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RESEARCH ARTICLE

Headspace volatile markers of *Sandesh*, a *chhana*-based delicacy stored at elevated temperatures

Karpurapu Uma[1], Narender Raju Panjagari[1] (✉), Rakesh Kumar Raman[1], Ashish Kumar Singh[1], Lal Chand Sharma[1], Sangita Ganguly[1], Rajan Sharma[2] and Vivek Sharma[2]

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Abstract: The present study explores the headspace volatiles of *Sandesh* (dessert made from heat acid coagulated milk) that contribute to the product quality during storage. *Sandesh* is packaged in clear and dark-coloured glass containers and stored at 30 °C and 45 °C in an incubator. *Sandesh* quality during the storage was estimated by biochemical, microbiological and sensory analysis. The concentration of head-space volatiles was simultaneously determined by employing headspace solid-phase micro-extraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS). The identified volatiles were pertaining to various functional groups, which include acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. As a result, acetic acid, propanoic acid, valeric acid and butyric acid were suggested as headspace freshness markers, while spoilage markers were identified as 1-hexanol 2-ethyl-; 1-hexanol; 3-aminomethyl-3,5,5-trimethylcyclohexanol trans-; 1-propanol, 2-amino-; pentane, 2-methyl; hexane, 2,4-dimethyl-; hexane, 3-methyl; n-hexane; acetone; 2-heptanone; 2-pentanone. The obtained volatile markers are essential to develop the intelligent packaging systems for monitoring the product's quality

Keywords: *Sandesh*, Headspace, Volatile organic compounds, Solid-phase micro-extraction, Quality change, Spoilage markers

[1]Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal-132001, India
[2]Dairy Chemistry Division, ICAR-National Dairy Research Institute, Karnal 132001, India

Narender Raju Panjagari (✉)
Dairy Technology Division
ICAR-National Dairy Research Institute
Karnal-132001, Haryana
pnr.ndri@gmail.com

Introduction

India is a unique and traditionally rich country in terms of producing a variety of region-specific dairy products. Among several such region-specific dairy products, *Sandesh* is one of the most popular products of acid-coagulated hot milk (*Chhana*, which is similar to cottage cheese but contains sugar). It has a smooth texture with a firm body, which consists of a high amount of milk proteins, fat, sucrose, and fat-soluble vitamins (Aneja et al. 2002; Khamrui and Solanki, 2010). It is highly popular in the eastern and northeastern states of India but gaining huge popularity across India and overseas due to its flavour and palatability. Flavour is composed principally of the sensation of smell and taste and it is the most important factor, which governs our appreciation of the food that we consume. Volatile constituents of the food can determine its aroma and flavour. Previous reports indicated identification of nine volatile carbonyl compounds namely formaldehyde, acetaldehyde, propionaldehyde and /or acetone, butyraldehyde, pentan-2-one, heptan-2-one, benzaldehyde, octan-2-one, nonan-2-one in fresh samples of *chhana* using the Gas-Liquid Chromatography technique (Kumar and Srinivasan, 1984). The major volatiles found in Queso blanco cheese, which is similar to *Sandesh* (but contains salt instead of sugar) are acetone, acetaldehyde, butanol, isopropanol, formic acid, acetic acid, propionic acid and butyric acid, which contribute to the cheese's flavour and aroma (Torres and Chandan, 1981).

Volatile constituents of a product are not only responsible for determining its flavour and aroma, but also play a pivotal role in the estimation of its shelf life. The volatile compounds can generate during storage due to various microbial and biochemical reactions in the product (Cheng, 2010). The profiling of headspace volatiles of food products has been extensively studied for the determination of spoilage markers. In an earlier report (Li et al. 2022), potential volatile compounds associated with deteriorating raw milk were investigated and suggested monitoring 2-ethyl-5-methylpyrazine, 2-pentanone, pyridine, 2-butanone, n-butyraldehyde, and 2,3 butanedione as possible raw milk deterioration indicators. Similarly, Song et al. (2021) identified three headspace volatile compounds namely ethanol, 2,3-butanediol and 2-ethyl-1-hexanol as decay markers of minced pork by characterizing headspace volatiles during storage. In this regard, several researchers (Anagnostopoulos et al. 2022; Martín-Gómez et al. 2022; Opara et al. 2022; Pavlidis et al. 2021; Sarnoski et al. 2010; Waghmode et al. 2021; Wierda et al. 2006; Yang et al. 2022) reported various spoilage and freshness markers in food products by using volatolomics approach.

The headspace solid-phase microextraction (HS-SPME) technique combined with Gas chromatography-mass spectrometry is an advanced and potential tool to evaluate the quantitative and qualitative headspace volatiles of foods. Pawliszy and co-workers developed the SPME method, which describes the preparation of samples without using solvents for chromatographic analysis. In the SPME method, volatiles are adsorbed on the reusable fibre, which is layered with a stationary phase (Arthur and Pawliszy, 1990). In recent studies, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibres have been reported to be most effective for extracting a wide range of analytes with different polarities and molecular weights. Further, identification and quantification of the compounds are achieved by injecting the fibre extracts into a gas chromatography-mass spectrometer (GC-MS). The combination of SPME and GC-MS has been employed to examine the flavour of various dairy products (Balasubramanian and Panigrahi, 2011). To the best of our knowledge, the investigation on determining headspace volatiles of most of the traditional Indian dairy products has not been conducted so far using advanced techniques, except for *ghee* (Duhan et al. 2020; Wadodkar, 2003). The main purpose of this study is to evaluate the range of volatiles produced during storage under various conditions using GC-MS technique coupled with headspace solid-phase microextraction (HS-SPME) to identify the key quality markers of *Sandesh*.

Materials and methods

Preparation of *Sandesh*

Cow's milk and cream were procured from the Experimental Dairy of ICAR-National Dairy Research Institute, Karnal, India. Milk was standardized to 4% milk fat and 8.2% milk solids-not-fat (MSNF) in the laboratory. Soft grade *(Narampak) Sandesh* was prepared by following the standard protocol given by Sen and Rajorhia, 1990. The standardized milk was subjected to heating at 90°C and coagulation with 1% citric acid solution until clear whey. The obtained coagulated mass (pH 5.2-5.4) strained using a muslin cloth for whey draining for 30 min. The obtained mass (*Chhana*) was kneaded to get fine consistency and sugar (30% by weight of *chhana*) was added to it. Later, the mixture was heated to 70°C for 15 min. and further heating continued to 60°C for about 10 min with continuous stirring. Then the mixture was moulded (22g / piece) in a mould and cooled (37°C) to attain *sandesh*.

Proximate composition analysis of *Sandesh*

Sandesh samples were analysed for moisture and fat as per BIS (1981) procedure. The protein and ash content were estimated by using AOAC (2016) methods. The total carbohydrate percentage of the sample was calculated by the difference of the sum of the moisture, fat, protein and ash from the total weight (*Sandesh*).

Packaging and storage of samples

A large portion of *Sandesh* sold in market in plastic containers. However, glass packaging materials were chosen to determine headspace volatiles. The reason was to avoid migration and scalping of headspace volatiles from the container. Freshly prepared *Sandesh* was packaged in two types of packaging materials such as clear glass vials (CG) and dark-coloured glass vials (DG) (M/s Labco, India, Cat No: 993/4). They were stored at 30 °C and 45 °C (Fig. 1a) in an incubator. Initially, 8 g of sample was weighed into each vial of 30 mL for headspace volatiles determination. Later, 150 g of sample was weighed in CG and DG containers (300 mL) (Yera Airtight Manufactures, India) to determine the biochemical, microbial and sensory changes during storage. The analysis was carried out at 24 h interval until visible mould growth (Fig. 1d).

Extraction and measurement of headspace volatile compounds by HS-SPME-GC/MS

The head-space volatiles from the *Sandesh* samples were extracted by using a headspace solid-phase microextraction (HS-SPME) device consisting of a fibre (Supelco cat. No: 57348-U, Bellefonte, PA, USA) with a film thickness of 50/30µm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and a manual holder (Supelco cat. No: 57330-U, Bellefonte, PA, USA). The SPME syringe was introduced into the vial's headspace, followed by the extension of the fibre (2 cm) into the vial (Fig. 1b). The gap between the fibre tip and the product surface in the bottle was 0.5 cm. The extraction time of samples stored at 30 °C and 45 °C for 40 and 45 min, respectively, were optimized by conducting experiments (Fig. A1& Fig. A2). After extraction of volatiles, the fibre was placed into the manual holder and inserted into the heated injection port of the GC-MS for thermal desorption of the samples for 15 min (Fig. 1c). GC-MS (TQ-8030, Shimadzu Corporation, Kyoto, Japan) instrument located at the National Referral Centre for Milk Quality and Safety, ICAR-NDRI, Karnal, India was employed in this study. Chromatographic separation was performed on an Equity-1 column (Capillary column; 30 m X 0.32 mm i.d. X1.0 µm film thickness, Supelco cat. No: 28057-U, Bellefonte, PA, USA). The injector, detector and interface were operated at 270 °C, 220 °C, and 260 °C, respectively. GC oven was initially set at 270 °C for 11 min, then ramped linearly at a rate of 10 °C/min to 300 °C and it was kept for 5 min at the same temperature. Flow rate of helium

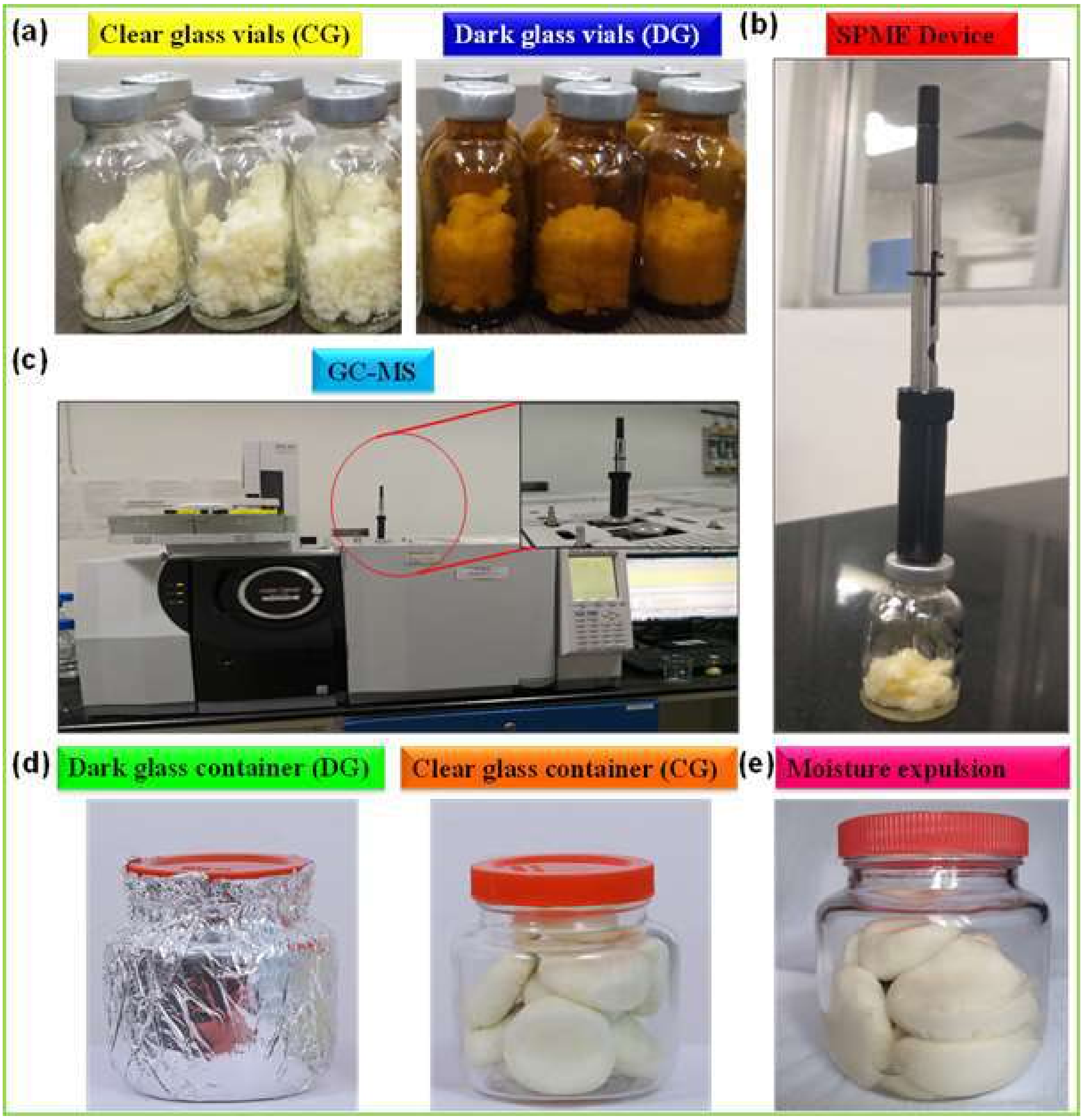


Fig. 1 **(a)** Clear glass vials and dark coloured glass vials for headspace volatiles analysis, **(b)** Headspace volatiles collection using SPME device, **(c)** GC-MS coupled with SPME, **(d)** Clear and dark (aluminium wrapped) glass containers for quality analysis, **(e)** Moisture expulsion from samples during storage

carrier gas was maintained at 2.33 mL/min under splitless mode. The standard ionization energy of 70 eV was maintained in Electron Ionization (EI) under full scan mode in a range of 50-500m/z to analyse the volatiles. The identification of volatile compounds was accomplished by using NIST 17 and NIST 14S (National Institute of Standard and Technology, New York, USA) mass spectral library. The results from the headspace volatile analysis were expressed in terms of peak area (total ion concentration).

Analysis of biochemical changes

The titratable acidity of the sample was determined using the method described in BIS (1981). Sample pH was measured with the help of a calibrated pH analyser (Labndia, New Delhi, version 1). The free fatty acids content of *Sandesh* was estimated using Deeth et al. (1975) method. The extent of protein breakdown (proteolysis) was evaluated by employing the reported method of Jupfs (1973). The extent of oxidation of fat in terms of thiobarbituric acid (TBA) value was analysed using a method described by Strange et al. (1977).

Analysis of microbiological changes

Standard plate count (SPC) and, yeast and mould count (Y&M) were analysed according to the BIS (1981) procedure. Coliform count, aerobic spore count, proteolytic count, and lipolytic count were performed by using the specific methods given by Marshall (1992).

Analysis of sensory changes

Samples of *Sandesh* were evaluated for attributes such as colour and appearance, flavour, texture, and overall acceptability by a panel of eight semi-trained judges. The panel members were chosen based on their experience in judging traditional Indian dairy products. The panellists assessed the sensory parameters based on a 9-point hedonic scale, wherein “9” denoted extremely desirable and “1” denoted extremely undesirable.

Statistical analysis

Variations in biochemical, microbial and sensory parameters were investigated by analysis of variance (ANOVA) using IBM SPSS software (ver. 20). The results are represented in terms of mean and standard deviations (SD). To compare mean values, Duncan’s test (DMRT) was applied and the significant differences were identified. Principal component analysis (PCA) was performed using Origin pro software (ver. 2023) to determine significant volatile functional groups that affect the headspace of *Sandesh* during storage. Correlation analysis between headspace volatiles and quality attributes was expressed using polar heatmap (Origin pro software).

Results and Discussion

Chemical composition of *Sandesh*

The proximate composition of *Sandesh* (soft grade) revealed 26.90 ± 0.56% moisture, 22.27± 1.00% (*db* w/w) fat, 22.80 ± 0.56% (*db* w/w) protein, 53.40 ± 1.66% total carbohydrates and 1.48 ± 0.08% (*db* w/w) ash. The obtained composition was in agreement with the earlier reports (Khamrui and Solanki, 2010; Sen and Rajorhia, 1991).

Changes in headspace volatile of *Sandesh* stored under different conditions

Detected and identified headspace volatiles of *Sandesh* stored in two packaging materials such as CG vial and DG vial at 30°C and 45°C at each interval are represented in a polar heatmap (Fig. 2). Compounds with a peak area (total ion concentration) of more than 10^4 were considered for data analysis in the study. The peak area of detected compounds for all the samples is mentioned in Table A1 (supplementary materials). The identified volatiles are affirmatively confirmed to be acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. Approximately, 140 product-related headspace volatiles were successfully identified along with a small number (35-40) of fibre-related volatile contaminants, which include derivatives of hydrazine carboxamide, semicarbazide, silanediol, carbohydrazide and polysiloxane were noticed. However, these volatile contaminants were eliminated while processing the data as recommended in the earlier reports (Grimm and Champagne, 2001; Zhang et al. 2022).

Major volatiles

Acids, alcohols, alkanes, ketones and aldehydes have been noticed to be the largest functional group compounds that are present in the headspace of *Sandesh*. Forty acids were identified in *Sandesh* during the storage under various conditions. Among them, carboxylic acids with a chain length ranging from 1 to 20 (C_1-C_{20}) are the majority of compounds. The individual volatile acids possess a non-specific trend during storage due to the continuous degradation and production of acids. A similar non-specific trend of individual volatiles in fermented milk during storage was reported by Dan et al. (2017). However, the sum of the peak areas of all the acids (total peak area) has been considered to analyse the changes during storage. It can be observed from Fig. 3 that the total peak area of the volatile acids has decreased during the storage irrespective of various storage conditions. This could be due to the acid degradation by β-oxidation (saturated fatty acids) and auto-oxidation (unsaturated fatty acids) reactions (Cheng, 2010). The highest percentage of decrease (58.6%) in total peak area was observed in CG samples at both storage temperatures due to the susceptible property of packaging material (transparent) to auto-oxidation of fatty acids. It is believed that acetic acid, propanoic acid, valeric acid and butyric acid were responsible for the decrement in acids’ total peak area. Hence, these acids could be considered while determining the product’s freshness. In addition, we have identified a few more acids in *Sandesh* during storage such as cyacetacide, formic acid, hexanoic acid and stearic acid. The obtained acids have been earlier reported to be present in milk (Dursun et al. 2017). Despite this, carboxylic acids are essential aroma compounds which are also considered to be prominent precursors for methyl ketones, alcohols, aldehydes, alkanes, alkenes, and esters (Cheng, 2010).

In this study, twenty-two different alcohols are identified in *Sandesh* headspace. Ethanol and methanol were found to be the major alcohol compounds present in *Sandesh* (Table A1). Similar to earlier trend of non-specific peak area of acids, the alcohols have also followed the same trend at each storage interval among all the samples (Fig. 3). It can observe that the predominant total peak area has been recorded for alcohols at end of the shelf life in all the samples compared to fresh *Sandesh*. The observable non-

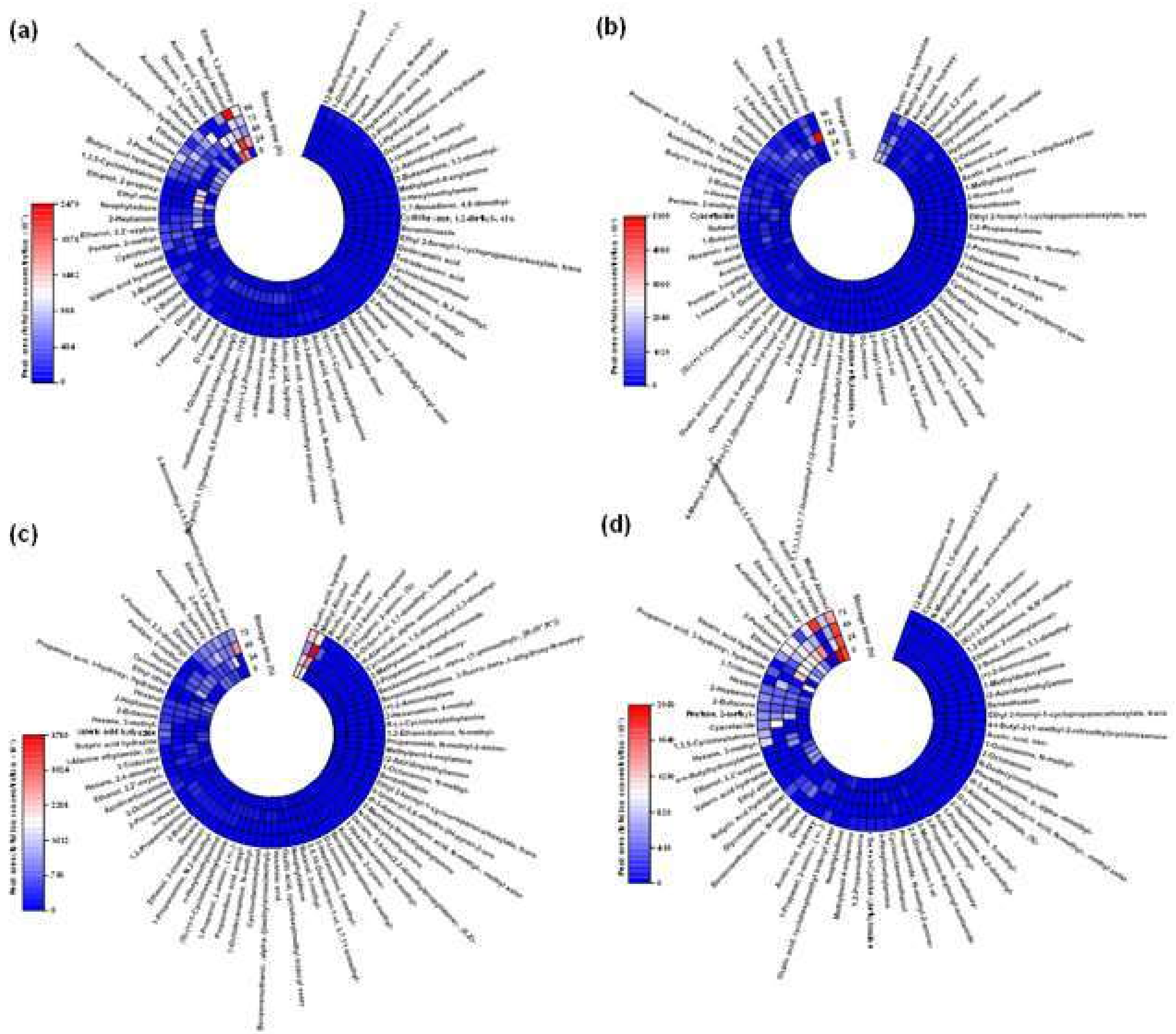


Fig. 2 Polar heat map of headspace volatiles of *Sandesh* during storage, **(a)** Samples stored at 30°C in dark glass vials, **(b)** Samples stored at 30°C in clear glass vials, **(c)** Samples stored at 45°C in dark glass vials, **(d)** Samples stored at 45°C in clear glass vials.

specific trend variations could have been due to various reasons, which include the production of alcohols from aldehydes and ketones reduction, fermentation of lactose, amino acid metabolism, and also partial esterification of resulting alcohols with acids (Yue et al. 2015). It can be believed that these specific compounds of 1-hexanol, 2-ethyl-; 1-hexanol; 1,1,3,3,5,5,7,7-octamethyl-7-(2-methylpropoxy) tetrasiloxan-1-ol; 2-propyl-1-pentanol in CG & 1-pentanol; 1-hexanol, 2-ethyl-; 2-propyl-1-pentanol; methyl alcohol in DG and, 3-aminomethyl-3,5,5-trimethylcyclohexanol, trans; 1-Propanol, 2-amino-, (.+/-.)-; Cyclooctanemethanol compounds in CG and 3-Aminomethyl-3,5,5-trimethylcyclohexanol , trans-; methanol compounds in DG are responsible for the peak area predominance in alcohols at 30°C and 45°C at the end of storage, respectively (Table A1). The observed specific alcohols of primary alcohols might have been originated from the oxidation of unsaturated fatty acids (Cheng, 2010). However, hexanol and pentanol are derived from the aldehydes such as hexanal and pentanal during lipid oxidation (Kilcawley et al. 2018). Rashid et al. 2019 findings revealed that 1-pentanol had appeared as the second-largest volatile compound in pasteurized milk at 10°C after 18 days of storage and 1-heptanol at the end of 16 days of storage. Urbach (1990) reported that ethanol, propan-2-ol and 3-methyl-butna-1-ol were major volatiles present in raw milk stored at 7 °C for 3 days. Therefore, the enhanced amount of alcohol compounds in stored samples of *Sandesh* in our present investigation could be attributed to auto-oxidation of unsaturated fatty acids.

Alkanes emerged as one of the important classes of volatiles in the headspace of *Sandesh* at the end of their shelf life, although they were absent in fresh *Sandesh*. However, aliphatic groups of alkanes with a chain length of C_2-C_{11} have been identified along with the detection of nineteen alkanes in the product . The total peak area of alkanes has been recognized to be enlarged with progressing storage time at 30°C irrespective of packaging materials till 48 h of storage and it has followed the non-specific trend further (Fig. 3). Furthermore, alcohol concentration was observed to be high in both the packaging materials at 45°C compared to *Sandesh* stored at 30°C till 24 h of storage. Eventually, the maximum total peak area was observed in samples stored in CG materials at 30 °C followed by samples in DG materials at the same temperature at the end of the shelf life. Usually, alkanes are secondary oxidation products that are formed from the decomposition of hydroperoxides during the auto-oxidation of unsaturated lipids (Kubow, 1992). However, an increasing trend of peak area has been conspicuously noticed, which was raised due to the presence of ethane, 1,2-diethoxy-; n-hexane; pentane, 3-methyl-; hexane, 3-methyl; pentane, 2-methyl-; heptane among all the alkanes. These results were substantiated by Frankel et al. (1982) who reported that the decomposition of 9- and 13-hydroperoxide from linoleic acid led to the production of pentane and ethane.

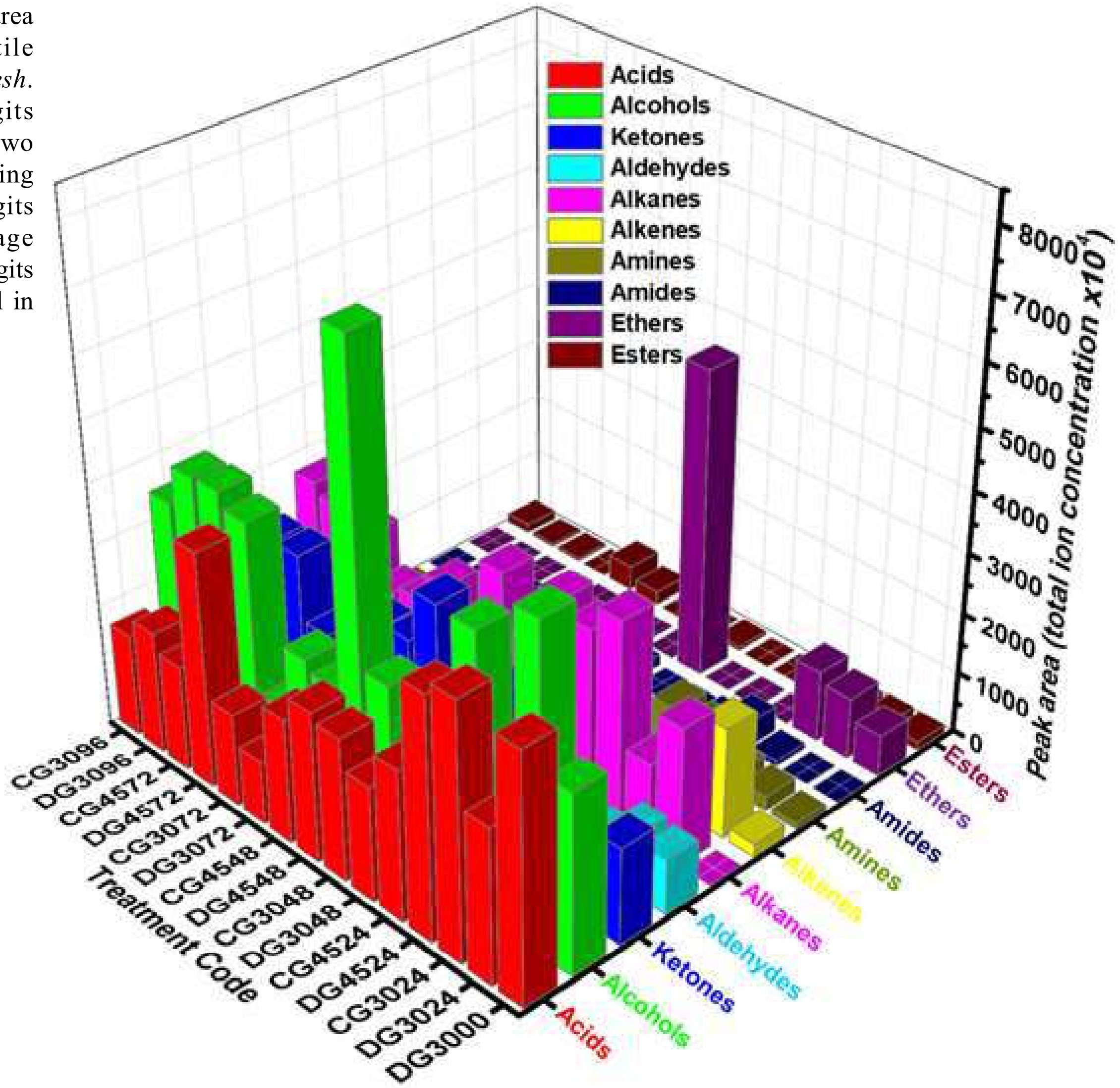
In addition, twelve different ketones were identified in *Sandesh* samples stored under different conditions and changes in their concentration are presented in Fig. 3. Among all the identified ketones, four compounds namely acetone, 2-pentanone, 2-heptanone and 2-butanone were shown highest peak area. These ketones were present in fresh and also formed during storage of *Sandesh* . The earlier studies (Clarke et al. 2020; Yue et al. 2015) demonstrated the presence of ketones in fresh milk and heated milk. Moreover, acetone, 2-heptanone and 2-pentanone have also been considered to be the major three ketones in microfiltered pasteurized milk (Yue et al. 2015). Kumar and Srinivasan (1984) reported that five ketones namely acetone, pentan-2-one, heptan-2-one, octan-2-one, nonan-2-one were identified in fresh samples of *chhana* prepared from cow's milk, buffalo's milk and samples procured from the market. In fresh *Sandesh,* these might have originated from milk and also due to the heat treatment during preparation. The total peak area of the ketones in both packaging materials was observed to be decreased during the first 24 h. Later on, it is increased gradually by the end of 48 h at 30 °C. The peak area of ketones increased slightly with progressing storage interval irrespective of packaging materials at 45 °C. Moreover, the maximum total peak area of the ketones was observed at end of the storage period among samples stored *Sandesh* at 30 °C, which indicates that the temperature played a significant role in ketones generation. Methyl ketones that are present in the product are typically liberated by decarboxylation of saturated fatty acids or by β-ketoacids decarboxylation (Cheng, 2010). Li et al. (2012) reported that 2-heptanone was one of the major compounds that enhanced with increasing storage time of milk powder. Li and Wang (2016) have also described 2-heptanone and 2-nonanone the typical volatiles of oxidized flavours in dairy products.

Aldehydes namely acetaldehyde, hydroxy-; butanal, 3-hydroxy-; glycolaldehyde dimer; hexanal; nonanal; butanal; butanal, 3-methyl-; 2,6-dodecadien-1-al have been determined in our present investigation. According to the earlier report (Yue et al. 2015), heated milk contains aldehydes significantly. The total peak area dynamics of aldehydes of *Sandesh* (Fig. 1e) revealed that the concentration of aldehyde presence has increased notably at the end of 72 h and 48 h of storage time in samples stored at 30 °C and 45 °C, respectively, in both the packaging materials. However, the maximum total peak area was estimated in the samples during the storage at 45 °C. Among all the aldehydes, we have observed the maximum total peak area of hexanal and acetaldehyde, which were present in both fresh and stored *Sandesh*. It is believed that the rapid production and spontaneous degradation of aldehydes into alcohols (Cheng, 2010) could be responsible for the total peak area dynamics of aldehydes. Earlier studies reported that aldehyde compounds can be emerged due to unsaturated fatty acids autoxidation, lactose fermentation and amino acid metabolism (Cadwallader and Singh, 2009; Cheng, 2010; Li et al. 2012).

Minor volatiles

Alkenes, amines, amides, esters and ethers were minor functional groups in the study of volatiles. However, alkene levels revealed that none of the samples exhibited any observable specific trend during storage except CG samples at 45 °C of storage. As alkanes, alkenes are also secondary oxidation products of milk fat during auto-oxidation (Kubow, 1992). From the earlier reports (van Beilen and Funhoff, 2007), the alkenes are preferably converted into alcohols, aldehydes and carboxylic acids due to microbial action. Amines were identified at low concentrations in fresh as well as stored *Sandesh* samples. Although, a maximum number of amines have been noticed in *Sandesh*, their concentration was less compared to acids, alcohols and alkanes (Fig. 2). The majority of amines present in *Sandesh* pertain to the aliphatic group. Usually, amines are familiar intermediate products of protein degradation and their further decomposition can lead to the formation of acids, aldehydes, alcohols, esters and sulfur compounds (Cadwallader and Singh, 2009). Like amines, amides were also generated from the protein degradation, but this class of compounds was present in negligible concentration among all the samples during storage. Also, we have observed eight esters in our present study. Esters corresponding to alcohols and acids in the product were identified to be the highest concentration at end of the shelf life among all the samples at 30 °C rather than the samples at 40 °C. Esters are commonly produced from alcohols and fatty acids through the enzymatic process. Ethers have been identified in the least concentration (Table A1).

Fig. 3 Changes in peak area of headspace volatile groups in stored *Sandesh*. Reference to six digits treatment code: first two digits indicate packaging material; next two digits indicate storage temperature; last two digits indicate storage interval in hours.



Changes in quality attributes of *Sandesh* stored under different conditions

We have carried out the analysis of quality changes in *Sandesh* stored under various conditions until satisfactory sensory scores have been recorded or visible mould growth

Biochemical changes

Biochemical changes of *Sandesh* samples stored under different conditions are presented in Table A2 (Supplementary materials). Present results displayed that the initial mean titratable acidity (% lactic acid) value of *Sandesh* significantly ($P<0.05$) increased from 0.46 to 0.56 and 0.58 at 30 °C; to 0.73 and 0.72 at 45 °C, respectively, in DG and CG during storage (Fig. 4a). Furthermore, ANOVA results revealed that the titratable acidity of *Sandesh* has been significantly ($P<0.05$) influenced by incubation temperature and non-significantly ($P>0.05$) by packaging materials. As evidenced, there was less improvement in the titratable acidity of *Sandesh* stored *at* 30 °C. The present observations were in good agreement with the earlier report (Yadu, 2014). After an initial pH of 5.89, *Sandesh* pH reduced to 5.69 and 5.76 at 30 °C; 5.73 and 5.71 at 45 °C, respectively in DG and CG during storage (Fig. 4b), which could be attributed to the production of lactic acid by spoilage microorganisms. Storage time shows its significant ($P<0.05$) impact on the pH of the product, whereas storage temperature and packaging materials had a non-significant ($P>0.05$) effect during storage. The decrease in pH of *Sandesh* samples has been earlier reported by Mandal (2019). In addition, the TBA value of all *Sandesh* samples enhanced significantly ($P<0.05$) with progressing storage time among all samples (Fig. 4c). The highest TBA value was observed in the CG container at 45 °C in the order of 0.229. The remarkable enhancement of TBA values could be attributed to the presence of a considerable amount of fat in *Sandesh*. This fat is highly susceptible to oxidation under higher temperature conditions.

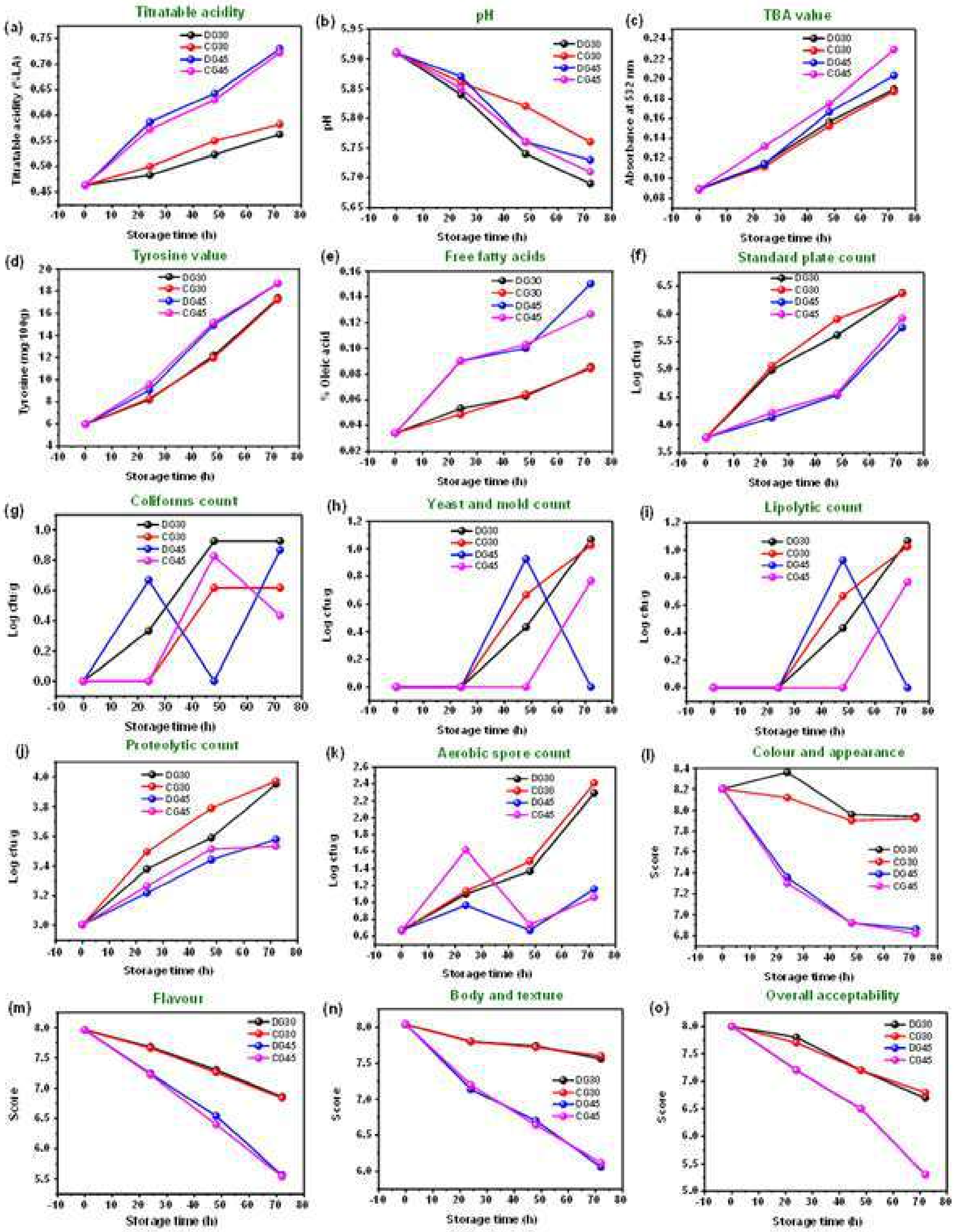


Fig. 4. Quality changes in *Sandesh* stored under various conditions **a)** Titratable acidity **b)** pH **c)** TBA value **d)** Tyrosine value **e)** Free fatty acids **f)** Standard plate count **g)** Coliforms count **h)** Yeast and mold count **i)** Lipolytic count **j)** Proteolytic count **k)** Aerobic spore count **l)** Colour and appearance **m)** Flavour **n)** Body and texture **o)** Overall acceptability. Reference to four digits treatment code: first two digits indicate packaging material; last two digits indicate storage temperature

Gargouri et al. (2015) reported that the rate of oxidation is influenced by many factors including temperature, oxygen and light. The significant increase in TBA values of samples stored in CG at 45 °C might be due to the combined effect of the storage temperature as well as light transmittance through the packaging material. Mandal (2019) has also observed a considerable enhancement in the TBA value (% malonaldehyde) of *Sandesh* during storage.

The tyrosine content (mg/100g) of *Sandesh* stored at 30 °C has been identified to be enhanced (P<0.05) from 5.98 to 17.38 and 17.24; to 18.66 and 18.60 in DG and CG, respectively at 45 °C. A significant amount of tyrosine content (P<0.05) in *Sandesh* is noticed at the end of the shelf life at 45 °C. However, non-significant (P>0.05) differences were observed in packaging materials. A similar increment in the tyrosine content of *Sandesh* during the shelf life has been mentioned in the earlier report (Yadu, 2014). The FFA values of *Sandesh* notably (P<0.05) varied from 0.034 to 0.15% oleic acid among all the samples (Fig. 4e). ANOVA revealed that the storage temperature substantially influenced the FFA value, but not packaging materials.

Microbiological changes

We have investigated the microbiological changes of *Sandesh* stored under different conditions. The standard plate count (SPC) (log cfu/g) of *Sandesh* exhibited a remarkable (P<0.05) increase from 3.76 to 6.37 when stored at 30 °C and to 5.92 at 45 °C. SPC of samples stored in DG container at 30 °C was estimated to be significantly (P<0.05) higher than samples stored at 45 °C, whereas non-significant (P>0.05) with samples stored in CG at 30 °C. However, the SPC of samples in both the packaging materials was found to be non-significantly different (P>0.05) at 72 h. The microbiological quality of all the samples stored under various conditions was found toexceed the prescribed standards (maximum limit 5.54 log cfu/g) recommended by FSSR (2011) after 48 hours.

It can be observed that coliforms were absent in fresh samples. Despite the presence of coliforms in the first dilution during storage, they did not exceed the standards set by FSSR (2011), indicating hygienic conditions were maintained during manufacturing and handling. Moreover, yeast and mould (Y&M) were not detected in the first dilution of the samples stored at 30 °C and 45 °C, respectively up to 48 h. On further storage, the count has been significantly (P<0.05) multiplied among all the samples (Fig. 4h). The Y & M count of *Sandesh* stored at 30 °C in both packaging materials exceeded the standards (2.17 cfu/g) prescribed by FSSR (2011). The count has been estimated to be substantially (P<0.05) greater than the samples stored at 45 °C in two packaging materials, which could be attributed to the favourable growth temperature and increased acidity of samples. The observations of enhanced Y&M count were also reported by the earlier worker, Yadu (2014). A similar trend was observed in the lipolytic bacteria count of *Sandesh* during the storage. However, the count was non-significantly (P>0.05) increased to 1.06 log cfu/g during storage (Fig. 4i). The proteolytic count of *Sandesh* samples has also been multiplied rapidly (Table A2) in storage time. In addition, aerobic spore count (log cfu/g) was found to substantially enhance from 0.66 to 2.40 among samples (Fig. 4k) but statistically non-significant (P>0.05) at the end of 72 h of storage. The increase in the count might be due to favourable temperature for the growth of aerobic spore count during prolonged storage.

Sensory changes

Sensory scores of *Sandesh* stored under different conditions for the stipulated period are displayed in Fig. 4. A non-significant (P>0.05) decrement in colour and appearance (C&A) scores of *Sandesh* was observed at 45 °C, whereas significant (P<0.05) decrement at 30°C in both the packaging materials. Further, it was observed that slight deformation of the shape of samples occurred due to the stacking of pieces in the container and the expulsion of moisture during storage, which might have contributed to reduced scores (Fig. 1e). However, no visible Y&M growth was observed even after a chemical or physical deterioration of samples when stored at 45 °C. Conversely, visible mould growth was observed in all the samples at 30 °C after 72 h of storage interval.

The flavour scores of *Sandesh* have been remarkably decreased in both the cases of DG and CG due to the production of off-flavours as a combined effect of deteriorative reactions. In this regard, storage conditions displayed a substantial effect on the flavour scores after 48 h of storage interval. The scores were noticed to be decreased rapidly in samples stored at 45 °C than 30 °C and also the formation of off-flavours was rapid at higher temperatures. Moreover, a drastic decline was observed in body and texture scores of *Sandesh* from 8.04 to 6.06 during storage at various conditions (Fig. 4n). Statistical analysis revealed that the samples stored at 30 °C exhibit substantial greater scores than those stored at 45 °C in both packaging materials, which could be due to the expulsion of moisture from the samples at 45 °C (Fig. 1e). The overall acceptability score decreased conspicuously (P<0.05) from 8.02 to 6.74 and 6.82 at 30 °C; to 5.32 and 5.30 at 45 °C DG and CG, respectively during the storage. The overall acceptability scores of *Sandesh* samples stored at 30 °C exhibited significantly higher values than the scores of samples at 45 °C (Fig. 4o). From biochemical, microbial and sensory results, the shelf life of *Sandesh* was estimated to be 72 h and 48 h at 30 °C and 45°C respectively, regardless of packaging materials.

Principal component analysis

The principal component analysis (PCA) was employed to determine the significant volatile functional groups that affected the headspace of *Sandesh during* storage and, also evaluate the

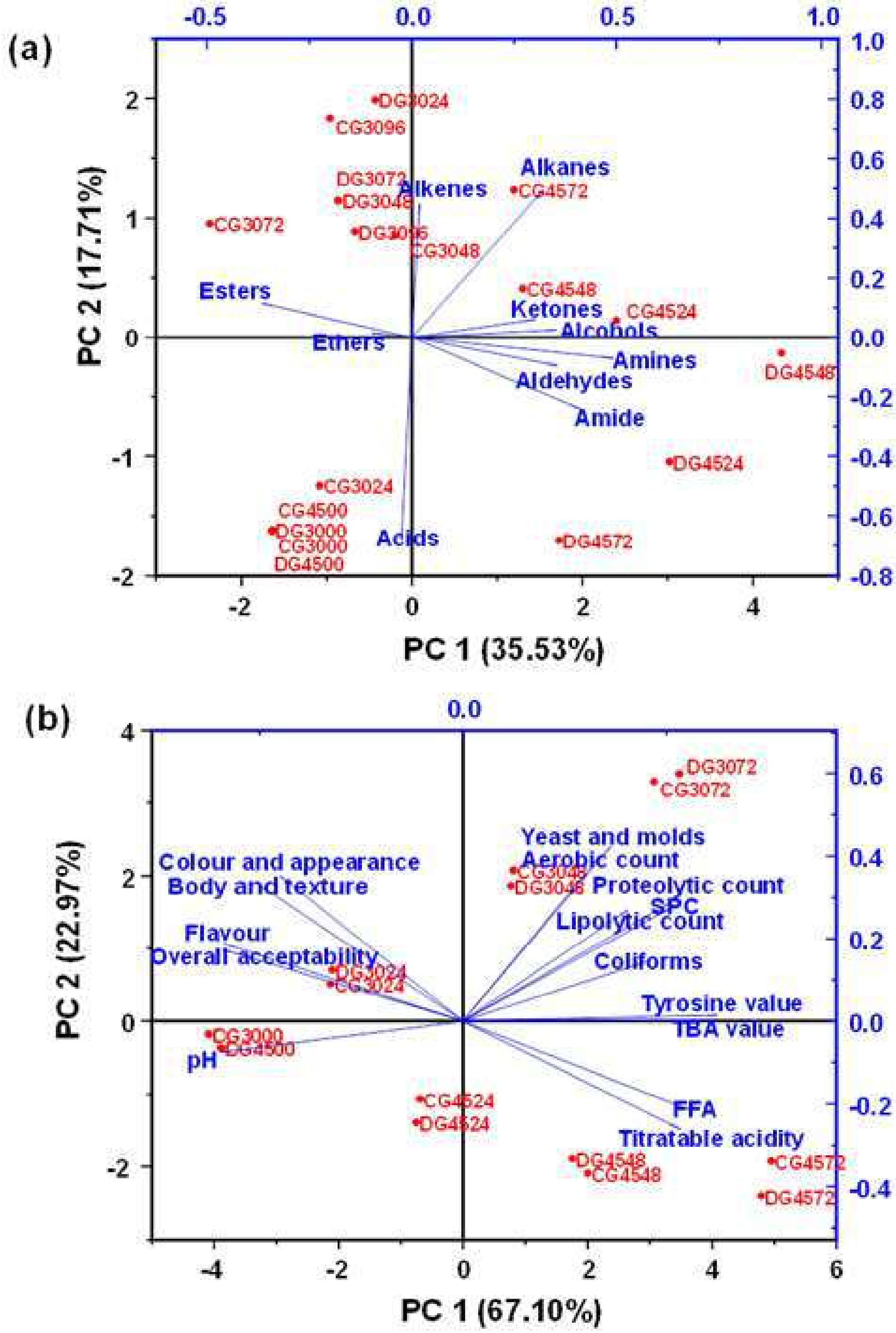


Fig. 5. Principal component analysis **(a)** Biplot for volatile components **(b)** Biplot for quality parameters. Reference to six digits treatment code: first two digits indicate packaging material; last two letters indicate storage temperature; last two digits indicate storage interval in hours.

considerable effect of various treatments of *Sandesh* on volatile functional groups. As a result of PCA, a biplot enabled us to visualize the interaction between the observations and variables (Fig. 5). In a biplot, the elongated line indicates greater variance in the variables, while the shorter line indicates less variance. The cosine angle between the lines in the biplot reflects the degree of correlation between their variables. The correlation between their variables decreases as the angle approaches 90 or 270°. However, a correlation of 1 or -1 is represented by an angle of 0 or 180 degrees, respectively (Kohler et al. 2005). We have observed that the first four principal components (PC) expressed approximately 78.21% of the variance between sample and headspace volatile functional groups. Of four principal components, PC1 is estimated to be 33.87% of the variance and is characterized by amines, amides and alcohols. PC2 was evaluated to be 17.98% of the variation and was defined by

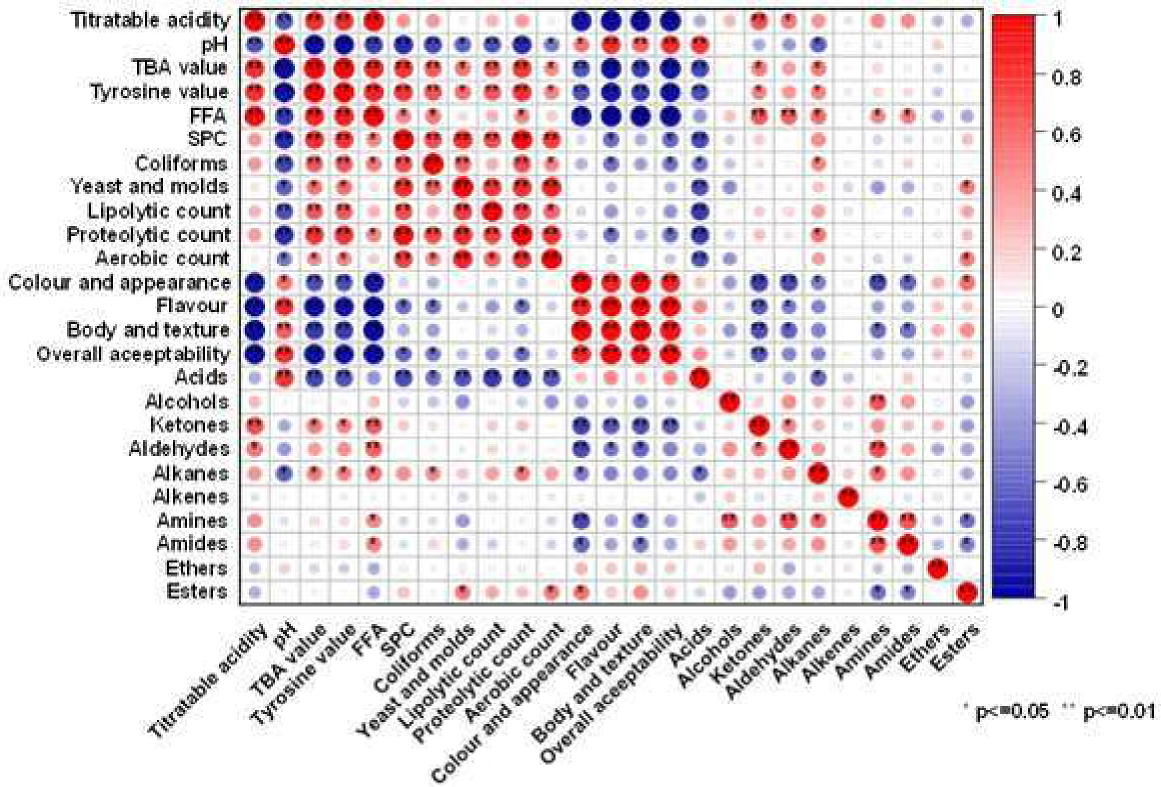


Fig. 6. Colour map of correlation analysis between headspace volatiles and quality attributes of *Sandesh* during storage

alkanes and opposed by acids. It revealed that amines, amides, alcohols and alkanes are increased with increasing shelf life and acids are inversely related. In the present study, loading with an absolute value of more than 0.7 indicates a strong influence on *Sandesh* quality during storage. From the results, fresh *Sandesh* has the highest number of acids. From the results, a greater total peak area of the acids is observed conspicuously in fresh *Sandesh* whereas the total peak area of alkanes, alcohols and ketones are recognized to be prominent in the treatments of DG4572, DG4548, and DG4524 samples. Moreover, amines, aldehydes and amides are noticed to be high in DG4572, DG4548 and DG4524 samples.

The major quality parameters (biochemical, microbial and sensory) of *Sandesh* during storage have been systematically evaluated from the PCA. According to the biplot of PCA (Fig. 2b), PC1 and PC2 were explained to be 67.10% and 22.97% of the total variance, respectively. Therefore, the total variance for the two factors can be explained as 90.07%. Furthermore, we have obtained meritorious levels of SPC, proteolytic count, aerobic count, lipolytic count and coliforms for DG3072, CG3072, DG3048 and DG3048 samples. Additionally, FFA and titratable acidity levels were observed to be more in CG4572, DG4572, DG4548 and CG4548 and also estimated the least sensory scores for CG4572, DG4572, DG4548 and CG4548.

Correlation analysis between the headspace volatiles and quality changes

The correlation analysis was systematically carried out to identify the association of the headspace volatile functional groups with quality attributes of *Sandesh* during storage under different conditions (Fig. 6). The correlation between volatile functional groups and quality changes revealed that the proteolytic count ($r=-0.774$, $P<0.01$), lipolytic count ($r=-0.765$, $P<0.01$), TBA value ($r=-0.726$, $P<0.01$), SPC ($r=-0.718$, $P<0.01$), tyrosine value ($r=-0.698$, $P<0.01$), Y&M count ($r=-0.696$, $P<0.01$), aerobic spore count ($r=-0.672$, $P<0.01$), coliform count ($r=-0.559$, $P<0.05$) were negatively correlated with acids, whereas pH ($r=+0.793$, $P<0.01$) was positively correlated with acids of *Sandesh* during storage. Moreover, titratable acidity ($r=+0.714$, $P<0.01$), FFA value ($r=+0.661$, $P<0.05$), TBA value ($r=+0.552$, $P<0.05$), tyrosine value ($r=+0.522$, $P<0.05$) were positively correlated with ketones, whereas colour and appearance ($r=-0.743$, $P<0.01$), body and texture ($r=-0.680$, $P<0.01$), flavour ($r=-0.657$, $P<0.01$), overall acceptability ($r=-0.649$, $P<0.01$) were negatively correlated with ketones of *Sandesh* during storage. FFA value ($r=+0.590$, $P<0.05$), proteolytic count ($r=+0.545$, $P<0.05$), TBA value ($r=+0.543$, $P<0.05$), tyrosine value ($r=+0.517$, $P<0.05$) and coliforms ($r=+0.500$, $P<0.05$) were positively correlated with alkanes, whereas pH ($r=-0.621$, $P<0.05$) and, colour and appearance ($r=-0.519$, $P<0.05$) were negatively correlated with alkanes of *Sandesh* during storage.

As we noticed, FFA value (r=+0.626, P<0.05) and titratable acidity (r=+0.563, P<0.05) were positively correlated with aldehydes since colour and appearance (r=-0.716, P<0.01), body and texture (r=-0.617, P<0.05) and flavour (r=-0.522, P<0.05) were negatively correlated with aldehydes of *Sandesh* during storage. It can be observed that FFA value (r=+0.514, P<0.05) was positively associated with amines, as colour and appearance (r=-0.701, P<0.01) and body and texture (r=-0.584, P<0.05) were negatively correlated with amines of *Sandesh* during storage. In amides, FFA value (r=+0.539, P<0.05) was positively correlated, whereas colour and appearance (r=-0.615, P<0.05) and, body and texture (r=-0.556, P<0.05) were negatively associated with amides of *Sandesh* during storage. We have also explored that colour and appearance (r=+0.562, P<0.05), Y&M (r=+0.543, P<0.05) and aerobic spore count (r=+0.541, P<0.05) were positively correlated with esters of *Sandesh* during storage.

Conclusion

In summary, we have investigated 140 product-related headspace volatiles for determining the product's quality markers. The identified volatiles were of various functional groups, which include acids, alcohols, ketones, aldehydes, alkanes, alkenes, amines, amides, esters and ethers. However, interestingly, among all the volatiles of functional groups, acids, alcohols, alkanes and ketones were found to be the largest in terms of peak area during the storage. The dynamics of volatile compounds revealed continuous degradation of produced metabolite volatiles, which lead to the production of new volatiles. Notably, we have determined the predominant total peak area for the alcohols at end of the shelf life compared to the fresh product. Nevertheless, the peak area of alkanes and ketones enhanced gradually during storage in most of the treatments. Also, as expected significant changes in biochemical, microbial and sensory parameters of *Sandesh* packaged and stored at various conditions were observed. As a result of these quality changes, the shelf life of *Sandesh* has been assessed to be 72 h and 48 h at 30 °C and 45 °C, respectively, irrespective of the packaging materials. Of all the groups, amines, amides, alcohols, alkanes and acids are noticed to be the most significant functional groups among all the classes according to principal component analysis. The obtained *Sandesh* quality factors have been observed to be well correlated with acids, ketones, alkanes and aldehydes during storage from the correlation studies. From this study, headspace freshness marker of acid group was recognized along with headspace key spoilage markers such as alkanes, alcohols and ketones, which consists of 1-hexanol, 2-ethyl-; 1-pentanol; 1-hexanol; 3-aminomethyl-3,5,5-trimethylcyclohexanol trans-; 1-propanol, 2-amino-, (.+/-.); pentane, 2-methyl; hexane, 2,4-dimethyl-; hexane, 3-methyl; n-hexane; acetone; 2-heptanone; 2-pentanone. The presence of these markers can evaluate the quality of *Sandesh* in both the ways such as freshness and spoilage. The present investigation could be used for quality control aspects in dairy industry and also in the development of intelligent packaging systems for monitoring the real-time quality of the packaged product.

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