



## **Physico-Chemical and Microbial Properties of Stored Mushroom Slices**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author BB designed the study, wrote the protocol, collected data, performed the statistical analysis, managed the literature searches, wrote the first draft of the manuscript and managed the field investigation. Authors LE and VSPB read, corrected and approved the final manuscript. Author GV supported financially this study. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Button mushroom (*Agaricus bisporus* L.) is extensively produced and consumed in the world. They are more perishable due to their high moisture content. Due to its short shelf-life, the mushroom is usually dehydrated for preservation. Hot air dried mushrooms result in losses in nutrients, colour degradation and deformation in structure. To overcome these problems, freeze-drying of mushroom slices was investigated. White button mushrooms after cleaning were vertically cut into 2, 4, 6 and 8 mm thick slices. Sliced mushrooms were frozen at  $-20^{\circ}\text{C}$  and then subjected to the freeze-drying at various heating plate temperatures of 10, 20, 30 and  $40^{\circ}\text{C}$ . The effect of slice thickness and heating plate temperature on physicochemical properties like rehydration, porosity, firmness, water activity, colour, ascorbic acid, protein and microbial properties like total bacterial, yeast and mould were evaluated during the storage. Increase in the storage period resulted in decrease in porosity (73.25%), colour  $L^*$  value (48.12), firmness (0.98 N), rehydration ratio (4.04), ascorbic acid content (14.47 mg/100 g) and protein content (19.15%), whereas the water activity (0.412) increased with the storage period. This may be due to the absorption of moisture during storage. Microbial analysis indicated by yeast count, mould count and total plate count was nil during the first three weeks of storage, whereas in the fourth week negligible growth was observed. So it is concluded that this may be due to the low water activity of stored mushroom slices.

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## 1. INTRODUCTION

Mushroom is a fungus with a hypogeous or epigeous fruiting body, large enough which can be seen with the naked eye and to be picked by hand [1]. For thousands of years mushrooms are used as both food and medicine. Mushroom is the oldest single cell protein food with protein content in between low-grade vegetable and high-grade meat protein. Mushrooms are a more valuable source of protein than cattle or fish and are valued for their characteristic meaty biting texture and flavour [2].

Button mushroom (*Agaricus bisporus* L.) is extensively produced and consumed in the world. Total country's and global production of it was about 90 and 40 per cent [3]. It consists higher amount of proteins, vitamins, minerals, polyphenols and polysaccharides.

Button mushrooms are more perishable due to their higher moisture content. The deterioration process starts, once the fruiting body matures and after some time mushrooms becomes inedible. Major factors contributing to quality losses are the browning and opening of veils. So, application of the suitable preservation technique to increase the shelf-life and to retain the quality of mushroom can play a key role in the commercialization of the mushroom. Amongst the various methods employed for preservation, drying is an energy-intensive operation by which the water is removed by the application of heat under controlled conditions. To produce a dried product of the required quality at a low cost, reducing enzymatic and microbiological reactions are the main aim of any drying method. Sun drying of mushrooms results in poor quality and unhygienic products [4].

Various drying methods were investigated for mushrooms. However, conventional air drying is still the most widely used method on a global scale. At the early stages of drying it is undoubtedly a very practical method but as the process progresses, it consumes more energy, the process slows down significantly and drying becomes difficult. Changes in color and deformation of the structure were observed in mushrooms dried by the hot air method. To reduce these problems, freeze drying has been implemented. Freeze-dried mushrooms are

found to be excellent in color, flavor and high rehydration capacity.

Freeze-drying is recognised as the most efficient method for drying of food products with minimum damage to nutrients available in food as compared to methods involving heat treatment. Sublimation is the main principle of freeze-drying, where the amount of free water present in the material is frozen to ice form and it is directly converted into vapour form without coming into a liquid state. It helps in preventing the enzymatic browning and microbial degradation in food products. Heat-sensitive components, main antioxidants in food products are better retained by freeze-drying.

During the peak season, there is a lack of availability of mushrooms in the market due to their perishable nature. Mushrooms have a shorter shelf-life than most ready-to-use vegetables because their respiration rate is rapid and they have no barrier to protect them from water loss or from microbial attack [5]. Therefore in order to minimise the wastage and maximize the storage an effort has made for development of freeze-dried mushroom slices with the objective to determine the physico-chemical properties. Very limited research work was done to determine the physicochemical properties of stored mushroom slices. The analysis was carried out at regular intervals of one week and mushroom slices were stored at room temperature ( $30 \pm 5$  °C) and.

## 2. MATERIALS AND METHODS

Button mushrooms having 92-94% moisture content (w.b.) were procured on daily basis from a local supplier in Pulivendula, YSR Kadapa district of Andhra Pradesh and stored in a refrigerator at 4-5 °C for further use. Mushrooms of suitable size were washed under tap water and graded to eliminate the variations in exposed surface area. Then, the samples were vertically cut into 2, 4, 6 and 8 mm thick slices with an vegetable slicer provided with adjustable cutting blade. The slices with specific shape were chosen for freeze drying experiments and were frozen in a blast freezer (NF Bioscience USA, Model No. 2513110) at -20 °C for 24 h. Frozen mushroom slices were freeze dried in a laboratory freeze dryer (Delvac Pumps Pvt. Ltd., Chennai: LYODEL-55) comprising of a drying

chamber, vacuum pump, condenser and a heater arrangement. The vacuum pump sucked the steam from the chamber which condensed into ice in a condenser. The Freeze-dryer heater arrangement consisted of three stage shelves located on a stand in the drying chamber. The heat required for the sublimation of moisture present in the mushroom slices was supplied by direct contact with heating plates. The drying process was done at heating plate temperatures of 10, 20, 30 and 40 °C with -40 °C condenser temperature and < 0.0150 mbar vacuum level. The weight loss was recorded every 30 min during the first 2 h, subsequently after every one hour until equilibrium moisture content was attained (change in weight < 0.01 g).

## 2.1 Physico-Chemical Properties of Mushroom Slices

### 2.1.1 Rehydration

Rehydration test is significant when the dried sample needs to be reconstituted before consumption. It is expected that the dried product on reconstitution is close to the fresh material in terms of moisture, colour and flavour. The rehydration ratio was evaluated by soaking a known weight of about 0.50±0.02 g of sample in sufficient volume of distilled water in a glass beaker at 20, 40 and 60 °C. At specific time intervals, samples were taken out of the water, blotted with tissue paper to remove surface water and reweighed. This process was continued till the sample weight became constant. Weight was recorded using an electronic balance (Essae-Teraoka Pvt. Ltd., Bangalore, AJ-220E). In order to minimize the leaching losses, a water bath was used for maintaining the defined temperature [6]. Rehydration ratio (RR) was computed as follows.

$$\text{Rehydration ratio, RR} = \frac{W_r}{W_d} \quad (1)$$

where,

$W_r$  = Weight of rehydrated sample, g  
 $W_d$  = Weight of dried sample used for rehydration, g

### 2.2 Bulk Density

The bulk density of mushroom slices was measured by gently filling them in a container and taking the weight using a digital balance (Essae-Teraoka Pvt. Ltd., Bangalore, AJ-220E).

The weight of the filled samples was divided by the volume of the container to give the bulk density of the samples [7].

### 2.3 True Density and Porosity

True density is defined as the ratio of the mass of a sample to its solid volume [7]. Mushroom slices (carpophores) volume was determined by using the liquid displacement method. Toluene ( $C_7H_8$ ) was used in place of water because it will be absorbed by carpophores to a lesser extent. Also, its surface tension is low, so that it fills even shallow dips in a carpophores and its dissolution power is low. Porosity of carpophores was calculated as follows.

$$\varepsilon = \left[ \frac{(\rho_d - \rho_y)}{\rho_d} \right] \times 100 \quad (2)$$

where,

$\varepsilon$  = Porosity in %  
 $\rho_y$  = Bulk density in  $kg\ m^{-3}$  and  
 $\rho_d$  = Particle density in  $kg\ m^{-3}$

### 2.4 Colour Analysis

Colour is one of the most important qualities of acceptance for products, reflects sensation to the human eye. Colour is important to consumer as a mean of identification, as a method of judging quality and for its basic aesthetic value. Dried products are usually darker in colour, but, darker colour does not mean better quality. Too dark may imply that the product is over dried. Hunter Lab Colorimeter (M/S Hunter Lab, Reston, VA, USA, CFLX-45) was used in the present investigation to determine the colour of mushroom slices.

A cylindrical glass sample cup (63.5 mm in diameter x 40 mm height) was placed at the light port. The hunter lab colorimeter was initially calibrated with a black as well as with a standard white plate supplied with the equipment. The measurement was done with an illuminate observer combination of D65/10°. The surface colour was quantified in terms of  $L^*$ ,  $a^*$  and  $b^*$  values of CIELAB colour space. The CIELAB colour space is organised in a cube form, where  $L^*$  axis runs from top to bottom with a minimum value of 0, representing black and a maximum of 100 representing white. The axis  $-a^*$ ,  $+a^*$  goes from green to red and the axis  $-b^*$ ,  $+b^*$  goes from blue to yellow. Hunter  $L^*$  value, which denotes the degree of whiteness, was chosen to represent the colour value of sample.

## 2.5 Water Activity

Water plays an important role in the stability of fresh, frozen, dried and stored foods. It acts as a solvent for chemical, microbiological and enzymatic reactions. Water activity can be defined as the ratio of the vapour pressure exerted by the food to the saturated vapour pressure of water at the same temperature.

Most bacteria do not grow at water activities less than 0.91, and most moulds cease to grow at water activities below 0.80 [8]. By measuring water activity, it is possible to predict which micro-organisms will or will not be potential sources of spoilage. The lower water activity of a dried product implies a better potential for storage.



**Pic. 1. Water activity meter**

Water activity was determined as a measure of storage stability using a water activity meter (Rotronic AG Bassersdorf, Switzerland, Hygrolab-3). Two gram sample was used to cover the filling indicator of the sample cup. The filled sample cup was kept in contact with sensor probe of water activity meter and values of water activity were recorded.

## 2.6 Firmness

The compression tests were carried out by using a force gauge (LUTRON, FG-20KG) of 20 kg capacity. A sample of sliced mushroom to be tested was placed horizontally on stationary hard surface. Load was applied by forcing the tip into the sample until the sample failed by cracking. Readings were noted which were displayed in negative magnitude for compression. Mean values of five measurements were taken for each trial.

## 2.7 Ascorbic Acid Content

Ascorbic acid also known as vitamin C is an antiscorbutic. Generally, it is present in all fresh vegetables and fruits. It is a water soluble and heat-labile vitamin. Ascorbic acid was

determined by the 2, 6 dichlorophenol-indophenols titrimetric method according to AOAC Method No. 967.21 [9].

Ascorbic acid reduces the 2, 6-dichlorophenol indophenol dye to a colourless leuco-base. The ascorbic acid gets oxidised to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acid medium. Oxalic acid is used as the titrating medium.

Solutions used were, oxalic acid 4%, dye solution (42 mg of sodium bicarbonate and 52 mg of 2,6-dichloro phenol indophenol was dissolved into a small volume of distilled water and made up to 200 mL with distilled water), stock standard solution (100 mg ascorbic acid was dissolved in 100 mL of 4% oxalic acid solution) and working standard (10 mL of the stock solution was diluted to 100 mL with 4% oxalic acid).

5 mL of the working standard solution was pipetted out into a 100 mL conical flask. 10 mL of 4% oxalic acid was added and titrated against the dye ( $V_1$  mL) and end point was the appearance of pink colour which persists for a few seconds. The amount of the dye consumed was equivalent to the amount of ascorbic acid.

One gram sample was mixed in 4% oxalic acid to make 100 mL volume. The solution was filtered and 5 mL of aliquot was taken, added 10 mL of 4% oxalic acid and titrated against the 2, 6-dichlorophenol indophenol dye ( $V_2$  mL) until a pink colour end point was reached which persisted for 15 seconds.

$$\text{Ascorbic acid (mg/100 g)} = \frac{0.5 \text{ mg}}{V_1 \text{ mL}} \times \frac{V_2}{5 \text{ mL}} \times \frac{100 \text{ mL}}{\text{Wt. of the sample}} \times 100 \quad (3)$$

## 2.8 Protein

Micro kjeldhal nitrogen distillation method was used to determine the protein content of mushrooms by estimating the nitrogen content of material and multiplying nitrogen value by 4.38. The nitrogen present in protein or any other organic material is converted to ammonium sulphate by sulphuric acid during digestion. This salt on steam distillation liberates ammonia, which is collected in boric acid solution and titrated against standard acid (0.1 N  $\text{H}_2\text{SO}_4$  or HCl) since 1 mL of 0.1 N acid is equivalent to 1.041 mg of nitrogen [10].

0.2 g of sample was weighed and transferred to micro kjeldhal flask to which 3 g of catalyst mixture (5 part  $K_2SO_4$  +1 part  $Cu_2SO_4$ ) and 10 mL concentrated  $H_2SO_4$  was added and digested at 420 °C for 1 h till it become colourless. Digested material was cooled and transferred to a distillation flask and diluted with 40 mL of distilled water. One sample was kept blank without test material. Ammonia was distilled by adding 10 mL of 40% NaOH and ammonia librated was received in a 4% boric acid containing methyl red and methyl blue indicator. Amount of ammonia librated was determined by titrated with 0.1 N HCl. The per cent nitrogen was calculated by using following equation.

$$\text{Nitrogen content (\%)} = \frac{(\text{Sample T.V.} - \text{Blank T.V.}) \times \text{Normality of HCl} \times 14}{\text{Weight of the sample (g)}} \times 100 \quad (4)$$

where,

Sample T.V. = Sample titre value (mL)

Blank T.V. = Blank titre value (mL)

## 2.9 Microbiological Analysis

Microbiological properties of sliced mushrooms before and after freeze drying and during storage were determined by total plate count, mould count and yeast count according to method described by AOAC [11].

### 2.10 Total Plate Count

Media was prepared with appropriate amount of plate count agar solution by autoclaving at 121 °C at 15 psi for 15 min. Then, 15 mL sterilized media was poured into the petri dishes and allowed to solidify in aseptic condition of laminar air flow chamber (Clean air, CAH 600). About 1 mg sample was transferred and spread over the solidified media plates. Then, the plates were kept in bacteriological incubator (Kemi, KIMS-6) at 37 °C for 48 h. The number of colonies were counted with digital colony counter and total plate count was determined in terms of cfu  $mg^{-1}$  using following equation.

$$\text{Colony forming unit (cfu/mg)} = \frac{\text{Number of colonies}}{\text{Dilution factor} \times \text{Volume plated}} \quad (5)$$

### 2.11 Mould Count

Media was prepared with appropriate amount of potato dextrose agar solution by autoclaving at 121 °C at 15 psi for 15 min. Then, 15 mL sterilized media was poured into the petri dishes

and allowed to solidify in aseptic condition of laminar air flow chamber. About 1 mg sample was transferred and spread over the solidified media plates. Then, the plates were kept in bacteriological incubator at 25 °C for 5 days. The number of colonies were counted with digital colony counter and mould count was determined in terms of cfu  $mg^{-1}$  using the Eq. 5.

### 2.12 Yeast Count

Media was prepared with appropriate amount of yeast extract peptone dextrose agar solution by autoclaving at 121 °C at 15 psi for 15 min. Then, 15 mL sterilized media was poured into the petri dishes and allowed to solidify in aseptic condition of laminar air flow chamber. About 1 mg sample was transferred and spread over the solidified media plates. Then, the plates were kept in bacteriological incubator at 25 °C for 5 days. The number of colonies were counted with digital colony counter and yeast count was determined in terms of cfu  $mg^{-1}$  using the Eq.5.

### 2.13 Statistical Analysis

Statistical analysis of all experimental data was carried out by the following standard procedures. Factorial completely randomized design (FCRD) was used and the effect of different independent variables on the dependent variables was analysed. Operational statistics (OPSTAT) software was used to analyse the data. Data presented to software were the averages of the results of three replicates with a standard error of less than 5%.

### 2.14 Packaging of Freeze-Dried Mushroom Slices

Freeze-dried mushroom slices were packed in pouches ( $25 \times 17 \text{ cm}^2$ ) made out of aluminium foil and then subjected for storage studies. All the pouches were sealed to make them leak proof and kept inside the jip lock pouches. The packed samples were kept for storage studies under room temperature ( $30 \pm 5 \text{ }^\circ\text{C}$ ) and the analysis was carried out at regular intervals of one week.

## 3. RESULTS AND DISCUSSION

### 3.1 Porosity

Porosity decreased in all samples during storage. The porosity of dried mushroom slices varied in the range of 82.57% to 73.25% after 28<sup>th</sup> day of

storage (Fig. 1). The lowest porosity was observed in 8 mm thick sample dried at 10 °C and highest was observed in 2 mm thick sample dried at 40 °C. This may be due to slight absorption of moisture during the storage.

### 3.2 Colour

It was found that the L\* values decreased with increase in storage period. The change in the L\* value of dehydrated samples was taken as a measurement of brightness during storage. The highest L\* value was observed as 50.57 in 2 mm thick sample dried at heating plate temperature of 10 °C whereas, the lowest value of 48.12 was observed in 2 mm thick sample dried at heating plate temperature of 40 °C after 28<sup>th</sup> day of storage (Fig. 2). Comparable trend was also noticed for white button mushrooms in an earlier investigation [12].

### 3.3 Water Activity

Water activity is an important factor in product preservation because physico-chemical and microbiological alterations that occur during processing and storing of a food product depend on it. Freeze dried mushroom slices had moisture content below 5.41% and water activity less than 0.311.

Water activity increased in all samples during storage. The water activity of dried mushroom slices varied in the range of 0.312 to 0.412 after 28<sup>th</sup> day of storage (Fig. 3). The lowest water

activity was observed in 2 mm thick sample dried at 40 °C and highest was observed in 8 mm thick sample dried at 10 °C. Water activity of freeze dried mushroom slices remained less than 0.6 in all the cases and hence, the samples were deemed to be safe from common microbial damage. Many research studies support that increasing the storage will increase the water activity [13,14].

### 3.4 Firmness

Studies indicated that there was a loss in firmness of mushroom slices irrespective of drying temperature and slice thickness throughout the storage period. The firmness force values decreased from 2.03 to 1.65 N, 5.55 to 4.74 N, 6.50 to 5.74 N and 8.98 to 8.54 N from the first day of storage to 28<sup>th</sup> day of storage for 2, 4, 6 and 8 mm thick slices dried at a heating plate temperature of 40 °C (Fig. 4). Similar reduction was also observed in remaining samples dried at a heating plate temperature of 30, 20 and 10 °C. The lowest firmness of dehydrated sample was observed as 0.98 N in 2 mm thick sample dried at heating plate temperature of 10 °C and highest was 8.54 N in 8 mm thick sample dried at heating plate temperature of 40 °C after 28<sup>th</sup> day of storage. Comparable trend was also noticed for white button mushrooms in an earlier investigation [12]. It was observed that samples became softer and had more soft texture due to increase in their moisture content during storage.

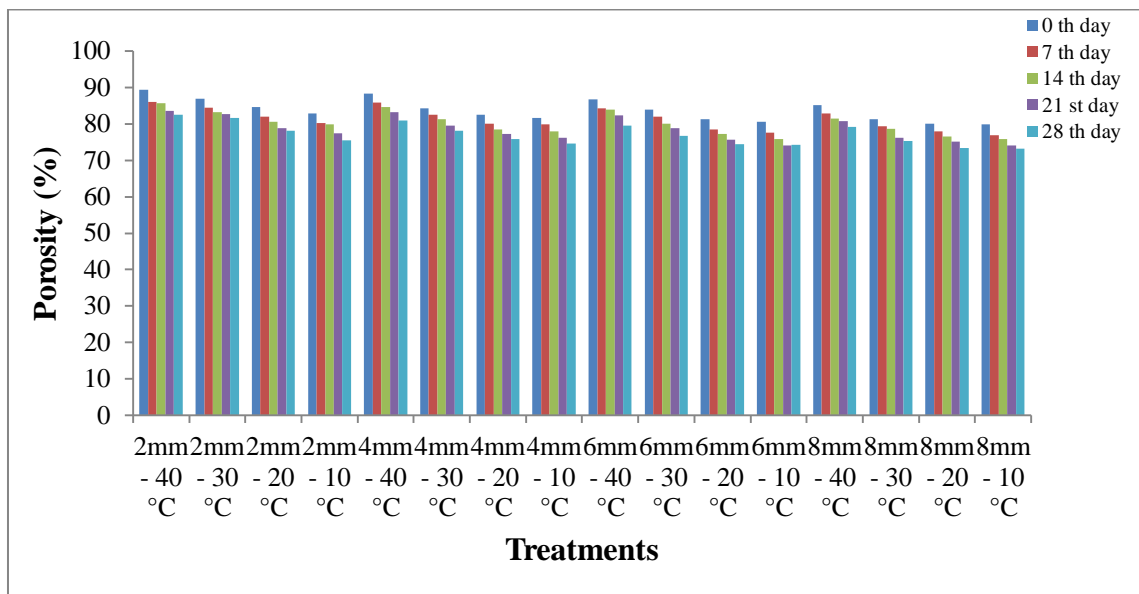


Fig. 1. Effect of storage period on porosity of freeze dried mushroom slices

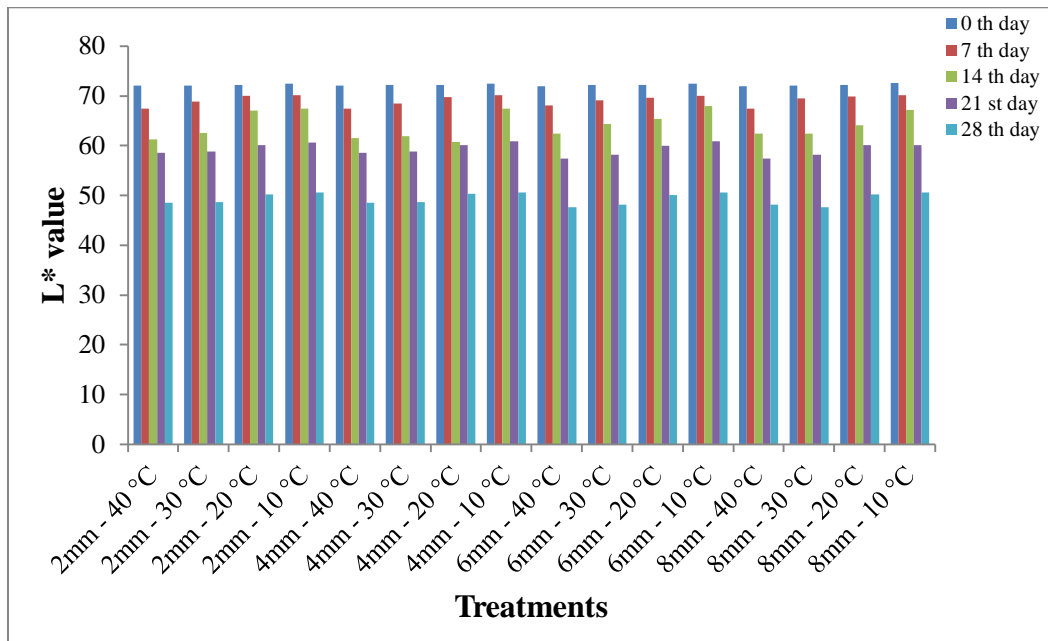


Fig. 2. Effect of storage period on L\* values of freeze dried mushroom slices

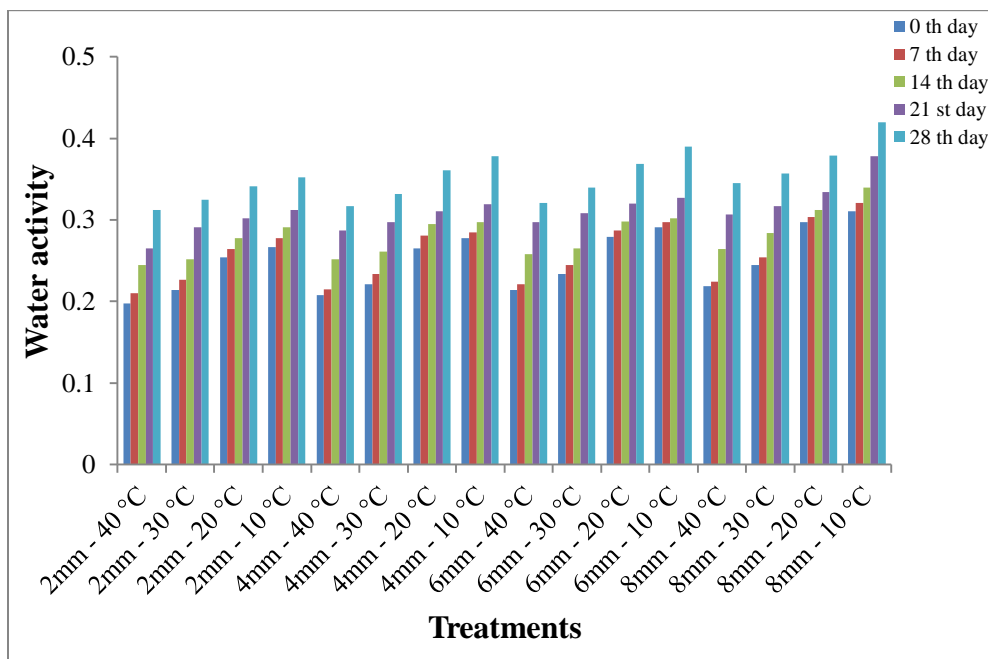


Fig. 3. Effect of storage period on water activity of freeze dried mushroom slices

### 3.5 Rehydration Ratio

The rehydration tests were conducted to evaluate the reconstitution qualities of dehydrated samples. In this study it was observed that rehydration ratio reduced with increase in storage period from first day of storage to 28<sup>th</sup> day of storage in all the samples (Fig. 5). This must be due to permeability of

moisture in packages during storage of freeze dried mushroom slices.

The lowest rehydration ratio of stored sample was observed as 4.04 in 8 mm thick sample dried at heating plate temperature of 10 °C and highest was 5.541 in 2 mm thick sample dried at heating plate temperature of 40 °C after 28<sup>th</sup> day of storage. Similar trends were observed for mushroom flakes [13].



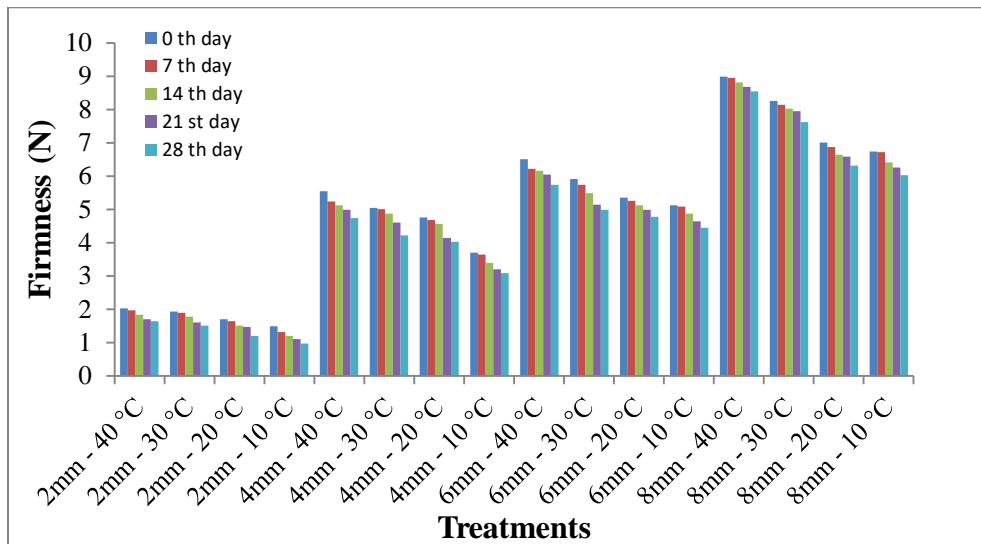


Fig. 4. Effect of storage period on firmness of freeze dried mushroom slices

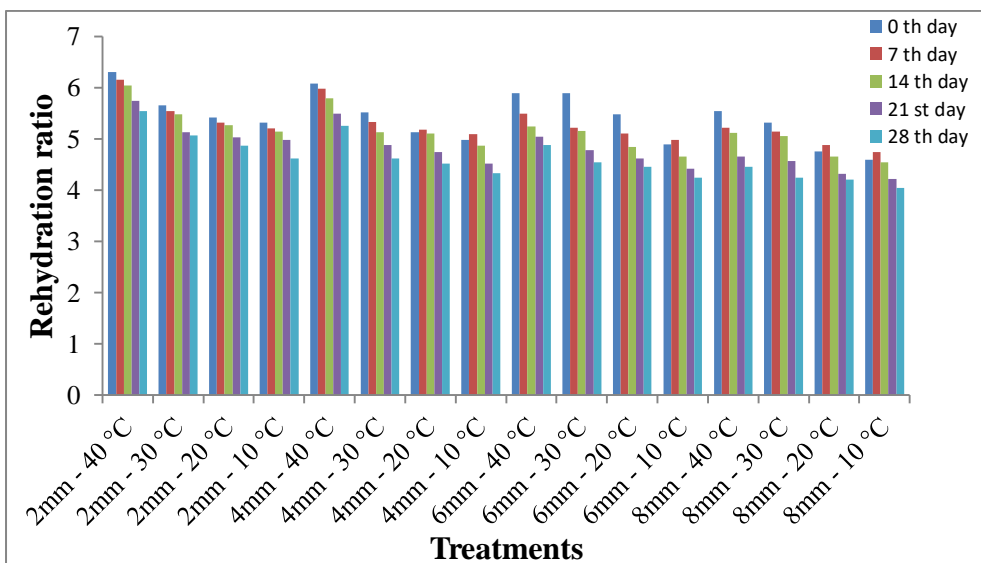


Fig. 5. Effect of storage period on rehydration ratio of freeze dried mushroom slices

### 3.6 Ascorbic Acid

The amount of vitamin C decreased in all samples during storage (Fig. 6). This was probably due to permeability of moisture inside the packaging material and oxidation reaction must have taken place in packages during storage of mushroom slices. Villota and Hawkes [15] had reported the destruction of vitamin C during thermal processing and storage.

The lowest ascorbic acid content of 14.47 mg/100 g was observed in 2 mm thick sample dried at heating plate temperature of 40 °C and

highest 22.98 mg/100 g for 8 mm thick sample dried at heating plate temperature of 10 °C after 28<sup>th</sup> day of storage respectively.

### 3.7 Protein

Protein content decreased with increase in the storage period. The lowest protein content of 19.15% was observed in 2 mm thick sample dried at heating plate temperature of 40 °C and highest protein content of 26.89% for 8 mm thick sample dried at heating plate temperature of 10 °C after 28<sup>th</sup> day of storage (Fig. 7). Eze and Akubor [16] had reported similar results for oven dried okra samples.



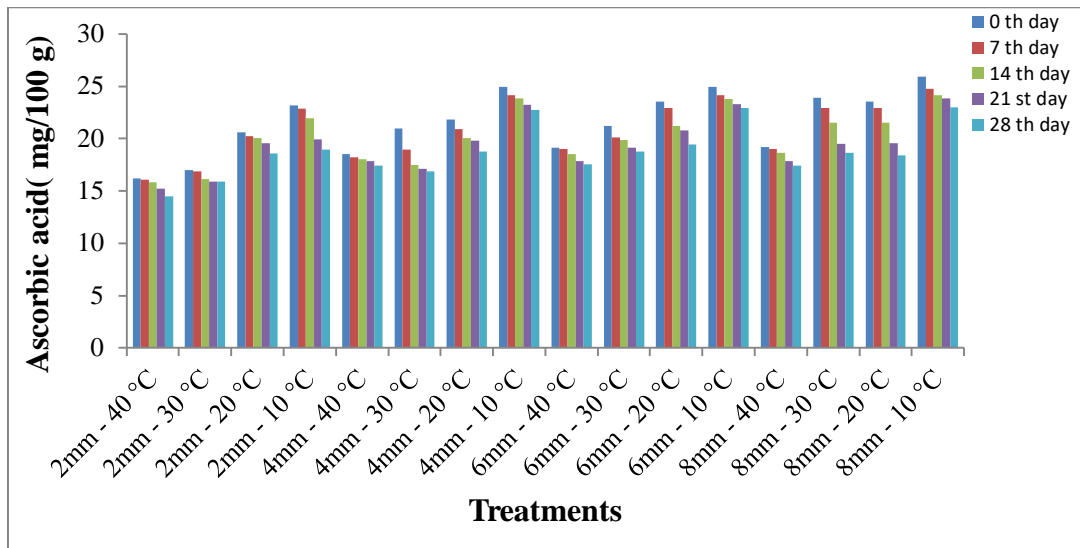


Fig. 6. Effect of storage period on ascorbic acid of freeze dried mushroom slices

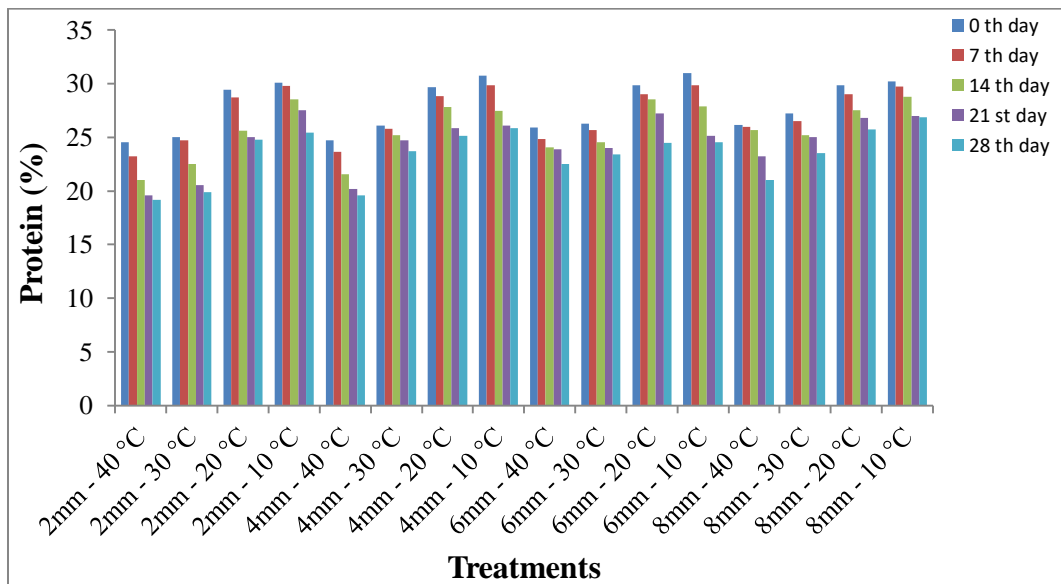


Fig. 7. Effect of storage period on protein of freeze dried mushroom slices

### 3.8 Microbiological Quality

Microbial count of the freeze dried stored product was nil during the first three weeks of storage, where as in the fourth week negligible growth was observed. Hence, the freeze dried mushroom slices are fit for consumption even after storage of four weeks at room temperature. Similar results were reported for mango powder [17].

### 4. CONCLUSIONS

Increase in the storage period resulted in decrease in porosity (73.25%), colour L\* value

(48.12), firmness (0.98 N), rehydration ratio (4.04), ascorbic acid content (14.47 mg/100 g) and protein content (19.15%), whereas the water activity (0.412) increased with the storage period. Microbial analysis indicated by yeast count, mould count and total plate count was nil during the first three weeks of storage, where as in the fourth week negligible growth was observed.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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