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Analytical Quality Assessment, Microbial Activity and Shelf Life Determination of Commercial Butter Samples in India

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ABSTRACT

This study aimed to train the master level students for carrying out the research work as a part of minor research project based learning. In this the quality assessment of butter samples obtained from the local super market in India was performed. Thirty different butter samples (commercially available) were analyzed. Quality assessment was carried out by calculation of the Reichert-Meissl (RM) value. Microbiological activity was also tested by storing the samples in two different temperatures: room temperature and refrigerated conditions. Based on the RM value and microbial activity data taken for a period of six consecutive months, self life of the butter samples were calculated to estimate the appropriate storage period and conditions suitable for consumption.

Keywords : Butter, Microbial activity, RM value, Analytical, quality assessment

I. INTRODUCTION

Since the year 1970, when the Indian government started the white revolution [1] moment, the dairy industry flourished here. Thousands of people were employed in the cattle feed industry as the White Revolution caused huge increment in production of milk and milk products and since then it is sold at competitive market prices. Today there are innumerous global as well as local brands of dairy products available across India. It is the world's highest producer as well as consumer of milk and has the world's biggest dairy herd [2]. Besides the whole milk, butter is one of the favorite's dairy products in India. Butter is basically water-in-oil emulsion. It has at least, 80% fat content, with water content 16% and non-fat milk solids 2%[3]. There is a considerable annual consumption of butter globally and the world production of butter is as high as 4.1 million tons per annum [4].A European Commission report mentioned that India will maintain high growth in the milk products processing and consumption in forth coming years. Annually, growth rate of butter production in India is 12% and the output has elevated by 4.4 lakhs tons since 1991. Furthermore, the report also claims that India will be able to sustain self-sufficiency in butter production and is the world's largest butter producing country. However, the increased competition at local and global levels had made these industries to adapt various unethical ways like adulterations to increase sales and profit. Therefore, food safety and sanitation methods play a key role to maintain the hygiene to improve shelf life and avoid food poisoning [5]. Hence, this study helps find the adulteration as of the various butter samples in the market using Reichert-Meissl (RM) value as a marker.

The number of milliliters of decinormal potassium hydroxide solution required to neutralize the volatile acid obtained by distillation of 5gm of oil or fat is called R-M value. This gives an estimate of the lower steam volatile fatty acids present in an oil or fat. It is

important or testing the purity of butter or ghee. The RM value of butter (from animal) is 20-30[6]. The value gets lowered upon adulteration. In the present work, 30 different butter samples were taken and there RM value was calculated. In further, the RM value was calculated daily (triplicated and average was taken) for six consecutive months with two different sets of same butter samples. Each sample of same brand was kept in two different physical conditions. One at room temperature (varied between 12^0C – 35^0C) and the other sample of same brand was refrigerated after opening as instructed on the pack. Refrigerator temperature was maintained about 4 to 5^0 C throughout the project. In addition to this, microbial activity and growth were also studied at the beginning and end of six months to check the effect of storage parameters on microbiological activity. According to the Beuro of Indian standards (BIS), the following are the standards prescribed for raw and pasteurized cream and butter [7].

Plate count/ ml (or g)	Grade
< 4 X 10^5	Very good
4 X 10^5 - 20X 10^5	good
20X 10^5 - 1X 10^6	fair
>1X 10^6	poor

Coliform count /ml (or g) : Not more than 100 Satisfactory. For Pasteurized cream, the plate count/mL (or g) should not be exceeding 60,000 and coliform count/mL (or g) should not be more than 10.

Standards and recommendations of Indian standards Institution for butter as follows

i) SPC. No standards have been suggested

ii) Coliform. The presence of more than 10cfu/mL butter is an index of insufficient pasteurization or contamination of butter from external source like wash water, equipment and other sources during manufacturing and packaging.

iii) Yeast & Molds (Y&M)

Y&M counts/ml	Quality
Less than 20	Good
21-50	Fair
51-100	Poor
More than 100	Very poor

The results from the performed microbial activity was compared with the given standards. Finally, the shelf life of the samples were predicted based on data observed with RM value and microbial activities. Over all, the project completed with analyzing the quality of 30 different brands of butters.

II. Experimental Section

2.1 Chemicals and materials

Butter sample, alcoholic sodium hydroxide (200mL) (6 g of KOH dissolve in 200 mL of rectified spirit), HCl (0.1 M), Phenolphthalein indicator, Sodium Carbonate crystals, petri plates, smear loop or inoculation loop, nutrient agar media from Himedia, incubator, laminar flow aseptic condition and all basic chemicals of analytical grade were used.

2.2 Procedure

2.2.1Determination of RM value

Step 1: Standardization of HCl. About 7 g of crystals of anhydrous carbonate is weighed accurately, dissolved in water and the solution is made upto 250Ml in standard flask. 20 mL of this solution is pipette out into a clean conical flask, 2 drops of methyl orange indicator are added and titrated against hydrochloric acid taken. The appearance of light pink color. The titration is repeated for concordancy and the strength of hydrochloric acid calculated[6].

Step 2: Determination o R-M value. About 2 g of the given butter is weighed accurately and taken in a 250 mL round bottom flask. Exactly 25mL of alcoholic KOH solution is added to it. The solution is refluxed on a water bath for 30 minutes. A blank is run simultaneously with the same quantity of alcoholic potash but without butter. Both the flasks are cooled and titrated against standard HCl solution using 1mL

phenolphthalein indicator. The end point is the disappearance of pink color[6].

Step 3: Calculation

Calculate the strength of HCl from the first titration.

Weight of butter taken= W g, volume of HCl required by the test solution= V1 mL, volume of HCl required by blank solution= V2 mL. Volume of alcoholic KOH reuired by the oil = (V2-V1) mL of HCl. R-M value is given by the equation 1.1 [6].

(V2-V1)/W * 5 * 0.1/ Strength of HCl ---------- 1.1

2.2.2 Microbiological activity

In order to study the microbial activity, thirty sterile and autoclaved Petri plates were filled with approximately 20mL of nutrient agar media[8]. Each petri plate was labeled with butter sample number to keep the identity of the brand tested. The agar was kept to solidify in aseptic sterile laminar flow condition. Inoculation or smear loop was sterilized keeping in flame, fresh samples of butter were taken, in aseptic laminar air flow sterile condition, the loop was rubbed slightly on butter slab and streaked (quadrant streaking)[9] over solidified nutrient agar plates. Petri plates were then incubated at 36^0C for 48 hours[8]. A negative control was also maintained with a plate of nutrient agar only. The plates were then placed on a colony counter and number of the bacterial colonies formed was counted [8]. Fungal growth was inspected by observing visually. This experiment was repeated again at the end of the storage period (six months) to analyze the microbial growth after. Both the data were compared to study the effect of storage condition on microbial growth. Finally, the RM values were accounted for determination of shelf life of the sample.

III. Results and Discussion

As per the described procedure, the butter samples of various brands were subjected to RM values calculation. Table 1A and 1B gives the average RM values of triplicated experiment. It was found that, amongst all the samples tested only the leading brands like Britannia, Amul, Nutralite, Mother diary, Milky Mist, Kwality, D'lecta, Nandini, Vijaya were found to have RM values within the appreciable limits. The other local brands had low RM values. However, to avoid the conflict, all the brand names are not mentioned and only sample numbers are given to denote them. For a period of six months, RM value of all the samples (same brands kept at two different temperatures, room temperature and refrigerator) were checked on the daily basis. The experiment was repeated thrice and average was taken. It was found that when the samples were refrigerated, RM value was within the acceptable limit for up to 40-60 days. Whereas, the same brand kept at room temperature showed erratic values and finally at the end of project duration it became inedible. Fig.1 shows a comparative graph of fresh sample and stored sample. Sample no. 1,2,3,4,5,6,7,8,11,12,15,22,23 and 27 shows good RM value for a period of 40-60 days when refrigerated. However, their counter parts kept at RT started deteriorating and fungal, mold growth started to take place right after 10-12 days. Since most of the samples showed fungal growth within 10 days of storage (RT), to compare the RM value on 5^{th} day was taken into consideration.

Table.2 shows the comparative data of the no. of bacterial colonies formed with the fresh and stale samples. The fresh samples showed microbial activity within prescribed limits whereas, stale samples (after six months at RT) showed innumerous colonies. It was also observed that fungus and molds developed on the sample. In further, the fresh samples upon streaking on petri dishes did not show any fungal growth. However, the stale samples not only gave bacterial colonies but also large fungal and mold formations.

Table.3 is the shelf life determination of all the samples based on RM and microbial activity. It was discovered that it is advisable to consume the butter only at refrigerated conditions within 30-60 days if stored hygienically at proper temperatures

(refrigeration) from the day of opening. However, the RT samples must be consumed within 2-3 days after opening.

IV. CONCLUSION

Overall, in the present study, various commercial butter samples were tested for quality assessment by their RM values and microbial activity. Same samples were subjected to different storage conditions. The effect of storage conditions on the quality was studied. Comparison of the data at the beginning with fresh samples to the stale samples after six months was done and the shelf life of the samples was predicted. It was discovered that few of the commercially local brands of fresh butter did not have the RM value within the range of 20-30 indicating the adulteration. When samples were opened and stored in refrigerator they were consumable maximum up to period of 40 days. However, the RT samples started showing fungal growth within 10 days. Based on all these observations, shelf life of all the samples was predicted. This work is an attempt of quality assessment of butter which is majorly consumed with an aim of giving the master students an experience of research based learning.

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