



# A Study on the Shelf life of the Spent Hen Meat Puffed Product (SHPP) Stored at Ambient Temperature

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## RESEARCH ARTICLE

# A Study on the Shelf life of the Spent Hen Meat Puffed Product (SHPP) Stored at Ambient Temperature

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**ABSTRACT:** Hot extruded spent hen meat puffed products (SHPP) are made of spent hen meat mixed with flours and additives subjected to a maturing and extrusion process. This study was undertaken to evaluate the hygienic quality, the organoleptic characteristics, physico-chemical, proximate and microbial status during shelf life of SHPP in ambient temperature. Growth models, developed and validated on hot extruded spent hen meat puffed products (SHPP) were used to predict the growth of microorganisms. Temperature data were obtained from retail and home refrigerators. Shelf life was greatly influenced by storage temperature, but initial microbial load had a smaller effect. The expiration date of hot extruded spent hen meat puffed products must be based only on the growth of the spoilage microorganisms. Only when product contamination with bacteria cell concentrations is high, the product fraction poses health risks for consumers. Sensitivity analysis confirmed that storage temperature and temperature variability were the most important factors for the duration of shelf life.

**Keywords:** Hot extrusion, Puffed meat product, Bacterial spoilage, Spoilage indicator, Shelf life

## INTRODUCTION

The meat from spent hen is generally tough, less tender and poor in functional properties, because of its increased collagen content and cross linkages (Bailey AJ, 1984). Unfortunately, the toughness prevents use of spent hens in whole meat food and reduces the market value (Sams AR, 1990; Nowsad *et al.*, 2000). This meat can be utilized by hot extrusion. Extrusion is a process in which material is pushed through an orifice or a die of given shape. The pushing force is applied using twin-screw extruder. Extrusion processing of food materials has become an increasingly important manufacturing method, and its application has broadened substantially in the last two decades (Godavarti, 1997). Among various classes of poultry birds, broilers are heavier in body weight and contain high fat (Kondaiah, 1990).

The shelf-life of meat and meat products is the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable bacterial level, or an unacceptable off-odour, off-flavour or appearance. The shelf-life depends on the numbers and types of microorganisms mainly, bacteria, initially present and their subsequent growth (Russell, 1996). During storage, environmental factors such as temperature, gaseous atmosphere, pH and NaCl will select for certain bacteria, and affect their growth rate and activity. The shelf-life of refrigerated meat and meat products may vary from days up to several months (Gill, 1997). The following presentation will focus upon bacteria able to grow and cause spoilage during the storage of meat (pork and beef) and cooked, cured meat products.

## MATERIALS AND METHODS

The present study on shelf life of hot extruded puffed product from spent hen meat was performed in various phases at the departments of Livestock Products Technology of Bombay Veterinary College and Fish Processing Department, Central Institute Fisheries Education, Varsova, Mumbai. In the initial stages of the experiment stable emulsion from spent hen was obtained after altering the process used for emulsion from broiler meat. The proper dough consistency was determined after several trials on various combinations of different types and levels of flours and spent hen meat emulsion to obtain a proper hot extruded puffed product. Various sieve sizes were also tried in the preliminary study and finally 2 mm diameter sieve size was used for extrusion. The moisture content of the feed, extrusion temperature and barrel temperature are very important for the manufacture of puffed products. Accordingly on the basis of preliminary trials the moisture content of the feed was maintained in the range of 14 to 22 percent for different treatments under study. The barrel temperature was maintained at 140°C to obtain the extrusion temperature 104°C (Chueachuaychoo, *et al.*, 2011). The steps / processes used in the preliminary trials were selected on the basis of suitable dough quality which was giving proper hot extruded puffed product. Thus the three treatments (T<sub>1</sub> – 15% emulsion + 85% flour mixture, T<sub>2</sub> – 20% emulsion + 80% flour mixture and T<sub>3</sub> – 25% emulsion + 75% flour mixture) as described above were finalized and were replicated four times (Kim, 1997).

Freshly prepared puffed products were divided into two lots. The first lot was subjected to sensory evaluation by the panel of expert judges and zero day analysis for physico-chemical, proximate and microbial status. Second lot was packaged in sterile food grade polyethylene pouch and was kept at ambient temperature to monitor physico-chemical and microbial changes during storage at the interval of ten days (Russell, 1995).

## 2.1 pH

The pH of fresh and stored puffed product was determined by the method of Trout GR (Trout, 1989). Five grams of sample was homogenized with 12.5 ml. of distilled water in a laboratory blender (Ahn, *et al.*, 1990). The pH of suspension was recorded with the help of digital pH meter (Model-HI 9916325 portable water proof meat pH meter from HANNA) in Department of VPH Bombay Veterinary College, Mumbai.

## 2.2 Thiobarbituric Acid Value

TBA number of fresh and stored puffed product was determined as per the method described by (Strange *et al.*, 1977) with little modification. Trichloroacetic acid (TCA) extract was prepared by blending 5 g of sample with 12.5 ml of precooled 20 percent TCA solution for two min. After homogenisation, the contents were transferred to a beaker by rinsing with 12.5 ml cold distilled water, mixed, centrifuged twice and filtered through Whatman filter paper No.42. Two ml aliquot of TCA extract was mixed with two ml of 0.01M 2-TBA reagent in a test tube. The test tubes were kept in a water bath at 100°C for 30 min. After cooling the tubes in running water for about 10 min, centrifuged once and the absorbance (A) at 532 nm was measured in spectrophotometer (Model no. EQ 820 with wavelength range of 350-950 nm, INDIA) (Witte, *et al.*, 1970).

## 2.3 Tyrosine Value

The procedure described by (Strange *et al.*, 1977) was used with slight modification. TCA extract of 2.5 ml was taken and mixed with equal amount of distilled water. The mixture was blended with addition of ten ml of 0.5 N NaOH to which three ml of diluted Folin and Ciocalteu reagent was added. The mixture after shaking was kept in dark at room temperature for 30 min for colour development. The optical density was measured at 730 nm using Spectrophotometer. Tyrosine value was calculated as mg tyrosine per 100 g of sample by referring to a standard graph, which was prepared as per the procedure described by Pearson (Pearson, 1968).

## 2.4 Sensory evaluation

The freshly prepared hot extruded puffed products were organoleptically evaluated by panel of 5 judges (Choudhry, *et al.*, 1992). The panel was comprised of trained academic staff of the institute. The puffed product were judged for various sensory attributes using nine point descriptive scale (Keeton, 1983). The scores of 5 judges were averaged and recorded as mean value for sensory score. The judges were also requested to give their critical comments for the products. Each panelist evaluated 3 samples (identified by codes) in a balanced sequential order. The entire fresh puffed product samples under treatment were served to panel of judges. They were also decoded differently at each time of judging for all four replications (Pooni, 1984).

## 2.5 Microbiological analysis

Total plate count of samples at all stages of storage and *Coliform* and *Salmonella* count at the end of storage study were estimated following the standard method of APHA (APHA, 1992).

### 2.5.1 Preparation of serial dilutions

Ten gram of aseptically packed puffed product were made into paste in a sterile mortar and fine suspension was prepared by adding 90 ml of sterile Normal saline solution using a sterile pestle for two min to get 10<sup>-1</sup> dilution. One ml of this dilution was transferred to nine ml of sterile NSS in a test tube and mixed uniformly to get 10<sup>-2</sup>(-2) dilution. All the dilutions were made under aseptic conditions.

### 2.5.2 Estimation of Total Viable Count (TVC)

For evaluating TVC, standard pour plate technique was followed where in 0.1 ml of inoculum from 10<sup>-1</sup> and 10<sup>-2</sup> dilutions was transferred to sterile empty petri plates in duplicate, in which 15-20 ml of molten nutrient agar having temperature around 43-45°C was poured. The inoculum was mixed thoroughly by rotating the plates five times each clockwise and anti-clockwise. After solidification of agar the plates were incubated at 37°C for 24-48 hrs (Sharma, *et al.*, 2002).

TVC was calculated by using the following formula:

$$CFU/gm = \frac{\sum c}{[n_1 + (0.1 \times n_2)] \times d}$$

Where:

$\sum c$  = Total no. of colonies developed on all the plates

$n_1$  = No. of plates of lower dilution

$n_2$  = No. of plates of higher dilution

$d$  = Dilution factor corresponding to lower dilution.

CFU = Colony Forming Unit

### 2.5.3 Coliform count

41.5 g of Violet Red Bile Agar (VRBA) was suspended in one litre of distilled water and boiled to dissolve the medium completely. Final pH of the medium was adjusted to 7.4±0.2. Precaution was taken not to autoclave the medium. One ml of suitable dilutions were placed in sterile petridishes and overlaid with molten agar. After solidification, the plates were incubated at 37 °C for 24 hrs. The number of red or purple colonies of 0.5 mm in diameter were counted and expressed as log<sub>10</sub> cfu/g of sample.

### 2.5.4 Salmonella count

For detection of *Salmonella*, pre-enrichment was done by suspending 25 g of sample in 225 ml buffered peptone water (Merck) followed by incubation at 37 °C for 16 to 20 hrs and selective enrichment was done by transferring 0.1 ml of pre-enrichment culture in 10 ml Rappaport-Vassiliadis broth (RVS broth) followed by incubation at 42 °C for 24 hrs and after incubation samples were streaked on modified XLD agar and incubated at 37 °C for 48 hrs. Black cantered colonies appeared on the plates were counted and expressed as log<sub>10</sub> cfu/g.

## RESULTS AND DISCUSSION

### 3.1 Physico-chemical changes of SHPP during storage

The spent hen puffed product (SHPP) manufactured under three treatments in the present study were monitored at ambient temperature for their physico-chemical and microbial changes during storage at an interval of 10 days for total period of 30 days (Gholam Reza Shaviklo, *et al.*, 2011).

### 3.2 Changes in pH of SHPP during storage

The values for the pH of freshly prepared spent hen puffed product (SHPP) and changes occur during storage are highlighted in Table 1 and depicted in Fig.1. Table of pH values depicts the changes in pH values of SHPP during the storage. The mean average values for pH during entire storage period were 6.45±0.07, 6.28±0.05 and 6.20±0.02 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively.

The freshly prepared SHPP had pH value of 6.84±0.01, 6.54±0.01 and 6.27±0.01 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively which significantly decreased to 6.22±0.01, 6.15±0.01 and 6.14±0.01, respectively for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> at the end of storage study (Yang CC, TC Chen, 1993). The decrease in the pH values as storage period advances could be ascribed to increase in the microbial load and TBARS value (Fletcher, 1995). The similar of decrease in pH value during storage of chicken snacks was also observed by (Kalra *et al.*, 1995).

### 3.3 Changes in Tyrosine value (mg/100g) of SHPP during storage

Table 2 depicts the values for tyrosine of freshly prepared spent hen puffed product and the changes during storage period; the same are depicted in Fig. 2. From the Table 2 it is clear that the average tyrosine value (mg/100g) for all the three treatments were 3.40±0.30, 3.19±0.28 and 2.49±0.15 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively, during entire storage period. The freshly prepared SHPP had tyrosine value of 2.04±0.01, 2.07±0.02 and 1.92±0.03 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. There was gradual increase in a tyrosine value with the advancement of storage period. The tyrosin values of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> exhibited significant increase from zero to 30th day of storage in all treatments. There was no significant difference in tyrosin values of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> up to 10th day of storage. The similar trend of increase in tyrosine value was reported by Kowle *et al.*, for spent hen meat noodles. This could be ascribed to the higher proportion of spent hen meat in the dough formulation.

### 3.4 Changes in TBARS value (mg of malonaldehyde / Kg) of SHPP during storage

The TBARS value of freshly prepared SHPP and changes during storage are highlighted in Table 3 and depicted in Fig. 3. The average TBARS value (mg of malonaldehyde / kg) during the storage period was 0.71±0.12, 0.70±0.12 and 0.70±0.13 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. The TBARS value of freshly prepared SHPP were 0.182±0.01, 0.166±0.05 and 0.221±0.01 for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively which reached to 1.29±0.01, 1.32±0.01 and 1.36±0.01 respectively, for T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> at the end of storage period. Significant difference in average TBARS values during the storage period among the treatments could be ascribed to proportionately decrease amount of spent hen meat in the dough formulations in respective treatments. Increase in meat proportion in the dough resulted in increased TBARS values. This increasing trend in the TBARS value with storage period was also reported by (Vaithyanathan *et al.*, 2008) for spent hen breast meat, (Singh *et al.*, 2001) for chicken snacks.

### 3.5 Changes in Total viable count (log<sub>10</sub> cfu/g) of SHPP during storage

The spent hen meat puffed products (SHPP) under all the treatments were subjected for total viable count (TVC log<sub>10</sub> cfu/g) at zero day and subsequently at the interval of 10 days upto the end of storage period. At the end of storage study all the samples under treatments were also analyzed for *coliform* and *Salmonella*. None of the samples was positive for *Coliform* or *Salmonella*. The values for TVC at zero day and during storage period are highlighted in Table 4.6 and depicted in Fig. 4.6. The TVC (log<sub>10</sub> cfu/g) of T<sub>1</sub> was 2.59±0.03 at zero day which increased to 3.41±0.008 at the end of 30th day with the average value of 2.93±0.10 during the entire storage period. The TVC value for T<sub>2</sub> was 2.65±0.05 at zero days, which increased to 3.53±0.01 at the end of 30th day with the average of 3.02±0.10 during entire storage period. T<sub>3</sub> had a TVC value of 2.55±0.07 at zero day which increased to 3.54±0.01 at the end of 30th day with the average of 3.03±0.11. TVC values in the products increased significantly (p≤0.05) as storage advanced. The trend observed in the present investigation for increase in TVC count with the storage period is in accordance with one reported for chicken chips (Singh, *et al.*, 2011).

## CONCLUSION

The present study clearly emphasizes the nature and pervasiveness of lipid oxidation in foods and in vivo and the challenges that face researchers to provide answers to oxidation issues. Shelf life was greatly influenced by storage temperature, but initial microbial load had a smaller effect. The decrease in the pH values as storage period advances could be ascribed to increase in the microbial load and TBARS value. Significant difference in average TBARS values during the storage period among the treatments could be ascribed to proportionately decrease amount of spent hen meat in the dough formulations in respective treatments. TVC values in the products increased significantly (p≤0.05) as storage advanced. At the end of storage study all the samples under treatments were also analyzed for *coliform* and *Salmonella*. None of the samples was positive for *Coliform* or *Salmonella*. Further studies on packaging techniques of puffed meat products are required to increase the shelf life.

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**Table 1: Average pH values observed in SHPP stored at room temperature**

Treatments	0 day	10 day	20 day	30 day	Mean ± S.E. for stor
T <sub>1</sub>	6.84±0.01 <sup>aA</sup>	6.46±0.01 <sup>bA</sup>	6.26±0.02 <sup>cA</sup>	6.22±0.01 <sup>dA</sup>	6.45±0.07
T <sub>2</sub>	6.54±0.01 <sup>aB</sup>	6.24±0.00 <sup>bB</sup>	6.19±0.01 <sup>cB</sup>	6.15±0.01 <sup>dB</sup>	6.28±0.05
T <sub>3</sub>	6.27±0.01 <sup>aC</sup>	6.22±0.00 <sup>bC</sup>	6.17±0.00 <sup>cB</sup>	6.14±0.01 <sup>dB</sup>	6.20±0.02

Means in the same row or column with different superscripts indicate significant difference ( $P < 0.05$ );  $n=3$

**NOTE: T<sub>1</sub> (Dough with 15% emulsion), T<sub>2</sub> (Dough with 20% emulsion), T<sub>3</sub> (Dough with 25% emulsion)**

**Table 2: Average Tyrosine (mg/100g) values observed in SHPP stored at room temperature**

Treatments	0 day	10 day	20 day	30 day	Mean ± S.E. for stor
T <sub>1</sub>	2.04±0.01 <sup>aA</sup>	2.91±0.02 <sup>bA</sup>	4.13±0.00 <sup>cA</sup>	4.53±0.01 <sup>dB</sup>	3.40±0.30
T <sub>2</sub>	2.07±0.02 <sup>aA</sup>	2.89±0.02 <sup>bA</sup>	3.14±0.01 <sup>cB</sup>	4.65±0.03 <sup>dA</sup>	3.19±0.28
T <sub>3</sub>	1.92±0.03 <sup>aB</sup>	2.17±0.02 <sup>bB</sup>	2.71±0.04 <sup>cC</sup>	3.18±0.02 <sup>dC</sup>	2.49±0.15

Values in the same row with the different superscript are differ significantly ( $P \leq 0.01$ )

**NOTE: T<sub>1</sub> - Dough with 15% emulsion, T<sub>2</sub>-Dough with 20% emulsion, T<sub>3</sub>- Dough with 25% emulsion**

**Table 3: Average TBARS (mg of malonaldehyde / Kg) values observed in SHPP stored at room temperature**

Treatments	0 day	10 day	20 day	30 day	Mean ± S.E. during stor
T <sub>1</sub>	0.182±0.01 <sup>dB</sup>	0.547±0.04 <sup>c</sup>	0.812±0.04 <sup>b</sup>	1.29±0.01 <sup>aC</sup>	0.71±0.12
T <sub>2</sub>	0.221±0.01 <sup>dA</sup>	0.524±0.06 <sup>c</sup>	0.744±0.03 <sup>b</sup>	1.32±0.01 <sup>aB</sup>	0.70±0.12
T <sub>3</sub>	0.166±0.05 <sup>dC</sup>	0.521±0.01 <sup>c</sup>	0.730±0.06 <sup>b</sup>	1.36±0.01 <sup>aA</sup>	0.70±0.13

Values in the same column with the different superscript are significantly different ( $P \leq 0.05$ ).

**NOTE: T<sub>1</sub> - Dough with 15% emulsion, T<sub>2</sub>-Dough with 20% emulsion, T<sub>3</sub>- Dough with 25% emulsion**

**Table 4: Average TVC (log<sub>10</sub> cfu/g) values observed in SHPP stored at room temperature**

Treatments	0 day	10 day	20 day	30 day	Mean ± S.E. during stor
T <sub>1</sub>	2.59±0.03 <sup>a</sup>	2.69±0.03 <sup>bB</sup>	3.04±0.02 <sup>cB</sup>	3.41±0.008 <sup>dB</sup>	2.93±0.10
T <sub>2</sub>	2.65±0.05 <sup>a</sup>	2.80±0.03 <sup>bA</sup>	3.10±0.02 <sup>cAB</sup>	3.53±0.01 <sup>dA</sup>	3.02±0.10
T <sub>3</sub>	2.55±0.07 <sup>a</sup>	2.86±0.03 <sup>bA</sup>	3.16±0.02 <sup>cA</sup>	3.54±0.01 <sup>dA</sup>	3.03±0.11

Means in the same row or column with different superscripts indicate significant difference ( $P < 0.05$ );  $n=3$

**NOTE: T<sub>1</sub> - Dough with 15% emulsion, T<sub>2</sub>-Dough with 20% emulsion, T<sub>3</sub>- Dough with 25% emulsion**

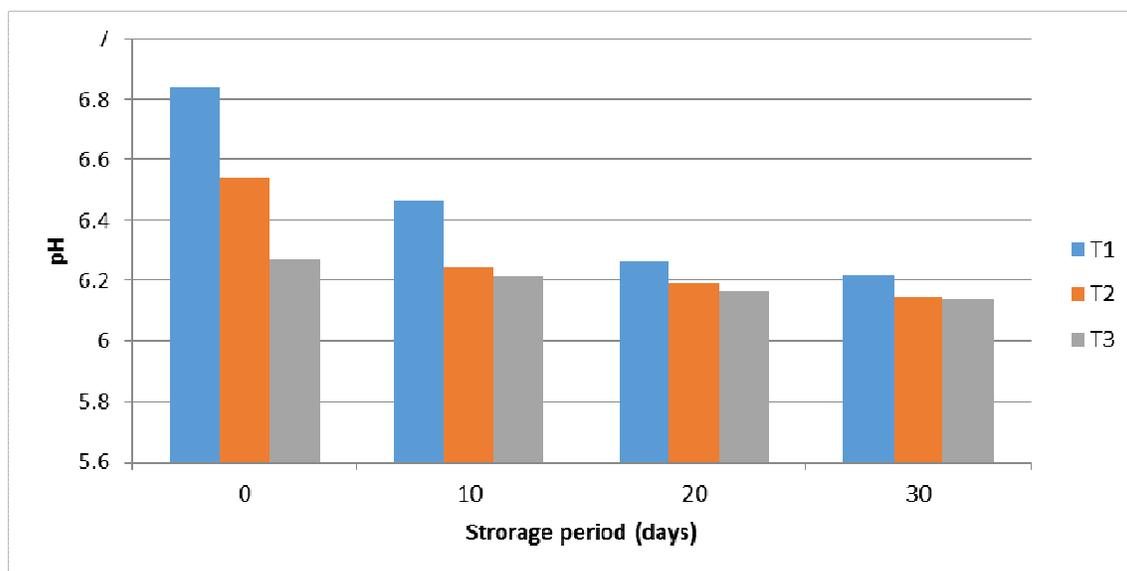


Fig 1: Average changes pH values observed in SHPP stored at room temperature

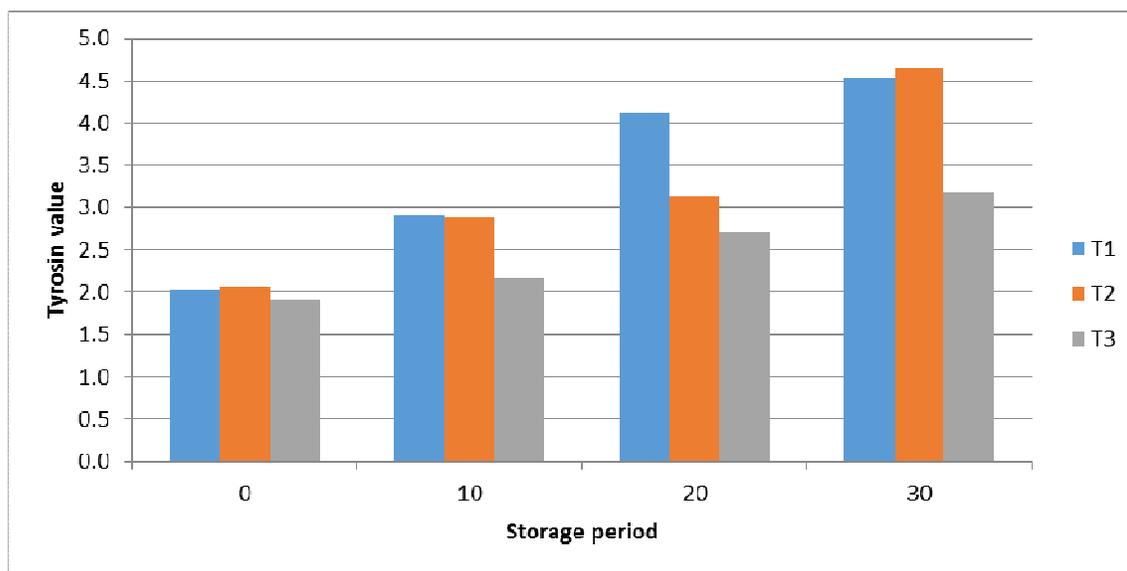


Fig 2: Average changes Tyrosine (mg/100g) values observed in SHPP stored at room temperature

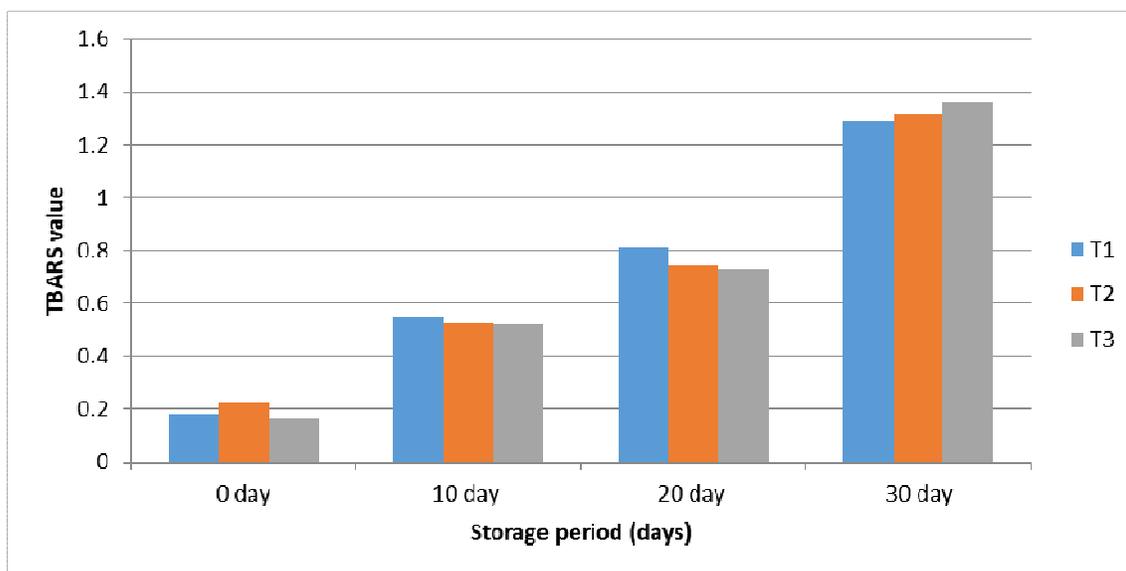


Fig 3: Average changes in TBARS (mg of malonaldehyde / Kg) values observed in SHPP stored at room temperature

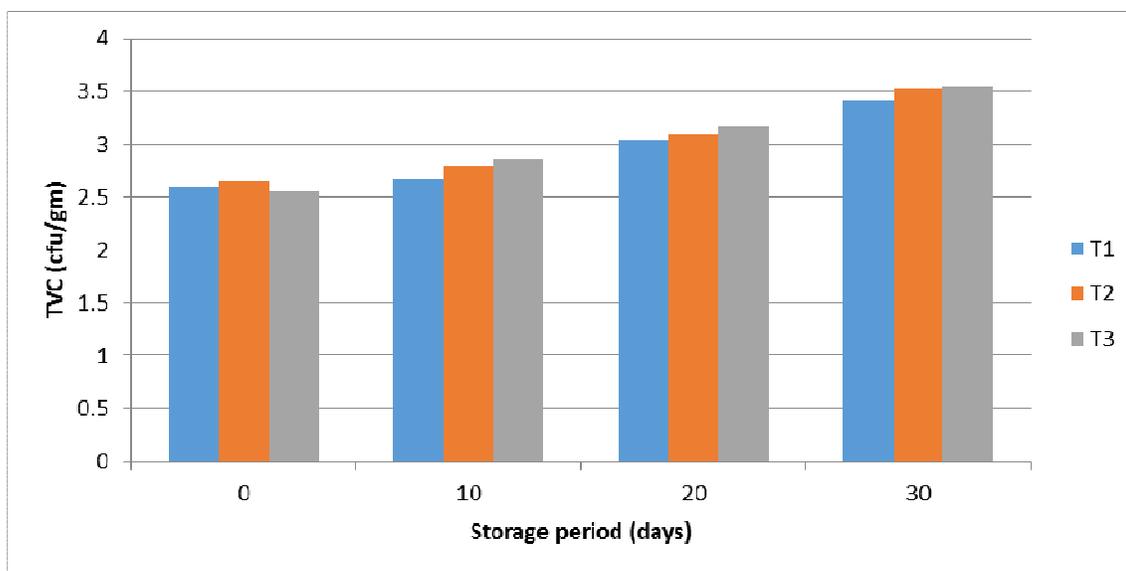


Fig 4: Average changes in TVC (log10 cfu/gm) values observed in SHPP stored at room temperature

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**CONFLICTS OF INTEREST**

“The authors declare no conflict of interest”.

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