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# Quality Characteristics of Traditional Hard Cheese (Oggtt) Packaged in Different Packaging Materials and Stored at Ambient and Refrigeration Temperature

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**Abstract:** This study, investigated the effects of ambient temperature (28±2°C) and refrigeration temperature (4±2°C) and packaging materials (LDPE, HDPE and PET) on the quality characteristics of fermented dried goat milk product, oggtt. Samples were analyzed for changes in pH, titratable acidity (% lactic acid), total viable bacteria count, lactic acid bacteria count, coliform bacteria count and yeast and mold count at 10 days interval during storage period. Temperature significantly ($p<0.05$) affected the titratable acidity (%), pH, total viable bacteria count and yeast and mold count and packaging significantly ($p<0.05$) affected the titratable acidity, total viable bacteria count and lactic acid bacteria count. However, titratable acidity (%), total viable bacteria count, lactic acid bacteria count and yeast and mold count significantly ($p<0.05$) increased and pH significantly ($p<0.05$) decreased during storage. Increase in CFU/g of total viable bacteria and lactic acid bacteria were more at 28±2°C as compared to 4±2°C. A direct relationship between microbial growth and titratable acidity was found. The samples packaged in PET and stored at refrigeration temperature (4±2°C) were more acceptable.

**Key words:** Packaging materials % Storage period % Refrigeration storage % Ambient temperature % Dairy products

## INTRODUCTION

Milk and dairy products have become the most important part of human's diet in various countries. Dairy products are extremely popular and the people are incorporating more dairy products in their daily diets due to its durability in hot weather compared with milk as well as for its convenience and nutritive value. Increasing shelf life has been the main purpose of dairy industry for many years to meet the demands for increasing distributions times and distances [1]. Oggtt is a local hard and sun dried cheese like dairy products made from goat's and/or sheep's milk by desert dwellers of Saudi Arabia [2]. The production of oggtt enables the surplus amount of milk in rural areas in Middle East to be preserved. The traditional production of oggtt is still of village technology and standardized protocol is not available. Fermentation and churning of fermented milk are basic steps involved in the preparation of oggtt. It is commonly hawked along the streets or displayed in the market. It has unique flavor which is formed as a result of fermentation process. It is known for its high calcium and protein content [2] making it an important food in the diet for both young and old people. It is important that manufacturer and food handlers keep food safe from pathogenic microorganisms as food eaten has a direct influence on health [3].

Approximately one fourth of the world's food supply is lost through microbial activity [4]. Milk and milk products serves as an excellent growth medium for a wide range of microorganisms. Microbial growth is dependent on various factors such as temperature, strain of mould, gas composition, water activity, substrate and the presence of chemical preservative and microbial interactions [5]. Right packaging and suitable temperature improves the shelf life and durability of products. So far studies were carried out with regard to quality characteristics and nutritive value of oggtt. However, hardly studies are being focused on the effect of storage temperature on the quality characteristics of oggtt. This study was designed to investigate the effect of ambient (28±2°C) and refrigeration (4±2°C) storage temperature on the quality characteristics of oggtt packaged in different packaging material.

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## MATERIALS AND METHODS

Oggtt and packaging materials (low density polyethylene (LDPE), high density polyethylene (HDPE) and polyethylene tetrapthalate (PET)) were purchased from local market (Saudi Arabia). Oggtt was aseptically weighed (150g/pack) and packaged in selected packaging materials (LDPE, HDPE and PET). Prior to packaging, all packaging materials were sterilized with the aid of ultra violet sterilizer (Liangliang, China). All packs were sealed with sealing machine (Double Leopards, Taiwan). Two sets of samples were prepared and stored in ambient (28±2°C) temperature and refrigeration (4±2°C) temperature respectively.

**Analysis:** Samples were drawn at regular intervals for analyzing physico chemical (pH, acidity) snd microbiological (total viable count, lactic acid bacteria count, coliform bacteria count and yeast and mold count) characteristics. Samples were transferred to a grinder (Philips, Brazil) and ground thoroughly to obtain a uniformly mixed sample which was then used for various physicochemical analyses. For microbiological analysis the packs containing samples were opened aseptically under sterile conditions. After analyzing the products on day 0, the remaining samples were stored at two storage temperature for subsequent analysis.

### Chemical Analysis

**pH:** Ten grams of sample was thoroughly homogenized with 70 ml distilled water in a blender jar (Philips, Brazil) at high speed for 2 minute and homogenate was used to determine the pH. The pH meter (Mettler Toledo, MP 220, Switzerland) was calibrated with standard buffers (4, 7 (BDH Laboratory, England)) before pH was measured.

**Titratable Acidity:** Acidity (% lactic acid) of the samples were determined according to the standard methods for examination of dairy products [6] using phenolphthalein (Riedel DE HAEN AG, Germany) as an indicator. Percent acidity was calculated by using the following expression:

Titratable acidity (%) = 0.0090 x volume of NaOH used x 100/ weight of the sample

**Microbiological Analysis:** Samples were examined according to the method described in the standard methods for the examination of dairy products [6]. Pour plate technique after serial dilutions in peptone water (0.1%w/v) was used in all tests. The first dilution was prepared by blending 25g of sample with 225 ml of 0.1% sterile peptone water and further dilutions were made according to the need. Duplicate plates were used for each dilution. Specifications of microbiological analysis are shown in Table 1.

Table 1: Microbiological Tests with specifications

| S. No. | Tests with specifications |
|---|---|
| 1 | Test: Standard Plate Count (SPC) |
| | Media: Plate count agar (PCA Oxoid, England) |
| | Incubation temperature and time: 32°C (±2°C) for 48 hours |
| 2 | Test: Lactic Acid Bacteria Count (LAB) |
| | Media: MRS agar (MRS Oxoid, England) |
| | Incubation temperature and time: 32°C (±2°C) for 24 hours |
| 3 | Test: Coli form Bacteria Count |
| | Media: Mac Conkey agar (Alpha Chemika, India) |
| | Incubation temperature and time: 32°C (±2°C) for 48 hours |
| 4 | Test: Yeast and Mould Count |
| | Media: Potato dextrose agar* with chloromphenicol** (Scharlau Chemie, Spain* and Fluka biochemika, Switzerland**) |
| | Incubation temperature and time: 25°C (±2°C) for 5 days. |

**Statistical Analysis:** The data obtained was subjected to statistical analysis by conducting analysis of variance (ANOVA), using SPSS (version 9). Significance differences of means were compared using Duncan's multiple range tests and each data in table was presented as average of replicates ± SD.

## RESULTS AND DISCUSSION

Statistically significant ($p<0.05$) decrease in pH at the end of storage was observed although it was not gradual (Fig. 1). Differences were noticed in the pH for treatments of packaging materials, which were in the order PET>HDPE>LDPE. Similar pattern was found in a previous study on oggtt stored at frozen temperature [7]. Decrease in pH of samples stored at ambient temperature (28±2°C) was significantly more ($p<0.05$) as compared to samples stored at refrigeration (4±2°C) temperature. The reason for increase in pH is unclear, however the decrease in pH recorded in all samples of oggtt at the end of storage period is a natural process which may be related to the metabolic activities of associated microbes which may have caused the release of some organic acids and other metabolites [8].

The changes in titratable acidity (% lactic acid) of samples during storage (0 to 30 days) at ambient (28±2°C) temperature and at refrigeration (4±2°C) temperature are shown in Fig. 2. Significant ($p<0.05$) effects of packaging materials were found on the acidity (%lactic acid).

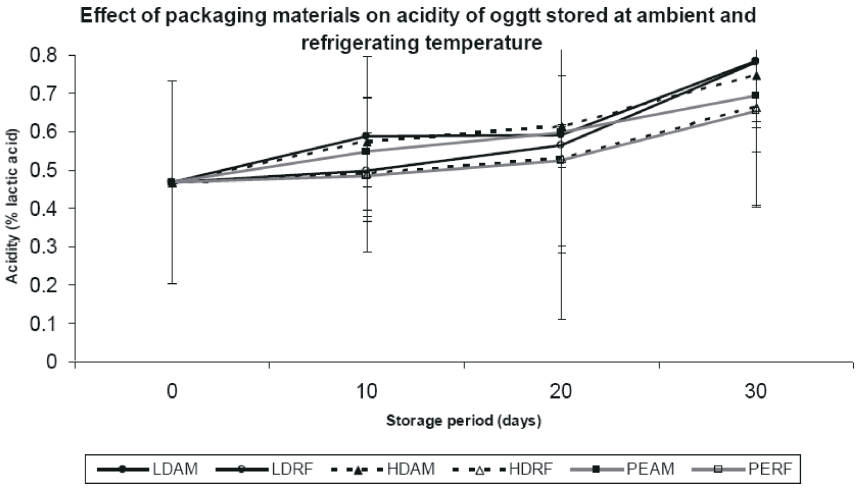


Fig. 1: Effect of packaging materials on acidity of oggtt stored at ambient and refrigerating temperature. Data are mean ± S.D of replicates.

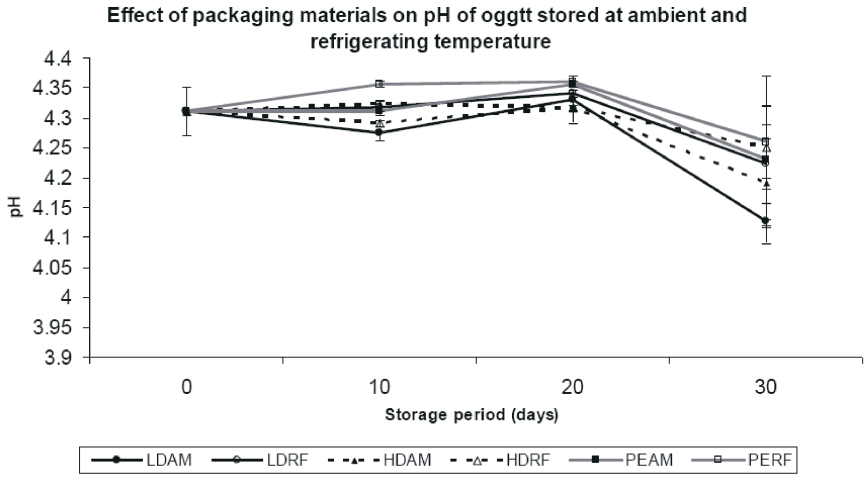


Fig. 2: Effect of packaging materials on pH of oggtt stored at ambient and refrigerating temperature. Data are mean ± S.D of replicates.

Difference in mean was significant ($p<0.05$) between LDPE and HDPE and LDPE and PET, while it was insignificant ($p>0.05$) between HDPE and PET. The titratable acidity of samples packaged in different packaging materials increased significantly ($p<0.05$) at both temperatures with the increase in storage time as compared to the control (value at zero days). The increase in acidity of samples stored at ambient (28±2°C) temperature was significantly ($p<0.05$) more compared to samples stored at the refrigeration (4±2°C) temperature which might be attributed to the fact that storage at ambient (28±2°C) temperature tends to increase the level of lactic acid due to activation of lactic acid forming bacteria by room (28±2°C) temperature, while the low acidity of the cheese stored at refrigerator (4±2°C) might be explained by the fact that low temperature slowed down growth and activity of lactic acid bacteria consequently lowering the rate of acid development [9]. This trend was same for all samples packaged in different packaging materials. However, whether at the room temperature or in the refrigerator, about 45-80% of increase in the acidity was mainly due to lactic acid formed by the generally predominating lactic acid bacteria which converted lactose into lactic acid and in turn increased acidity of oggtt [10,11]. Titratable acidity is calculated in terms of percent lactic acid. Less acidity value as compared to previous studies might be due to heat treatment. Lethal effect of prolong heating on lactic acid bacteria has been also reported earlier [12].

Table 2: Effect of packaging materials on microbiological quality of oggtt stored at ambient and refrigeration temperature

| | LDPE | | HDPE | | PET | |
|---|---|---|---|---|---|---|
| Storage period (days) | Ambient temperature | Refrigerator | Ambient temperature | Refrigerator | Ambient temperature | Refrigerator |
| Standard Plate Count | | | | | | |
| 0 | $1.15\times10^{2}\pm0.0707$ | $1.15\times10^{2}\pm0.0707$ | $1.15\times10^{2}\pm0.0707$ | $1.15\times10^{2}\pm0.0707$ | $1.15\times10^{2}\pm0.0707$ | $1.15\times10^{2}\pm0.0707$ |
| 10 | $3.75\times10^{4}\pm1.9374$ | $2.86\times10^{4}\pm1.0465$ | $2.38\times10^{4}\pm0.6010$ | $3.60\times10^{2}\pm1.6970$ | $5.40\times10^{2}\pm1.1314$ | $1.75\times10^{2}\pm1.3435$ |
| 20 | $1.46\times10^{7}\pm0.0368$ | $1.25\times10^{6}\pm0.0594$ | $2.02\times10^{5}\pm2.5244$ | $4.15\times10^{4}\pm1.6263$ | $2.00\times10^{5}\pm0.0000$ | $1.05\times10^{4}\pm0.0848$ |
| 30 | $9.90\times10^{7}\pm0.7587$ | $4.68\times10^{7}\pm0.7425$ | $7.21\times10^{7}\pm0.5232$ | $1.18\times10^{7}\pm0.0283$ | $9.38\times10^{6}\pm0.5374$ | $4.50\times10^{5}\pm0.9899$ |
| Lactic Acid Bacteria Count | | | | | | |
| 0 | $6.50\times10^{1}\pm0.7070$ | $6.50\times10^{1}\pm0.7071$ | $6.50\times10^{1}\pm0.7071$ | $6.50\times10^{1}\pm0.7071$ | $6.50\times10^{1}\pm0.7071$ | $6.50\times10^{1}\pm0.7071$ |
| 10 | $5.60\times10^{4}\pm0.8486$ | $1.00\times10^{4}\pm0.0000$ | $4.50\times10^{4}\pm0.0071$ | $2.89\times10^{3}\pm0.8555$ | $2.19\times10^{4}\pm0.3535$ | $1.40\times10^{2}\pm0.7071$ |
| 20 | $3.24\times10^{6}\pm0.0000$ | $3.87\times10^{4}\pm0.1626$ | $1.00\times10^{6}\pm0.9899$ | $2.20\times10^{4}\pm0.2828$ | $4.05\times10^{4}\pm0.7071$ | $6.43\times10^{3}\pm6.8942$ |
| 30 | $7.0\times10^{7}\pm1.9799$ | $3.47\times10^{7}\pm0.7779$ | $1.10\times10^{7}\pm1.5132$ | $4.17\times10^{5}\pm1.8809$ | $5.80\times10^{5}\pm1.4566$ | $1.97\times10^{5}\pm0.2828$ |
| Yeast and Mold Count | | | | | | |
| 0 | $2.75\times10^{2}\pm0.2121$ | $2.75\times10^{2}\pm0.2121$ | $2.75\times10^{2}\pm0.2121$ | $2.75\times10^{2}\pm0.2121$ | $2.75\times10^{2}\pm0.2121$ | $2.75\times10^{2}\pm0.2121$ |
| 10 | $2.76\times10^{4}\pm0.3253$ | $3.92\times10^{3}\pm2.6587$ | $1.14\times10^{5}\pm0.0877$ | $1.52\times10^{3}\pm0.1372$ | $5.35\times10^{3}\pm0.9192$ | $5.82\times10^{3}\pm3.3728$ |
| 20 | $1.95\times10^{6}\pm3.3729$ | $5.52\times10^{5}\pm1.4708$ | $3.62\times10^{6}\pm1.1597$ | $1.36\times10^{5}\pm0.0070$ | $2.50\times10^{4}\pm0.5657$ | $1.09\times10^{5}\pm0.8132$ |
| 30 | $1.44\times10^{10}\pm0.4525$ | $6.64\times10^{7}\pm3.6204$ | $9.71\times10^{9}\pm0.2333$ | $5.10\times10^{6}\pm5.7926$ | $5.54\times10^{8}\pm0.1979$ | $7.25\times10^{5}\pm3.4648$ |

Data are mean ± S.D of replicates.

Data presented in Table 2 shows the effect of packaging materials on total viable count, lactic acid bacteria, yeast and mold count in oggtt samples stored at ambient (28±2°C) and refrigeration (4±2°C) temperature. Total viable bacteria count was in the order LDPE> HDPE>PET. Increase in mean of bacteria count (bacteria and LAB) was insignificant ($p>0.05$) up to 20 days and thereafter significantly ($p<0.05$) increased as compared to zero time which indicates that injury and recovery time preceded growth [13]. Total viable bacteria in samples stored at ambient (28±2°C) temperature were significantly ($p<0.05$) more compared to samples stored at the refrigeration (4±2°C) temperature. Similarly increase in LAB count was also found to be in the order LDPE>HDPE>PET and statistically significant difference was found in all three treatments of packaging materials. CFU/g of lactic acid bacteria in samples stored at ambient (28±2°C) temperature were more compared to samples stored at the refrigeration (4±2°C) temperature. The discrepancy recorded in the microbial loads at ambient (28±2°C) and refrigeration temperature (4±2°C) might be due to change in respiration rate influenced by temperature changes [14]. Steady but gradual increase was observed for total viable bacteria and LAB count in all samples throughout the storage period. Barbo *et al.* [15] observed that during manufacture and handling; electrostatic charges can occur on the plastic. These charges attract air-borne materials such as dust and microorganisms which may be responsible for spoilage of food. The presence and increase in the number of micro flora may be attributed to the fact that microbes continued their activities during storage. Heat treatment does not destroy all pathogenic microorganisms, but reduces the number to a level at which they don't constitute a significant health hazard. Vyletelova *et al.* [16] reported that defects were detected in milk when microbial concentration reached $5 \times 10^{5}$-$5 \times 10^{7}$ cfu/ml. The comparison of data of titratable acidity and CFU of lactic acid bacteria shows direct relationship between microbial growth and acidity. Various factors such as pre-and post-processing treatments, handling, storage, display and application of an appropriate packaging are responsible for microbial growth [17, 18]. However, post production temperature also plays very important role for maintaining the quality, microbial growth and shelf life of milk products. Deposition of organisms on the food rather than their differential growth might be the reason for the presence of bacterial species and their differences in colony forming unit per gram. Lactic acid bacteria are group of gram positive bacteria and are very useful in producing fermented foods. Total viable bacteria count and LAB count reported in cows and sheep's jameed on the day of preparation was more than the counts observed in this study [12]. The difference in the total viable bacteria count and lactic acid bacteria count observed among the various packaging materials may be

attributed to their relative permeability to atmospheric gases such as oxygen, nitrogen, carbon dioxide and water vapor, which has been reported to affect the growth and survival of microorganisms in packaged foods [19, 20]. Polythene sachet is more permeable to air than glass and plastic bottles. Growth and survival of microorganisms in packaged food has been found to be due to permeability to various gases such as oxygen, carbon (IV) oxide and water vapor [21]. In a report the oxygen transfer rate and permeability characteristics of the packaging material was found to be in the order LDPE > HDPE > PET and water vapor transmission was found to be in order HDPE>PET>LDPE [22]. From Table 2 it is clear that LABs are by far the major microbial group in oggtt. Similarly, in previous studies on oggtt and jameed, LAB was reported to be the dominant microorganism [12, 7]. LABs are considered to be important components of the microbiota playing a large variety of health-promoting functions. Studies show that LABs have ability to produce antimicrobial compounds such as organic acids, hydrogen peroxide and bacteriocin which prevents the growth of spoilage and pathogenic bacteria [23, 24].

Yeast and Mold counts were in the order LDPE >HDPE > PET. Significant ($p<0.05$) effect of temperature and insignificant ($p>0.05$) effect of packaging material was found on the growth of yeast and mold in this study. Gradual increase was observed in yeast and mold count but this increase was insignificant ($p<0.05$) up to 20 days and after that slightly significant ($p>0.05$) increase was observed. Low pH and surface moisture is favorable for the growth of yeast [25]. Coliform bacteria were not detected in samples during storage period. Low pH is not suitable for the growth of coliform bacteria which may be attributed to the absence of coliform bacteria in the studied samples. At the end of study period change in colour, flavor and odor was observed in samples stored at ambient (28±2°C) temperature. It was also observed that samples under ambient (28±2°C) temperature undergo considerable and undesirable physicochemical and microbiological at the end of storage.

## CONCLUSION

Results of the present study show that refrigerated (4±2°C) storage of oggtt is better than storage at ambient (28±2°C) temperature as it resulted in better physicochemical and microbiological characteristics and shelf life, compared to ambient (28±2°C) temperature storage. Proper hygienic condition, right packaging materials and proper storage temperature plays significant role in reducing the survival of the microorganism's food contamination, spoilage and its implication in food borne disease.

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