EFFECT OF OPERATIONAL SPEED OF AN ENTOLETER AND PACKAGING MATERIAL ON MICROFLORA AND NUTRITIVE VALUE OF STORED WHEAT

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Abstract- Various physical and chemical methods for controlling microbes have been used for many years. The usage of mechanical techniques of controlling microorganisms in stored food stuffs and other products is advantageous, as no left over toxicity risk is involved and the microbes doesn't evolve any resistance to these techniques. In the present study, a physical method of controlling microorganisms was effectively carried out, with the help of an Entoleter. Experiments were conducted in the developed Entoleter, with a speed varying from 500-1500 rpm for wheat grains. Wheat samples were passed through the Entoleter three times (first pass, second pass, third pass) and examined for microbial load of the samples. Results revealed significant ($p<0.05$) decrease in microbial load with the increase in speed and number of passes. Maximum decrease was observed at 1500 rpm in third pass. In modern new age, food packaging has become very crucial because of preservation of product from contamination by macro and micro-organisms and their filth, preclusion from loss or gain of moisture, protection of product from oxygen and to assist handling. Wheat samples (5kg) were packaged in PET containers, Steel containers and PP bags and evaluated for microbiological quality and quality attributes, to find the best packaging material for storage. Results suggested that, there was a significant increase in microbial load (total plate count, fungi and coliform count) throughout the storage. Increase was more profound in PP bags followed by PET and steel containers. Quality attributes were analyzed after an interval of 15 days. Results showed a significant decrease in carbohydrate, protein, fat, fiber and ash content, while moisture content increased throughout the storage. Maximum decrease was observed in PP bags. Hence, steel containers best maintained the microbiological quality and nutritive value of the stored samples.

Keywords: **Entoleter, Microbial load, Packaging material, Quality attributes**

INTRODUCTION

Wheat is a vital and key cereal for global population. It is a primary crop consumed cosmically (Breiman and Graur 1995). Different varieties of wheat are sown worldwide according to different climates. The two main types of crop are spring and winter wheat. In India, wheat production is 96.64 million tonnes (Anonymous 2016). Wheat grown in dry areas is hard type, while wheat of humid areas is softer. Hard wheat has strong gluten and 11-15 % of protein content, while soft wheat has weak gluten and 8-10 percent of protein content. There is a need to assess the microbiological quality of wheat grain at the start of the flour supply chain due to increased frequency of food borne disease caused by flour products. Coliforms and *E. coli* species are usually used as safety indicators in many food industries to assess the microbiological safety of environments and foods. Food storage plays an important role in maintaining the nutritional value of the food. The usage of mechanical techniques of controlling microorganisms in stored food stuffs and other products is advantageous. The physical method which has proved effective is an Entoleter, which employs the effects of percussion and centrifugal force. Entoleter's acceptance is based on its effectiveness, solidity and simplicity of operation. A fast speed rotor is the only restless part in the processing place of the Entoleter mill. Entoleter is assisted with inlet located at the top of the mill, through which material is entered. Material which is entered through the inlet is thrown outward due to centrifugal force of the spinning rotor to conveyors or bins. The security of flour-products has been cooperated long back, not only by pathogenic bacteria, but also by fungal contamination, particularly by mycotoxin producing molds. Mashinini and Dutton (2006) reported that species producing mycotoxin belong to the genera *Fusarium, Penicillium* and *Aspergillus* were commonly observed in wheat retail products. According to FAO when grain was stored for three months or longer, effects of storage environment such as fungi caused substantial loss. Food storage plays an important role in maintaining the nutritional value of the food. The storage of food products must be done under conditions that are not detrimental to the safety and quality of a particular food product.

MATERIAL AND METHODS

This study was conducted in the laboratories of Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana. The material and methods used have been presented under the followings headings:

Procurement of material

Wheat samples were purchased from the local market.

Effect of operational speed of an Entoleter on microbiological quality of wheat grains

Experiments were conducted in the developed Entoleter (Jindal 2017) with speed varying from 500-1500 rpm for wheat grains. This speed was chosen with a goal of maximum mortality of insects and minimum change in size, moisture content, broken percentage of grains (Jindal 2017). The samples which were not passed through the Entoleter were marked as control. Half of the

samples were passed through the Entoleter three times and were marked as first pass, second pass and third pass. These samples were then evaluated for the effect of speed and number of passes on microbial load of wheat. The media, Plate Count Agar (PCA) used for total plate count, Potato Dextrose Agar (PDA) for fungi, Bacillus Cereus Agar Base (BCA) for *Bacillus cereus* (spread plating), MacConkey Agar (MCA) for coliforms and Lactose Broth for *Escherichia coli*. Control and passed samples (all three passes) of wheat (10g) were weighed and suspended in 90 ml of Ringer's solution. Pour plating on respective culture media was done using serial dilution technique. Serial dilutions were made and 1 ml of the appropriate dilution was poured. Plates were incubated at 37°℃ for 24 h for enumeration of the total microbial load under anaerobic condition. Developed colonies were counted and expressed as colony forming units/ gram (cfu/g) of sample.

Effect of packaging material and storage duration on microbial load of wheat grains

Untreated (samples not passed through the Entoleter) and Machine Treated (samples passed through the Entoleter) 5kg each were packaged in three different packaging materials i.e. Steel containers, PET containers and Polypropylene (PP) bags for three months storage at ambient conditions. The stored samples were regularly monitored for microbial load and quality attributes. Microbial analysis was done using serial dilution technique using different media i.e. Plate Count Agar (PCA) for total plate count, Potato Dextrose Agar (PDA) for fungi, Bacillus Cereus Agar for *Bacillus cereus* (spread plating), MacConkey Agar (MCA) for coliforms and Lactose Broth for *Escherichia coli* using pour plate technique. Microbial analysis was done after every 15 days in duplicate as discussed earlier.

Effect of packaging material and storage duration on physicochemical characteristics of wheat grains

Various physico-chemical characteristics such as moisture, carbohydrate, crude protein, fat, crude fiber and ash content of wheat samples were determined after an interval of 15 days of three months storage.

Moisture content

The moisture content was determined by the Hot Air Oven Single Stage Method (AOAC 2000). Five grams of each sample was kept in an oven at 130°C for 1hour in an already cooled and weighed dish with cover. After 1hr the sample dishes were kept in a dessicator to bring the sample to room temperature and weighed. The dish was placed back in the oven at 60°C after half hour intervals till a constant weight is achieved. The formula used to calculate the moisture content was:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (gms)}}{\text{Wt. of sample (gms)}} \times 100$$

Carbohydrate content

The values of moisture content, protein, fat, crude fiber and ash were added and subtracted from 100 (AOAC 2000). The difference gave the value of available carbohydrates.

Crude protein

Crude proteins were determined by Kjeldahl's method as recommended by AOAC (2000). **Reagents:**

For digestion:

(i) Concentrated sulphuric acid

(ii) Digestion mixture – prepared by mixing the following in a pestle and mortar

Cupric sulphate ($CuSO_4.5H_2O$) 500g

Potassium sulphate (K_2SO_4) 30g

Mercuric oxide red 3g and selenium powder (Se) 1g

For distillation:

(i) **4% boric acid solution-** 4g boric acid powder dissolved in water to make total volume 100ml.

(ii) **40% sodium hydroxide solution-** 40g sodium hydroxide dissolved in water to make total volume 100ml.

(iii) **Mixed indicator solution-** 0.33g methyl red+ 0.60 g bromocresol green in 100ml of 95% alcohol.

(iv) **Boric acid-** mixed indicator solution- 20ml of mixed indicator solution was dissolved in one liter of 4% boric acid solution. The color of boric acid mixed indicator was adjusted to purple blue by adding 1ml of 40% NaOH in 10 ml distilled water.

For titration: Standard H_2SO_4 solution (0.1N H_2SO_4): This solution was prepared by dissolving 2.6 ml of concentrated H_2SO_4 in distilled water to make volume to 100ml.

Procedure:

a. Digestion

1. Took 0.5g sample and transferred it to digestion tube. To this, 0.02g digestion mixture and 4ml concentrated sulphuric acid were added.
2. The digestion tubes with different samples were kept in digestion apparatus and the contents were heated at 150°C for 2 hours after which, the temperature was increased to 250°C at which the contents were heated for 1-1.5 hours. Finally, the temperature was increased to 400°C and the samples were heated at this temperature for 30 minutes more after the color of the contents turned bluish green.
3. The digestion tubes were removed from the digestion apparatus and were cooled for 30-40 minutes. The volume of the contents was made to 50ml with distilled water.

b. Distillation:

1. Took 5g boric acid-mixed indicator solution in the conical flask which was placed beneath the condenser tube of the distillation apparatus so that the tip of the condenser dipped in the boric acid- mixed indicator solution (the water supply was provided and the heater was switched on well in advance to avoid suction of boric acid – mixed indicator solution into the condenser.
2. Pipette out 10ml digested sample and 10 ml NaOH solution into the boiling flask and quickly attach to the condenser through the kjeldahl trap and let the steam enter the boiling flask.
3. Continued distillation till the distillate amounted to almost 30ml in the conical flask containing boric acid – mixed indicator solution. Lowered the flask and allowed the washing down of the condenser tip off the contents.

c. Titration

1. Removed the conical flask and titrated its contents with standard H_2SO_4 to a faint pink end point. Recorded the volume of H_2SO_4 used in the titration.
2. A reagent black was run simultaneously to account for the nitrogen present in the reagents and that absorbed from the atmosphere.

Nitrogen % calculated was then multiplied with a factor of 5.95 to obtain % crude protein. Crude protein was calculated using the formula:

Dilution factor= Total volume made of digested sample/ volume taken for distillation

1ml of 1N H_2SO_4 = 0.014g N

$$\% \text{ Nitrogen} = \frac{\text{Volume of 0.1 N } H_2SO_4 \text{ used} \times 0.0014}{\text{Wt. of sample}} \times 100$$

$$\% \text{ crude protein} = \% \text{ Nitrogen} \times 5.95$$

Fat content

For the estimation of crude fat content Soxhlet method (AOAC 2000) was used. Two grams of moisture free sample was weighed and transferred to thimble. Thimble with porosity was used to permit rapid passage of ether. Mouth of thimble was plugged with fat free absorbent cotton and placed in soxhlet assembly. Petroleum ether (40-60 ºC) was taken in the flasks (200ml) and apparatus was fit into condenser to water tap for cold water circulation. Extraction period may vary from 4 hour at condensation rate of 5-6 drops to 16 hour at 2-3 drops. The extract was dried for 30 minutes at 100 ºC and then was cooled and weighed. The fat content of the samples were calculated as:

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat (gm)}}{\text{Weight of sample (gm)}} \times 100$$

Crude fiber

Crude fiber is defined as loss on ignition of dried residue remaining after digestion of sample with 1.25 % sulphuric acid and 1.25 % sodium hydroxide solution under specific conditions (AOAC 2000). Five grams of moisture and fat free sample was weighed and 200 ml of 1.25 % sulphuric acid was added to it. This solution was then boiled for 30 minutes and then filtered through Buchner funnel and filtration apparatus. The residue left behind was washed with water till it was acid free and residue was transferred to a beaker. Then, 200 ml of 1.25 % sodium hydroxide solution was added and again boiled for 30 minutes. The residues were again filtered and washed with water. Residues were then transferred to the pre-weighed crucible and dried to a constant weight was recorded. Crude fiber was calculated using the formula:

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{weight of ash after ignition}}{\text{Wt. of sample (g)}} \times 100$$

Ash content

Five grams of the sample was taken in a previously weighed crucible. Crucibles were then placed in a muffle furnace at 550 ºC for 4 hours or until light grey ash resulted (AOAC 2000). The residue left was weighed after cooling it to room temperature in a dessicator. The formula used to calculate ash content was:

$$\text{Ash (\%)} = \frac{\text{Weight of ash (gms)}}{\text{Wt. of sample (gms)}} \times 100$$

Statistical analysis

Data was collected in duplicate and analysis of variance (ANOVA) technique was used to analyze the data and to compare the mean difference of samples. The statistically significant differences were defined as $p < 0.05$.

RESULTS AND DISCUSSION

Effect of operational speed and number of passes on microbiological quality of wheat grains

Total plate count, coliforms and fungi associated with wheat samples decreased significantly ($p<0.05$) with an increase in the operational speed and number of passes, as shown in Table 1. Maximum decrease was observed, when the samples were exposed to 1500 rpm in the third pass. Total plate count in wheat grain samples decreased from 66.5 to 39.5 cfu/g, coliform count decreased from 9.5 to 4.0 cfu/g, fungal count decreased from 27.0 to 16.5 cfu/g. This decrease may be attributed to an increase in the impact force of the Entoleter (Bryan and Elvidge 1977). *Escherichia coli* and *Bacillus cereus* was not detected in wheat grain samples. However, all the counts were within the microbiological limits as given by Food and Drug Administration, 2013.

Table 1: Effect of operational speed of the Entoleter and number of passes on microbiological quality of wheat grains

Total plate count cfu/g (×10^2)					**Microbiological Specifications***
	Passes			**Control**	
Operational speed (RPM)	I	II	III		
500	64.5	62.0	60.5	66.5	<10^6 cfu/g
1000	57.5	54.5	51.0		
1500	47.0	43.0	39.5		
CD speed= 1.26		CD pass= 1.08			
	CD speed*pass= 2.17				
Fungi count cfu/g (×10^2)					
		Passes		**Control**	
Operational speed (RPM)	I	II	III		
500	26.5	26.0	25.5	27.0	<10^4 cfu/g
1000	24.5	23.5	22.5		
1500	20.5	18.0	16.5		
CD speed= 1.14		CD pass= NS			
	CD speed*pass= NS				
Coliform count cfu/g (×10^2)					
		Passes		**Control**	
Operational speed (RPM)	I	II	III		
500	9.0	8.5	8.0	9.5	<10^{2}cfu/g
1000	7.5	7.0	6.5		
1500	6.0	5.0	4.0		
CD speed= 1.14		CD pass= NS			
CD speed*pass=NS					

CD ($p<0.05$)
Values are mean of two replications
Control- sample not passed through the Entoleter
E.coli <3.0 MPN/g in wheat grains sample
B.cereus was not detected in wheat grain sample (25g)
* Food and Drug Administration (FDA), 2013

Effect of packaging material and storage duration on microbiological quality of wheat

Total plate count and coliform count indicate effectiveness and efficiency of the food chain process and give information regarding the shelf life and organoleptic changes during storage of the food stuff (Batool *et al* 2012). The results indicated a significant ($p<0.05$) increase in total plate count during storage of wheat grains (Table 2). Total plate count in wheat grains increased from 38.5 to 91.0 cfu/g in PET containers, 38.5 to 84.0 cfu/g in steel containers and 38.5 to 94.0 cfu/g in PP bags. Bacterial count increased significantly ($p<0.05$) throughout the storage period. Fungal count of wheat grains increased from 5.5 to 22.0 cfu/g in PET containers, 5.5 to 21.5 cfu/g in steel containers and 5.5 to 24.0 cfu/g in PP bags. Coliform count, plate count and *E. coli* are important, as they give an indication of the hygienic properties of the food. The results indicated significant ($p<0.05$) increase in coliform count during storage of wheat grains. Coliforms were not observed at the start of storage, but appeared after 30 days of storage of wheat grains. This was because coliforms grow at specific moisture content. This may be due to an increase in moisture content during storage. Increase was more profound in PP bags than in PET containers and least in steel containers. This might be attributed to the permeability of the packaging materials to atmospheric gases such as oxygen, carbon dioxide and water vapour. Therefore, the results indicated that wheat grains stored in steel containers would best maintain microbial stability for three months. These results are in agreement with the reports of Uchechukwu-Agua (2015), that the microbial load of cassava flour stored in plastic container for a period of 12 weeks was influenced by moisture content, water activity and pH.

Table 2: Effect of packaging material and storage duration on microbiological quality of wheat grains

Total plate count cfu/g (×10^2)	**Microbiological specifications ***

			Packaging material				
	PET Containers		Steel Containers		PP bags		
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	
0	38.5	33.5	38.5	33.5	38.5	33.5	$<10^6$
15	53.5	48.0	52.5	46.5	57.0	53.0	
30	65.0	60.0	59.0	56.0	65.5	62.5	
45	74.5	71.0	73.0	65.0	77.5	68.0	
60	83.5	78.5	82.0	72.0	85.5	79.5	
75	90.5	85.5	88.5	80.0	93.5	88.5	
90	98.0	91.0	96.0	84.0	100.5	94.0	
CD packaging material = 1.32		CD treatment= 1.08			CD storage= 2.02		
CD P*T= NS		CD P*S= NS		CD T*S= NS		CD P*T*S= NS	
Fungi count cfu/g ($\times 10^2$)							
			Packaging material				
	PET Containers		Steel Containers		PP bags		
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	
0	9.5	5.5	9.5	5.5	9.5	5.5	$<10^4$
15	11.5	7.0	11.0	8.5	12.5	9.5	
30	13.0	9.5	12.5	9.0	14.0	10.5	
45	15.5	10.0	14.0	11.0	16.5	13.5	
60	17.0	11.5	18.0	14.0	18.5	15.5	
75	19.5	13.0	19.0	15.0	20.0	18.5	
90	22.0	17.5	21.5	16.5	24.0	20.0	
CD packaging material = 0.60		CD treatment= 0.49			CD storage= 0.92		
CD P*T= 0.86		CD P*S= NS		CD T*S= NS		CD P*T*S= NS	
Coliform count cfu/g ($\times 10^2$)							
			Packaging material				
	PET Containers		Steel Containers		PP bags		
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	
0	0.0	0.0	0.0	0.0	0.0	0.0	$<10^2$
15	0.0	0.0	0.0	0.0	0.0	0.0	
30	0.0	0.0	0.0	0.0	0.0	0.0	
45	3.0	2.5	3.5	2.5	4.5	3.5	
60	6.5	4.5	4.5	4.0	6.0	5.5	
75	9.0	6.0	5.5	5.0	10.0	8.5	
90	12.5	9.5	8.5	8.0	13.5	11.5	
CD packaging material = 0.39		CD treatment= 0.31			CD storage= 0.59		
CD P*T= 0.55		CD P*S= 1.03		CD T*S= 0.84		CD P*T*S= NS	

CD ($p<0.05$)
Values are mean of two replications
Untreated sample- sample not passed through the Entoleter
Machine treated sample- sample passed through the Entoleter
E.coli <3.0 MPN/g in wheat grains
B.cereus was not detected in wheat grains (25g)

* Food and Drug Administration (FDA), 2013

Effect of packaging material and storage duration on physicochemical characteristics of wheat grains

Whiteley (1970) stated that moisture content of wheat varies from 11 to 15%, depending upon the storage conditions. Moisture content of wheat grains showed significant ($p<0.05$) increasing trend throughout the storage (Table 3). According to Butt *et al* (2004), the increase in the percentage moisture content of stored grains can be attributed to the hygroscopic properties of the stored grain and might be due to the fact that at a high humidity, the vapour pressure may have increased which aids water absorption into the samples. Moisture content of wheat grains increased from 7.23 to 10.37% in steel containers, 7.23 to 10.68 % in PET containers and 7.23 to 11.08% in PP bags. Maximum increase was observed in PP bags and minimum increase was observed in steel containers. The increases in moisture content can be attributed to aerobic respiration of the stored wheat. Aerobic respiration results in complete oxidation of hexose and this yield CO_2, H_2O and energy (McKenzie *et al* 1980). Consequently, the loss in mass and an increase in moisture content of the grain occurs.

The carbohydrate, protein, fat, fiber and ash content of wheat decreased significantly ($p<0.05$) during storage, regardless of the packaging material. Statistical analysis conducted, using ANOVA at the 5% level of significance showed that carbohydrate content of wheat grains decreased from 64.18 to 63.40% in PP bags, 64.18 to 63.45% in PET containers and 64.18 to 63.47% in steel containers. The protein content of wheat decreased significantly ($p < 0.05$) during storage. The protein content of wheat before storage was 12.62 %. During storage, the highest rate of decrease of protein content of the stored wheat was observed in PP bags. The highest protein content deterioration rate of wheat in PP bags can be attributed to high humidity conditions. Fat content of wheat grains decreased from 1.96 to 1.21% in PP bags, 1.96 to 1.33% in steel containers and 1.96 to 1.28% in PET containers. Crude fiber content of wheat grains decreased from 2.35 to 1.76% in steel, 2.35 to 1.72% in PET containers and 2.35 to 1.70% in PP bags. Steel containers generally have good barrier against moisture, but PP bags had higher water vapour permeability, compared with PET containers. Therefore, the steel containers best maintained the microbiological quality and quality attributes of stored wheat grains.

Table 3: Effect of packaging material and storage duration on physicochemical characteristics of wheat grains

Moisture (%)						
			Packaging material			
	PET Containers		**Steel Containers**		**PP bags**	
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**
0	7.29	7.23	7.29	7.23	7.29	7.23
15	7.88	7.83	7.87	7.69	8.13	7.90
30	8.36	8.34	8.33	8.17	8.54	8.38
45	8.91	8.86	8.86	8.74	8.98	8.94
60	9.55	9.38	9.35	9.16	9.66	9.43
75	10.02	10.01	9.98	9.88	10.33	10.24
90	10.68	10.44	10.37	10.23	11.08	10.97
CD packaging material = 0.004		CD treatment= 0.004			CD storage= 0.007	
CD P*T= 0.0069		CD P*S= 0.013		CD T*S= 0.011		CD P*T*S= 0.018
Carbohydrate (%)						
			Packaging material			
	PET Containers		**Steel Containers**		**PP bags**	
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**
0	64.10	64.18	64.10	64.18	64.10	64.18
15	63.93	64.11	63.96	64.13	63.91	64.09
30	63.84	64.07	63.87	64.10	63.82	64.04
45	63.72	64.03	63.75	64.06	63.70	63.98
60	63.65	64.00	63.66	64.02	63.62	63.91
75	63.53	63.96	63.54	63.99	63.51	63.86
90	63.45	63.92	63.47	63.95	63.40	63.81
CD packaging material = NS		CD treatment= 0.016			CD storage= 0.031	
CD P*T= 0.029		CD P*S= 0.055		CD T*S= 0.044		CD P*T*S= NS
Protein (%)						
			Packaging material			

	PET Containers		Steel Containers		PP bags	
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**
0	12.66	12.62	12.66	12.62	12.66	12.62
15	12.57	12.57	12.56	12.58	12.53	12.55
30	12.47	12.51	12.48	12.52	12.45	12.49
45	12.41	12.49	12.42	12.50	12.39	12.47
60	12.34	12.46	12.38	12.48	12.32	12.42
75	12.30	12.43	12.30	12.44	12.28	12.37
90	12.25	12.41	12.27	12.41	12.22	12.34
CD packaging material = 0.0058		CD treatment= 0.0047			CD storage= 0.0088	
CD P*T= NS		CD P*S= NS		CD T*S= 0.012		CD P*T*S= 0.022

Fat (%)

			Packaging material			
	PET Containers		**Steel Containers**		**PP bags**	
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**
0	1.99	1.96	1.99	1.96	1.99	1.96
15	1.80	1.92	1.88	1.93	1.78	1.89
30	1.71	1.86	1.76	1.91	1.70	1.84
45	1.59	1.83	1.62	1.88	1.56	1.81
60	1.51	1.77	1.54	1.85	1.48	1.75
75	1.43	1.74	1.46	1.84	1.42	1.71
90	1.28	1.69	1.33	1.82	1.21	1.64
CD packaging material = 0.0043		CD treatment= 0.0034			CD storage= 0.0065	
CD P*T= 0.006		CD P*S= 0.015		CD T*S= 0.009		CD P*T*S= .016

Fiber (%)

			Packaging material			
	PET Containers		**Steel Containers**		**PP bags**	
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**
0	2.31	2.35	2.31	2.35	2.31	2.35
15	2.22	2.28	2.24	2.31	2.20	2.25
30	2.14	2.24	2.17	2.27	2.12	2.22
45	2.03	2.20	2.06	2.23	2.00	2.17
60	1.92	2.16	1.96	2.19	1.90	2.12
75	1.81	2.12	1.85	2.15	1.77	2.09
90	1.72	2.07	1.76	2.11	1.70	2.04
CD packaging material = 0.0076		CD treatment= 0.0062			CD storage= 0.011	
CD P*T= NS		CD P*S= 0.020		CD T*S= 0.016		CD P*T*S= NS

Ash (%)

			Packaging material			

	PET Containers		Steel Containers		PP bags	
Storage days	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**	**Untreated UT**	**Machine treated MT**
0	1.45	1.43	1.45	1.43	1.46	1.43
15	1.37	1.41	1.39	1.41	1.33	1.40
30	1.30	1.38	1.32	1.40	1.29	1.36
45	1.24	1.37	1.26	1.39	1.22	1.32
60	1.17	1.34	1.21	1.38	1.15	1.29
75	1.11	1.32	1.16	1.37	1.11	1.25
90	1.06	1.31	1.09	1.36	1.03	1.23
CD packaging material = 0.0034		CD treatment= 0.0028			CD storage= 0.0053	
CD P*T= 0.0049		CD P*S= 0.009		CD T*S= 0.0074		CD P*T*S= 0.013

CD ($p<0.05$)
Values are mean of two replications
Untreated sample- sample not passed through the Entoleter
Machine treated sample- sample passed through the Entoleter

LITERATURE CITED

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