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Analytical Methods

Comparison of five analytical methods for the determination of peroxide value in oxidized ghee

Bhavbhuti M. Mehta [a,*], V.B. Darji [b], K.D. Aparnathi [a]

[a] Dairy Chemistry Department, SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India
[b] Department of Agricultural Statistics, College of Agriculture Information Technology, AAU, Anand, India

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ABSTRACT

In the present study, a comparison of five peroxide analytical methods was performed using oxidized ghee. The methods included the three iodometric titration viz. Bureau of Indian Standard (BIS), Association of Analytical Communities (AOAC) and American Oil Chemists' Society (AOCS), and two colorimetric methods, the ferrous xylenol orange (FOX) and ferric thiocyanate (International Dairy Federation, IDF) methods based on oxidation of iron. Six ghee samples were stored at 80 °C to accelerate deterioration and sampled periodically (every 48 h) for peroxides. Results were compared using the five methods for analysis as well as a flavor score (9 point hedonic scale). The correlation coefficients obtained using the different methods were in the order: FOX (−0.836) > IDF (−0.821) > AOCS (−0.798) > AOAC (−0.795) > BIS (−0.754). Thus, among the five methods used for determination of peroxide value of ghee during storage, the highest coefficient of correlation was obtained for the FOX method. The high correlations between the FOX and flavor data indicated that FOX was the most suitable method tested to determine peroxide value in oxidized ghee.

1. Introduction

Ghee, clarified butterfat, has an important place in Indian diet because of its characteristic flavor and pleasing aroma. It is a good source of fat-soluble vitamins (A, D, E and K) and essential fatty acids (Chand, Sree Kumar, Srinivasan, Batish, & Chander, 1986). The chemistry of ghee flavor is very complex; more than 100 compounds responsible for its flavor have been identified (Wadhwa, 1998; Wadhwa & Jain, 1990).

Ghee undergoes deterioration, mainly oxidation, under ambient conditions of storage (Kuchroo & Narayanan, 1973; Rangappa & Achaya, 1974). Autocatalytic oxidation results in product loss, which has significant economic impact. In addition, oxidation also leads to generation of toxic substances (Etsuo, Yasukazu, Yoshiro, & Noriko, 2005; Kanner, 2007; Logani & Davies, 1980; Rangappa & Achaya, 1974; Srinivasan & Anantakrishnan, 1964). Being a costly dairy product, and in view of the fact that India is its prime producer and exporter, oxidative spoilage of ghee is of major concern. The acceptability of ghee largely depends on the extent to which the oxidative deterioration has occurred. Several chemical methods have been developed to measure the oxidative changes in oils and fats (Gray, 1978). Based on extent of oxidation, one can select antioxidants to delay the rate of oxidative deterioration.

The methods reported to monitor oxidative deterioration of various oils and fats are based on chemical changes taking place at different stages, i.e., primary and secondary stage of oxidation. The first compounds formed during oxidation are peroxides, especially hydroperoxides, also called primary oxidation products. Peroxide value (PV) is used most commonly as an indicator of the early stages of oxidation in fats and oils. Many chemical methods have been developed to quantify oxidative deterioration with the object of correlating data with off-flavor development (Shahidi & Wanasundara, 2002). Sensory evaluations rely largely on humans to assess the acceptability and sensory properties of a product. It is difficult for instruments to replicate or replace the human taste, and sensory evaluation is, therefore, of importance in a quality assessment system for food products. Sensory evaluation is, generally, considered to be the most reliable indicator of rancidity (Malcolmson, 1995; Warner & Frankel, 1985).

Although ghee is one of the major dairy products in the diet of Indian consumers, little or no attention has been given to selection of suitable analytical methods for monitoring oxidative deterioration in this important fat-rich dairy product. There are five potentially useful methods; the Bureau of Indian Standard (BIS; IS: 3508-1966), the Association of Analytical Communities International (AOAC, 2000), and the American Oil Chemists'

* Corresponding author. Mobile: +91 9825807454.
E-mail address: bhavbhuti5@yahoo.co.in (B.M. Mehta).

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Society (AOCS; Cd 8b-90; 1996) are all based on iodometric titration method. The ferrous oxidation-xylenol orange method (FOX; Shantha & Decker, 1994) and ferric thiocyanate method (International Dairy Federation, IDF; 74A: 1991), based on oxidation of iron, have also been reported (Ronald, 2005; Shahidi & Wanasundara, 1996, 2002; Shantha & Decker 1994). Only one report is available comparing peroxide value obtained using the IDF method with changes in organoleptic quality of ghee stored at 37 °C (Ashok & Bector, 1985). Thus, there is a need to undertake a systematic study to generate data that could be valuable for monitoring the quality of ghee. Our study compared the performance of BIS, AOAC, AOCS, IDF and FOX methods for monitoring the peroxide formation in ghee against sensory evaluation.

2. Materials and methods

2.1. Preparation of ghee samples

White butter was procured from the Vidya dairy, a commercial dairy plant, located in Anand (Gujarat, India). Ghee was prepared by the creamery butter method in our laboratory from fresh butter. The butter was clarified into ghee with continuous stirring at a temperature of 120 °C/5 min. The clarified fat (i.e., ghee) was filtered through four folds of muslin cloth, and used for subsequent analysis. A total of six batches of ghee samples were prepared. All of the fresh ghee samples were weighed into 100 ml beakers and stored in a hot air oven maintained at 80° ± 2 °C. The stored ghee was sampled at regular interval (48 h), and peroxide value determined using the five methods and scored for flavor by sensory evaluation using the nine-point hedonic scale (Elizabeth, 1977) until flavor scores fell below an acceptable level, i.e., less than five.

2.2. Determination of peroxide value of ghee

Peroxide values were determined by the five different methods as described in BIS method (IS:3508, 1966), AOAC (2000), AOCS (Cd 8b-90; 1996), FOX (Shantha & Decker, 1994) and IDF (74A:1991), which are briefly described below. The dye (xylenol orange sodium salt) was purchased from S.D. Fine-Chem. Limited (Mumbai, India). Other chemicals used in this study were procured from Fisher Scientific (Mumbai, India). All chemicals and reagents were of analytical grade unless otherwise specified.

2.2.1. Iodometric methods

Briefly, for the BIS method (IS:3508, 1966), 1 g ghee, 1 g KI (powdered) and 20 ml solvent mixture comprising of acetic acid and chloroform (2:1) were mixed, while for the AOAC method 5 g ghee, 0.5 ml saturated KI solution and 30 ml solvent mixture comprising of acetic acid and chloroform (3:2) were combined. The AOCS method used 5 g ghee, 0.5 ml saturated KI solution, but the solvent mixture (30 ml) comprised of acetic acid and isooctane (3:2). Titration was carried against 0.002 mol/l $Na_2S_2O_3$ (for BIS method) or 0.1 mol/l $Na_2S_2O_3$ (for AOAC and AOCS methods) using 1% starch indicator.

2.2.2. Colorimetric methods

In the IDF method, the sample (⩽0.01–0.30 g) was mixed in a disposable glass tube with 9.8 ml chloroform–methanol (7 + 3, v/v) on a vortex mixer for 2–4 s. Ammonium thiocyanate solution (50 μL) was added, and the sample was mixed on a vortex mixer for 2–4 s. Then, 50 μL iron(II) solution was added, and the sample was mixed on a vortex mixer for 2–4 s. After 5 min incubation at room temperature, the absorbance of the sample was determined at 500 nm against a blank that contained all the reagents except the sample by using a spectrophotometer. The entire procedure was conducted in subdued light and completed within 10 min.

The FOX method (Shantha & Decker, 1994) was similar to the IDF method, except that 0.01 mol/l xylenol orange sodium salt solution in water was used as the complexing dye instead of ammonium thiocyanate. Absorbance was determined at 560 nm after 5 min of incubation at room temperature.

To construct the curve of Fe^{3+} concentration v/s absorbance, a standard solution of iron(III) chloride (10 μg Fe/ml) was prepared for both the methods.

2.3. Sensory evaluation of ghee

All the samples of ghee made in laboratory were evaluated for their sensory characteristics on a 9 point hedonic scale by a panel of 10 experience judges. Sensory evaluation was developed considering the rancidity of ghee. The 10 judges who were familiar with rancidity (off-flavor) of ghee were academic staff (between 30 and 56 years of age) at SMC College of Dairy Science, Anand Agricultural University (Anand, India). Each judge evaluated the ghee for flavor (i.e., rancidity); 9-like extremely, 5-neither like nor dislike and 1-dislike extremely.

The comparison between various methods used for changes in peroxide value, along with changes in flavor score of ghee, during storage were plotted and trends were examined.

2.4. Statistical analysis

The collected data were subjected to statistical analysis. Data were analyzed by completely randomized design and critical difference test at 5% level of significance ($P < 0.05$) to determine the differences between the methods and their correlation with flavor scores. Relationships between peroxide values, as measured by the five different methods, and flavor score were established using correlation analysis (Snedecor & Cochran, 1967).

3. Results and discussion

3.1. BIS method

The BIS method is based on iodometric titration. The peroxide values obtained for six samples stored at 80° ± 2 °C and analyzed every 48 h using the BIS method are given in Table 1. The initial peroxide value of fresh ghee samples ranged from 1.20 to 4.00 meq O_2/kg fat (data are not shown) with an average of 1.79 ± 0.44 meq O_2/kg fat. The peroxide value increased significantly ($P < 0.05$) on the second day of storage, declined slightly on the fourth day of storage, and reached a maximum of 6.38 ± 0.65 meq O_2/kg fat on the eighth day of storage where it remained up to 10 days (end of analysis).

The peroxide value of fresh ghee samples obtained by BIS method was reported as an average of 3.73 meq O_2/kg of fat by Parmar, Kaushik, Devaraja, and Singh (2013). Thus, the average peroxide value of fresh ghee samples obtained in the present study was lower. Parmar et al. (2013) stored the ghee at 80° ± 1 °C and found that the PV increased to 45.33 meq O_2/kg of fat after 2 days (and the product becomes totally oxidized and sensorial unacceptable). This difference might be due to initial quality of ghee used in the studies. Achaya (1949) studied the rancidity of ghee and found the peroxide values in the range 0.7–71.6 meq O_2/kg fat (average 34.7 meq O_2/kg fat) in buffalo ghee whereas cow ghee had 15.0–48.5 meq O_2/kg fat (average 32.5 meq O_2/kg fat) in rancid sample stored in between 15 and 20 °C over a period of three years in diffused daylight. Thus, the increase in peroxide values of ghee samples observed in this study was less than those reported by the