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Antioxidant and antimicrobial analysis of spreadable cheese incorporate with button mushroom powder

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Abstract---The present study aimed at the determining the physio-chemical characteristic of fortified cheese prepared from cow milk and to study about microbial analysis of spreadable cheese fortified with mushroom powder. Also, Fourier transform infrared (FTIR) Spectroscopy was used to identified the functional group present in fortified cheese. Obtained the result was PH value of T0 (5.24%) and T1 (5.28%), the Titrable acidity value of T0 (0.28%) and T1 (0.42%), while the content of salt was T0 (0.69%) and T1 (0.87%). The fat value was T0 (28.09%) and T1 (32.22%) and the content of protein was T0 (22.45%) and T1 (36.43%). During examine shelf-life major components like a protein, fat, TSS, ash has major changes to microbial growth. At the 21 days storage period the self-life of fortified cheese was maximum to control spreadable cheese sample. Mushroom powder incorporated with spreadable cheese was prevent from microbes and help to enhance the self-life of fortified cheese, so the spreadable fortified cheese stored for 21 days. This result indicate that our fortified cheese sample was reduce the microbial growth.

Keywords---physio-chemical, FTIR spectroscopy, microbial analysis, fortified cheese, shelf-life.

Introduction

Nowadays, After COVID-19 Pandemic, People demands for healthy food and immunity boost food products. So, Food industry has to manufacture product with health benefits. In the last years post COVID-19, People has been demanded for functional or fortified foods due to their beneficial health effects. Fortified food has fulfilled the basic nutrients and have a potential positive impact on people health [1].

In general, Milk and dairy products have turn out to be a major part of the human food plan in many countries over many years. About a 3rd of the world's milk production is used in cheese manufacture and cheese is highly nutritious food, which is convenient and versatile and gives a variety of flavors and textures. In the world's context, cheese is highly demanding dairy product. The cheese market in India exhibited strong growth during 2015 – 2020[17]. Cheese is wealthy within the fats and casein parts of milk, which can be retained within the curd all through manufacture, and it Carries exceedingly small amounts of the water-soluble constituents (whey proteins lactose, and water-soluble nutrients), Which partition especially into the whey. Like most dairy Merchandise, cheese is a wealthy supply of minerals, protein, vitamin, Fats and carbohydrate [2]. There are hundreds of different types of cheese to be had throughout the globe and the unhappy element isn't one sort of categorization can encompass these types of cheese types. Cheese may be Classified on the basis of their united states of foundation, fats content material (milk type), and texture, production approach, physical Look like form or moisture content material. In this newsletter we May have a have a look at a few normally used cheese varieties and examine greater about them [3].

Mushrooms have been proved to be one of the most effective resources producing a massive and diverse range of bioactive metabolites with tremendous antimicrobial activities. The medicinal bioactive compounds gift in mushroom consists of: polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, tri-terpenoids, lectins, lipids and their derivatives. The antimicrobial residences of positive mushrooms offer human disorder manipulate that is normally safe and effective. Numerous mushrooms have verified green antibacterial activity as well as antifungal hobby against resistant human pathogens. White button mushroom has been explored to combat easy and more than one drug resistant isolates of Eschrichia coli, Staphylococcus epidermidis, S. Aureus and species of Candida Streptococcus, Enterococcus [4,5,6].

Mushrooms have a great potential because of having high and right quality proteins (20 to 40% on dry weight basis), nutrients (nutrition B- complicated) and minerals. Mushrooms may be dried and transformed into powdered form, which can be used for fortification in baked merchandise like cheese, bread, biscuits, and so forth [16]. The study was undertaken the see of the effect of fortification of mushroom powder on spreadable cheese, to examine the chemical analysis of spreadable cheese fortified with mushroom powder and study about microbial analysis of spreadable cheese and fortified cheese. The main aim of the

study was to know about effect of storage in the chemical composition of fortified cheese

Materials and Methods

The experiment was carried out at the laboratory of Food Science and Technology, Babasaheb Bhimrao Ambedkar University Lucknow. In this research 2 liter milk used as raw material. The edible mushroom (button mushroom), vinegar (acetic acid), salt and pepper powder taken for research were purchased from the local market of the south city Lucknow

Preparation of mushroom powder and spreadable cheese fortified with mushroom powder

Mushroom was cleaned, cut into slices and after put it solar drying for 2 days and finely powdered. The development of spreadable cheese, milk was standardized to 4.2% fat and pasteurized to 72°C for 15 seconds, then after stored overnight at a cooling at less than 10°C temperature. The next day milk was warmed to below 20°C. When the milk temperature rise to 50°C, add 150ml acetic acid with continuous agitation to pH-5.9. Then mix thoroughly with a stirrer. After 5-10 minutes a firm curd was obtained then stirring was done. After that whey was drained and curd was washed in tap water for 5-10 minutes, molded to shape then after grinding cheese in mixer. Prepared spreadable cheese (200gram) incorporated with 45gram mushroom powder. Spreadable fortified cheese was packed in air tight glass container.

Chemical Analysis

The chemical analysis of laboratory of food science and technology made spreadable cheese were carried out to determine the contents, moisture, ash, fat and protein according to AOAC [7] methods.

Determination of Acidity

For determination of titrable acidity, the titrimetric method was used according to the AOAC [7]. 5g of sample take and dispersed in 100 ml of distilled water in 250 ml conical flask, after which six drops of phenolphthalein indicator have been brought. The sample turned into then titrated with 0.1N sodium hydroxide until a stable pink color changed into fashioned. The titrable acidity changed into expressed as % lactic acid from the subsequent formula.

$$\text{Titrable acidity (\%)} = \frac{\text{ml of Na OH} \times T \times \text{dilution factor} \times 100}{\text{Weight of sample} \times 1000}$$

Where T: is the amount of lactic acid reacted with 1.0 ml of 0.1N sodium hydroxide.

Determination of PH

The pH of mozzarella cheese samples were determined using digital pH meter (MT-103). The pH meter was calibrated with buffers of pH 4 and 7. The cheese samples were stirred and the pH value was recorded according to AOAC [7].

Determination of TSS

The Total solid content of the spreadable cheese samples become determined in according to AOAC [7]. Three grams of samples have been weighed into a dry easy crucible, and then heated in water bath for 10- 15 minutes. The dish was then placed in an oven at 80°C in a single day (16 hours) cooled down in desiccators and weighed. The total solid content was calculated from the subsequent equation:

$$\text{Total solid content (\%)} = W1 \times 100/W2$$

Where:

W1 = weight of sample after drying

W2 = original of sample

Determination of Salt

The salt content of Spreadable cheese samples and fortified cheese sample changed into determined according to AOAC, [7]. Where in five gm samples have been weighed and placed into 250 ml conical flask, additionally 100 ml boiling water were brought. Then, swirled for 10 min and cooled to 50 - 55°. Titrated against silver nitrate until the colour of indicator potassium chromate changed from faded yellow to buffered color and titration determine changed into recorded (V). Calculation of salt % w/w as follows:

$$\text{Salt \% w/w} = 58.45 \times V \times N \times 100/W \times 100$$

N = Normality of silver nitrate.

W = weight of sample.

V = weight of silver nitrate.

FTIR analysis of fortified cheese

The functional group of fortified cheese is determine by analysis using Fourier transform infrared (FTIR) spectrometer. FTIR analysis was performed at instrument research Centre of Babasaheb Ambedkar university in Lucknow (UP). The FTIR was Model no is Nicolet 6700, Make: Thermo-scientific, USA. The spectral range is 4000-500cm⁻¹. Sample with practical size <0.15mm was employed for analysis.

Microbiological analysis

Sample of spreadable cheese were prepared for microbiology analysis according to (Marshall, 1992)[8] the method described in the standard methods. Control cheese sample and Fortified sample was examined for the standard plant count method at $37 \pm 1^\circ\text{C}$ for 24, 48 and 72 hrs interval. The result expressed as colony forming unit (CFU)/g. the media used were in a dehydrated form and prepared according to the manufactures instruction. Total viable bacteria were enumerated by pour plate method using standard plate count agar method [9,10] and plate were incubated at 37°C for 24 hours.

Determination of antioxidant activity of Fortified Cheese

The antioxidant activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) is calculated via spectrophotometer (Tekao et al. 1994)²¹ with small modifications (Kumarasamy et al. 2007)²². In methanol, the color of DPPH is dark blue. In its reduced form, the antioxidant compound changes color from purple to yellow, allowing DPPH to gain electrons. DPPH shows strong absorption at 517 nm, determined by 2, 2-diphenyl-22-pyridyl hydroxylase (DPPH). Briefly, 0.1 ml DPPH solution was mixed with 1ml of Fortified cheese prepared in various concentration (4,8,12,16 $\mu\text{g/ml}$). A control sample of 1 ml of methanol was prepared and incubated in darkroom for 30 minutes at ambient temperature. After incubation, the absorbance of the sample was read at 517 nm using a UV Visible spectrophotometer methanol use as a blank. Reduction in the absorbance value, shows high activity in scavenging free radicals (Srishti Tripathi and Sunita Mishra)¹⁹.

It was measured as a percentage of DPPH scavenging activity by using the following formula given below:

$$\text{DPPH scavenging activity} = (A_{\text{control}} - A_{\text{Sample}}) / A_{\text{control}} \times 100$$

Note: The test tube was covered with brown paper as DPPH is very sensitive to light.

Statistical Analysis

Data obtained were assessed by analysis of variance (ANOVA) as shown by Sendecor and Cochran [11] and means were compared using Duncan's multiple-range test [12] with probability $p \leq 0.05$.

Result and discussion

Chemical composition of the laboratory made spreadable cheese and fortified cheese product

Figure (1) show the PH value of control cheese sample and fortified cheese. The PH of the control cheese was 5.24 ± 0.1 , this value is similar to that[2] (Moneim, 2012), who reported a value $5.2 \pm$ in cow's milk mozzarella cheese. The PH of the fortified cheese was 5.28 ± 0.1 . There is no significant difference was found in PH of control and fortified cheese sample. Figure (1) also shows the results to acidity as lactic acid for spreadable cheese fortified with mushroom powder. The titrable

acidity of control cheese sample was $0.28 \pm 0.01\%$, this value was higher than [2], who reported value is $0.203 \pm 0.01\%$ in cow milk mozzarella cheese. The titrable acidity value of fortified cheese was $0.42 \pm 0.01\%$. However little bit difference was found in fortified cheese sample to control sample.

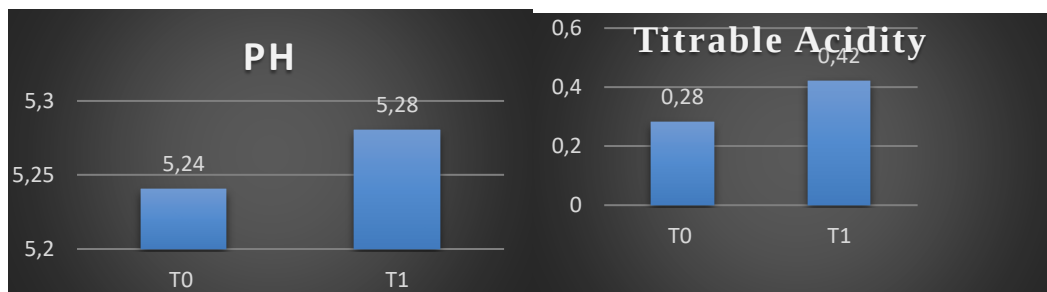


Fig (1) The PH value and Titrable acidity of control cheese sample (T0) and fortified cheese (T2)

Fig (2) shows the salt content during of control sample of spreadable cheese and fortified cheese. Salt content of control cheese was $0.69 \pm 0.01\%$. However salt content of fortified cheese was $0.87 \pm 0.01\%$ was significantly higher than control cheese sample. Fig (2) also show the total solid content of fortified cheese and control cheese sample. The total solid content of control cheese sample was $44.01 \pm 0.002\%$ and fortified cheese sample was $48.65 \pm 0.003\%$ which higher than control cheese sample.

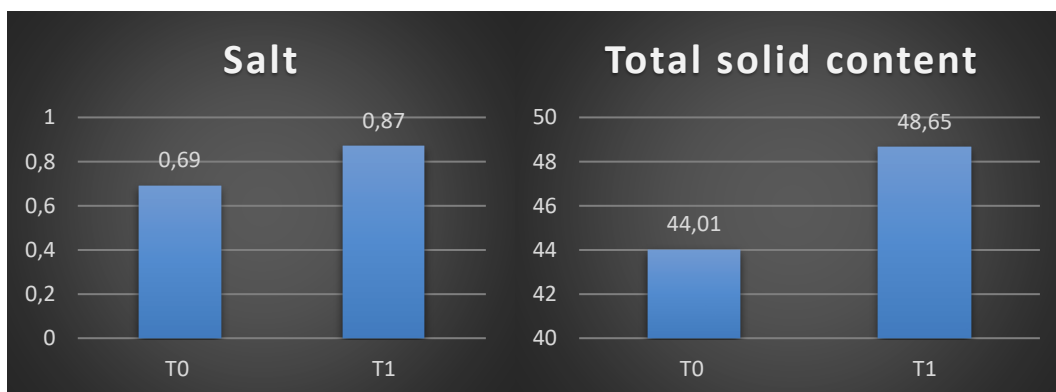


Fig (2) show the salt and total solid content of fortified cheese and control cheese sample

Fig (3) show the moisture content of spreadable cheese and fortified cheese. The moisture content of control spreadable cheese was $52.36 \pm 0.001\%$ which is lower value of 52.67% obtained by [13] and higher than the value of $49.866 \pm 0.01\%$ obtained by [2]. The moisture content of fortified cheese was $58.66 \pm 0.01\%$ which is higher value of control spreadable cheese.

Ash content of spreadable cheese and fortified cheese as illustrated in fig (3). The ash content control cheese sample was $2.72 \pm 0.001\%$ which is higher value than of 2.23% obtained by [13] and also higher value than CMMC of $2.04 \pm 0.01\%$

obtained by[2]. The ash content of fortified cheese was $3.95\pm0.01\%$ which higher value of control spreadable cheese.

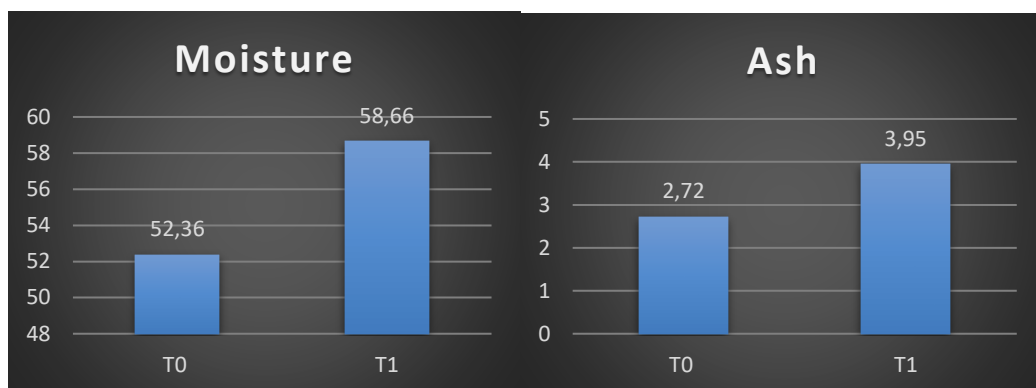


Fig (3) show the moisture and ash content of spreadable cheese sample (T0) and fortified cheese (T1)

Fig (4) show the protein content of spreadable cheese and fortified cheese product. The protein content of spreadable control cheese was $22.45\pm0.01\%$ which is higher value than of 22.28% obtained by[13]. The protein content of fortified cheese was $29.08\pm0.01\%$ which is higher than value of spreadable control cheese product. The fat content of spreadable cheese in fig (4) was $28.9\pm0.05\%$ higher than value obtained by[13]who found that fat content of mozzarella cheese were (24.67%). The fat content of fortified cheese was $32.22\pm0.01\%$ which is higher vale than control spreadable cheese.

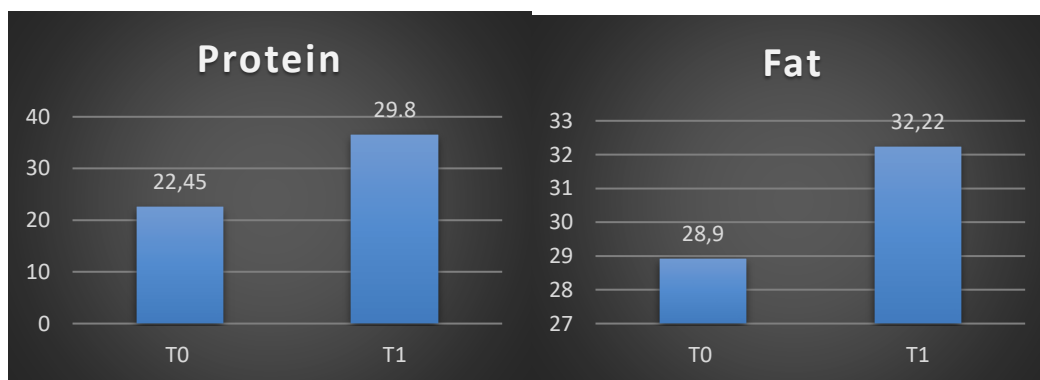


Fig (4) Show the protein and fat content of spreadable cheese (T0) and fortified cheese (T1)

FTIR analysis of fortified cheese

The infrared spectrum of fortified is proven in fig.5. Consequently, the peak at 3455.3 cm^{-1} where represent O-H stretching within the hydroxyl groups. This area in our result turned into the range of 3600 to 3700. The interval of $3000\text{--}2800\text{ cm}^{-1}$ from FTIR spectra offers the absorption bands which characteristic to symmetrical and asymmetrical vibration in fatty acid [14]. The interval $1800\text{--}1600\text{ cm}^{-1}$ constitute C=O of acids and ester and

exhibited versions within the wave number variety (1744.7 cm^{-1} , 1655.4 cm^{-1}) along with absorption from ester of fatty acid this can be attributed to differences in the degree rate of lipolysis at special ranges of ripening [14]. The interval $1600\text{--}1390\text{ cm}^{-1}$ represent Amide I and amide II of protein. This area in our end result became in the variety of 1542.8 cm^{-1} , 1459.6 cm^{-1} as shown fig 5. The interval $1200\text{--}800\text{ cm}^{-1}$ represent bands feature to the C-C hyperlink and to the vibration link C=O [14,15].

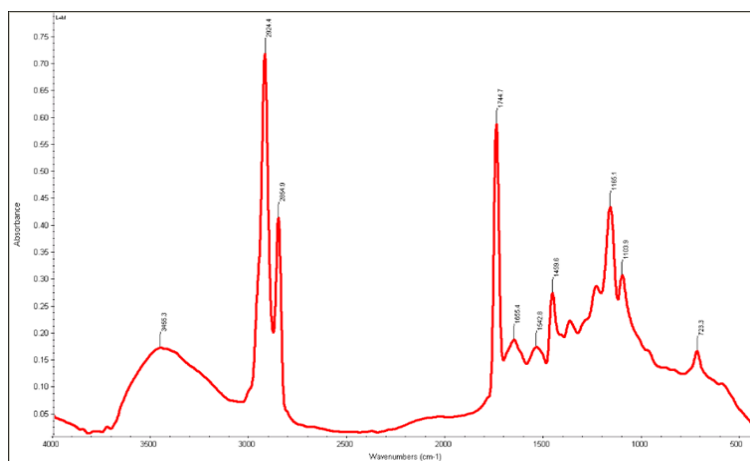


Fig 5. FTIR spectrum of fortified cheese

*peak at 3455.3 cm^{-1} , 2924.4 cm^{-1} , 2854.9 cm^{-1} , 1744.7 cm^{-1} , 1655.4 cm^{-1} , 1542.8 cm^{-1} , 1459.6 cm^{-1} , 1165.1 cm^{-1} , 1103.9 cm^{-1} , 723.3 cm^{-1} .

DDPH Activity of Fortified cheese

Cheese product have antioxidant properties so considered a good choice for the the growing of its natural antioxidant by fortification by mushroom powder. DPPH is the most suitable way to determine the antioxidant property of a sample (Tripathi 2021)²⁰. The colour of the sample changes from purple to yellow as DPPH free radicals are scavenged by antioxidant chemicals. Figure 3.3.1 depicts the relationship between fortified cheese sample concentration (mg) and antioxidant activity (%). Figure 3.3.1 revealed that fortified cheese treatment has value higher than control, and this value increased by increasing concentration of solution. The obvious antioxidant properties of fortified cheese could be as consequence of high content of antioxidant component in mushroom powder.

By using a spectrophotometer, the optical density of a sample and the optical density of the control can be calculated to determine DPPH value a sample. According to (Fridianny et al. 2014)²³, if DPPH value is below $50\text{ }\mu\text{g/ml}$ it has a very strong antioxidant property, if it lies between $5\text{--}10\text{ mg/ml}$ it has strong antioxidant property and if it is above 20.048 mg/ml it has weak antioxidant property. The antioxidant activity of fortified cheese sample at different concentrations ($4, 8, 12, 16\text{ }\mu\text{ml}$) was evaluated and the result obtained were illustrated in Fig. 3.3.1. According to these result, spreadable cheese concentration increases up to 23.960 mg .

The equation generated on the above curve was used to determine the IC₅₀ value, where y was replaced with 50 and value of x was calculated which indicated the concentration of extract at which 50% DPPH radicals were scavenge.

Obtain extracted solution (ml)	Concentration mg/ml	Absorbance (A)	Inhibition %	ic50
Control	0	0.409		
1	4	0.392	4.156	0.320984
2	8	0.360	11.980	4.36205
3	12	0.327	20.048	1.50652
4	16	0.311	23.960	2.099289

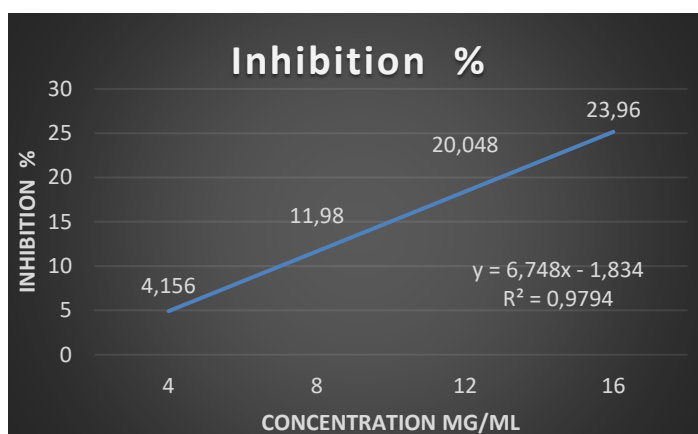


Fig: 3.3.1 shows DDPH activity of fortified cheese

Microbiology analysis of spreadable fortified cheese

Changes in microbiological quality during storage of spreadable cheese fortified with mushroom powder. During examine shelf life major components like a protein, fat, TSS, ash has major changes to microbial growth. As we shown during shelf life period treatment T0 was more contaminated compared to T1. Mushroom powder incorporated with spreadable cheese was prevent from microbes and help to enhance the self-life of fortified cheese, so the spreadable fortified cheese stored for 21 days

Table (3.4.1.) the microbiological quality (log₁₀cfu/gm) of spreadable cheese during storage period from manufacture under study

Microbiology count					
(log ₁₀ cfu/gm)					
Total bacteria count					
Storage period					
Treatment	24hrs	48hrs	72hrs	15 days	21 days
T0	00	2.15	2.45	3.45	3.76

T1	00	2.61	2.75	2.97	3.15

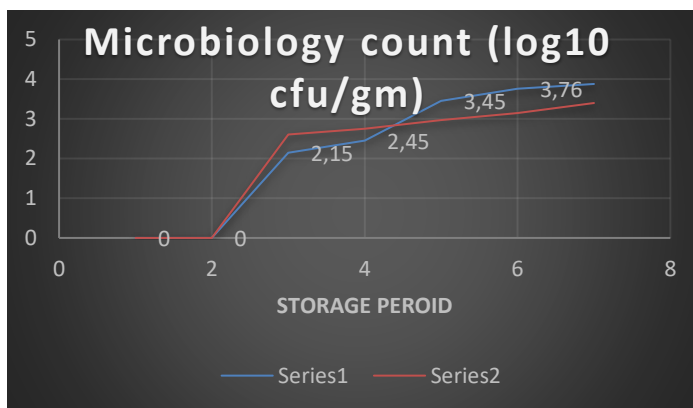
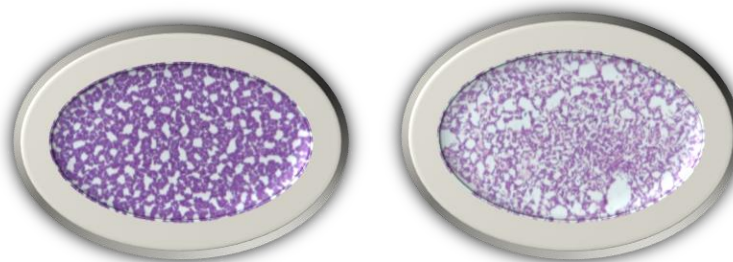
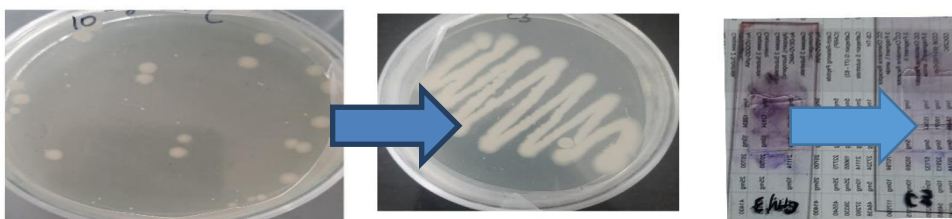


Fig (3.4.1) show microbiological count (total bacterial count) storage period and identify the bacteria in spreadable cheese and fortified cheese



Control cheese (T0)



Fortified cheese (T1)

Self-life evaluation of spreadable cheese table 6. Showed different growth of micro-organism at 21 days storage. Microbial growth in the sample T0 was (3.76cfu/g) and sample T1 was (3.15cfu/g). and Fig (6) shown the sample control cheese was shown gram stain of L.GARVIEA SRB NIV-1 stain with clusters and short chains of gram-positive cocci and staphylococcus epidermids and E.coli might be present in control cheese sample. And In fortified cheese samples slide shown gram positive. Its rod shaped bacterium shown so Eisco Clostridium Botulinum may be present in fortified cheese. Microbial growth of T0 did show maximum number of total plate count (TPC) in the sample T1. This result indicate that our fortified cheese sample was reduce the microbial growth

Conclusion

The spreadable cheese was successfully developed by enriched mushroom powder. The fortified cheese gave the highest PH and total solid throughout all the storage period. However, the moisture content, fat, protein, Titrable acidity and salt recoded the highest content as compare to spreadable cheese during at 21 storage period. The enriched improved the nutritional value of spreadable cheese. The microbial load to develop fortified cheese was decrease due to incorporation of mushroom powder. Mushroom powder incorporated with spreadable cheese was prevent from microbes and help to enhance the self-life of fortified cheese, so the spreadable fortified cheese stored for 21 days. This result indicate that our fortified cheese sample was reduce the microbial growth and this study is that the fortified cheese has good antioxidant activity and antimicrobial properties. Thus, the incorporating mushroom powder in the product could be beneficial from nutritional, microbial and health perspective.

Declaration

The author declare that they have no known competitive financial interests or personal relationship that may affect the work reported in this paper.

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Competing interests

Author have declared that no competing interests exist.

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