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RESEARCH ARTICLE

Comparative appraisal of antioxidant profile & shelf life of ghee obtained from cow, goat & buffalo milk

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Abstract: This study aims to evaluate the antioxidant potential, predict a shelf life using the Rancimat method, and examine the chemical changes that take place during accelerated storage at 80°C in ghee made from goat milk, cow milk and buffalo milk by direct heating cream method. To achieve this, we procured fresh raw milk from goats, cows, and buffaloes within the *Amreli* region, specifically in the *Saurashtra* region of Gujarat, India. Antioxidant potential assessment was done by calculating %RSA (DPPH and ABTS) and induction period measurement at 130°C. %RSA by DPPH and ABTS reagent showed that goat ghee (5.34±0.57, 3.32±0.58%) possessed least antioxidant potential compared to cow ghee (9.24±0.83, 4.42±0.42%) and buffalo ghee (6.19±0.53, 3.40±0.43%). Induction period at 130 °C was higher in cow ghee (7.05±0.43 hr) compared to goat ghee (3.14±0.20 hr) and buffalo ghee (6.46±0.16 hr). The extrapolation-based calculation of shelf life for various ghee samples was conducted using the Rancimat's built-in software, considering different IP values obtained at both 130°C and 140°C. At 37°C, in terms of months, the calculated shelf life of goat, cow, and buffalo ghee were 3.24±0.51, 8.92±0.54, and 7.25±0.45, respectively, while at 80°C, predicted shelf life in days were 4.53±0.51, 11.44±0.67, and 9.82±0.35, respectively. Primary and secondary chemical changes during accelerated storage (at 80 °C) of ghee samples evaluated using peroxide value, %CD, TBA, and P-AnV. This chemical analysis at 3 days interval showed that higher rate of oxidized metabolite formation in goat ghee compared to cow and buffalo ghee. Overall study indicated that antioxidant potential and shelf life of goat ghee was lower compared to cow and buffalo ghee. PCA analysis exhibited 72.95% and 9.42% of variance on PC1 and PC2, respectively. The results of PCA could assist manufacturers in developing strategies to improve the antioxidant properties of ghee, leading to a longer shelf life and increased customer satisfaction.

Key word: Anti-oxidant potential, shelf life, goat ghee

Abbreviation

DAHD= Department of Animal Husbandry and Dairying, FSSAI= Food Safety and Standards Authority of India, AOAC= Association of Official Analytical Chemist, AOCS=American Oil Chemists' Society, BIS= Bureau of Indian Standards IP=Induction Period, DPPH= 2,2-diphenylpicrylhydrazyl, ABTS= 2,22 -azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid, PUFA= Poly unsaturated fatty acid, MUFA=Mono unsaturated fatty acid, %RSA = Radical Scavenging Activity, OD= Optical Density, CD= Conjugated Dienes, TBA= Thiobarbituric acid, MDA= Malondialdehyde , p-AnV= p-Anisidine Value, Q_{10} = temperature coefficient, PCA=Principle Component Analysis.

Introduction

In the fiscal year 2021-22, India achieved a total milk production of 221.06 million tonnes, indicating an annual growth rate of 5.29% (Selokar et al. 2023; DAHD, 2022-23). The primary sources of milk for the dairy industry in India are buffalo, cow, goat and sheep. Among these, goat milk accounts for 3% of the nation's total milk output. Goat milk typically contains 12.2 % total solids, approximately 4.0-4.5% fat, 3.2% protein, and around 4.6% lactose content (Lima et al. 2018). Goat milk has more Conjugated Linoleic Acid (linked to potential anticancer properties) than cow milk, and it's also high in selenium (13.7 ng/mL), which makes it often used for treating dengue fever (Ceballos et al. 2009; Yuce et al. 2023; Van Dael et al. 1992). Ghee is Indian traditional premium edible fat, made by the process of heating and clarifying milk cream or butter, is acclaimed as the prime selection for cooking and frying, ranking as the second most great in demand dairy

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product after fluid milk (Gandhi et al. 2013, 2018). Approximately 27.5% of India's total milk supply is used to produce butter and ghee (Atanu Jana[a], 2018). Goat milk fat is rich in medium-chain fatty acids, which are easily digested and absorbed by the body. It can help improve digestion, reduce inflammation in the gut, and promote the growth of beneficial gut bacteria (Gupta and Kumari, 2018).

This research initiative is encouraged by remarkable findings on a commercial website, indicating a considerable price difference of 4-4.5 times higher for goat ghee compared to traditional ghee. The noteworthy aspect is the observed shelf life of 6 to 8 months for regular ghee (Bekele and Kassaye, 1987) even though manufacturers declare a 12-month shelf life for goat ghee without any preservatives. This is particularly significant as research indicates that goat milk fat, abundant in PUFA (6.16%) and MUFA (26.78%) prone to oxidation (Sbihi et al. 2015), raises concerns about the precision of labeling and potential implications for consumer health. The latest FSSAI-2023 standards provide detailed physicochemical specifications for traditional ghee but lack specificity regarding ghee produced from milk of which species ? .The outdated or limited data available on the characteristics of ghee made from Indian goat milk further emphasizes the necessity of this study. After the COVID-19 pandemic, there is an increasing inclination in nations such as India towards premium natural choices such as goat milk and milk products, indicating that they give greater importance to health and quality even with a considerably higher price tag. Keeping in mind above facts, in this research, ghee from non-ruminant and ruminant animal milk was prepared and were analysed for antioxidant potential, prediction of shelf life using Rancimat and chemical changes taken places during accelerated storage.

Materials and Methods

Ghee preparation

The pooled milk sourced from Goats (*Gohilwadi*), Cows (*Gir*), and Buffaloes (*Jafarabadi*) were collected at regular intervals every two months (January to November 2022) from Amreli region of *Saurashtra*, Gujarat. Ghee was then made using the direct cream heating method, as suggested by Atanu Jana[b](2018).

Antioxidant potential

The antioxidant potential of prepared ghee samples were evaluated by induction period at 130 ^{0}C using Metrohm Rancimat Model 892 (Herisau, Switzerland), DPPH method (Espin et al. 2000), and ABTS method (Re et al. 1999) and. Induction period was measured as per method given by AOCS Cd 12b-92 (1999).

Rancimat Model 892 was used to measure the IP of different ghee. The operating parameters were set: like temperature at 130 ^{0}C, airflow rate 20 L/hr and sample size 8 g. Prior to use of instrument, the glassware were thoroughly washed and cleaned as per the instruction given by manufacturer. 8.0 g of fully melted and thoroughly mixed ghee was accurately weighed into each of the glass sample vessels. The glass vessels were then placed in the heating block of apparatus and connected to the vessels via a thin white plastic pipe. Around 60 mL of deionized water was taken into each of the measuring vessels containing the electrodes, and the vessels were placed in the apparatus. Rancimat apparatus was started to measure IP at 130 ^{o}C until the end points reached.

1.0 ml of melted ghee sample diluted to 9.0 ml in ethyl acetate. Twenty micro liter of diluted ghee (1:10) was added to 3.8 mL of ethyl acetate to make 4 mL of the mixture, followed by addition of 1 mL of DPPH solution. Tubes were kept in dark for 10 min, then OD was measured at a wavelength of 520 nm using a spectrophotometer (Shimadzu UV Vis Spectrophotometer UV 1900, Tokyo, Japan). The reference sample was made by mixing 1 mL of DPPH solution in 4 mL ethyl acetate. %RSA activity was calculated using following formula.

$$\%\ RSA\ per\ 0.02\ ml\ = \frac{Control\ OD - Sample\ OD}{Control\ OD} x100$$ 1 ml of ghee was dissolved in 5-8 mL of methanol and made up to a final volume to 10 mL with same solvent. 3.0 mL of ABTS working solution was taken in a cuvette and OD was adjusted to 0.70±0.02 against methanol. Twenty micro litres of the diluted ghee sample (1:10) was added to ABTS working solution as well as in the blank (cuvette with 3 mL methanol). The contents were mixed and OD at 734 nm was recorded after 6 min after keeping tubes in dark using a double beam spectrophotometer (Shimadzu UV Vis Spectrophotometer UV 1900, Tokyo, Japan). %RSA was calculated by using the formula.

$$\%\ RSA\ per\ 0.02\ ml\ = \frac{Control\ OD - Sample\ OD}{Control\ OD} x\ 100$$

Shelf life predication of ghee

The shelf life of ghee at 37^{0}C and 80^{0}C was predicted using Rancimat by extrapolating induction period (IP) of ghee sample obtained at 130 ^{0}C and 140 ^{0}C, respectively. The shelf life was calculated by in-built StabNet1.0 software in Metrohm Rancimat Model 892.

Evaluation Chemical changes taken place in ghee during accelerated storage at 80.0±2 ^{o}C:

The chemical change include primary and secondary oxidation taken place during accelerated storage was tested at 3 days of interval by Peroxide value (BIS, 1981), CD value (AOAC, 1971), TBA value (Patton and Kurtz, 1951) and p-AnV (AOCS, 2009). Simultaneously, the flavor evaluation of ghee samples was also conducted by 10 panelists using 9 hedonic scales.

Peroxide value

The peroxide value of ghee was determined by iodometric method, as described in fourth reprint of BIS (1981).

TBA value

3.0 g melted ghee precisely weighed and placed into stoppered test tube. To this, 10.0 ml of carbon tetrachloride used and then 10.0 ml TBA reagent (0.76 g 2-Thiobarbituric acid in 100 ml distilled water) was mixed. The test tube was then mixed vigorously for 4 min. The test tube kept undisturbed until two apparently separate layers formed. Next, 5.0ml of supernatant transferred to second test tube, and then incubated in boiling water bath for 30 minutes. Simultaneously, a blank sample was made by 3.0ml solvent instead of ghee. The OD was measured at 532 nm.

CD value

0.1 g melted ghee sample was measured and placed into a 100ml volumetric type flask, which was then filled with isooctane solvent. The resulting mixture of isooctane and samples were then checked for OD at 233 nm against a solvent blank (isooctane only).

$$CD\ value\ =\ 0.91\ x\ \left(\frac{OD\ of\ diluted\ ghee}{Cell\ length\ in\ cm}\ x\ M - 0.03\right)$$

Where M = mass of ghee sample in one lit of final dilution used for the OD measurement

p-AnV

Well mixed and completely melted 0.5 to 4.0 g was mixed with isooctane in a volumetric flask to obtain 25 ml volume. The OD of the resulting fat solution was measured at wavelength set to 350 nm. A volume of 5.0 ml of the fat solution was then thoroughly mixed with 1 ml of a p-anisidine reagent (0.25% in acetic acid, w/v). After the interval of ten minutes, OD of content recorded at 350 nm wavelength against a blank (Isooctane). The p-AnV was calculated using mathematical equation.

The p-AnV was given by the given formula

$$p - AnV\ =\ \frac{25\ x(1.2\ As - Ab)}{m}$$

Where,

As = OD for solution of fat after its chemical reacted with p-anisidine reagents; Ab = OD for blank, m=grams of sample used for the analysis

Statistical Analysis

All the results were expressed as mean ± S.E. The data was analyzed by one-way analysis of variance (ANOVA), followed by Turkey's multiple comparison test in GraphPad Prism 5. A p-value less than 0.05 was considered significant.

To understand the connections between parameters and data trends reported during the analysis of ghee samples, a Principle Component Analysis (PCA) was performed using PAST 4.2 software.

Result s and Discussion

To achieve a more comprehensive measurement, three indices DPPH, ABTS, and IP were utilized to evaluate antioxidant activity accurately. IP at 130 °C of goat ghee (3.14±0.20 hr.) was significantly (P<0.05) lower than cow ghee (7.05±0.43 hr.) and buffalo ghee (6.46±0.16 hr.). Pawar et al. (2014) examined the IP at 130 °C of ghee was 4.10 hr for 9.0 g sample. The variation in IP at 130°C in this study may be attributed to sample size, animal species, ghee preparation method, and the specific Rancimet model used for analysis. Additionally, the DPPH activity revealed that the %RSA value of 0.02 ml goat ghee (5.34±0.57) and buffalo ghee (6.19±0.53) were significantly (P<0.05) lower compared to cow ghee (9.24±0.83). However, Non-significant (P>0.05) differences was observed in ABTS activity of goat ghee (3.32±0.58), cow ghee (4.42±0.42) and buffalo ghee (3.40±0.43). All three antioxidant parameter (DPPH, ABTS and IP at 130 °C) showed similar trend in data. The antioxidant potential of milk fat was influenced by the presence of fat-soluble vitamins, namely vitamin E but particularly alpha-tocopherol, as well as vitamin A and beta-carotene (Celi, 2011; Kaneai et al. 2012; Sunariæ et al. 2012).

Table 1: Antioxidant potential of species wise ghee sample (n=6)

Type of ghee	IP per 8.0 g at 130°C (hr)	% RSA per 0.02 ml by DPPH	% RSA per 0.02 ml by ABTS
Goat ghee	3.14±0.20[a]	5.34±0.57[a]	3.32±0.58
Cow ghee	7.05±0.43[b]	9.24±0.83[b]	4.42±0.42
Buffalo ghee	6.46±0.16[b]	6.19±0.53[a]	3.40±0.43

Means with different superscript (a-c) letters are significantly different in column. P value less than 0.05 was considered as significant;%RSA=radical scavenging activity; IP= Induction period

Peroxide value of fat/oil indicates the extent of its primary oxidation during storage. Initially, fresh cow and buffalo ghee sample showed a no peroxide value, however, the goat ghee possessed 0.29±0.05. On third and six day analysis (Table 2.0), goat ghee achieved peroxide value significantly (P<0.05) higher than cow and buffalo ghee samples. This showed that peroxide formation rate was higher in goat ghee compared to cow and buffalo ghee during accelerated storage.

CD value in fat/oil represents the early stage of oxidation which absorbs wavelength at 233 nm (Abeyrathne et al. 2021). It was observed that mean CD value of fresh goat ghee (1.42±0.10) was higher than cow (0.83±0.01) and buffalo ghee (0.77±0.02). This value was increased significantly (P<0.05) for goat ghee compared cow and buffalo ghee during 3rd and 6th analysis. The CD values for fresh cow ghee and buffalo ghee varied between 0.7713 to 0.7913 (with an average of 0.7828) and 0.6541 to 0.6872 (with an average of 0.6670), respectively (Gosewade et al. 2017). Therefore, the findings of this study indicate that the initial stages of oxidation occurred more rapidly in goat ghee compared to cow and buffalo ghee.

TBA test is used to measure a secondary oxidation product by quantifying MDA from fats /oil. This value was significantly (P<0.05) higher in goat ghee at initial day and non-significant (P>0.05) difference observed between cow and buffalo ghee. TBA value was increased in all the samples during storage. However, it was observed that in goat ghee TBA value significantly (P<0.05) increase from 0.122 to 0.393 within 6 of storage at 80 °C. This result suggested that goat ghee might have higher PUFA than cow and buffalo ghee. Mehta et al. (2015) reported an average TBA value of ghee was increased from 0.03 to 0.23 during 6 days of accelerated storage (80 ^{0}C); our analysis data of cow and buffalo ghee for TBA was in general agreement.

p-AnV measures saturated and unsaturated carbonyl compounds with high molecular weights produced from PUFA present in TAG. The p-AnV was significantly (P<0.05) higher in goat ghee, however, in cow and buffalo ghee, it was nil. After six day of

Table 2: Chemical changes in different ghee samples during accelerated storage at 80 °C (n=6)

Days	Parameter	Goat ghee	Cow ghee	Buffalo ghee
0 day	Peroxide value (meq O_2/kg fat)	0.29±0.05^{aA}	0.00±0.00^{aA}	0.00±0.00^{aA}
	CD (µmol hydroperoxides/g)	1.42±0.10 ^{bA}	0.83±0.01 ^{aA}	0.77±0.02^{aA}
	TBA value (mg of MDA per kg)	0.122±0.01 ^{bA}	0.030±0.01 ^{aA}	0.048±0.004^{aA}
	p-AnV (mmol/kg)	0.50±0.06^{bA}	0.00±0.00^{aA}	0.00±0.00^{aA}
	Flavor score	7.95±0.20^{aC}	9.19±0.11^{bB}	8.75±0.12^{abB}
3day	Peroxide value (meq O_2/kg fat)	3.04±0.62^{aB}	1.56±0.23^{aA}	1.84±0.23^{aA}
	CD (µmol hydroperoxides/g)	2.27±0.06 ^{bB}	0.99±0.06^{aA}	0.89±0.02^{aA}
	TBA value (mg of MDA per kg)	0.283±0.03^{cB}	0.056±0.01^{aA}	0.123±0.004^{bA}
	p-AnV (mmol/kg)	1.70±0.17^{bB}	0.69±0.15^{aB}	1.52±0.17^{bB}
	Flavor score	6.92±0.09^{aB}	8.81±0.11^{bB}	7.67±0.10^{aA}
6 day	Peroxide value (meq O_2/kg fat)	9.68±0.76^{aC}	1.84±0.23^{bB}	2.37±0.38^{bB}
	CD (µmol hydroperoxides/g)	3.02±0.15^{bC}	1.04±0.01^{aA}	1.02±0.03^{aA}
	TBA value (mg of MDA /kg)	0.393±0.15^{cC}	0.104±0.01^{aB}	0.219±0.02^{bB}
	p-AnV (mmol/kg)	6.37±0.22^{cC}	0.68±0.09^{aB}	1.94±0.39^{bB}
	Flavor score	3.67±0.36^{aA}	7.54±0.17^{bA}	7.00±0.19^{bA}

Data were presented as means±S.E. Means within row with different superscript (a-c) was significantly different from each other. Means within column with different uppercase superscript (A-C) are significantly different (p<0.05) from each other.

Table 3 Predicted shelf life of ghee using Rancimat analysis

Sr.No	Parameter	Goat ghee	Cow ghee	Buffalo ghee
1.	IP-130 °C	3.14±0.20^{a}	7.05±0.43^{b}	6.46±0.16^{b}
2.	IP-140 °C	1.55±0.22^{a}	3.39±0.21 ^{b}	3.15±0.09 ^{b}
3.	Q_{10} value	2.03±0.03^{a}	2.08±0.01^{a}	2.05±0.01^{a}
4.	Shelf life of ghee at 37°C (month)	3.24±0.51^{a}	8.92±0.54^{b}	7.25±0.45^{b}
5.	Shelf life of ghee at 80°C (days)	4.53±0.51^{a}	11.44±0.67^{b}	9.82±0.35^{b}

Data are presented as mean±S.E . Means with different superscript (a-b) are significantly different (P<0.05) from each other in row.

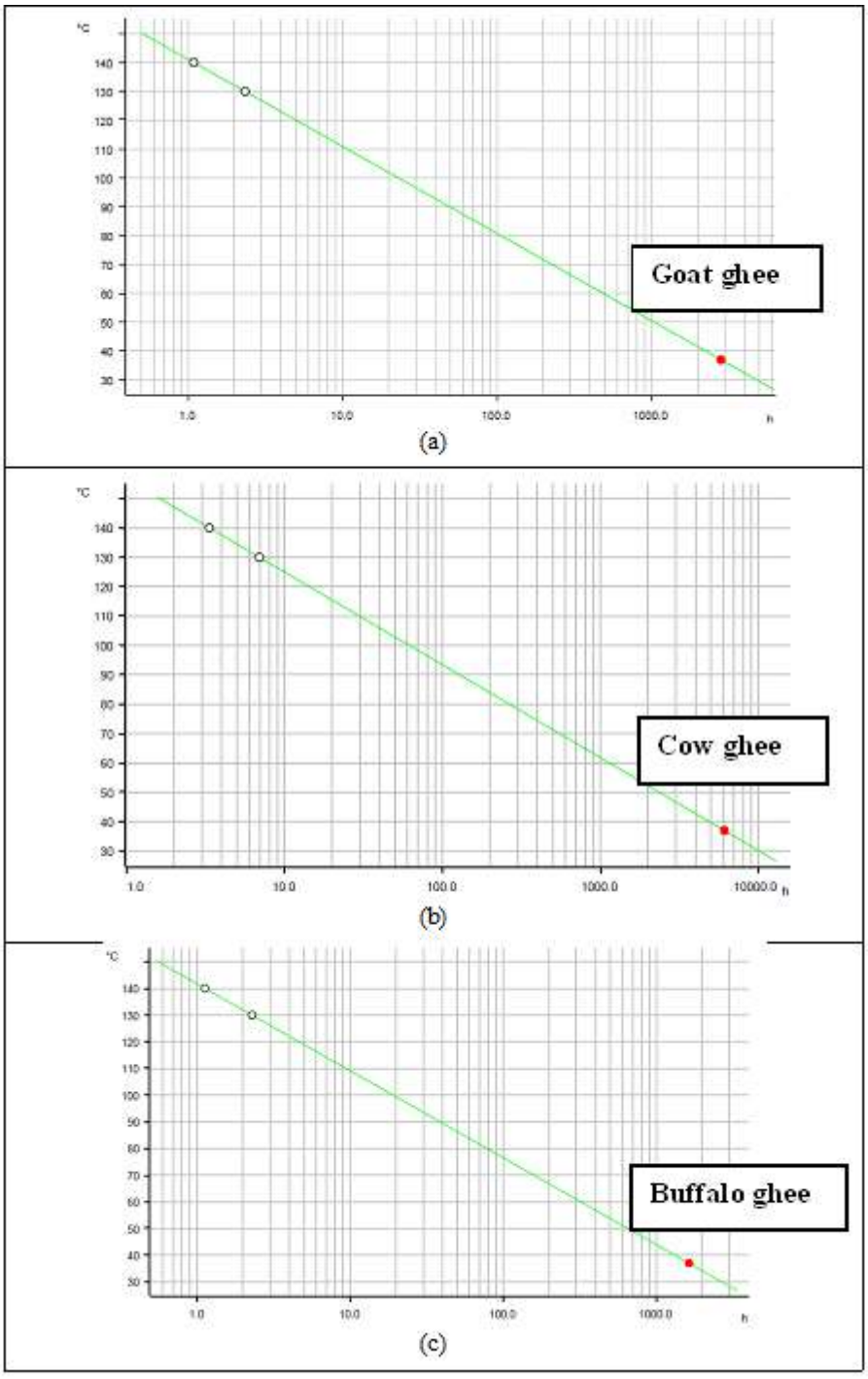

Fig. 1 Extrapolation graph of shelf life calculation of different ghee samples

storage goat ghee (6.37±0.22) attained significantly (P<0.05) higher value compared to cow (0.68±0.09) and buffalo ghee (1.94±0.34). This data indirectly suggest that formation rate of saturated and unsaturated carbonyl compounds was higher in goat ghee during accelerated storage. Mehta et al. (2015) reported that mean p-AnV in ghee samples ranged from 0.55 to 2.60 within six days of storage; the results obtained in the present investigation regarding cow and buffalo ghee were in general agreement except for goat ghee.

Flavor is one of the most significant aspects affecting the acceptance of edible oil. The flavor of various ghee samples during accelerated storage was evaluated using a 9-point hedonic scale. Ghee is popularly known for its pleasing, nutty and lightly caramelized flavor with good body and texture. A present study

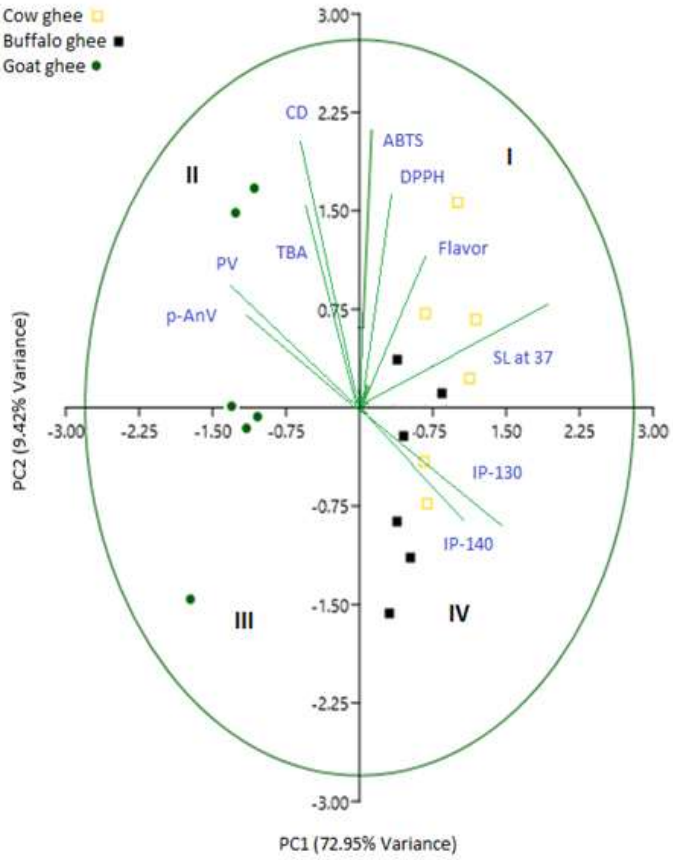

Fig. 2 Biplot and scatter plot of PCA analysis

revealed that pure goat ghee possessed lightly cooked, pleasant and slightly typical gotty flavor with white light greenish in color with large grains, as noticed by sensory panels. Where as in case of cow and buffalo ghee were apparently pale golden and white to greenish in color with fine grain, respectively. The typical gotty flavor of goat ghee may be due to the presence of high levels of fatty acids C10:0 and C12:0 compared to cow and buffalo ghee (Bindal and Wadhwa, 1993). The flavor score decreased for all the samples as the days of accelerated storage increased. On the third day, a slight oxidized flavor was perceived, and on the sixth day, it was intensely off flavor, in the case of goat ghee only. Significantly ($P<0.05$) higher flavor scores were obtained for cow (7.54±0.17) and buffalo ghee (7.00±0.19) compared to goat ghee (3.67±0.36) after six days of storage. Goat ghee became unacceptable (flavor score < 5). The panel of judges made the remark that the samples of cow and buffalo ghee did not have oxidized flavors on the sixth day but lacked the unique flavor that was perceived on the first day.

The Rancimat analysis output for the predicted shelf life calculation at different temperature (130 °C and 140 °C is shown in Table 3 and extrapolation graph illustrated in Fig 1. It can be noticed that the average shelf life of goat ghee (3.24 month at 37 °C, 4.53 day at 80 °C) was significantly ($P<0.05$) lower compared to cow ghee (8.92 month at 37 °C, 11.44 days at 80 °C) and buffalo ghee (7.25 month at 37 °C, 9.82 days at 80 °C). The IP value of goat at 140°C remained significantly ($P<0.05$) lower compared to cow ghee and buffalo ghee. IP at 140 °C value of all the samples was lower compared to IP at 130 °C. The higher shelf life obtained in cow and buffalo ghee might be due to the method of manufacture employed in present investigation, i.e. direct heating cream; such ghee possesses higher antioxidant potential. The predicted shelf life at different temperatures, as determined by the Rancimat method, showed that goat ghee has a significantly shorter shelf life compared to cow and buffalo ghee.

PCA analysis

PC1 (Fig 2) gained a much higher percentage of variance explained (72.95%) compared to PC2 (9.42%), indicating that PC1 captures the majority of the variation in the dataset. The positive loadings on parameters such as IP-130, IP-140, SL (shelf life) at 37 °C, PV (Peroxide value), CD, TBA, and p-AnV suggest that PC1 primarily represents the oxidative stability and chemical properties of ghee. Therefore, efforts to improve oxidative stability and minimize oxidation products could enhance the overall quality of ghee products. Based on Fig 2, it is evident that there exists an inverse relationship between IP values and the chemical parameters (PV, CD, TBA, and p-AnV). In other words, a higher IP value correlates with lower formation of oxidation products. Upon examination of the quarter I, it became apparent that both ABTS, DPPH, and flavor score exhibit a positive correlation with the shelf life of ghee (SL at 37°C). This trend in the data indirectly implies that samples with higher antioxidant potential would likely have an extended shelf life for ghee. Understanding these relationships between various parameters can enlighten strategies for adopting ghee production method and ensuring product quality and consumer satisfaction.

Conclusion

From the above study, it could be concluded that the antioxidant potential of goat ghee was lower compared to cow and buffalo ghee. The deterioration rate during accelerated storage of ghee was much higher in goat ghee, which indirectly suggested that goat ghee might have a higher amount of PUFA than cow and buffalo ghee. The Rancimat analysis report showed that the calculated shelf life of goat ghee was also lower than that of other species of ghee samples. PCA results suggest that oxidative stability and antioxidant potential are key factors influencing the quality of ghee.

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