS
		M & T	0.221	0.632**
	10^{-4}	Months	0.101	0.289**
		Treatments	0.054	NS
		Months & treatments	0.143	0.409**
Bacteria	10^{-5}	Months	0.147	0.421**
		Treatments	0.079	NS
		Months & treatments	0.209	0.596**
	10^{-6}	Months	0.146	0.417**
		Treatments	0.078	NS
		Months & treatments	0.206	0.589**
Moulds	10^{-4}	Months	0.100	0.287**
		Treatments	0.054	NS
		Months & treatments	0.142	0.405**
	10^{-5}	Months	0.081	0.232**
		Treatments	0.043	NS
		Months & treatments	0.115	0.328**

*NS = Non significant; ** significance at 1%.*

Table 5: Cultural characteristics of QPM flour on Rose Bengal Chloramphenicol Agar plate (MP640) observed after 6 months of storage LDPE polyethylene cover and Plastic box at room temperature

Organisms (ATCC)	C		B	
	Treatments/Dilution		Treatments/Dilution	
	$C/10^{-4}$	$T/10^{-4}$	$C/10^{-4}$	$T/10^{-4}$
Aspergillus brasiliensis	1.20 ±0.5	0	1.5±0.58	0
Bacillus subtilis	1.25±0.5	0	1.0±0.0	0
Saccharomyces cerevisiae	1.25±0.5	0	1.0±0.0	2.0±0.0
E. coli	0	0	0	0
Enterococcus faecalis	0	0	0	0

Values are the mean of four replications with along with standard deviation.* ***P* *= LDPE polyethylene cover;* ***B*** *= Plastic Box;* ***C****= Without Treatment/control;* ***T*** *= With BHA Treatment.*

Table 6: Cultural characteristics of QPM flour on Hitouch HexaCrome Flexiplate (FL031) observed after 6 months of storage at room temperature

Organisms (ATCC)	P		B	
	Treatments/Dilution		Treatments/Dilution	
	$C/10^{-4}$	$T/10^{-4}$	$C/10^{-4}$	$T/10^{-4}$
E. coli	0	0	0	0
Proteus mirabilis	2.0±0.0	1.0±0.0	2.25±0.50	1.25±0.50
Klebsiella pneumoniae	1.25±0.50	0	2.5±0.58	1.0±0.0
Pseudomonas aeruginosa	0	0	0	1.0±0.0
Staphylococcus aureus	0	0	0	0
Enterococcus faecalis	0	0	0	0

Values are the mean of four replications with standard deviation.* ***P* *= LDPE polyethylene cover;* ***B*** *= Plastic Box;* ***C****= Without Treatment/control;* ***T*** *= With Treatment.*

Table 7: Cultural characteristics of QPM flour on Hitouch Ogye Floze Flexiplate (FL032) observed after 6 months of storage LDPE polyethylene cover and Plastic box at room temperature

Organisms (ATCC)	P		B	
	Treatments/Dilution		Treatments/Dilution	
	$C/10^{-4}$	$T/10^{-4}$	$C/10^{-4}$	$T/10^{-4}$
Aspergillus niger	0	0	1.25±0.50	1.0±0.0
Candida albicans	0	0	1.0±0.0	2.25±0.50
E. coli	0	0	0	0
Saccharomyces cerevisiae	1.0±0.0	1.0±0.0	2.0±0.0	2.0±0.0

Values are the mean of four replications with along with standard deviation.* ***P* *= LDPE polyethylene cover;* ***B*** *= Plastic Box, C = Without Treatment/control;* ***T*** *= With Treatment.*

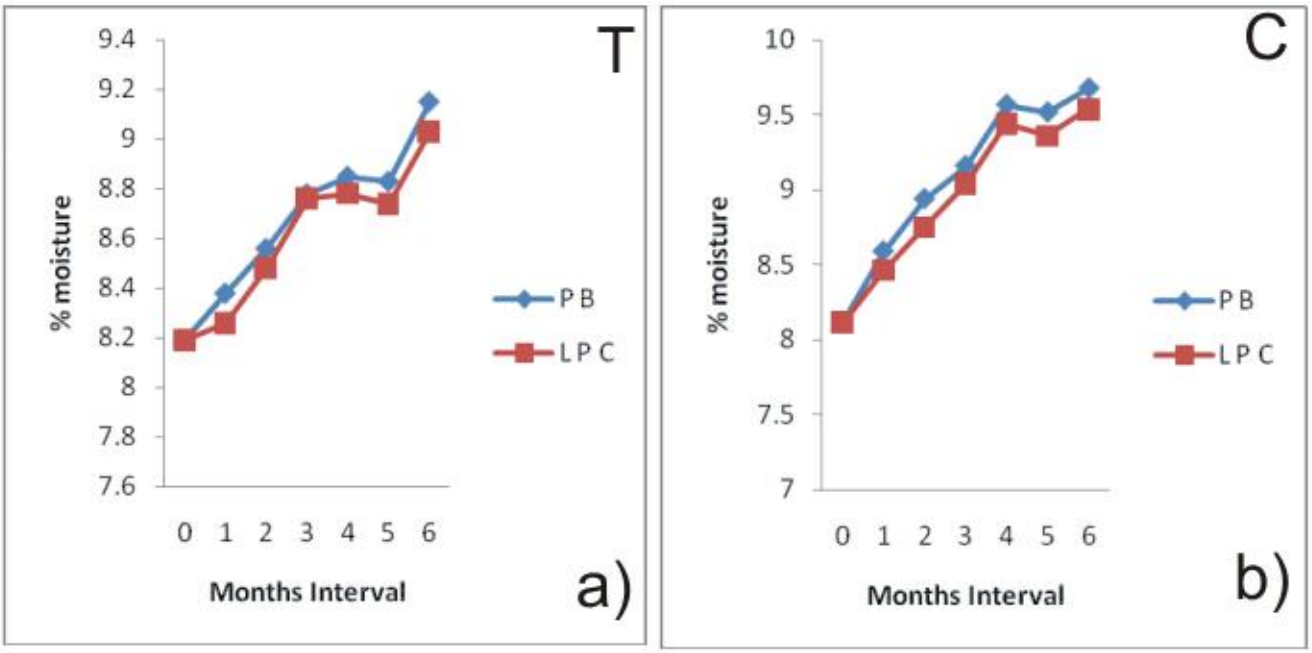

Figure 1: Effect of packaging and antioxidant on the moisture content of qpm flour.
T -With BHA Treatment; C-without treatment/Control

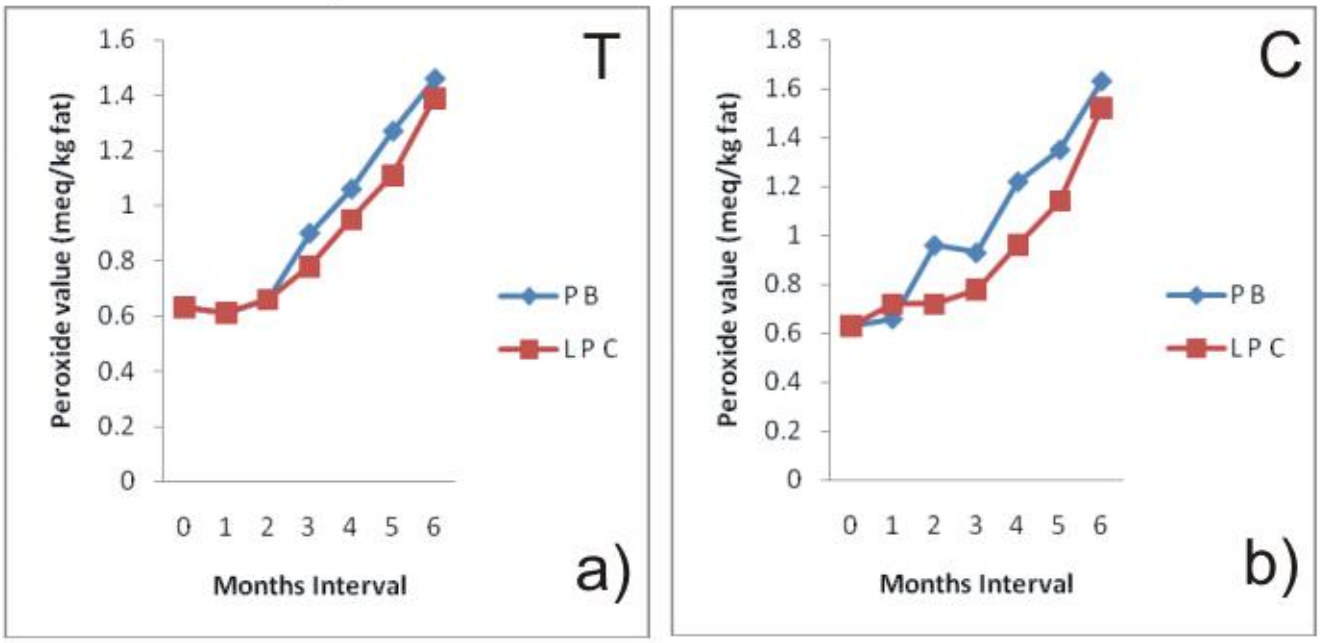

Figure 2: Effect of packaging and antioxidant on the peroxide value of qpm flour.
T -With BHA Treatment; C-without treatment/Control

is the most important member of the coli form group and its presence in large numbers in food is generally taken to indicate fecal contamination (Jay 1987). *E coli* food poisoning was noticed when the volunteers were fed with foods contaminated at levels of 10^6-10^8 organisms/g or ml and *Klebsiella spp* have been implicated in histamine associated poisoning (Wentworth *et al* 1956).

Colonies indicating of *Aspergillus niger* having light blue colour with black spores and green coloured *Candida albicans* (Table 7) were absent in polyethylene packs irrespective of the treatment, but the same were present in box sample *(A. niger*1.25±0 .5x10^{-4}; CB and 1.0±0.0 x10^{-4}; TB; *Candida* 1.0±0.0 x$10^{-4;}$ C B and 2.25±0.5 x10^{-4} TB).These *Candida spp* have been implicated in food spoilage especially refrigerated food and disease of humans (Jay, 1987). Colorless colonies describing *S cerevisiae* were present in both the samples in the range of 1.0±0.0 $10^{-4;}$ CB, $^{1.0}$ ±0.0 x10^{-4} ; TP and 2.0±0.0 x10^{-4} ,CB and 2.0±0.0 x10^{-4} TB, indicating both the samples were contaminated either during handling ,pouring or inherent organisms present in the sample itself in small numbers. Similar kind of study conducted by Eleazu *et al.*, (2011) indicates that the total viable and fungal counts of 10 cassava flour varieties ranged from 4.5 x 10^5 and 0.5 x10^3 respectively. Never the less these microorganisms could be eliminated at cooking temperature, and the microbial counts were not high enough to produce effective dose to cause food spoilage and food poisoning. The more widely distributed the organism, the higher is the chances of organisms to be associated with the sample rather than contamination from analysis. Our results obtained were consistent with FAO (2003) standards up to 3 months in treated polythene covers; 0.50±0.6x10^5 (bacteria), 0.5±0.6x10^3 (fungi), as well as in treated boxes (0.25±0.0x10^5 and 0.5±0.0x10^3) indicates that the antioxidant along with good manufacturing practices would extend the shelf life of the product, and packaging had a very little effect on the microbial load. Therefore the predominating organisms observed at the end of 6 month storage period were *Bacillus subtilis, Saccharomyces cerevisiae, Proteus mirabilis, Klebsiella aeruginosa* in flour of both the packages, irrespective of the treatment and was not high enough to produce food poisoning.

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