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CENTRAL OFFICE : Indian Dairy Association, IDA House, Sector IV, R.K. Puram, New Delhi-110022. Phones: 011-26170781, 26165237, 26165355. Email: idahq@rediffmail.com /www.indiandairyassociation.org

ZONAL BRANCHES & CHAPTERS : **South Zone:** Dr. Satish Kulkarni, Chairman, IDA House, NDRI Campus, Adugodi, Bangalore-560 030. Ph.: 080-25710661 Fax: 080-25710161. **West Zone:** Dr.J.B. Prajapati, Chairman; A-501, Dynasty Business Park, Andheri-Kurla Road, Andheri (East), Mumbai 400059 Email: chairman@idawz.org / secretary@idawz.org Ph.: 91 22 49784009 **North Zone :** Dr. Rahul Saxena, Chairman; c/o IDA House, Sector IV, R.K. Puram, New Delhi - 110 022 Phones: 011-26170781, 26165355. **East Zone:** Shri Sudhir Kumar Singh, Chairman, c/o NDDB, Block-DK, Sector-II, Salt Lake City, Kolkata-700 091 Phones: 033-23591884-7. **Gujarat State Chapter:** Dr. Amit Moolchand Vyas, Chairman; c/o SMC College of Dairy Science, AAU Campus, Anand-388110 Gujarat. Email: idagscac@gmail.com **Kerala State Chapter:** Dr. S.N. Rajakumar, Chairman; c/o Prof. and Head, KVASU Dairy Plant, Mannuthy, E mail: idakeralachapter@gmail.com **Rajasthan State Chapter:** Dr. Karun Chandalia, Chairman; 418-419, Fourth Floor, Sunny Mart, New Atish Market, Jaipur-302 020 E-mail: idarajchapter@yahoo.com **Punjab State Chapter:** Dr. Inderjit Singh, Chairman, H.No. 1620, Sector-80, SAS Nagar, Mohali-140 308 (Punjab) Email: secretaryidapb2023@gmail.com **Bihar State Chapter:** Shri D.K. Srivastava, Chairman; c/o Former Managing Director, Mithila Milk Union; House No. 16 Mangalam Enclave, Baily Road, Near Saguna SBI, Patna-801503 (Bihar). E-mail: idabihar2019@gmail.com **Haryana State Chapter:** Dr. S.K. Kanawjia, Chairman; c/o D.T. Division, NDRI, Karnal-132 001 (Haryana). Ph.: 09896782850 Email: skkanawjia@rediffmail.com. **Tamil Nadu State Chapter:** Shri Kanna K.S., Chairman; c/o Department of Dairy Science, Madras Veterinary College, Vepery, Chennai-600007. **Andhra Pradesh Local Chapter:** Prof. Ravi Kumar Sreebhashyam, Chairman; c/o College of Dairy Technology, Sri Venkateshwara Veterinary University, Thirupathi - 517502 Email: idaap2020@gmail.com **Eastern UP Local Chapter:** Dr. Arvind, Chairman; Assistant Professor, Department of Dairy Science & Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005 Ph.: 7007314450 Email: arvind1@bhu.ac.in **Western UP Local Chapter:** Dr. Ashok Kumar Tripathi, Chairman; c/o Flat no. 1003/8, Zen Spire, Ramprastha Greens, Vaishali, Ghaziabad-201010 (UP). Email: ttreddy@arvinddairy.com **Jharkhand Local Chapter:** Shri Pavan Kumar Marwah, Chairman; c/o Jharkhand Milk Federation, FTC Complex, Dhurwa Sector-2, Ranchi, Jharkhand-834004 Email: jharkhandida@gmail.com **Telangana Local Chapter:** Shri Rajeshwar Rao Chalimeda, Chairman; c/o Dodla Dairy Ltd Corporate Office, # 8-2-293/82/A, 270/Q, Road No 10-C, Jubilee Hills, Hyderabad – 500 033 Telangana. **Karnataka State Chapter:** Shri N.B. Marathe, Chairman; c/o Dairy Science College, Mahagaon Cross, Kalaburagi-585316. Mobile: 9483124271 Email: ida.kar.chapter@gmail.com

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RESEARCH ARTICLE

Feasibility assessment of sensorial accepted novel dairy based dip during storage

Subhadip Manik[1], Anindita Debnath[2](✉), Shamim Hossain[1], Partha Pratim Debnath[2], Kuntal Roy[2], Pinaki Ranjan Ray[3] and Lopamudra Haldar[4]

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Abstract: In recent era, the inclination of people towards convenient food products has increased due to health consciousness and growing urbanization as well as rapid change in social and cultural practices. The preparation of sauce or dip like products with some diversification and enhanced nutritional as well as functional value have become an area of research. Therefore, an attempt was made to develop a novel dairy-based dip like product from heat-acid induced milk gel and whey. Based on the sensory analysis during preliminary trails, level of cream (40% milk fat), whey, common salt, glycerol monostearate, trisodium citrate and sodium hexametaphosphate were optimised. A good quality of dairy based dip could be prepared using heat-acid induced milk gel from bovine skim milk, cream (40% fat), whey, common salt, trisodium citrate, sodium hexametaphosphate and glycerol monostearate @ 52.67%, 14.70%, 31.73%, 0.42%, 0.16%, 0.16% and 0.16%, respectively. The developed product was stored at refrigeration temperature (4±1 ºC) in PET bottles. It was stable up to 11 days in in the aspect of physico-chemical, sensorial and microbiological point of view. For the first time, a novel dairy based dip was formulated in a stable form using direct application dairy byproducts (whey and skim milk).

Keywords: Dairy based dip; Heat-acid induced milk gel; Whey; Novel; Sensory

Introduction

Numerous studies have been focused nowadays to design new food products that can provide better health, eating habit and nutrition. It can also provide positive impact on human health. Changes in food formulation, food service procedures, and eating customs have resulted from this. Currently, a lot of work is being done on dietary solutions to meet the increasing demands of the ageing population. Food design must therefore include the needs of contemporary populations, especially ageing populations, taking into account convenience, enjoyment, health and nutrition (Sun-Waterhouse et al. 2021). Heat-acid induced milk gel (also known as *Chhana* in India) offers enormous scope in the development of new dairy products due to its immense popularity and nutritive value among all age groups people. Several works have been done to develop spread like products from heat-acid induced milk gel (Dixit, 2006; Chappalwar et al. 2010; Kumar et al. 2016) to cope up with the demand for both low fat but nutritious and diversified foods with ethnic flavour. However, no reference is available in the literature on the development of sauce or dip like product from heat-acid induced milk gel.

Dip has a thinner consistency than spread but thicker than sauce. It is served in separate container in cold condition, while sauce can be served both in warm or cold conditions (IFIS, 2009). Additionally, the market for cheese sauces and similar product was US$ 1438.39 million in 2020 and same is expected to grow up to US$ 2218.38 by 2030 with a CAGR of 4.5 % as reported by Prophecy Market insights (2023). Demott *et al.* (1977) developed chip dip (solid content 13.07-13.30%) from cottage cheese whey by adding xanthan gum at the rate of 1.2 - 1.4% followed by slow blending and was stored 4°C. Saad *et al.* (2016) developed a processed cheese sauce (25% dry matter and 40% fat on dry matter basis) from ras cheese by blending it with milk protein, butter fat, nisin, stabilizer (admixture of guar gum and corn starch), NaCl, and emulsifying salt. The effect of milk protein from different sources such as milk protein concentrate, total milk proteinate, UF-retentate curd, skim milk powder and soy protein concentrate were evaluated. The final products were found acceptable in

[1] Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India.
[2] Department of Dairy Technology, [3] Department of Dairy Chemistry, [4] Department of Dairy Microbiology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences, Mohanpur, Nadia, West Bengal, India

Anindita Debnath(✉)
Department of Dairy Technology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences, Mohanpur, Nadia-741252, West Bengal, India.
Email: aninditadebnathwbuafs@gmail.com; Contact No: +91-9163384369

terms of sensorial properties and shelf life but the most acceptable was the product made using UF-retentate curd. Shalaby *et al.* (2017) developed plain processed cheese sauce by admixing whey protein concentrate and acid casein curd. The effect of essential oils was evaluated from different sources such as turnip, shallots, capsicum and cardamom on the sensorial quality of the cheese sauce with an aim of providing improved flavour to the product. Dixit (2006) reported the manufacturing process of heat-acid induced milk gel spread by blending with salt, whey and preservatives, packaged, and stored at 5±1°C. Gamay *et al.* (2011) observed that whey protein contribute mouthfeel as well as texture of cheese sauce, while the viscosity and texture of were largely influenced by the presence of gum like stabilizers such as sodium alginate, guar gum, xanthan gum. Flavour and texture profile of the product was provided by phosphate salt and common salt. Hine (1994) observed that desired quality cheese sauce can be obtained by using either natural or unmodified food-grade starch (e.g. rice starch, tapioca starch and potato starch) as ingredient. Spanier (1986) suggested to use either maltodextrin or corn syrup solids as filler material for improving the texture of cheese sauce. Bansal et al. (2017) prepared a cheese dip using 8.82% protein blend (whey protein concentrate-70:sodium caseinate = 80:20), 6% Cheddar cheese and 9.72% cream. During preparation of cheese dip, trisodium citrate, carboxy methyl cellulose and glycerol monostearate were used. Bansal et al. (2021) also evaluated the storage (4±1 ºC) induced changes in developed product with and without spices in PET bottles for 30 days. This study revealed that blending of spices prevented the deterioration in terms of sensorial, physico-chemical and microbiological aspect. Furthermore, inter-relationship of food quality and its storage stability plays a vital role for the introduction of new products. In order to estimate the shelf life of food product, package material choice, and storage conditions necessary to preserve the quality of food products, it is strategically important to investigate changes in the sensory, physico-chemical, textural, and microbiological characteristics of foods. A suitable shelf life also confirms that consumers can obtain high-quality food (Bansal et al. 2021).

In view of the opportunity of bringing diversification in food products through the use of heat-acid induced milk gel and to cater to the need of health-conscious consumers, an attempt was made to develop a dip like product. Production of such dairy based dip with lower fat content using heat-acid induced milk gel from skim milk and whey will not only meet the demand of health-conscious consumers, but also improve the scale of economy in dairy sector through the utilization of surplus skim milk and whey and help in product diversification. Till date, not a single research study reported that advocate the development and estimation of storage stability of heat and acid coagulated gel-based dip. The utilization of dairy by products i.e., whey and skim milk were also focused in this study. So that, a feasibility assessment was done in this current study using dairy based dip for the first time.

Materials and Methods

Materials

Fresh cow milk was acquired from the farm of West Bengal University of Animal and Fishery Sciences (Mohanpur Campus, West Bengal, India). Skim milk and cream were separated using a centrifugal cream separator. The fat percentage in skim milk and cream was standardized at 0.5 and 40%, respectively. Whey obtained during cow skim milk heat-acid induced milk gel preparation, was pasteurized to 72°C for 15 s and cooled to room temperature. Glycerol monostearate (GMS) was procured from Tripathi Products Pvt. Ltd., New Delhi. Common salt was procured from Tata chemicals Ltd., Mumbai. Food grade citric acid and trisodium citrate (TSC) were procured from Urban Platter, New Delhi. Sodium hexametaphosphate (SHMP) was obtained from Choice Organochem LLP, Hyderabad.

Preparation of heat-acid induced milk gel

The method for heat-acid induced milk gel preparation as suggested by Kumar et al. (2016) was followed with some modification. The cow milk after receiving was filtered, separated and standardized to 0.5% fat and 8.5% SNF. The standardized skim milk was heated to 90°C followed by immediate cooling to coagulation temperature 65°C. The coagulation was done with citric acid solution of 2% strength till a clear whey separation. Finally, whey was drained out using a muslin cloth and rest part was hung for 30 min to obtain the heat-acid coagulum i.e., heat-acid induced milk gel.

Preparation of dairy based dip

Dairy based dip was prepared using heat-acid induced milk gel from cow skim milk and whey (Figure 1). Here, level of pasteurized whey, sodium hexametaphosphate, tri-sodium citrate and common salt of the total of heat-acid coagulated gel was optimised on the basis of sensory analysis during preliminary trails. Similarly, pasteurized cream (40% fat) and salt level were also optimised. The optimised level of pasteurized whey, sodium hexametaphosphate, tri-sodium citrate and common salt were 60.26%, 0.3%, 0.3% and 0.8% on total weight of heat-acid induced milk gel and they were blended with heat-acid induced milk gel thoroughly with a domestic hand blender (Philips Hand Mixer Model: HR3705, equipped with two kneading hooks) at speed control level 5 (1200 rpm), respectively. After that, pasteurized cream (40% fat) and glycerol monostearate were also added into that homogeneous slurry at the level of 27.92% and 0.3% on the basis of total weight of heat-acid induced milk gel. The entire mixture was blended to obtain a product with homogeneous consistency. The product was heat treated for 5 min at 65 °C. It was then cooled to room temperature. After that, the dairy based dip was filled in a PET bottle and stored under refrigeration (4±1 ºC) for further analysis.

Physico-chemical analysis

The moisture, fat, protein, lactose, salt, and ash of the optimized product were estimated using AOAC (2005). Acidity of dairy based dip was estimated using the standard method of IS: SP (1981). Adopting the described method by Juffs (1973), tyrosine value for the dairy based dip estimated. While, 2-thiobarbituric acid (TBA) value was measured as the standard method described by Tarladgis et al. (1960). Similarly, free fatty acid of the optimised product was estimated using Deeth et al. (1975). The pH of the optimised product during entire storage period was recorded using a pre-calibrated pH meter at 25 ± 1 ºC.

Sensory analysis

The sensory evaluation conducted in this study received ethical approval and participant consent, adhering to the ethical standards of both the institutional and national research committees. This process also complied with the principles outlined in the 1964 Helsinki declaration and its subsequent amendments or equivalent ethical guidelines. A panel of 7 trained judges (they have prior experience in sensorial analysis of milk and milk products and trained for 6 h before the sensory analysis of dairy based dip) from the Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences (Mohanpur Campus, West Bengal, India) evaluated dairy based dip samples for sensory characteristics through 9-point hedonic scale. Flavour, body and texture, colour and appearance (CA) and overall acceptability (OA) are the sensory attributes of dairy based dip sample. Panel members carried out sensory evaluation in individual booths where 50 g of sample in a glass container was given to the judges at 20ºC. Ethical consent was obtained from all trained panellist of this study.

Microbiological analysis

Standard plate count (SPC), coliform and yeast and mold (YM) count were conducted as per the standard method outlined by Bansal et al. (2021). Here, obtained results were represented in total number of colonies in each gram of sample. Required agars were obtained from HiMedia Laboratories Pvt. Ltd. (Maharashtra, India).

Statistical analysis

Obtained results were analysed using one-way analysis of variance (ANOVA) in IBM SPSS program (version 25) with α=0.05 and Tukey HSD was used as a post hoc test for comparison the means. While, descriptive statistics was used to analyse chemical composition of dairy based dip.

Results and Discussion

Chemical composition of dairy based dip

The optimized product contained 72.59 ± 0.26% moisture, 8.34 ± 0.05% fat, 12.20 ± 0.08% protein, 0.71 ± 0.01% salt, 5.98 ± 0.38% lactose and 0.11 ± 0.002% ash, respectively. The optimised product

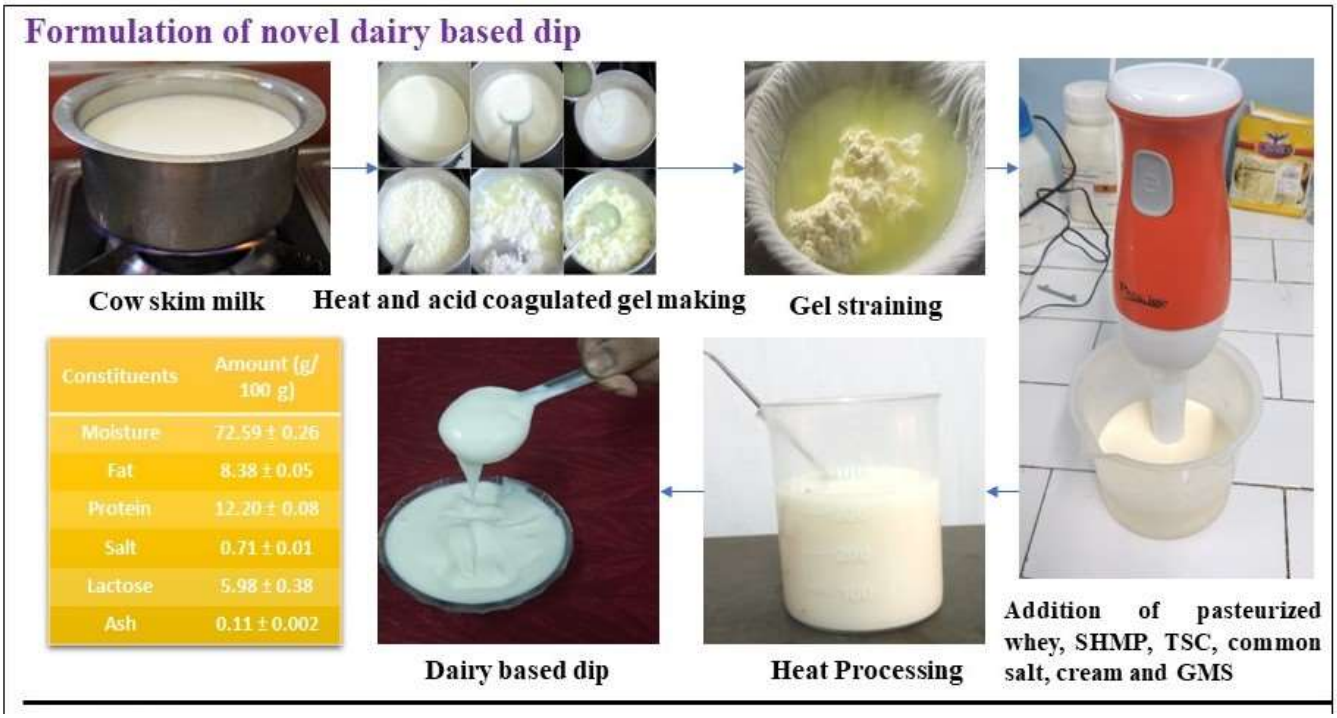


Fig. 1 Process flow diagram for novel dairy based dip and graphical representation of current study

contained lower moisture, fat, salt and ash content over cheese dip (76.21%, 11.60%, 1.06% and 2.54%, Bansal et al. 2017). While higher protein and lactose content was observed in dairy based dip than cheese dip (8.12 and 0.46%, Bansal et al. 2017).

Physico-chemical changes in dairy based dip during storage at 4±1°C

The moisture content of dairy based dip sample decreased from 72.57% at 1st day to 72.47% at 11th day of storage. The difference between the moisture content of the product at 1st and 11th day of storage was significant ($p<0.05$) (Figure 2a.). The increase in total solids content in paneer spread with progression of storage was observed by Dwivedi et al. (2014). The result was in accordance with the present result. Moreover, decline of moisture content of the product with the progression of storage was attributed due to the free water evaporation in the product (Bansal et al. 2021). Similar trend was observed with earlier work (Rafiq and Ghosh, 2018; Bansal et al. 2021).

The acidity of dairy based dip increased from 0.426% lactic acid at 1st day to 0.580% lactic acid at 11th day of storage. The acidity of dairy based dip sample from 1st day to 3rd day increased significantly ($p<0.05$) but with progression of storage, the acidity from 3rd day to 11th day increased non-significantly ($p>0.05$) (Figure 2b.). The increase in titratable acidity in dairy based dip was probably due to the growth of lactic acid producing bacteria, resulted in lactic acid production. The present result is in accordance with the result was obtained during storage study of paneer spread by Dwivedi et al. (2014). The increase in acidity in paneer spread during storage was observed.

The pH value of dairy based dip decreased from 6.30 at 1st day to 6.10 at 11th day of storage. The pH of the product decreased non-significantly ($p>0.05$) with progression of storage (Figure 2c) The increase in lactic acid content and free fatty content during storage might be the reason of lowering pH in all samples. Saad *et al*. (2015) also observed the lowering of pH with progression of storage day in processed cheese sauce. It was proposed that hydrolysis of lactose, activity of resistant enzymes present in sauces, presence of polymerized phosphate of emulsifying salts and their interaction with proteins was responsible for pH reduction (Saad et al. 2015).

The tyrosine value of dairy based dip increased from 0.217 mg/100 mL at 1st day to 0.881 mg/100 mL at 11th day of storage. The tyrosine value of sample increased significantly ($p<0.05$) at the middle of the storage period (Figure 2d.). The proteolysis

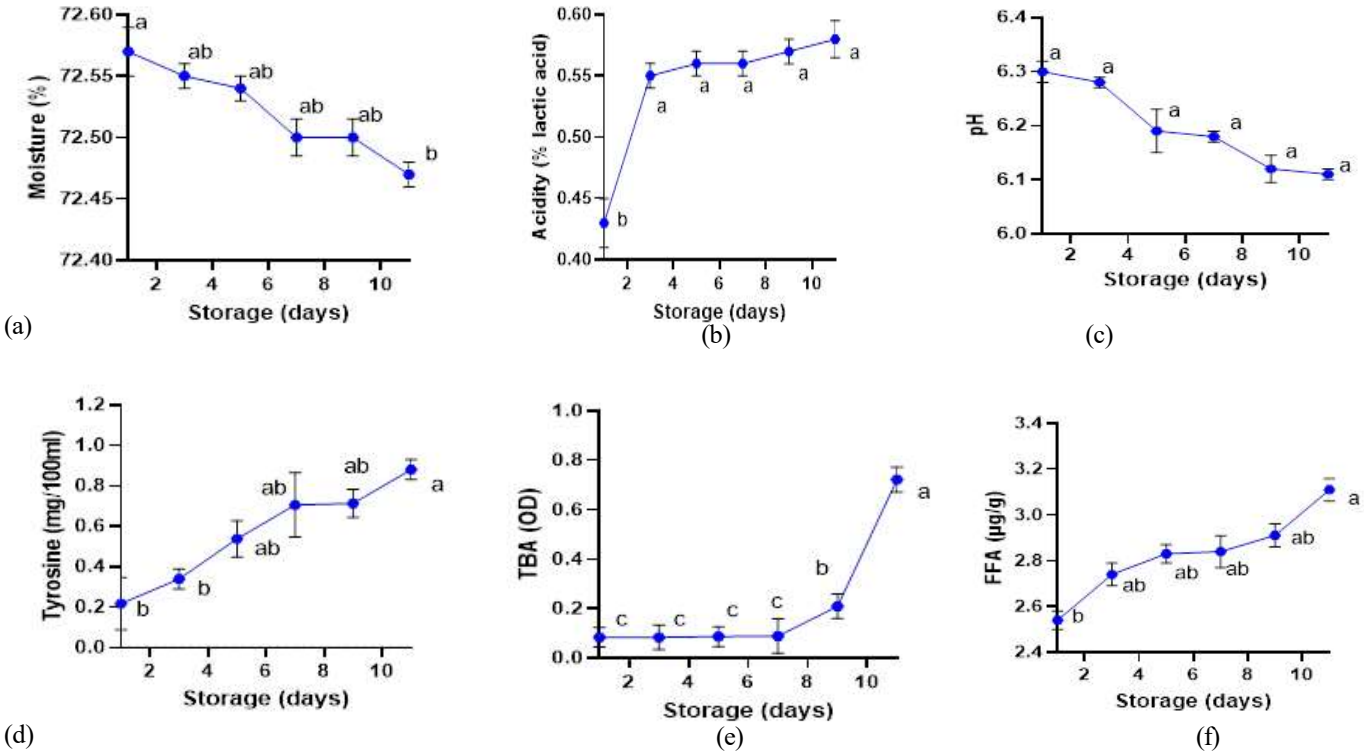
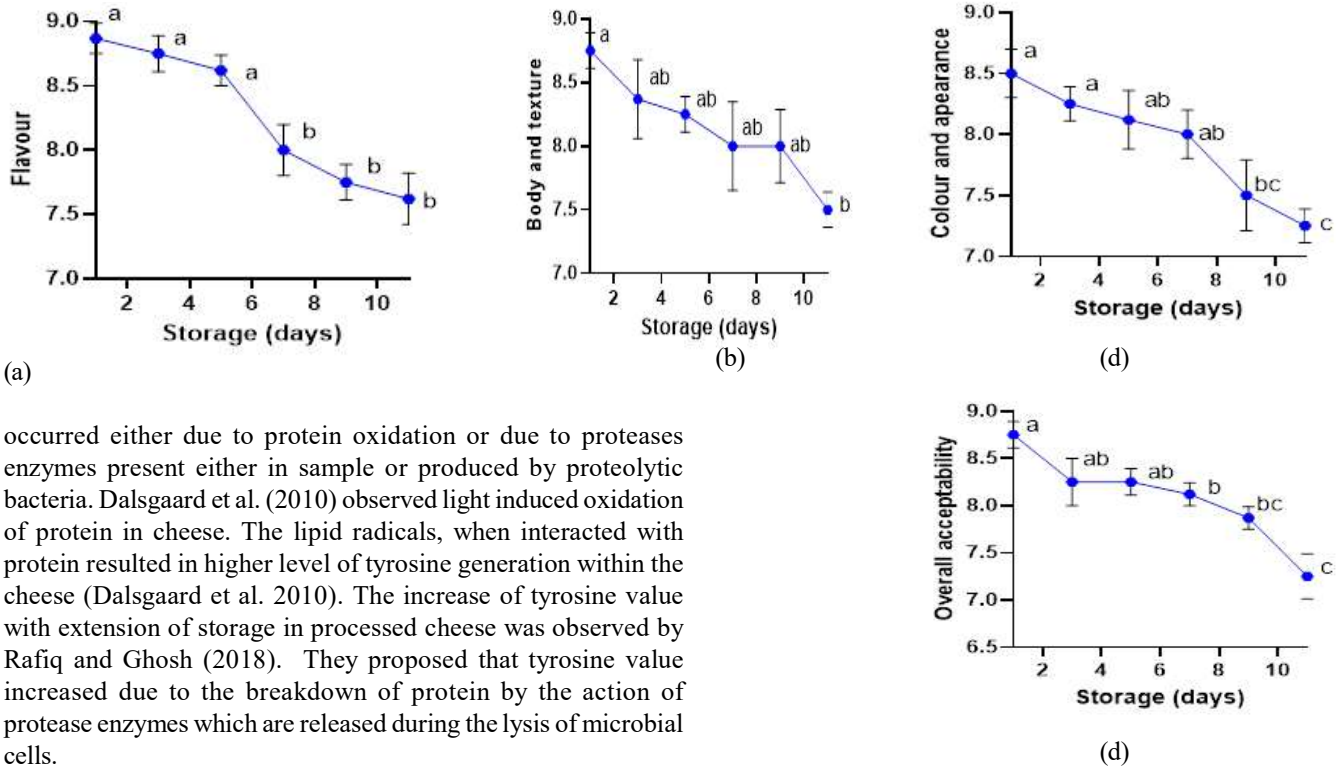


Fig. 2 Physico-chemical changes in dairy based dip during storage at 4±1°C. (a) Moisture (%), (b) Acidity (% lactic acid), (c) pH, (d) Tyrosine value (mg/ 100 g), (e) TBA value (OD), (f) FFA (µg/g). Values are the mean of the three replicates (n=3) with SD (standard deviation). a, b, c: Different superscripts lowercase letters denote significant ($p<0.05$) differences between the storage days within the same sample



(a) (b) (d) (d)

occurred either due to protein oxidation or due to proteases enzymes present either in sample or produced by proteolytic bacteria. Dalsgaard et al. (2010) observed light induced oxidation of protein in cheese. The lipid radicals, when interacted with protein resulted in higher level of tyrosine generation within the cheese (Dalsgaard et al. 2010). The increase of tyrosine value with extension of storage in processed cheese was observed by Rafiq and Ghosh (2018). They proposed that tyrosine value increased due to the breakdown of protein by the action of protease enzymes which are released during the lysis of microbial cells.

The TBA value measures the degree of oxidation. The TBA value of dairy based dip increased from 0.084 OD at 1st day to 0.722 OD at 11th day of storage. The TBA of the product increased significantly ($p>0.05$) at later stage of storage (Figure 2e). Oxygen in the packed product headspace and oxygen diffusing through the PET bottles may be the cause of the TBA value increase during storage. Earlier research works related to milk fat also advocated the increment of lipid oxidation during progression of storage (Olmedo et al. 2013; Smet et al. 2008; Pettersen et al. 2005). Bansal et al. (2021) also reported that TBA value of cheese dip increased during storage. So that, significant ($p<0.05$) increase in TBA value of dairy based dip could be attributed due to the oxidation of milk fat.

One of the primary chemical processes that reduces shelf life and deteriorates food quality in storage is lipolysis. The extent of lipid lipolysis can be measured by FFA content. The FFA value of dairy based dip increased from 2.54µg/g at 1st day to 3.113 µg/g at 11th day of storage. The FFA value of the product increased significantly ($p<0.05$) with progression of storage (Figure 2f.). The FFA content of the processed cheese also increased with progression of storage (Rafiq and Ghosh, 2018). Bansal et al. (2021) reported that yeast and mould proliferation significantly ($p<0.05$) increased FFA content in the final product and the same was attributed in cheese dip. So that, increase in yeast and mould count of dairy based dip could be attributed the increase of FFA throughout the storage period.

Sensorial changes in dairy based dip during storage at 4±1°C

Fig. 3 Sensorial changes in dairy based dip samples during storage at 4±1°C. (a) Flavour, (b) Body and texture, (c) Colour and appearance, (d) Overall acceptability. Values are the mean of the three replicates (n=3) with SD (standard deviation). a, b, c: Different superscripts lowercase letters denote significant ($p<0.05$) differences between the storage days within the same sample

The flavour score of dairy based dip decreased from 8.87 at 1st day to 7.62 at 11th day of storage. The flavour score of the product decreased significantly ($p<0.05$) with progression of storage (Figure 3a). The higher lactic acid concentration, proteolysis, production of free fatty acids through lipolysis as well as oxidation of fat affected the flavour of dairy based dip adversely. The body and texture score of dairy based dip decreased from 8.75 at 1st day to 7.50 at 11th day of storage. The body and texture score of the product decreased significantly ($p<0.05$) with progression of storage (Figure 3b). The decrease in moisture content, the occurrence of lipolysis, lipid and protein oxidation, protein degradation might affect the body and texture of dairy based dip during storage. The CA score of dairy based dip decreased from 8.50 at 1st day to 7.21 at 11th day of storage. The CA score of the product decreased significantly ($p<0.05$) with progression of storage (Figure 3c). The OA score of dairy based dip decreased from 8.75 at 1st day to 7.37 at 11th day of storage. The OA score of the product decreased significantly ($p<0.05$) with progression of storage (Figure 3d.). The deterioration of sensorial quality of processed cheese with progression of storage also was observed

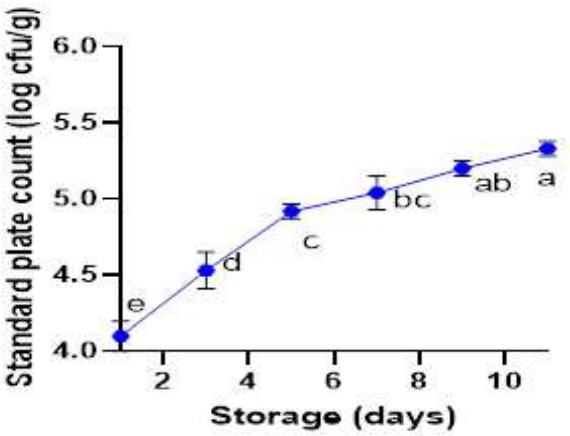


Fig. 4 Standard plate count changes in dairy based dip samples during storage at 4±1°C. Values are the mean of the three replicates (n=3) with SD (standard deviation). a, b, c, d, e: Different superscripts lowercase letters denote significant ($p<0.05$) differences between the storage days within the same sample

by Saad et al. (2015) in processed cheese sauce and Desouky et al. (2019) in camel milk powder incorporated cheese sauce.

Microbiological changes in dairy based dip during storage at 4±1°C

The standard plate count (SPC) of dairy based dip sample increased from 4.10 log cfu/g at 1st day to 5.33 log cfu/g at 11th day of storage. The SPC of the product increased significantly ($p<0.05$) with progression of storage (Figure 4). The result was in agreement with the previous study reported by Smigic et al. (2018), where authors found an increase in aerobic count of processed cheese with progression of storage period. Similarly, Bansal et al. (2021) also reported an increase in SPC count in cheese dip. Change in quality parameters could be affected by the growth of microorganisms.

The presence of yeast and mold, and coliform in dairy based dip was not detected with progression of storage of 11 day. The result indicated the good quality of the product. Bansal et al. (2021) reported that cheese dip had no coliform and yeast and mold at 1st day of storage, Although, presence of yeast and mould was detected during 10th day of storage and same was increased gradually. This work clearly indicates the good hygienic practice during formulation of dairy based dip.

Conclusions

A good quality of dairy based dip was formulated using heat-acid induced milk gel from cow skim milk, cream (40% fat), whey, common salt, trisodium citrate, sodium hexametaphosphate and glycerol monostearate @ 52.67%, 14.70%, 31.73%, 0.42%, 0.16%, 0.16% and 0.16%, respectively. Current study showed the stability of dairy based dip at refrigeration temperature in the aspect of physico-chemical, sensorial and microbiological point of view. The product showed a promising market potentiality as consumers are ready to pay for nutritious, healthy and diversified food product. In a nutshell, direct utilization of dairy byproducts (whey and skim milk) to formulate a dairy based novel product was done for the first time. The product showed a promising market potentiality as consumers are ready to pay for nutritious, healthy and diversified food product.

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Conflict of interest

There was no conflict of interest for this article.

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RESEARCH ARTICLE

Optimization of frozen yoghurt based on fat content and freezing temperature for superior sensorial attributes

Arijit Ray(✉), PS Minz, Chitranayak, Hima John and HK Rohit

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Abstract: A beloved dairy desert with a long history, frozen yoghurt has garnered praise for its creamy and tangy flavour profile and is marketed as a refreshing substitute for traditional ice cream. The interaction of fat content and freezing temperature is acknowledged as a key factor influencing the sensory characteristics of frozen yoghurt. Even though these characteristics are very important, there is a major research gap since not much has been done to explicitly examine the best fat content and freezing temperature combination for frozen yoghurt. This study intends to close this gap by optimizing frozen yoghurt depending on fat content and ultimate temperature utilizing factorial design capabilities. For every sensory property, the model demonstrated great significance and accuracy (R^2e"88.96% and $p<0.05$). The model's forecast and the optimized frozen yoghurt were closely matched ($p>0.05$), also had the greater sensory qualities (more than 8.2). The optimized product was cohesive and chewy, with good springiness and mild firmness. It also confirmed microbiological safety criteria with a adequate total plate count (2.1×10^8 cfu/g) and non-detectable levels of mold, yeast, and coliform. The research offers a comprehensive grasp of the aspects impacting product quality, such as consistent sensory qualities, textural features, and microbiological safety, which is essential information for the industry. The results open the door to improved frozen yoghurt quality, satisfying customer demands, and promoting industrial improvements.

Dairy Engineering Division, ICAR-National Dairy Research Institute, Karnal-132001, India
Arijit Ray(✉)
Dairy Engineering Division, ICAR-National Dairy Research Institute, Karnal-132 001, India
E-mail: rayarijit300@gmail.com

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Introduction

Within the category of frozen desserts, frozen yoghurt has become a popular dairy delicacy with a long history and a growing following as a preferred pleasure. Frozen yoghurt was first introduced as a healthier substitute for regular ice cream. Its creamy and tangy flavour profile has captured the attention of customers, and its living cultures are often commended for their apparent health advantages. With its delicious flavour and healthy benefits, this adaptable frozen treat has grown to be a popular option, reflecting changing consumer tastes for decadent but healthful sweets.

Owing to the bioactive peptides produced during fermentation, yoghurt intake has a variety of health advantages. These peptides have many benefits, such as immunomodulatory, antioxidant, and antihypertensive properties. Interestingly, yoghurt has been associated with increased resistance to respiratory infections and has shown effectiveness in treating gastrointestinal disorders including acute gastroenteritis and diarrhoea, as well as in avoiding common diseases like the cold and influenza (Gouda et al. 2021).

The texture and stability of frozen yoghurt are largely shaped by the freezing process. Low temperatures are given to the yoghurt product during the freezing process, which causes a phase shift from a liquid, gel network, or colloidal suspension to a mixed phase with ice crystals and a supersaturated solution (Alinovi et al. 2021). At lower freezing temperatures, there is less water activity and molecular mobility, which slows down the kinetics of deteriorative events such proteolysis and oxidation (Verdini and Rubiolo 2002). For frozen yoghurt to remain stable overall and to minimize quality losses, temperature and freezing rate must be carefully balanced. The sensory qualities of frozen yoghurt are also significantly impacted by freezing temperature, which is necessary for managing the size and formation of ice crystals. Frozen yoghurt's smoothness and general attractiveness by consumers are significantly impacted by the production of ice crystals (Giroux et al. 2023).

Frozen yoghurt has gained popularity as a healthier substitute for traditional ice cream in recent years due to growing consumer awareness of balanced diets. Its market presence has been facilitated by its nutritional advantages and sensory qualities, such as its low-fat content and live bacteria (Skryplonek et al. 2019).

Particularly in India, the dairy industry is essential to the country's economy. For rural areas, the dairy business provides a vital source of revenue due to its significant contributions to GDP and agricultural output. With a predicted rise from USD 1.69 to 2.14 billion at a CAGR of 3.5% from 2021 to 2025, the global frozen yoghurt industry is expected to flourish in this context. Similarly, from 2021 to 2026, the Indian market for frozen and flavoured yoghurt is expected to rise at a notable pace of 21.3% (Sajeev 2022).

Despite the increasing popularity of the frozen yoghurt market, one important aspect i.e. the optimization of important variables that affect product quality has gotten little attention. In particular, the interaction between fat percentage and the ultimate freezing point continues to be crucial in determining the sensory characteristics of frozen yoghurt. This study intends to close this gap by examining the relationship between fat content and ultimate freezing temperature and how best to produce frozen yoghurt of superior quality.

This study aims to provide important insights since there are now no regulatory criteria for the fat content of frozen yoghurt and little research on the best freezing conditions. To close the knowledge gap on the variables affecting frozen yoghurt quality, a thorough understanding of these elements has to be provided. This study aims to optimise fat content and freezing temperature, which might eventually help to improve the overall quality and market appeal of frozen yoghurt.

Materials and Methods

Preparation of yoghurt

In order to create varying fat content frozen yoghurt, several types of milk from the (From ICAR-National Dairy Research Institute, Model Dairy Plant, Karnal, Haryana, India) were initially employed in the yoghurt-making process, including skim milk (fat 0.5%), double toned (fat 1.5%), toned (fat 3.0%), standardized (fat 4.5%), and full cream milk (fat 6.0%). After that, the temperature of the milk was raised to 80 to 90°C. The milk was gradually cooled to 37°C, creating the perfect environment for the microbial colonies. The milk was then inoculated with cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Freeze-dried Direct Vat Set Culture) culture obtained from a local supplier), and vigorous stirring ensured uniform dispersion (Tripathi et al. 2022). The resultant mixture was poured in a 240 ml frozen yoghurt mould which had 4 individual compartment of 60 ml, then it was placed in an incubator and allowed to ferment at 37°C for six hours (Ray et al. 2024).

Experimental procedure

After the yoghurt mix was fermented, the yoghurt mould was put into a domestic refrigerator for freezing, and the freezer was set to -23°C. Pt100 sensors (Thermonic Stainless Steel Temperature Sensor, Model: TH-PT100-D) were placed in the frozen yoghurt samples. the sensors were connected to data logger to obtain the temperature of samples. The yoghurt was frozen to various temperature ranging from -4 to -20°C at 4°C interval, which took almost 96 to 190 minutes to freeze in a domestic refrigerator.

Sensory analysis

Each frozen yoghurt samples of different trials were evaluated by 10 semi trained assessors panel. 9-point hedonic scale was used to evaluate the samples based on the different sensory attributes like colour, appearance, body and texture, flavour, melting resistance and overall acceptability. The frozen yoghurt samples were removed from the moulds by briefly contacting the mould walls with running tap water at normal temperature. This method facilitated the easy removal of the samples from the moulds. After removal, the samples were presented to the judges for evaluation.

Optimization process of frozen yoghurt

Factorial design was used for the optimization of frozen yoghurt based on sensory attributes using statistical software Design Expert Version 13. Full factorial design was used to observe the effect of the two factors on the sensorial attributes. The two factor, fat and freezing temperature coded as A and B. Their range and level are presented in Table 1. 25 trials were suggested by the design and different sensory attributes were taken like colour, appearance, body and texture, flavour, melting resistance and overall acceptability to get optimized frozen yoghurt based on these properties. Factorial model (2FI) was fitted for each of the responses. To obtain the optimized solution, the desirability of the factors was set within the specified range, and the desirability of the responses was configured to the maximum weight of importance. This approach ensures that the optimization process prioritizes achieving the best possible product characteristics and sensory attributes. The optimization method is aimed at improving the end product's sensory appeal within a quantitative goal range, which was defined by the lower and upper bounds for these variables.

Texture profile analysis (TPA) and microbial analysis of the optimized frozen yoghurt

For Texture Profile Analysis (TPA), TA.HDplusC (Stable Micro Systems, UK) with a 60 kg load cell and a perplex cylindrical probe with a diameter of 25 mm was used. The pretest speed was

set at 2.0 mm/sec, the test speed at 5.0 mm/sec, the post-test speed at 5.0 mm/sec, and the distance was set at 6 mm for the measurements (Hussain et al. 2016). The frozen yoghurt samples were taken out of the mould and placed in a rectangular plate for TPA. Using the technique described by researchers (Cappuccino and Sherman 2011), the frozen yoghurt sample that had been improved was also put through microbiological investigations for total plate count, yeast and mold count, and coliform count.

Results and Discussion

Diagnostic check of the fitted model

Sequential regression analysis was used to create the 2FI models for the many sensory metrics, including colour, appearance, body and texture, flavour, melting resistance, and overall acceptability. Table 2 displays the partial coefficients of regression for these responses, expressed as correlations between the values of two components. The fitted 2FI model explained more than 88.96 to 95.76% of the variance in the experimental data, as shown by the significant model F-value for the characteristics and coefficient of determination (R^2) for all the responses ranged between 0.8896 to 0.9576. The relevance of the model in precisely predicting the replies was shown by the model F-value for each response which ranges from 28.77 to 44.76. For every answer, the adequate precision value, which calculates the signal-to-noise ratio, is more than four, which is extremely desired. For each answer, the model's P-value was less than 0.05, indicating that it was a significant predictor of the responses.

Effect on colour

The frozen yoghurt's colour score (Fig 1. a) varied from 4.5 to 8.7. The standard deviation was 1.37 and the average colour score was 6.41. At a freezing temperature of -8ºC and 0.5% fat content, the minimal colour score was achieved. In contrast, frozen yoghurt that was made with 6% fat content and a freezing temperature of -20 ºC obtained the highest colour score. The model was very significant, as shown by the R^2 of 0.9487 obtained from the regression analysis of the data in Table 2. Richer and more vivid colour was a result of the higher fat content. The milk matrix's fat globules were essential for light scattering, which gave the product an appearance of greater depth and opacity (Cheng et

Table 1 Full factorial design and sensory acceptance scores for different attributes of frozen yoghurt

Run	A:Fat	B:Freezing temperature	Colour	Appearance	Body and texture	Flavour	Melting resistance	Overall acceptability
	%	Degree Celsius						
1	3	-12	6.5	6.6	6.3	6.7	6.6	6.2
2	4.5	-12	7.5	7.7	7.5	7.5	7.3	7.5
3	0.5	-20	4.7	4.6	4.7	4.5	4.7	4.7
4	3	-4	5.4	5.9	5.5	5.5	5.5	5.6
5	0.5	-12	4.5	4.5	4.3	4.5	4.4	4.2
6	6	-8	7.4	7.4	7.4	7.6	7.6	7.5
7	3	-8	6.6	6.7	6.4	6.3	6.6	6.5
8	6	-4	7.2	7.3	7.3	7.3	7.4	7.4
9	6	-20	8.7	8.8	8.5	8.6	8.5	8.3
10	3	-20	6.6	6.3	6.2	6.4	6.4	6.5
11	4.5	-4	6.4	6.2	6.4	6.3	6.3	6.4
12	0.5	-8	4.5	4.3	4.3	4.3	4.4	4.8
13	0.5	-4	4.5	4.4	4.2	4.2	4.3	4.7
14	4.5	-20	8.6	8.6	8.6	8.7	8.5	8.8
15	1.5	-20	5.7	5.4	5.6	5.5	5.7	5.7
16	1.5	-16	5.8	5.5	5.7	5.4	5.5	5.6
17	1.5	-8	5.6	5.3	5.5	5.4	5.5	5.6
18	6	-12	8.4	8.4	8.4	8.5	8.2	8.2
19	4.5	-16	7.7	7.5	7.6	7.8	7.4	7.8
20	1.5	-12	5.5	5.8	5.7	5.6	5.6	5.6
21	6	-16	8.4	8.8	8.5	8.4	8.3	8.7
22	1.5	-4	5.5	5.5	5.4	5.9	5.5	5.6
23	3	-16	6.5	6.5	6.5	6.3	6.7	6.3
24	4.5	-8	7.5	7.3	7.6	7.6	7.4	7.4
25	0.5	-16	4.6	4.5	4.5	4.7	4.3	4.6

al. 2019). Emulsified fat gave the frozen yoghurt a smooth and creamy texture that visually enhanced the overall colour profile.

Colour = 6.41-1.85A[1]-0.792A[2]-0.092A[3]+1.13A[4]-0.612B[1]-0.092B[2]+0.068B[3]+0.188B[4]

Where $A[1]$ to $A[4]$ and $B[1]$ to $B[4]$ are the specific levels of the fat content and product temperature which were used in the factorial design.

Moreover, the development of ice crystals within the frozen yoghurt structure was impacted by the usage of higher freezing temperatures. A rougher and less aesthetically pleasing texture may result from bigger ice crystals forming after freezing at a lower temperature. Higher freezing temperatures, on the other hand, may encourage the development of smaller ice crystals, giving the texture a creamier, smoother consistency (Mo et al. 2019). The frozen yoghurt had an appealing look due to its rich colour and smoother texture, which were both attributed to its increased fat content.

Effect on appearance

Frozen yoghurt had an appearance score (Fig 1. b) ranging from 4.3 to 8.8. 6.39 was the average appearance score, with a 1.43 standard deviation. At a freezing temperature of -8ºC and 0.5% fat content, the minimal appearance score was achieved. Conversely, the frozen yoghurt that was produced at a freezing temperature of -20 ºC and 6% fat content received the highest appearance score. The model was very significant, as shown by the R^2 of 0.9359, which was found in the regression analysis of the data in Table 2. Additional statistical analysis revealed that the model provided a good fit to the data; the model's F-value of 28.92 suggests that it is significant ($p<0.05$). The increased fat level makes the creaminess more noticeable and gives the frozen yoghurt a luxurious and enticing look. Higher fat content also affected how light is reflected and absorbed, giving the hue a deeper, more brilliant appearance that improved the overall appearance (Huppertz et al. 2020). This creamy quality from the higher fat level together with its refined texture added a great deal to the frozen yoghurt's overall visual appeal. The final appearance equation, as determined by the coefficient table, is

Appearance = 6.39-1.93A[1]-0.892A[2]+0.008A[3]+1.07A[4]-0.532B[1]-0.192B[2]+0.208B[3]+0.168B[4]

Effect on body and texture

The frozen yoghurt's body and texture scores (Fig 1. c) varied from 4.2 to 8.6. 6.34 was the average body and texture score. At a freezing temperature of -4ºC and 0.5% fat content, the minimal body and texture score was achieved. On the other hand, frozen yoghurt made with 4.5% fat content and a freezing temperature of -20 ºC obtained the highest body and texture score. The model was very significant, as shown by the R^2 of 0.9572 obtained from the regression analysis of the data in Table 2. Additional statistical analysis revealed that the model provided a good fit to the data; the model's F-value of 44.76 suggests that it is significant ($p<0.05$). The creaminess and stability of the frozen yoghurt's body and texture were further enhanced by the increasing fat content. As an emulsifier, fat improved mouthfeel overall and keeps water from separating during freezing, which may cause unwanted ice crystallization (Mu et al. 2022). The combination of fat content and freezing temperature guaranteed a finished product with a cohesive and well-balanced texture. The body and texture final equation is based on the coefficient table is

$$Body\ and\ texture = 6.34 - 1.94A[1] - 0.764A[2] - 0.164A[3] + 1.2A[4] - 0.584B[1] - 0.104B[2] + 0.096B[3] + 0.216B[4]$$

Effect on flavour

The frozen yoghurt's flavour score (Fig 1. d) varied from 4.2 to 8.7. The flavour score had an average of 6.38 and a standard deviation of 1.43. At a freezing temperature of -4ºC and 0.5% fat content, the lowest flavour score was achieved. On the other hand, frozen yoghurt made with 4.5% fat content and a freezing temperature of -20 ºC earned the highest flavour score. The R^2 for the data in Table 2's regression analysis was 0.8896, indicating that the model was very significant. Additional statistical analysis revealed that the model provided a good fit to the data; the model's F-value of 40.29 suggests that it is significant ($p<0.05$). The likelihood of noise producing a model F-value this high was about 0.01%. Increased fat content in frozen yoghurt, which was typically attained by using higher fat content ingredients like full cream milk, enhanced the product's mouthfeel and richness. Fat is recognized as a carrier of flavour compounds, acting as a medium for the dissolution and dispersion of aromatic substances thereby contributing to the overall taste qualifications. In addition to giving the yoghurt a creamy texture, the increased fat content enhanced and interacted with its natural tastes (Dias et al. 2020; Mohan et al. 2021). This is especially important for frozen yoghurt since the freezing process may often make tastes seem less intense. The fat enhanced the flavour of the frozen yoghurt by giving it a smoother, more prominent taste profile, which increased the indulgence and pleasure of the dessert as a whole. Based on the coefficient table the final equation for flavour is

$$Flavour = 6.38 - 1.94A[1] - 0.82A[2] - 0.14A[3] + 1.2A[4]$$

Effect on melting resistance

The melting resistance score (Fig 1. e) of frozen yoghurt varied from 4.3 to 8.5. The average melting resistance score was 6.34. The minimal melting resistance score was achieved at 0.5% fat content and ultimate temperature of -4ºC. Whereas, frozen yoghurt manufactured with process parameters 4.5% fat content and ultimate temperature of -20 ºC got greatest melting resistance

score. The model was very significant, as shown by the R^2 of 0.9487 obtained from the regression analysis of the data in Table 2. Further the statistical analysis found that the model matched the data well, the model F-value 42.87 suggests that the model is significant ($p<0.05$). When exposed to greater temperatures, frozen yoghurt retained its structural integrity for a longer duration due to the synergy between increased fat content and higher freezing temperatures. Consumers can indulge in frozen yoghurt for longer without sacrificing its quality because of the product's improved resistance to melting, which was produced by the stabilized emulsion that fat provides and the regulated ice crystal formation at higher freezing temperatures (Sitnikova and Tvorogova 2019; Zhao et al. 2023). Based on the coefficient table the final equation for melting resistance is

$$\begin{aligned} Melting\ resistance\ = \ & 6.344 - 1.924A[1] - 0.784A[2] \\ & + 0.016A[3] + 1.036A[4] - 0.544B[1] \\ & - 0.044B[2] + 0.076B[3] + 0.096B[4] \end{aligned}$$

Frozen yoghurt had an overall acceptance (Fig 1. f) score ranging from 4.2 to 8.8. 6.41 was the average overall acceptability score. With a freezing temperature of -12ºC and a fat content of 0.5%, the minimal overall acceptability score was achieved. The greatest colour and overall acceptability score were found in frozen yoghurt manufactured with process parameters of 4.5% fat content and a freezing temperature of -20 ºC. The R^2 for the data in Table 2's regression analysis was 0.935, indicating that the model was very significant. Additional statistical analysis revealed that the model provided a good fit to the data; the model's F-value of 28.77 suggests that it is significant ($p<0.05$). A harmonic balance between creaminess, smoothness, and taste intensity was ensured by the combination of greater freezing temperatures and increased fat content. Together, these elements affected how well-liked frozen yogurt is by customers, who generally choose products with a flavour profile that is balanced, a texture that is attractive, and a resistance to unfavourable

Effect on overall acceptability

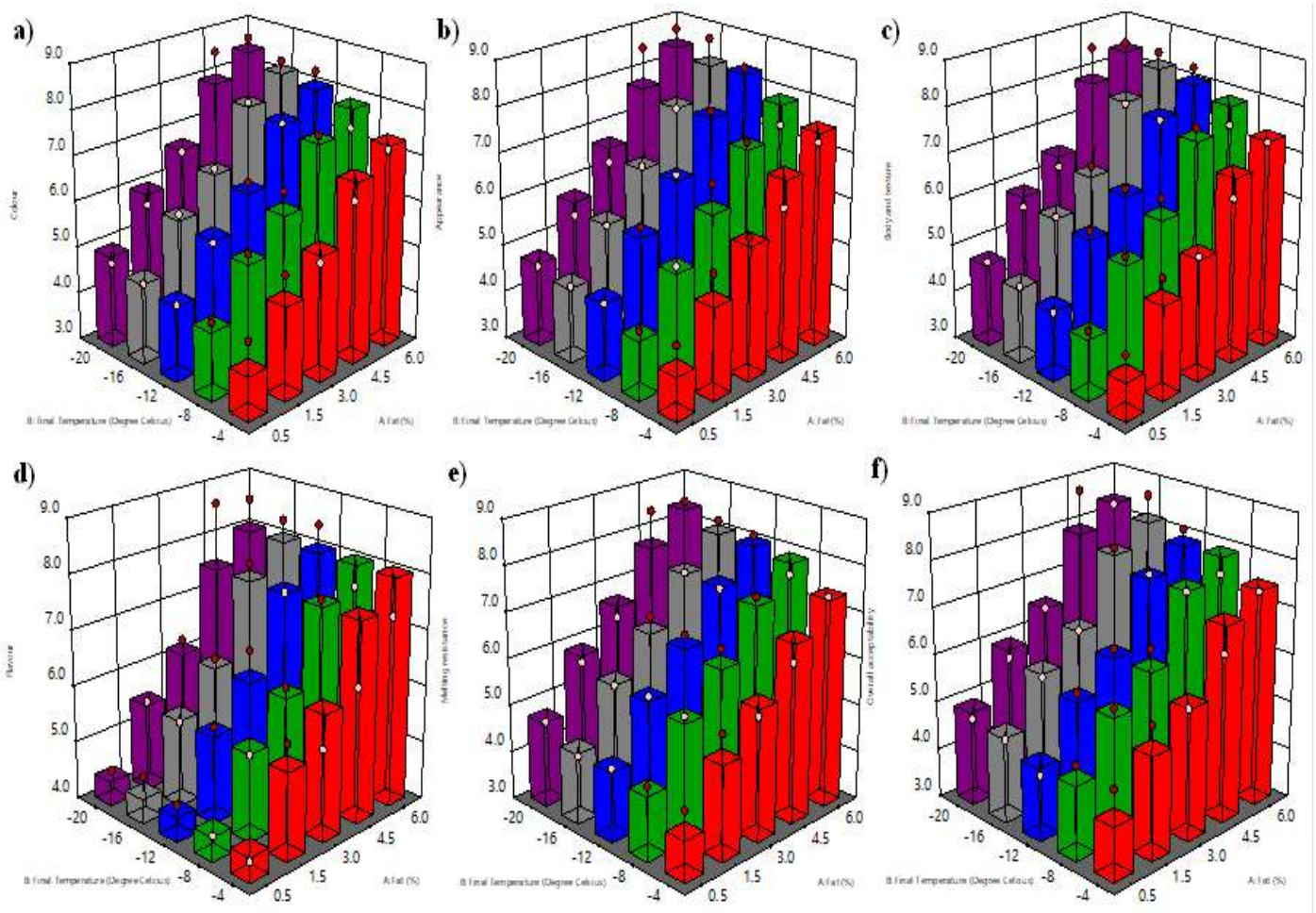

Fig 1. 3D surface plots for different sensory attributes as influenced by the two factors

qualities like iciness or melting quickly. Based on the coefficient table the final equation for overall acceptability is

$$\text{Overall acceptability} = 6.408 - 1.808A[1] - 0.788A[2] - 0.188A[3] + 1.172A[4] - 0.468B[1] - 0.048B[2] - 0.068B[3] + 0.192B[4]$$

Optimized solution for frozen yoghurt

The main objective (Table 3) was to make sure that the fat content and freezing temperature were within the designated limits (0.5 to 6% fat content and -4 to –20ºC freezing temperature, respectively), with each element having equal weight. In order to get the required frozen yoghurt qualities, this suggests a balanced consideration of these aspects.

With a weight of 5, these characteristics were given a greater relevance level, indicating their crucial involvement in influencing the frozen yoghurt's overall quality and acceptability by consumers.

Based on the requirements, an optimized solution with a freezing temperature of -20ºC and a fat content of 6% was provided for frozen yoghurt. With a desirability of 0.93 (Fig. 2), the optimized

Table 2 Regression coefficient and ANOVA of fitted model for sensorial attributes of frozen yoghurt

Partial coefficient	Colour	Appearance	Body and texture	Flavour	Melting resistance	Overall acceptability
Intercept	6.412	6.392	6.344	6.38	6.344	6.408
A[1]	-1.852**	-1.932**	-1.944**	-1.94**	-1.924**	-1.808**
A[2]	-0.792**	-0.892**	-0.764**	-0.82**	-0.784**	-0.788**
A[3]	-0.092**	0.008**	-0.164**	-0.14**	0.016**	-0.188**
A[4]	1.128**	1.068**	1.196**	1.2**	1.036**	1.172**
B[1]	-0.612**	-0.532**	-0.584**		-0.544**	-0.468*
B[2]	-0.092	-0.192**	-0.104**		-0.044**	-0.048*
B[3]	0.068**	0.208**	0.096**		0.076**	-0.068*
B[4]	0.188**	0.168**	0.216**		0.096**	0.192*
Model F-value	37.01	28.92	44.76	40.29	42.87	28.77
R^2	0.9487	0.9353	0.9572	0.8896	0.9554	0.935
APV	19.828	17.0111	21.3233	15.5892	21.3463	16.7855

** $p < 0.05$, * $0.05 \leq p > 0.1$ and A[1] to A[5] and B1[to B[5] represents the specific levels of the fat content and product temperature

Table 3 Goal set for constraints for optimized production of frozen yoghurt

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Fat	is in range	0.5	6	1	1	3
B: Freezing temperature	is in range	-4	-20	1	1	3
Colour	maximize	4.5	8.7	1	1	5
Appearance	maximize	4.3	8.8	1	1	5
Body and texture	maximize	4.2	8.6	1	1	5
Flavour	maximize	4.2	8.7	1	1	5
Melting resistance	maximize	4.3	8.5	1	1	5
Overall acceptability	maximize	4.2	8.8	1	1	5

Table 4 Comparison of predicted and observed sensorial attributes (scores) of frozen yoghurt

Parameters	Predicted	Actual	t-test value
Colour	8.5	8.7	0.423 (NS)
Appearance	8.5	8.7	0.423 (NS)
Body and texture	8.4	8.3	0.728 (NS)
Flavour	8.1	8.2	0.728 (NS)
Melting resistance	8.4	8.3	0.728 (NS)
Overall acceptability	8.4	8.3	0.728 (NS)

*NS means the predicted and actual scores are not significantly different

Table 5 Texture profile analysis (TPA) and microbial count of optimized frozen yoghurt

Analysis	Parameter	Value
TPA	Hardness (g)	51.63±0.15
	Cohesiveness	0.39±0.01
	Adhesiveness (g)	-1.34±0.04
	Springiness (mm)	1.03±0.02
	Gumminess (g)	25.77±0.1
Microbial	Total plate count (cfu/g) $\times 10^8$	2.1
	Yeast and mold (cfu/g) $\times 10^4$	ND
	Coliform count	ND

trial performed very well in terms of obtaining tightly matched data. The frozen yoghurt was prepared using the optimized approach, which demonstrated that the predicted values nearly matched the real values for every sensory metric (Table 4). This suggests that the expected and actual results for these sensory qualities are very consistent. According to the t-test results, there was no statistically significant difference between these groups ($p>0.05$). Overall concordance between expected and actual values suggests that the sensory qualities of the frozen yoghurt were well anticipated by the optimization experiment. Thus, it was determined that the ideal freezing temperature and fat content level for creating frozen yoghurt with the best sensory qualities was -20°C and 6% respectively.

TPA and microbial count analysis of optimized frozen yoghurt

The combined TPA parameters (Table 5) provide a comprehensive description of the texture characteristics of the frozen yoghurt. The measured results pointed to a moderately hard frozen yoghurt with a balanced resistance to compression. A sample that rebounded from deformation to its original condition was indicated by a good springiness rating, which enhanced the eating experience. The cohesive and chewy texture of the frozen yoghurt was further highlighted by the stated gumminess value.

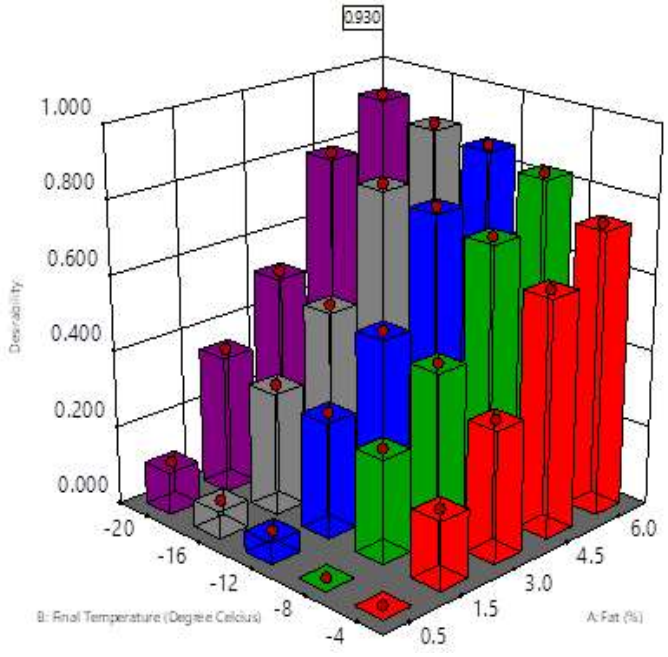

Fig 2. Desirability plot for optimized solution for frozen yoghurt

The overall plate count of 2.1 $\times 10^8$ cfu/g (Table 5) indicated a reasonably adequate microbial load in the frozen yoghurt in terms of microbiological quality. Moreover, coliform counts, yeast, and mold were reported as non-detectable levels. The frozen yoghurt had been made under sanitary settings and satisfied microbial safety criteria, as evidenced by the collective microbiological parameters.

Conclusion

In summary, the goal of this study was to maximize the sensory qualities of frozen yoghurt by examining the relationship between fat content and freezing point. Significant prediction was shown by the 2FI models produced by stepwise regression analysis for a number of sensory measures, such as colour, appearance, body and texture, taste, melting resistance, and overall acceptability ($R2 \geq 88.96\%$, $p<0.05$). Dairy foods, especially frozen yoghurt, have a greater fat content, which is responsible for improving colour and appearance. Higher freezing temperatures simultaneously affected the creation of ice crystals, which changed the texture. Larger ice crystals with a rougher structure resulted from lower freezing temperatures. A larger fat level improved the creaminess and brilliance of the colour of the frozen yoghurt, adding to its visual appeal. The sense of natural tastes was enhanced by the higher fat content, resulting in a smoother and more distinct flavour profile. The frozen yoghurt exhibited improved resistance to melting due to its structural integrity being preserved by the combination of a higher fat content and freezing temperatures. The equilibrium

of smoothness, flavour intensity, and creaminess all contributed to the frozen yoghurt's general appeal. The highly consistent ($p>0.05$) predicted and actual results of the improved frozen yoghurt validated the optimization trial's (Fat: 6%; Freezing temperature: -20ºC) efficacy in correctly improving the frozen yoghurt's sensory qualities. The product had a cohesive, chewy texture, good springiness, and a moderate level of hardness, according to the TPA values. microbiological examination demonstrated non-detectable amounts of mold, coliform, and yeast, along with a reasonable total plate count (2.1×10^8 cfu/g), demonstrating that frozen yoghurt is produced under hygienic circumstances and in compliance with microbiological safety regulations. Thus, by taking into account the relationship of fat content and ultimate freezing temperature, this study effectively improved frozen yoghurt, improving both product quality and consumer satisfaction.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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RESEARCH ARTICLE

Application of response surface methodology in preparation of spinach paneer

Rachana B Rathwa[1], Chetan N Dharaiya[2] (✉) Suneeta V Pinto[3], Ajay J Gokhale[4] and Mital Kathiriya[5]

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Abstract: The objective of current study was to optimize the level of milk fat, rate of addition of whey protein concentrate -70 (WPC-70) and rate of addition of spinach paste to prepare spinach paneer using response surface methodology. Milk fat content, rate of addition of WPC-70 and spinach paste were used as variables while sensory attributes of paneer viz., flavour, body & texture, colour & appearance and textural attributes of paneer viz., hardness and chewiness as well as compositional parameters such as moisture content and fat content on dry matter basis are used as responses. On the basis of the results, RSM suggested the milk fat content, rate of addition of WPC-70 and spinach paste to be 3.08%, 0.96% and 10.11% respectively with desirability of 0.92. The experimental spinach paneer was prepared as per the suggestions from RSM and compared with control paneer prepared from standardized milk containing 4.5% milk fat and 8.5% MSNF. Moisture, fat and carbohydrate content of spinach paneer were significantly different from control paneer while protein and ash content were statistically similar. Textural and sensory characteristics were statistically similar. Hence, RSM can be a useful tool for optimization of spinach paneer from spinach paste.

Keywords: Spinach paneer; Whey protein concentrate-70 (WPC); response surface methodology; sensory parameters; textural characteristics

Dairy Microbiology Department, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India

Chetan N Dharaiya (✉)
Dairy Technology Department, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India
Email: chetandharaiya@gmail.com
Mobile #: +91 75730 13697

Introduction

Paneer, a popular heat and acid coagulated traditional Indian dairy product, is an unripened variety of soft cheese. Paneer is a rich source of animal fat and protein to the vegetarian population. It is also considered as an appreciable source of essential amino acids. The biological value (BV) of protein in paneer is in the range of 80 to 86. Ideally, paneer should have a marble white appearance with a firm, cohesive and spongy body and a close-knit texture. It should have a clean, pleasing, boiled milk flavour (Chaudhari et al. 2023).

Spinach (*Spinacia oleracea*) is a plant that is grown all over the world as a cool-season annual green leafy crop. It serves as an effective ingredient in a new product with excellent nutritional and biological benefits because it is a good source of protein, fibre and minerals. Major micronutrients including iron, manganese, zinc and magnesium are abundant in spinach, which also contains trace amounts of vitamin E, A, C, K, folate, thiamine (B1), pyridoxine (B6), and riboflavin (B2). Incorporation of processed spinach in food products enhances nutritive value and sensory profile of food products. Different forms of spinach have been added in yoghurt (Havaty et al. 2014), cheese (El-Sayed, 2020) and paneer (Pallavi et al. 2020) as well as extruded snack like product (Mangaraj et al. 2018), bread (Waseem et al. 2021) and biscuits (Galla et al. 2017).

Response Surface Methodology (RSM) has been widely used in recent years for the development of new products as well as improvement in existing products. RSM delineates the effect of the independent variables on responses of importance and is regarded as an effective method to optimize the new product formulations. It is a robust tool for data analysis that focuses on an adequate approximation relationship between input and output variables and determines the best operating circumstances for a system (Dean et al. 2017).
With a changing lifestyle and increasing awareness towards health and nutrition, consumers are now moving towards low-fat diet to reduce the risk of obesity, coronary heart disease, atherosclerosis and hypertension (Dharaiya et al. 2021). High fat diet is also linked with psychiatric disorders (Jeong et al. 2019). Fat, being a costliest constituent in milk, increases the cost of

final product and make the product unaffordable by low-income group people. However, reduction in fat content of paneer influences sensory and textural characteristics of the product. Incorporation of spinach will make up for the deterioration taken place in the quality of paneer by reduction of fat along with improvement in the nutritional quality of the final product. Hence, in current investigation, spinach has been incorporated in low-fat paneer.

Materials and Methods

Fresh, raw mixed (cow and buffalo) milk was procured from Livestock Research Station (LRS) of the University and calculated quantity of whole milk was subjected to cream separation in order to obtain skimmed milk. Citric acid (edible grade), supplied by Loba-Chemical Pvt. Ltd., Mumbai was used as a coagulant. Whey protein concentrate-70 (WPC), containing 77.8 per cent protein, supplied by Saisukrithkar supplements Pvt. Ltd., Bengaluru was used as fat replacer. Spinach leaves were procured from local market and were packed in 12 µ polyester + 50 µ LDPE/LLDPE laminated pouches.

The fat and total solids content in milk, moisture, fat, protein and ash content in paneer as well as pH and acidity of paneer was estimated by methods described by FSSAI (2016). Lactose content was calculated by difference of all constituents in paneer.

Preparation of paneer

Paneer was prepared as per the method suggested by Paul et al. (2019) with minor modifications. The detailed method is as follows:

Milk was standardized to 3.0 per cent fat and 8.5 per cent SNF and utilized for spinach incorporated paneer. Standardized milk was heated up to 85ºC for 10 minutes followed by addition of spinach paste at the rate of 8.0 per cent of the quantity of milk and cooled down rapidly to the coagulation temperature (75ºC). Citric acid (1 per cent solution) was heated to 75°C and was added to the milk till stable coagulation was achieved. The coagulum formed was left as it is in the whey for 2 min to ensure the proper acidification. The whey was filtered and separated from the coagulum with the help of clean sterilized muslin cloth. The coagulum, collected within the muslin cloth, was immediately filled into paneer hoops and pressed for 30 minutes under pneumatic paneer pressed at a pressure of 2.5 kg/cm^2. After dehooping the paneer block was immersed into pasteurized chilled water (4 ºC) for about 30 min to obtain desired firmness. Spinach paneer blocks were packed in metallized laminated pouch (12 µ high optical density metalized polyester + 50 µ LD/LLDPE) followed by refrigerated storage at 7±1°C.

Texture Profile Analysis: Compression testing of paneer samples was done with Lloyd Instrument, Hampshire, UK (Model No. 01/2962) using 5 KN probe which moved at a speed of 20 mm/min. The paneer samples were taken for texture measurement after tempering at 23±1°C for 1 h. All the textural measurements were conducted in a room maintained at 23±1°C temperature and 65±1 per cent RH. Cubic samples of the experimental paneer, with edges of 20 mm, were placed in the compression support plate in uniform direction. The cubic samples were compressed up to 70% of their initial size. Five paneer samples were used for each experimental paneer under study and the average value of these readings was reported.

Sensory evaluation of paneer: Each block of paneer was cut into approximately 25 g rectangular pieces. The paneer samples were tempered to 15±2°C before judging. Sensory analysis of paneer samples was performed in isolated booths illuminated with incandescent light maintained at 23±2°C. The sensory panel (n=10) was composed of faculty members of the institute who have basic idea of the product. The paneer samples were evaluated using 100- point scale as described in Indian Standards (IS: 15346, 2003).

Statistical analysis: A Central Composite Rotatable Design (CCRD) of the Response Surface Methodology (RSM) technique was adopted for the optimization of milk fat, rate of addition of WPC-70 and spinach paste. The minimum and maximum levels of milk fat, WPC-70 and spinach paste were selected as 1.5 and 4.5 per cent, 0.5 and 1.0 per cent as well as 5 and 15 per cent respectively, on the basis of preliminary trials. The CCRD of three factors contained 20 combinations, including lower and upper limits, along with their responses for sensory attributes, textural characteristics and compositional parameters are displayed in Table 1. The data generated for different responses were analyzed using Design Expert® software (13.0.2 version) (Stat-Ease, Inc., 2021 E. Hennepin Avenue, Minnepolis, USA). A general polynomial equation given below was fitted for each response.

$$Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_{12} + a_{22}x_{22} + a_{33}x_{32} + a_{12}x_1x_2 + a_{23}x_2x_3 + a_{13}x_1x_3 + \text{Error term}$$

where Y represents the predicted response; a_0 the constant coefficient; a_{11}, a_{22} and a_{33} denote quadratic coefficients; a_{12}, a_{23} and a_{13} denote interaction coefficients; x_1, x_2 and x_3 denote the level of milk fat, WPC-70 and spinach paste respectively.

Adequacy of the model was evaluated using coefficient of determination (R^2) and statistical significance was examined by F value. The effect of independent variables and individual responses was described at $P<0.05$. t-test for two samples assuming equal variance was applied using Microsoft Excel for comparison of predicted values with the actual values of the responses. The variation between control sample prepared using standardized milk (4.5% fat and 8.5% SNF) and spinach paneer prepared from toned milk (3.0% fat and 8.5% SNF) was analyzed using independent t-test.

Results and Discussion

The optimization of the level of milk fat, rate of addition of WPC-70 and spinach paste was carried out on the basis of sensory attributes, textural characteristics and compositional parameters of spinach paneer. The paneer samples were prepared as per the suggestions of RSM and the responses were recorded (Table 1). The successive regression analysis of the responses produced the quadratic models for each response. The variation in the experimental data of fitted quadratic model was given by coefficient of determination (R^2) which ranged between 85 and 92 per cent (Table 2). The model F-value of the fitted quadratic model for all responses was found to be significant. The sufficient accuracy for predicting all response variables of spinach paneer prepared from any combinations of variables within the range was evaluated by non-significant lack of fit. These indicate that the obtained quadratic model fitted the data strongly. The signal to noise ratio called *Adequate precision value (APV)* for a well fitted model should be more than four. This measure also fulfilled for the obtained mode with APVs ranging between 7.83 and 9.89. All these results firmly recommended that the model could be used to develop spinach paneer from spinach paste.

Influence of variables on colour and appearance of paneer: Colour and appearance is the first sensory parameter which is observed during sensory evaluation and the impression lasts through out during sensory evaluation. The colour and appearance score of the product ranged between 7.12 and 9.14. The minimum colour and appearance score was given to the product prepared with milk containing 3.0 per cent milk fat, 0.75 per cent WPC and 18.4 per cent spinach paste while the maximum score was obtained by paneer sample prepared with milk containing 3.0 per cent milk fat, 0.75 per cent WPC and 10.0 per cent spinach paste. Spinach paste significantly ($P<0.05$) improved the colour and appearance of the product at linear level while it deteriorated colour and appearance at quadratic level. Milk fat content and rate of addition of WPC failed to exert impact on colour and appearance of the spinach paneer. The intense dark colour of spinach paneer, when spinach was added at higher rate, was disliked by the judges. Chaudhari et al. (2022) reported similar results for paneer incorporated with spinach powder.

Table 1: Design matrix showing factors and their responses for the development of spinach paneer

Milk ʿat ıtent ⁄%)	B: Rate of addition of WPC-70 (%)	C: Rate of addition of spinach paste (%)	Response 1: Flavour	Response 2: B&T	Response 3: C&A	Response 4: Hardness, N	Response 5: Chewiness, Nmm	Response 6: Moisture, %	Response 7: Fat on Dry Matter basis, %
.50	1.00	15.00	35.57	30.24	8.05	18.89	43.45	61.38	15.23
.00	0.75	01.59	38.14	30.41	7.39	17.89	43.21	58.13	20.08
.50	1.00	15.00	35.24	31.05	8.14	18.85	44.23	59.53	19.81
.00	0.75	10.00	41.56	32.11	9.06	17.56	43.48	58.65	18.59
.50	0.50	05.00	36.25	27.54	7.19	18.53	43.18	56.27	17.51
.50	1.00	05.00	37.41	32.16	7.51	18.41	41.24	60.19	22.56
.50	0.50	15.00	36.85	28.61	8.31	16.48	44.12	56.98	21.89
.00	0.75	10.00	41.26	32.14	9.14	17.85	43.18	57.92	18.60
.50	0.50	15.00	36.87	28.68	8.09	18.78	43.13	57.48	16.28
.47	0.75	10.00	34.52	30.26	9.04	19.02	43.85	56.02	3.76
.00	0.75	10.00	40.63	32.45	8.19	17.45	42.38	58.81	18.42
.00	1.17	10.00	40.71	31.54	8.22	17.46	40.26	61.64	17.62
.00	0.75	10.00	41.05	31.24	8.17	17.69	42.26	58.55	18.58
.00	0.75	10.00	41.81	32.35	8.25	17.85	43.18	58.71	18.64
.00	0.75	18.40	37.45	29.31	7.12	18.10	43.34	59.91	13.36
.00	0.75	10.00	41.64	32.01	7.98	17.56	43.48	58.86	18.71
.50	0.50	05.00	41.23	28.14	7.45	17.86	43.21	56.99	23.61
.52	0.75	10.00	38.21	31.62	8.81	18.53	42.86	56.45	21.96
.00	0.32	10.00	40.29	32.19	8.71	17.56	41.12	55.41	20.32
.50	1.00	05.00	36.54	32.27	7.23	18.42	41.28	59.98	16.64

Influence of variables on flavour of the paneer: Flavour is the most important sensory characteristics for paneer. The flavour score of spinach paneer varied from 34.52 to 41.81. The minimum flavour score was obtained when paneer was prepared from the milk containing 0.47 per cent fat, 0.75 per cent WPC and 10.0 per cent spinach paste while the maximum score was obtained when the paneer was prepared from milk containing 3.0 per cent fat, 0.75 per cent WPC and 10.0 per cent spinach paste. Milk fat and spinach paste significantly (P<0.05) improved flavour of the paneer at linear level. The interaction of milk fat and spinach paste also had significantly (P<0.05) positive impact on flavour of the experimental paneer. At quadratic level, milk fat significantly (P<0.05) improved flavour while WPC and spinach paste significantly (P<0.05) deteriorated the flavour. A typical rich and pleasant flavour provided by milk fat was appreciated by judges. A slight salty taste provided by spinach paste was liked by judges at lower level but was disliked at higher level. Addition of WPC resulted in more water binding and ultimately higher moisture in paneer which yielded flat flavour which was also disliked by judges. Al-Bedrani et al. (2021) reported a progressive increase in flavour scores of low-fat soft cheese with increase in level of WPC up to 2.0 per cent level. Paul et al. (2018) also observed that flavour score of herbal paneer samples (4.5% mint extract and 1.5 to 3.0% milk fat, 8.5 % SNF) increased with increase in fat per cent in milk.

Influence of variables on body and texture of paneer: Body and texture is an important sensory criterion for paneer. The body and texture score of spinach paneer ranged between 27.54 and 32.45. The minimum body and texture score was obtained when milk contained 1.5 per cent fat, WPC was added at the rate of 0.5 per cent and spinach paste was added at the rate of 5.0 per cent while the maximum score was obtained when milk contained 3.0 per cent fat, WPC was added at the rate of 0.75 per cent and spinach paste was added at the rate of 10.0 per cent. Addition of WPC significantly (P<0.05) improved body and texture characteristics at linear level while at quadratic level milk fat significantly (P<0.05) improved it but addition of WPC deteriorated it at quadratic level. Milk fat yielded soft velvety texture in paneer which was appreciated by the judges. WPC reduced chewiness when added at lower level but resulted in loose and weak body at quadratic level which was rejected by the experts. Pinto et al. (2014) also reported an improvement in body & texture of low-fat paneer with increase in fat content in milk.

Influence of variables on hardness of paneer: Hardness, the force necessary to attain a given deformation, is one of the important factors in determining paneer texture as well as acceptability of the paneer. The hardness of paneer varied between 16.48 and 19.02 N. The paneer sample prepared from milk containing 4.5 per cent milk fat as well as WPC and spinach paste were added at the rate of 0.5 per cent and 15.0 per cent respectively displayed minimum hardness while the maximum hardness was observed when milk fat content was 0.47 per cent as well as rate of addition of WPC and spinach paste were 0.75 per cent and 10.0 per cent respectively. Increasing milk fat content significantly (P<0.05) increased hardness at linear as well as quadratic level which could be attributed to higher melting point of milk fat. The interaction of milk fat and spinach paste significantly (P<0.05) reduced hardness of the experimental paneer while WPC-70 and spinach paste could not influence hardness of the paneer. Singh et al. (2015) also observed as increase in hardness with increase in fat content of paneer.

Table 2: Regression coefficients and ANOVA fitted quadratic model for the responses of spinach paneer

Partial Coefficients	Flavour	Body & texture	Colour & appearance	Hardness, N	Chewiness, Nmm	Moisture, %	FDM, %
Intercept	41.03	31.86	8.35	17.66	42.81	58.95	18.57
A-Milk fat	0.93*	0.26	0.37	0.82*	-0.98*	-0.29	1.81*
B-WPC-70	-0.43	0.91*	0.12	0.51	0.29	1.43*	-0.62*
C-Spinach paste	0.70*	-0.26	0.59*	-0.13	-0.25	0.18	-0.52*
AB	0.40	1.29	0.79	0.11	-0.03	0.42	0.56
AC	0.86*	1.78	1.07	-0.65*	0.22	0.12	0.61
BC	0.13	-0.50	0.74	0.06	0.33	0.51	0.16
A^2	1.53*	13.2*	0.49	1.44*	-1.56*	-0.46	2.52*
B^2	-1.22*	-1.52*	0.36	-0.26	0.89*	1.98*	-1.12*
C^2	-1.15*	0.57	-1.29*	-0.24	0.30	0.50	-1.18*
Model Fit Statistic							
Lack of fit	0.062	0.058	0.069	0.060	0.055	0.064	0.065
Model F value	15.41	12.96	14.46	17.81	15.56	14.84	15.61
R^2	0.89	0.86	0.91	0.90	0.85	0.92	0.91
APV	9.63	8.82	9.06	7.83	8.91	9.89	9.51

Influence of variables on chewiness of paneer: Chewiness is the energy required to chew a solid food product to a state where it is ready for swallowing (Berta et al. 2016). The chewiness of paneer ranged from 4026 Nmm to 44.23 Nmm. The paneer samples with 3.0% milk fat, 1.17% WPC-70 and 10.0% spinach paste had minimum chewiness while the one with 4.5% milk fat, 1.0% WPC-70 and 15.0% spinach paste had maximum chewiness (Table 1). Milk fat significantly ($P<0.05$) reduced chewiness at linear and quadratic level. WPC-70 failed to exert impact on chewiness at linear level while significantly ($P<0.05$) increased it at quadratic level. Spinach paste had no significant influence on chewiness. Suthar et al. (2020) also reported reduction in chewiness of paneer with increase in fat content of milk.

Influence of variables on moisture content of paneer: Moisture content of low-fat paneer should not be more than 60% (w/w) (FSSAI, 2006). Moisture content of the experimental paneer varied between 55.41 per cent to 64.64 per cent. The paneer sample with 3.0 per cent fat in milk, 0.32 per cent addition of WPC-70 and 10.0 per cent addition of spinach paste contained minimum moisture while the sample with 3.0 per cent milk fat, 1.17 per cent WPC-70 and 10.0 per cent spinach paste showed maximum moisture content. WPC-70 significantly ($P<0.05$) increased moisture content of paneer which could be attributed to higher water holding capacity of whey protein. Milk fat, spinach paste as well as the interaction between all the variables failed impact moisture content of final product. Pinto et al. (2014) reported similar results for low-fat paneer.

Influence of variables on FDM content of paneer: Fat content on dry matter basis (FDM) should not be more than 20% (w/w) for low-fat paneer (FSSAI, 2006). The FDM content of paneer varied from 3.76 per cent to 23.61 per cent. The paneer sample prepared from milk containing 0.47 milk fat and added with 0.75 per cent WPC-70 and 10.0 per cent spinach paste had minimum FDM content while the one prepared from milk with 4.5 per cent fat and added with 0.5 per cent WPC-70 and 5.0 per cent spinach paste showed maximum fat content on dry matter basis. Fat content of milk significantly ($P<0.05$) increased FDM content of paneer at linear as well as quadratic level while rate of addition of WPC-70 and spinach paste significantly ($P<0.05$) reduced FDM content of paneer. Addition of WPC-70 significantly ($P<0.05$) increased moisture content of paneer resulting in reduction of fat content while addition of spinach paste increased yield of paneer without contributing fat and ultimately resulted in reduction of FDM content. Pinto et al. (2014) also observed an increase in FDM content of paneer with increase in fat content of milk. Addition of WPC resulted in reduction of fat content in paneer (Gawande et al. 2023).

Optimization of variables for preparation of spinach paneer

The optimization of different variables such as milk fat content, rate of addition of WPC-70 and rate of addition of spinach paste was carried out using numerical optimization technique. The criteria used for optimization are summarized in Table 3. Among the variables, spinach paste was maximized while milk fat and WPC-70 were kept in range. Among the responses, sensory

Table 3: Goal set for constraints to optimize spinach paneer

Constraint	Goal	Lower limit	Upper limit
Milk fat, %	In range	1.50	4.50
WPC-70, %	In range	0.50	1.50
Spinach paste, %	Maximize	5.00	15.00
Flavour	Maximize	34.52	41.81
Body & texture	Maximize	27.54	32.45
Colour & appearance	Maximize	7.12	9.14
Hardness, N	Range – 17 to 18	16.48	19.02
Chewiness, Nmm	Range – 42 to 44	40.26	44.23
Moisture, %	Range – 55 to 59	55.41	61.64
FDM, %	Range – 17 to 19	3.76	23.61

Table 4: Comparison for predicted values and observed values for spinach paneer

Attributes	Predicted value	Observed value	t-value
Flavour	41.04	41.10	NS
Body & texture	31.96	31.91	NS
Colour & appearance	8.53	8.57	NS
Hardness, N	17.64	17.57	NS
Chewiness, Nmm	42.76	42.70	NS
Moisture, %	58.75	58.78	NS
FDM, %	18.86	18.89	NS

Table 5: Comparison of spinach paneer with control paneer

Parameter	Control paneer	Spinach paneer	t-value
Chemical composition			
Moisture, %	53.26±0.71	58.78±0.36	21.98*
Fat, %	25.61±0.65	18.89±0.23	17.68*
FDM, %	58.55±0.79	32.13±0.27	20.66*
Protein, %	17.09±0.57	17.52±0.56	NS
Carbohydrates, %	2.32±0.17	4.90±0.25	8.98*
Ash, %	1.72±0.11	1.87±0.18	NS
Rheological characteristics			
Hardness, N	17.72±0.91	17.57±0.62	NS
Cohesiveness	0.39±0.05	0.37±0.09	NS
Springiness, mm	9.38±0.18	9.18±0.15	NS
Gumminess, N	6.91±0.15	6.50±0.12	NS
Chewiness, Nmm	64.81±1.27	59.67±1.07	NS
Adhesiveness, N	0.84±0.06	0.87±0.05	NS
Sensory characteristics			
Flavour	41.16±2.12	41.10±1.62	NS
Body & texture	31.51±1.71	31.91±1.53	NS
Colour & appearance	8.65±0.69	8.57±0.61	NS
Total score*	86.32±2.72	86.58±2.21	NS

Full score for Packaging (5) is added in Total score

parameters such as flavour, body & texture and colour & appearance were maximized while textural characteristics viz. hardness and chewiness as well as compositional parameters viz. moisture as FDM content were kept in range. The range for textural parameters was decided on the basis of textural characteristics of control paneer while the range for compositional parameters was decided as per the legal requirements. RSM suggested milk fat content, rate of addition of WPC-70 and spinach paste to be 3.08 per cent, 0.96 per cent and 10.11 per cent respectively with desirability of 0.92. Spinach paneer was prepared keeping milk fat content, WPC-70 and spinach paste as suggested by RSM. The predicted values for flavour, body & texture, colour & appearance, hardness, chewiness, moisture content and FDM content were 41.04, 31.96, 8.53, 17.64 N, 42.76 Nmm, 58.75 per cent and 18.86 per cent respectively. It is evident from table 4 that the observed values were not significantly ($P>0.05$) different from predicted values with respect to all attributes. Therefore, it was confirmed that the selected level of milk fat, rate of addition of WPC-70 and spinach paste is most suitable for the preparation of spinach paneer with optimum sensory, textural and compositional parameters.

Analysis of spinach paneer

Spinach paneer was analyzed and compared with control paneer for its compositional, textural and sensory attributes and analyzed statistically using t-test. Moisture and carbohydrate content of experimental paneer was significantly ($P<0.05$) higher due to addition of WPC-70 and spinach paste while fat and FDM content were significantly ($P<0.05$) lower than control. Protein and ash content of control and experimental paneer were statistically similar. Similarly, no significant difference was observed between textural and sensory characteristics of control and experimental paneer (Table 5).

Conclusion

Spinach-based low-fat paneer was prepared using response surface methodology and the milk fat content as well as rate of addition of WPC-70 and spinach paste were optimized to obtain sensorially and legally acceptable product with similar textural characteristics to those of control. At linear level, increase in milk fat content improved flavour of the paneer, increase hardness and FDM content while reduced chewiness and moisture content. Increasing the rate of addition of WPC-70 improved body & texture, increased moisture and reduced FDM content. Increasing spinach paste improved flavour and colour & appearance and reduced FDM content of experimental paneer. At quadratic level, milk fat improved flavour and body & texture, increased hardness and FDM content and reduced chewiness while WPC-70 had negative impact on flavour and body & texture, increased chewiness and moisture content and reduced FDM content. Spinach paste also deteriorated flavour and colour & appearance and reduced FDM content. On the basis of the outcomes, RSM suggested to prepare low-fat spinach paneer with milk containing 3.08 per cent fat, 0.96 per cent WPC-70 and 10.11 per cent spinach paste. The final product was highly acceptable. Hence, an acceptable quality low-fat spinach paneer can be developed by using response surface methodology.

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RESEARCH ARTICLE

Optimization and quality characterization of *Aloe vera* enriched flavored whey beverage

S. Sharath Kumar(✉)[1], S. Kartikeyan[2], G. Swarnalatha[3] and S. Swathi[4]

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Abstract: Whey is a by-product from cheese, channa and paneer industries containing valuable nutrients such as, proteins, vitamins, phosphorus and calcium etc. *Aloe vera* is often called "miracle" plant due to its antiseptic, antibacterial, antiviral, anti-diabetic, anti-carcinogenic, anti-inflammatory, natural healer, improves human immune system and digestive system. So the present study was undertaken to develop functional beverage blend using whey and *Aloe vera* juice. In the present investigation, paneer whey has been used as a base material to prepare flavoured whey beverage by incorporating *Aloe* vera juice. Different concentrations 0, 5, 10, 15 and 20 per cent of *Aloe vera* juice were optimized to prepare whey beverage. The effect of *Aloe vera* juice incorporation on sensory, proximate composition and physicochemical characteristics of control and whey samples were studied. The treatment samples were observed significant different and comparable to control samples on "9 point Hedonic sensory evaluation score card. The overall acceptability of experimental samples was between 7.12-7.40 which was on par with control. From organoleptic scores and colour data, it was observed that no significant difference in colour and taste were perceived in experimental products. Total solids, fat, protein and carbohydrate constituents were reduced marginally due to dilution effect. Titratable acidity and viscosity increased in treatment samples, whereas pH was decreased slightly. However, the incorporation of 15% *Aloe vera* juice showed organoleptically most liked *Aloe vera* enriched flavored whey beverage in terms of sensory quality without adversely affecting the physicochemical properties.

[1,4] Department of Dairy Technology, College of Dairy Technology, P. V. Narsimha RaoTelangana Veterinary University, Kamareddy,
[2], Department of Dairy Technology,College of Dairy Science and Food Technology, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Raipur,
[3], Department of Dairy Chemistry,
College of Dairy Technology, P. V. Narsimha RaoTelangana Veterinary University, Kamareddy

(✉) Department of Dairy Technology, College of Dairy Technology, P. V. Narsimha RaoTelangana Veterinary University, Kamareddy.
Email: Sharathkumar10dairy@gmail.com

Keywords: *Aloe vera* juice, Whey beverage, Sensory, Viscosity, Acidity and pH

Introduction

Whey is a nutritious by-product from *cheese, channa and paneer* industries containing a range of nutrients, such as protein (serum albumin, immunoglobulins, β-lactoglobulin, α-lactoalbumin), vitamins (thiamine, and riboflavin), phosphorus, magnesium, and calcium. Additionally, whey proteins are fast proteins because of their ability to quickly deliver nourishment to muscles (Bindu Naik et al. 2023). Around 80% of whey comes from *Channa* and *Paneer,* out of which only 2-3 per cent is utilised and rest is drained. Whey constitutes 45-50% of total milk solids, 70% of milk sugar (lactose), 20% of milk proteins and 70-90% of milk minerals and most importantly, almost all the water soluble vitamins originally present in milk (Alane et al. 2017). The conversion of whey in to beverages through fermentation or without fermentation is one of the common methods for the utilisation of whey and can be used for consumption of baby, geriatric and athletic drink. By adding some simple ingredients like sugar, colour, flavours to whey, we can improve the nutritive value, taste and acceptability. Whey contains a diverse range of components, some with high nutritional value and biological activity, which has intensified interest in its utilization (Miloradovic et al. 2025).

The Aloe barbadensis miller is botanical name of *Aloe vera* and it belongs to Asphodelus (Liliaceae) family. Aloe barbadensis Miller is reported to the most biologically active species. *Aloe vera* is well known as a miracle plant. *Aloevera* juice consists of about 97±0.17 % water (Kamble et al. 2022). AV is available in different forms including fresh whole leaf, fresh gel (pulp), juice (sap and extract), and dried gel. It contains over 200 biologically active substances, such as anthraquinones (barbaloin, isobarbaloin, anthranols, aloetic acid), hydrosoluble and liposoluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids (kaur S and Bains K. 2024).*Aloe vera* works as antiseptic, antibacterial, antiviral, anti-diabetic, anti-carcinogenic, anti-inflammatory and natural healer, improves human immune and digestive system (Asif et al. 2022**).** *Aloe vera* has been used as a functional and therapeutic food, especially for the preparation

of health food drinks and other beverages. Nitesh et al (2022) formulated the different types of whey beverages and estimate the sensory quality of whey beverages which prepared from camel and goat milk (70%, 30%). The formation of whey beverages blended in various combinations of whey, alovera juice (5%, 10% and 15%), coconut water (5%, 10% and 15%), honey (5%) and black salt (1%).

Materials and methods

Dairy and non-dairy ingredients

Fresh Buffalo milk was procured from Dairy farm at Serikhedi, National Highway -6, Raipur,(C.G).Fresh *Aloe vera* leaves were procured from Centre of Excellence on Medicinal & Aromatic plants and NTFP's of Indira Gandhi KrishiVishwavidyalaya, Raipur (C.G).Good quality cane sugar,orange flavour, orange colour and black salt were purchased from the local market of Raipur.

The samples were analyzed for various physicochemical parameters. The pH of beverage samples was determined by using "Digital pH analyzer" as per the procedure. Specific gravity was determined by finding out the weights of a certain volume of sample and of the same volume of distilled water at the same temperature taken in a specific Gravity bottle.The viscosity of the whey, *Aloe vera* and beverage sample was determined by Ostwald viscometer. The fat content of whey, *Aloe vera* juice and beverage sample was estimated by Gerber method. Titratable acidity was titrated against the standard 0.1 N NaOH solution using phenolphthalein as an indicator and the acidity was calculated by using following formula and expressed in terms of per cent lactic acid. Total solids measured by Gravimetric method, protein contents estimated as per the procedure SP: 18, Part XI (BIS, 1961) using the Pelican Kel plus nitrogen estimation system. The A.O.A.C (2005) procedure was adopted for estimating the ash content and was calculated using follows:

$$\text{\% of ash} = \frac{\text{Weight of crucible dish after ashing - Weight of empty crucible dish}}{\text{Weight of the sample}} \times 100$$

Preparation of *Aloe vera* juice and paneer

First, the fresh *Aloe vera* leaves were washed and then peeled. After that, its gel was completely homogenized by a mixer. The gel was pasteurized with a water bath at temperature of 70°C for 30 minutes. (Fig. 1) (Nooshi Manjili et al. 2024).

Paneer was prepared by standardizing buffalo milk to 6.0 per cent fat and 9.0 % SNF using the procedure (Kumar S et al. 2014). The whey (Fig.2) obtained during paneer process was used for preparation of control whey beverage (T_0) and Aloe vera based whey beverage (Fig.3).

The treatment combinations used for preparation of Whey based *Aloe vera* beverage as depicted in Table 1. The *Aloe vera* juice (AVJ) was incorporated at different levels (0%, 5%, 10%, 15%, 20%), sugar incorporated at constant level @ 10 % (based on preliminary trials, the amount of *Aloe vera* juice incorporation was restricted to a maximum of 20% for final study).

Results and Discussion

Effect of *Aloe vera* juice incorporation on sensory attributes of whey beverage

The sensory characteristic of whey beverage prepared with incorporation of *Aloe vera* is presented in Table 2. The sensory attributes for different treatment samples were evaluated following the 9-point hedonic scale rating. On this scale, 'like extremely' was given the highest score of '9' and 'dislike extremely' is given the lowest score of '1'. A judgment panel of 25 participants comprised of postgraduate students and faculty of 20-60 age group people those who are expertise in judging and grading of food & dairy products of the College of dairy and food technology raipur.

It is evident that *Aloe vera* incorporation did not have any significant influence on colour and appearance (Fig 4) and taste of the product. However, the incorporation had significant ($P<0.05$) effect on flavour, consistency and overall acceptability of the product. The control had the highest score of 7.54 and differed significantly from T2 and T4 samples but was on par with T1 and T3. Among experimental samples, T3 had the highest

Table 1. Treatment Details and proportions of ingredients

	Per cent				
Treatments/ingredients	T0	T1	T2	T3	T4
Whey	89.3	84.3	79.3	74.3	69.3
Aloe vera	0	5	10	15	20
Sugar	10	10	10	10	10
Black salt	0.5	0.5	0.5	0.5	0.5
Colour	0.1	0.1	0.1	0.1	0.1
Flavour	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100

Fig 1. Aloe vera gel and Aloe vera juice

Fig 2. Buffalo milk, *Paneer* coagulum and whey

Fig. 3 Preparation of *Aloe vera* enriched flavored whey beverage

flavor score of 7.50, while the T4 had the lowest score of 7.19. As compared to control, the lower flavour score in experimental sample might be due to carry over of residual Aloin and Aloe-emod in content from *Aloe vera* juice in to the samples, which might have reduced the flavour score as the level of incorporation of *Aloe vera* juice increased. Similar results found that control beverage had highest score in flavour in whey based ready-to-serve therapeutic beverage from *Aloe debrana* juice developed by Mohammed et al (2021).

Incorporation of *Aloe vera* juice had a significant influence on the consistency score of whey beverage and the values are given in Table 2. It could be seen that T0 (Control) had significantly ($P<0.05$) lowest value of 7.23 for consistency, while the T4 had the highest value of 7.49 for consistency. Among the experimental samples the consistency value ranged from 7.27(T1) to 7.49 (T4). The T1 was on par with the control (T0) in its consistency and differed from the rest of the samples. A significant increase in the consistency score was recorded by addition of above 10 % *Aloe vera* juice incorporation in the beverage and was found significant from their onwards.

The observed difference in the consistency value of experimental beverage could be accounted to increase in viscosity as AVJ had higher viscosity (3.21 cp) as compared to whey (1.80 cp) which was very well reflected in the mouth feel as recognized by panel of judges. The increase in consistency score might also be due to presence of polysaccharides which has the tendency to increase the viscosity and there by the consistency of the product. The value on consistency attributes obtained in this study were similar to the Elbandy et al (2014), prepared mango nectar by incorporating *Aloe vera* juice showed increased consistency from 18.6 to 19.2 (out of 20) as the level of *Aloe vera* juice incorporation was increased.

The *Aloe vera* juice incorporation had significant ($P<0.05$) effect on the overall acceptability scores. The control had the highest overall acceptability score of 7.40 and was par with T1, while T4 had lowest overall acceptability score of 7.12. Among the

experimental samples, the T1 overall acceptability score was on par with T2, T3. Though the level of *Aloe vera* juice incorporation increased, the overall acceptability was not statistically significant up to 15 % incorporation. The lowest score in T4 is very well corroborated to its lower score in its colour and appearance, flavor and taste. Sharma Chand Nitesh et al (2022) developed whey beverages with a composition of 79% whey and 15% aloevera juice (T_1A_3) had obtained maximum overall acceptability (7.54±0.050).

Effect of *Aloe vera* juice incorporation on the Physico-chemical composition of whey beverage:

The observations on effect of *Aloe vera* juice incorporation on the physico-chemical properties of whey beverage are displayed in Table 3. From the Table, it is evident that *Aloe vera* juice incorporation had significant effect on specific gravity, viscosity, ash, total solids (TS), total carbohydrate (TCH), acidity and pH of the experimental whey beverage samples.

It can be observed that specific gravity of the beverage decreased with increase in *Aloe vera* juice incorporation. The control had the highest specific gravity of 1.035 and was on par with T1 and T2. The lowest specific gravity was recorded in T4 beverage (1.024) and significantly ($P<0.05$) differed from T1, T2 and T3.

The decrease in specific gravity value in beverages might be associated to lower total solids in *Aloe vera* juice, that resulted in dilution of whey beverage as the level of AVJ incorporation increased at and above 15 %.

A significant contribution by incorporation of *Aloe vera* juice on viscosity was also observed. The control (T0) had the lowest viscosity of 2.86 cp, while samples T4 had the highest viscosity of 4.11 cp. As the level of *Aloe vera* juice incorporation increased, the viscosity of the beverage samples also increased. The increase in viscosity might be due to the presence of mucilaginous substances and polysaccharides present in *Aloe vera* juice. The addition of *Aloe vera* juice increased the viscosity in yoghurt samples (Ahmed S et al. 2023).

Table 2. Effect of *Aloe vera* juice incorporation on the sensory quality of fresh beverage

Treatments	Colour and appearance (C&A)	Flavour	Taste	Consistency	Overall acceptability(OA)
T0	7.44	7.54^{A}	7.38	7.23^{A}	7.40^{A}
T1	7.35	7.44^{AB}	7.36	7.27^{A}	7.34^{AB}
T2	7.34	7.41^{B}	7.33	7.38^{B}	7.26^{B}
T3	7.51	7.50^{AB}	7.36	7.44^{C}	7.24^{B}
T4	7.39	7.19^{C}	7.31	7.49^{D}	7.12^{C}
F-values	1.38	14.17	0.145	6.88	9.97
SE(m)	0.020	0.074	0.003	0.041	0.045
CD$^{\$}$	NS	0.11	NS	0.05	0.10

$^{\$}$ indicates significant at 5 per cent level
The superscript A, B, C indicate the comparison of variables with respect to *Aloe vera*juice incorporation levels based on the CD values.

Table 3. Effect of *Aloe vera* juice incorporation on the physic-chemical quality of fresh beverage

	Per cent								
Samples	Specific gravity	Viscosity (cp)	Fat	Protein	Total solids	Carbohydrates	Ash	Acidity	pH
T0	1.035^{A}	2.86^{A}	0.11	0.41	15.52^{A}	14.40^{A}	0.59^{A}	0.22^{A}	5.08^{A}
T1	1.035^{A}	3.12^{B}	0.09	0.40	15.20^{B}	14.16^{AB}	0.53^{B}	0.24^{B}	4.98^{AB}
T2	1.033^{A}	3.37^{C}	0.08	0.37	14.93^{C}	13.96^{BC}	0.52^{B}	0.26^{C}	4.96^{B}
T3	1.028^{B}	3.62^{D}	0.08	0.35	14.65^{D}	13.69^{CD}	0.51^{BC}	0.27^{D}	4.84^{C}
T4	1.024^{C}	4.11^{E}	0.07	0.34	14.41^{D}	13.43^{D}	0.48^{C}	0.29^{E}	4.73^{D}
F-Values	22.826	42.865	0.766	14.603	28.629	15.812	9.272	53.597	16.291
SE(m)	0.00	0.922	0.001	0.002	0.765	0.586	0.006	0.003	0.072
CD$^{\$}$	0.003	0.226	NS	NS	0.252	0.297	0.040	0.019	0.103

$^{\$}$ indicates significant at 5 per cent level
Values are average of four replications

Table 4. Cost analysis of *Aloe vera* enriched flavored whey beverage

Ingredients	Quantity required(g) for 1000 g of beverage					Rate in Rs./ kg	Cost in Rs				
	T0	T1	T2	T3	T4		T0	T1	T2	T3	T4
Paneer whey	893.0	843.0	793.0	749.0	693.0	2.0	1.79	1.69	1.59	1.49	1.39
Aloe vera Juice @ 0, 5, 10, 15,20	0	50.0	100.0	150.0	200.0	25.0	0.0	1.25	2.50	3.75	5.0
Sugar @ 10%	100.0	100.0	100.0	100.0	100.0	40.0	4.0	4.0	4.0	4.0	4.0
Black salt @ 0.5%	5.0	5.0	5.0	5.0	5.0	30.0	0.15	0.15	0.15	0.15	0.15
Color @ 0.1%	1.0	1.0	1.0	1.0	1.0	400.0	0.40	0.40	0.40	0.40	0.40
Flavour @ 0.1%	1.0	1.0	1.0	1.0	1.0	400.0	0.40	0.40	0.40	0.40	0.40
Total weight (g)	1000.0	1000.0	1000.0	1000.0	1000.0	Cost Rs./lit	6.74	7.89	9.04	10.19	11.34

It could be seen from Table 3, the control whey beverage (T0) showed higher values of carbohydrate and pH of 14.40 and 5.08% respectively on par with T1 sample whereas, the total solids (TS) and ash of control differed significantly ($p<0.05$) when compared with fortified whey samples. Among experimental samples T1 had the highest TS, carbohydrate, ash and pH than other treatments. The significant decrease ($p<0.05$) of constituents in *Aloe vera* incorporated whey beverage might be due to the dilution effect by addition of *Aloe vera* juice which had lower values in its chemical constituents.

The fat and protein was shown no significant affect by the incorporation of *Aloe vera* juice. *Aloe vera* juice contain tiny amount of 0.02 %fat and 0.12 % protein (Kamble et al. 2022). Acidity increased with increase in *Aloe vera* juice incorporation in whey beverage. Control had the lowest acidity of 0.22 per cent LA, while T4 had the highest acidity of 0.29 % LA .The highest acidity in *Aloe vera* incorporated samples could be attributed to the fact that initially the *Aloe vera* juice itself had higher acid. Mudgil et al (2016) reported that titratable acidity of cultured buttermilk showed an increasing trend with increase in level of *Aloe vera* juice fortification. Sasikumar and Deka (2015) developed Low calorie therapeutic *Aloe vera* RTS beverage and reported that there was an increase in titratable acidity from 0.30 to 0.38 per cent LA and pH values decreased as the increase in *Aloe vera* juice incorporation increased.

As the level of *Aloe vera* juice incorporation increased the pH of the beverage samples decreased 5.08, 4.98, 4.96, 4.84 and 4.73 for T0, T1, T2, T3, and T4 respectively. The control had the highest pH score of 5.08 and was on par with T1, while the lowest pH value was observed in T4 sample. The significant decrease in the above constituent might be due to the addition of *Aloe vera* juice which had lower side in pH value. Kamble et al (2022) reported *Aloe vera* juice had pH 4.47 % which is lower than whey pH. Similar observations were recorded by Biswas S et al (2016) who Development and Quality Evaluation of *Aloe Vera* and Pineapple Juice Blended Beverage.

Cost analysis of *Aloe vera* enriched flavored whey beverage

The Table 4 shows the various ingredients used and their cost in the manufacture of beverage.

Cost of Whey based *Aloe vera* herbal beverage was estimated simply by considering the price of each ingredient. The cost estimates include only the raw materials cost incurred in the preparation of 1000 gm of final beverage. *Aloe vera* was procured at Rs 10.0 per Kg and was converted in to juice as per the procedure. The yield of AVJ was about 40 %. The cost of AVJ was calculated as Rs 25.0 per kg. This AVJ was used for manufacture of beverage by incorporating at different levels. The cost per kg of whey based *Aloe vera* herbal beverage was calculated to be Rs 6.74 (T0), 7.89 (T1), 9.04 (T2), 10.19 (T3) and 11.34 (T4). The higher difference in the cost of experimental samples was attributed to the fact that the raw material *Aloe vera* juice itself had higher cost per liter (Rs 25/-). Though the costs of AVJ incorporated beverages are higher than control, the health benefits derived in terms of therapeutic value from *Aloe vera* juice can be offset.

Conclusions

It can be concluded that *Aloe vera* juice can be utilized for incorporation in whey based beverages. *Aloe vera* juice can be incorporated @ 15 per cent to produce organoleptically good quality *Aloe vera* enriched flavored whey beverage without adversely affecting the sensory and physico-chemical properties of the product. The technologies generated may be explored for value addition to paneer for preparing a product from the by product whey. Though the cost of AVJ incorporated beverages are higher than control, the health benefits derived in terms of therapeutic value from *Aloe vera* juice can be offset.Due to the continuous growth of the dairy industry, large quantities of by-products are produced, mainly whey. Lactose, fat, and proteins constitute the main fraction of the organic load. In the absence of sustainable practices, whey is considered the most important environmental pollutant of the dairy industry because a large amount of whey is disposed of as wastewater and is associated

with serious environmental hazards. The disposal of whey also represents a significant loss of potential nutrients and energy, so in order to utilize the nutritional value of whey and at the same time mitigate the harmful effects of disposal in the environment, it is important to direct whey management towards a cost-effective and sustainable way of utilization and directing it into the production of novel valuable products at the same time.

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RESEARCH ARTICLE

Effect of potential adjunct culture on physico-chemical and ripening parameters of goat milk white brined cheese

Sapna Tomar[1], Nitika Goel[1] (✉), Namita Rokana[1], Veena N[1,2], Pranav K. Singh[1], S. Sivakumar[1]

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Abstract: This study aimed at assessing the potential Lactobacillus strain which could survive conditions prevalent in goat milk white brined cheese (GM-WBC). Six single strains of Lactobacillus were screened for acidification, salt tolerance (3-9%), proteolytic activity, lipolytic activity and compatibility with starter cultures. *Lactiplantibacillus plantarum* was most tolerant to salt (7% NaCl) followed by *Lacticaseibacillus casei*. All strains exhibited good proteolytic activity with maximum being observed for *L. casei*. Two strains i.e. *L. casei* and *L. plantarum* were selected and added individually into GM-WBC to study their effect on physico-chemical and biochemical characteristics during ripening. Free fatty acids and water-soluble nitrogen increased significantly throughout ripening for 60 days in all samples with highest in case of GM-WBC with *L. casei*. SDS-PAGE of cheese extract showed unknown bands in GM-WBC with *L. casei* sample from 15th day of ripening depicting higher proteolytic activity of *L. casei compared to L. plantarum*. Thus, *L. casei* could be used as a potential adjunct culture for manufacture of GM-WBC.

Keywords: Adjunct cultures, White brined cheese, *Lactobacillus species*, Ripening period, Physico-chemical properties

[1]College of Dairy Science & Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

[2]Department of Food Safety and Quality, DSLD College of Horticultural Engineering and Food Technology, UHSB, Devihosur, Haveri, Karnataka, India

(✉) Email: nitika.gadvasu@gmail.com, Mobile: +91-8146314448

Introduction

Survivability of goats under harsh conditions entitles them as poor man's cow. The higher digestibility, alkalinity, high buffering capacity and therapeutic benefits are the key biological properties possessed by goat milk (de Almeida Júnior et al. 2015). Mediterranean and Balkan regions are known for their brined cheeses which often lack a rind and taste salty and mildly acidic due to ripening by lactic acid bacteria and preservation in a thick brine (12 to 18% NaCl). Salt and acid are the crucial factors for preserving these cheeses during ripening (Hayaloglu et al. 2008).

Starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB) rule the cheese microbiota. SLAB is responsible for acid development while NSLAB (adjunct cultures) helps in improving organoleptic properties (Blaya et al. 2018). NSLAB tend to grow after ripening due to their tolerance to heat or acid treatment which helps in bacterial autolysis during later stages. Such process facilitates release of enzymes (Lazzi et al. 2016). The potential ability of NSLABs is to survive extreme conditions like low pH, presence of salt, low moisture and depleted nutrition helps to increase their count after few months of ripening (Settanni and Moschetti, 2010). Major species in NSLAB family include facultative heterofermentative mesophilic *Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus* etc. Other genera are *Pediococcus*, *Micrococcus* and *Leuconostoc* (Bozoudi et al. 2016).

Proteolysis results in protein breakdown and causes changes in texture, water activity, pH, amino acid profile, etc. It also develops cheese flavour and sometimes off-flavour (bitterness) by production of peptides and free amino acids by different pathways (Sousa et al. 2001). Proteolysis causes changes in textural attributes like increased softening and reduction in water activity of cheese curd due to changes in water binding activity by formation of new carboxylic acid and amino groups (Fox et al. 2004).

To prevent the adventitious growth of NSLAB, off flavours and monitor the consistency of cheese prepared, it is important to select the potential NSLAB strains. In this study, six

Lactobacillus strains were screened based on their ability to survive conditions of white brined cheese (WBC). White brined cheese was prepared from goat milk using selected adjunct cultures and its effect on physico-chemical and biochemical attributes was also studied.

Materials and Methods

Materials

Pooled goat milk of Beetal breed was collected from Experimental Dairy Plant of College of Dairy Science & Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Microbial rennet (FROMASE-2200 TL granulate) from *Rhizomucor miehi* was procured from Essdee Marketing, Pune, Maharashtra, India. Blend of thermophilic and mesophilic (*Streptococcus thermophilus*, *Lactococcus lactis* and *Lactococcus cremoris*) starter culture was procured from Chr. Hansen (India) for making GM-WBC. Six single strains (*Lactobacillus acidophilus* (NCDC-15), *Lacticaseibacillus casei* (NCDC-17), *Lactobacillus helveticus* (NCDC-288), *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*) were collected from National Collection on Dairy Cultures (NCDC) and Molecular Biology Laboratory of National Dairy Research Institute, Karnal, Haryana, respectively.

Compositional analysis of milk

Raw goat milk was analysed for fat, solid-not-fat and protein content using Milkoscreen (IndiFoss, Ahmedabad, India). Acidity, ash and moisture content were determined according to the method described in AOAC (2005). pH was recorded using handheld pH meter (Cole Parmer, Mumbai, India). Casein content was determined using Pyne's method (Pyne, 1932).

In-vitro screening of adjunct cultures

Six single strains (*L. acidophilus* (NCDC-15), *L. casei* (NCDC-17), *L. helveticus* (NCDC-288), *L. delbrueckii* subsp. *bulgaricus, L. plantarum* and *L. rhamnosus*) were screened based on their ability to survive conditions of white brined cheese.

The compatibility of *Lactobacillus* strain with starter culture was determined by spotting 10 µl of each adjunct culture alongside the starter culture on nutrient agar (pH 6) followed by incubation at 37°C for 48 hrs. Zone of inhibition depicted incompatibility of adjunct culture with starter culture.

Acidification activity was measured for their ability to reduce pH with reference to Meng et al. (2018). Adjunct cultures were sub-cultured in 25 ml sterile reconstituted skim milk (10% w/v) aseptically and pH was recorded at 0, 3, 6, 18 and 24 h interval of incubation at 37°C.

Salt tolerance was determined by the method of da Silva Ferrari et al. (2016) at different salt concentration (3, 5, 7 and 9% w/w) via their growth in the MRS media containing 0.017% bromocresol purple solution. A change in colour from purple to yellow was observed which was the evidence of cell growth and positive result for salt tolerance.

Proteolytic activity was determined as per de Almeida Júnior et al. (2015) on skim milk agar (1% skim milk powder to nutrient agar) for formation of transparent halo zone around the spots was considered positive for proteolytic activity.

Lipolytic activity was determined as per Albayrak and Duran (2021) method using nutrient agar with 0.01% (w/v) $CaCl_{2.}$ and 1% (v/v) of Tween 80 and observed for visual precipitation zones around colonies due to precipitation of $CaCl_2$ and fatty acids on lipolytic agar which indicated the ability of strains to lipolyze lipids.

Production of white brined cheese (WBC) using adjunct cultures

White brined cheese (WBC) was prepared from goat milk standardized to casein to fat ratio of 0.69-0.72 as per the method described by Zaravela et al. (2021) with slight modifications. Goat milk was batch pasteurized at 63°C/30 min followed by cooling at 35°C. Starter culture (calculated quantity mentioned on the DVS sachets, blend of thermophilic and mesophilic culture) was added along with 0.5% (v/v) of individual adjunct culture to cheese milk. Milk was left undisturbed for period of 60 min to allow culture to activate and hydrate itself in cheese milk. Later, 0.01% (w/v) of $CaCl_2$ and 13 mg/L of rennet was added at 35°C. Milk was allowed to set into firm curd for 50-60 min followed by cutting the curd into 2 cm^3 cubes. Cubes were heated at 35°C for 20 min with gentle stirring for every 5 min to avoid shattering of curd and further prevent lump formation and then transferred to perforated moulds to promote whey drainage. Once the curd was moulded, it was then pressed under its own weight. Curd mass was inverted every 1 hr interval for first 3-4 hrs followed by overnight whey drainage at 16-18°C in stability chamber till pH reached in the range of 4.6 to 4.8. The next day, cheese block was dry salted with 2.5% (w/w) of NaCl and left at 16-18°C in stability chamber (Macro Scientific Works, Delhi, India) for 24 h. The cheese blocks were then dipped in 7% brine solution containing 0.2% $CaCl_2$ and 5% citric acid until the pH reached to 4.6 and kept for ripening under brine at 4°C for 60 days. Control GM-WBC sample was prepared without addition of adjunct cultures while experimental samples were prepared using selected adjunct cultures such as *L. plantarum* (GM-WBC(P)) and *L. casei* (GM-WBC(C)) based on salt tolerance, acidification, proteolytic and lipolytic activity. The control and experimental samples were analysed for biochemical and physico-chemical characteristics at 15 days interval from 0 to 60 days.

Compositional and biochemical analysis of WBC

Fat, moisture, ash and acidity of GM-WBC samples were analysed as per AOAC (2005) method. pH of grated cheese (2 g mixed with 2 ml of distilled water) was measured using handheld pH meter (Cole Parmer, Mumbai, India). Salt content was analysed using Mohr's method. Total protein was calculated by Kjeldahl method (AOAC, 2005).

Free fatty acid (FFA) content was determined by extraction-titration method given by Deeth and Fitz Gerald (1976). Procedure of Kuchroo and Fox (1982) was adopted for determining water soluble nitrogen (WSN) content.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method given by Jin and Park (1996) to separate the protein fractions at different stages of ripening. Cheese samples for electrophoresis were prepared by the method given by Mbye et al. (2021). The protein in prepared sample was quantified in the sample using Nanodrop One (Thermo Fisher Scientific, Madison, USA). Broad range molecular weight markers of the range 3.5 to 205 KDa (GeNei, Bangalore, India) were used.

Statistical analysis

Statistical Software Package IBM SPSS version 26 was used to compare the results using two-way ANOVA and significant differences between groups was evaluated by Post hoc Tukey's test with a significance level $P<0.05$ throughout the study. The data was presented as mean ± standard deviation in Microsoft excel.

Results and Discussion

The average compositional analysis of goat milk used for preparing white brined cheese is given in Table 1.

***In vitro* screening of Lactobacillus adjunct cultures**

All *Lactobacillus* strains were compatible with blend starter culture since no zone of inhibition was observed (Fig. 1). Our results agreed with Kandola (2018) who demonstrated that cell free extracts of yoghurt culture *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb-09 and Lb-305) and *Streptococcus thermophilus* (ST-311 and ST-74) were compatible with *L. acidophilus* (LA-13).

Result for acidification activity is shown in Figure 2. Fastest reduction in pH was observed in case of *L. acidophilus* (pH

Fig 1. Spot assay of *Lactobacillus* species and blend of thermophilic and mesophilic starter culture (RST: Blend of thermophilic and mesophilic starter culture; P: *L. plantarum*; C: *L. casei*; R: L. *rhamnosus*; B: *L. delbruekii* subsp. *bulgaricus*; A: *L. acidophilus*; H: *L. helveticus*)

3.55) while *L. delbreukii* subsp. *bulgaricus* showed the slowest reduction in the pH value (5.84) from an initial pH of 6.55 after a period of 24 h. Nieto-Arribas et al. (2009) reported similar results with *L. plantarum* strains that showed low acidifying capacity than *L. paracasei* subsp. *paracasei* strains due to its heterofermentative nature. Herreros et al. (2003) also found that *L. plantarum* and *L. brevis* showed significantly lower acidification than most strains of *L. casei* subsp. *casei*. Strains of *L. casei* (TAUL 1522 and TAUL 1580) developed acidification at similar rates as of *Lactococci* after 24 h.

The growth of all six *Lactobacillus* species was inhibited at 9% salt concentration. Only *L. plantarum* and *L. casei* were able to tolerate till 7% salt concentration by showing growth which indicated by colour change while *L. bulgaricus* and *L. acidophilus* were unable to tolerate even 3% salt concentration (Fig. 3). *L. rhamnosus* and *L. helveticus* started to show inhibition at 5% salt concentration. Yao et al. (2020) suggested that two strains of *L. plantarum* isolated from fermented Chinese Dongbei kimchi showed no growth above 8% NaCl concentration. While strain of *L. plantarum* D_{31} was able to survive at 8% salt concentration. Nath et al. (2020) also reported that *L. plantarum*

Table 1. Composition of goat milk

Fat (%)	Solids-not-fat (%)	Protein (%)	Lactose (%)	Ash (%)	pH	Acidity (% lactic acid)
4.0 ± 0.2	8.7 ± 0.05	3.7 ± 0.08	4.0 ± 0.04	0.84±0.02	6.45±0.06	0.17 ± 0.01

strain GCC_19M1 isolated from fermented milk sample was able to survive till 7.5% salt concentration beyond which viability decreased.

All *Lactobacillus* strains exhibited proteolytic activity by forming halo zone around them while *L. casei* showed the maximum activity (Fig. 4). Meng et al. (2018) reported proteolytic activities in case of 9 *L. paracasei* and 6 *L. rhamnosus* strains isolated from goat milk semihard cheeses. Rahmati (2017) reported that 6 strains belonging to *L. casei*, *L. plantarum* and *S. cerevisiae* exhibited proteolytic activity with zone diameter ranging between 15-19 mm on agar plates. Ma et al. (2012) also stated that 36 strains of *Lactobacilli*, isolated from Chinese fermented milks exhibited proteolytic activity in the range 17-48 mg Gly/L milk. Suresh and Nampoothiri (2022) observed that two strains, *L. plantarum* FCW2 and *L. plantarum* FCW4, showed clear zones on MRS agar plates added with skim milk.

For lipolytic activity, precipitation was only observed around colonies of *L. rhamnosus*, *L. bulgaricus* and *L. casei* on lipolytic agar (Fig. 5). The results were in agreement with Rahmati (2017) who found only 5 strains out of 16 isolated from Iranian traditional dairy products (*L. casei* GYL1, *L. casei* AKL2, *L. casei* DDL2, *L. plantarum* ACL4 and *S. cerevisiae* DDy2) which could hydrolyse fat where *L. plantarum* possessed poor lipolytic activity. The results of Meng et al. (2018) are contradictory with ours' as they reported no lipolytic activity in *L. paracasei* and *L. rhamnosus* on tributyrin agar.

Based on these results, *L. plantarum* and *L. casei* were selected and used as adjunct culture in WBC from goat milk.

Compositional analysis of GM-WBC

Addition of adjunct cultures retains more moisture during ripening when compared to cheese made without adjunct culture. The moisture content of all samples increased significantly ($P<0.05$) during cheese ripening (Table 2). This might be due to the diffusion of brine water into the cheese matrix until an equilibrium was reached. Kocak et al. (2020) reported a significant ($P<0.05$) increase in moisture content with increased ripening period. Though, no significant ($P>0.05$) effect was reported by use of adjunct cultures. The increase in moisture content of white brined cheese was reported to be due to an increase in the water uptake at cold storage at low salt concentration (<20%) where migration of water occurred from brine to cheese. An increase in moisture is an indicative of formation of ionic bonds which have a water binding capacity by breaking of peptide bonds (Creamer and Olson, 1982). No significant difference ($P>0.05$) was observed in fat content between the samples (Table

Table 2. Composition of goat milk white brined cheese (GM-WBC) during ripening

Parameters	Days	Cheese samples		
		GM-WBC	GM-WBC(C)	GM-WBC(P)
Moisture (%)	0	61.70 ± 0.05^{dA}	62.15 ± 0.07^{dA}	62.33 ± 0.07^{cA}
	15	63.84 ± 0.03^{cB}	64.23 ± 0.09^{cA}	64.24 ± 0.01^{bA}
	30	64.09 ± 0.06^{bB}	64.32 ± 0.03^{bcA}	64.22 ± 0.01^{bA}
	45	64.23 ± 0.02^{aB}	64.44 ± 0.03^{abA}	64.25 ± 0.01^{bB}
	60	64.33 ± 0.04^{aC}	64.51 ± 0.02^{aA}	64.42 ± 0.02^{aB}
Fat (%)	0	15.62 ± 0.19^{aA}	15.64 ± 0.06^{aA}	15.63 ± 0.12^{aA}
	15	15.60 ± 0.11^{abA}	15.61 ± 0.15^{bA}	15.62 ± 0.08^{aA}
	30	15.58 ± 0.03^{bcA}	15.59 ± 0.14^{bcA}	15.58 ± 0.15^{bA}
	45	15.57 ± 0.07^{bcA}	15.58 ± 0.09^{cA}	15.58 ± 0.13^{bA}
	60	15.56 ± 0.02^{cA}	15.58 ± 0.07^{cA}	15.58 ± 0.11^{bA}
Protein (%)	0	14.63 ± 0.05^{aA}	14.64 ± 0.11^{aA}	14.63 ± 0.19^{aA}
	15	14.36 ± 0.06^{bA}	14.22 ± 0.16^{bB}	14.33 ± 0.13^{bA}
	30	14.24 ± 0.20^{cA}	14.05 ± 0.05^{cB}	14.24 ± 0.10^{cA}
	45	13.76 ± 0.18^{dA}	13.58 ± 0.05^{dB}	13.74 ± 0.12^{dA}
	60	13.34 ± 0.05^{eA}	13.24 ± 0.16^{eB}	13.32 ± 0.13^{eA}
Salt (%)	0	4.06 ± 0.08^{cA}	4.07 ± 0.10^{cA}	4.09 ± 0.05^{bA}
	15	4.02 ± 0.05^{dA}	4.02 ± 0.07^{dA}	4.02 ± 0.06^{cA}
	30	4.27 ± 0.04^{bA}	4.26 ± 0.05^{bA}	4.28 ± 0.08^{aA}
	45	4.28 ± 0.06^{abA}	4.29 ± 0.09^{aA}	4.28 ± 0.08^{aA}
	60	4.30 ± 0.05^{aA}	4.31 ± 0.02^{aA}	4.32 ± 0.07^{aA}

Values (mean ± SD, n = 3) with different superscript 'abcde' within column indicates the days of ripening, has significant ($P<0.05$) effect on dependent variables.
Values (mean ± SD, n = 3) with different superscript 'ABC' among row indicates the type of adjunct culture, has significant ($P<0.05$) effect on dependent variables.

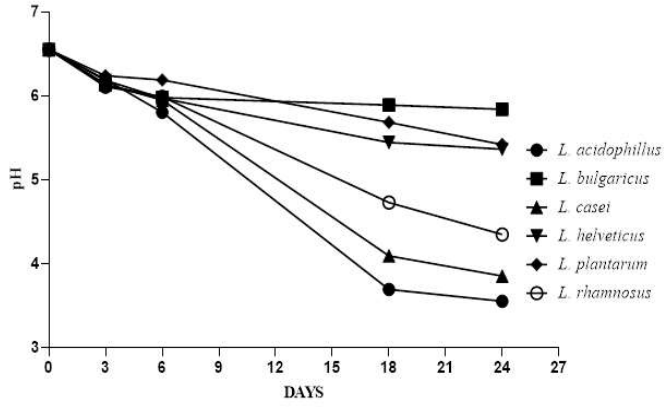

Fig 2. Acidification activity of different *Lactobacillus* species

Fig 3. Proteolytic activity of different Lactobacillus species (P: *L. plantarum*; C: *L. casei*; R: L. *rhamnosus*; B: *L. delbruekii* subsp. *bulgaricus*; A: *L. acidophilus*; H: *L. helveticus*)

Fig 4. Lipolytic activity of *Lactobacillus* species (a) *L. rhamnosus* (b) *L. delbruekii* subsp. *bulgaricus* (c) *L. casei*

2). While decrease in fat content (P<0.05) was observed during ripening period in all samples due to increase in moisture content which affected the overall composition of WBC. Lipolysis might also contribute a decreased fat content of cheese. The protein content of all the samples decreased significantly (P<0.05) during the ripening due to protein degradation (Table 2). Smiljanić et al. (2014) reported higher levels of protein loss in white brined cheese from goat or ovine milk than from cow milk due to compact structure of cow milk cheese. However, Protein content of control and GM-WBC(P) showed no significant difference (P>0.05). Salt content showed a significant (P<0.05) increase in all samples due to diffusion of salt inside the curd to balance the osmotic pressure of cheese and brined water. However, no significant difference was observed between treatments throughout storage period. Kaminarides et al. (2019) also reported a significant (P<0.05) increase in salt content of sample stored under brine (7% NaCl) at 4°C due to diffusion of water from brine to cheese to attain osmotic pressure equilibrium.

Physicochemical analysis

A sudden increase in the pH value for all the samples during initial days of ripening (at 15th day) could be attributed to the onset of proteolysis which are basic in nature (Poullet et al. 1991). Thereafter, pH decreased significantly (P<0.05) due to adaptation of lactic acid bacteria and production of lactic acid (Table 3). The results of change in pH during ripening were in accordance with Mahmoudi et al. (2012), who claimed significant (P<0.05) increase and then decrease in pH of the of Iranian white brined cheese containing starter culture (*L. lactis* subsp. *lactis*, *L. lactis subsp. cremoris*, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) and in sample prepared using *Bifidobacterium animalis* during 60 days of ripening. GM-WBC(C) and GM-WBC(P) samples had

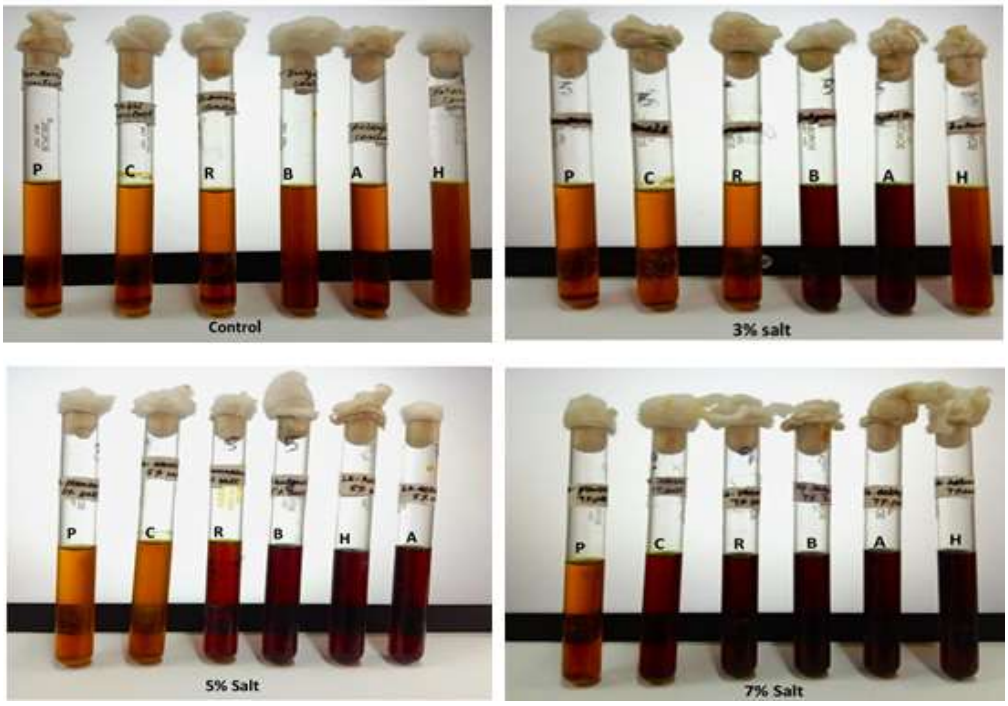

Fig 5. Salt tolerance of Lactobacillus species at different salt concentrations (control (0), 3, 5 and 7%) (P: *L. plantarum*; C: *L. casei*; R: *L. rhamnosus*; B: *L. delbruekii* subsp. *bulgaricus*; A: *L. acidophilus*; H: *L. helveticus*)

Table 3. Physicochemical parameters of goat milk white brined cheese (GM-WBC) during ripening

Parameters	Days	Cheese samples		
		GM-WBC	GM-WBC(C)	GM-WBC(P)
pH	0	4.75 ± 0.01^{cA}	4.60 ± 0.01^{dC}	4.65 ± 0.01^{bB}
	15	4.81 ± 0.01^{aB}	4.84 ± 0.01^{aA}	4.81 ± 0.01^{aB}
	30	4.74 ± 0.01^{cA}	4.72 ± 0.01^{bB}	4.70 ± 0.01^{bC}
	45	4.72 ± 0.01^{dA}	4.67 ± 0.01^{cB}	4.70 ± 0.01^{bA}
	60	4.77 ± 0.01^{bA}	4.62 ± 0.01^{dB}	4.40 ± 0.01^{dC}
Acidity (% lactic acid)	0	0.535 ± 0.04^{cB}	0.765 ± 0.05^{bA}	0.734 ± 0.02^{cA}
	15	0.556 ± 0.03^{bcC}	0.813 ± 0.05^{abA}	0.746 ± 0.05^{abB}
	30	0.565 ± 0.02^{bcC}	0.834 ± 0.06^{abA}	0.746 ± 0.09^{abB}
	45	0.587 ± 0.01^{bC}	0.860 ± 0.04^{aA}	0.797 ± 0.05^{bB}
	60	0.627 ± 0.02^{aC}	0.898 ± 0.08^{aB}	1.308 ± 0.02^{aA}
Water soluble nitrogen (%)	0	0.21 ± 0.10^{dA}	0.23 ± 0.06^{dA}	0.20 ± 0.10^{cA}
	15	0.22 ± 0.09^{dB}	0.29 ± 0.07^{cA}	0.24 ± 0.06^{bcB}
	30	0.26 ± 0.08^{cB}	0.35 ± 0.08^{bA}	0.26 ± 0.08^{bB}
	45	0.32 ± 0.07^{bC}	0.44 ± 0.10^{aA}	0.36 ± 0.04^{aB}
	60	0.38 ± 0.07^{aB}	0.46 ± 0.05^{aA}	0.39 ± 0.09^{aB}
Free fatty acids (% oleic acid)	0	0.441 ± 0.14^{dB}	0.569 ± 0.07^{dA}	0.551 ± 0.11^{dA}
	15	0.445 ± 0.11^{dC}	0.663 ± 0.14^{cB}	0.767 ± 0.14^{cA}
	30	0.792 ± 0.14^{cB}	1.180 ± 0.07^{bA}	0.781 ± 0.12^{cB}
	45	0.845 ± 0.14^{bC}	1.185 ± 0.07^{abA}	0.871 ± 0.11^{bB}
	60	0.981 ± 0.13^{aB}	1.208 ± 0.06^{aA}	0.992 ± 0.09^{aB}

Values (mean ± SD, n = 3) with different superscript 'abcd' within column indicates the days of ripening, has significant ($P<0.05$) effect on dependent variables.
Values (mean ± SD, n = 3) with different superscript 'ABC' among row indicates the type of adjunct culture, has significant ($P<0.05$) effect on dependent variables.

lower pH values than control due to high acidification activity of adjunct cultures. A significant ($P<0.05$) increase in titratable acidity of cheese added with adjunct cultures compared to control with ripening period was observed. GM-WBC(P) showed a sudden increase in the acidity after 60th day of ripening due to the better adaptability of *L. plantarum* to salt at later stages (Table 3).

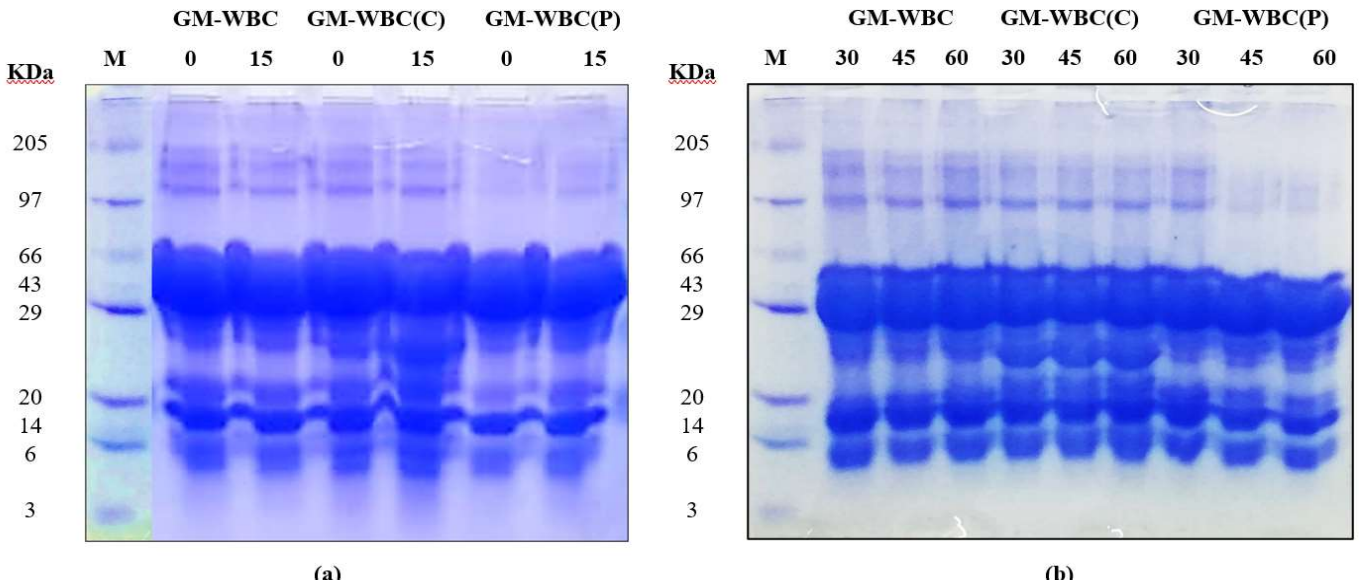

Fig 6. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins of goat milk white brined cheese (GM-WBC) with or without adjunct culture ripened for (a) 0 and 15 days (b) 30, 45 and 60 days (M - Molecular weight marker ranging from 3 to 205 KDa).

Dabevska-Kostoska et al. (2015) reported a significant increase ($P<0.05$) in the titratable acidity due to addition of *L. casei* as adjunct culture in white brined cheese compared to control sample.

Water soluble nitrogen (WSN) is used as an index for maturation or cheese ripening. WSN consists of high, medium and low molecular weight peptides along with free amino acids (Fox et al. 2004). Significant increase ($P<0.05$) in the levels of WSN was observed in all samples during ripening. Highest WSN content was observed in GM-WBC(C) due to more proteolytic activity of *L. casei* (Table 3). Kocak et al. (2020) reported similar results with significant ($P<0.05$) increase in WSN content from 0.12 to 0.67% at 90th day of ripening of WBC prepared using *L. casei* as adjunct culture. Addition of *L. plantarum* MU12 and *L. plantarum* S6-4 increased WSN-total nitrogen values from 4 to 21% and 4.8 to 32%, respectively from 30 to 90 days in Cheddar cheese (Duan et al. 2019). The Free fatty acids (FFA) content of cheese samples showed a significant ($P<0.05$) difference among control and treated samples throughout ripening period. FFA content was highest for GM-WBC(C) than the GM-WBC(P) and control which might be due to lipolytic nature of *L. casei* (Table 3). Kumar et al. (2015) reported a significant ($P<0.05$) increase in the levels FFA content during ripening. Highest FFA (4.23µg/g) was reported at 60th day in case of feta cheese made with *L. helveticus*. A consistent increase in FFA with ripening period was reported due to increase in intracellular lipases produced by *Lactobacilli species*.

Electrophoresis

Figure 6 represents the SDS-PAGE results of the GM-WBC samples at 15 days interval during ripening for 60 days under brine. The results of electrophoresis suggests that there had been some degradation of α and β fractions of caseins especially in GM-WBC(C) sample after 15th day ripening which was shown by formation of extra bands (Fig. 6). However, no such extra bands were observed in control as well as GM-WBC(P) sample after 15 days of ripening which represented minimal degradation of casein. This could be due to inhibition of hydrolysis of casein especially β-casein by rennin or pepsin due to presence of salt. Plasmin activity is minimal due to pH and salt conditions in white brined cheese (Hashemi et al. 2009). Our results were in agreement with Tarakci and Tuncturk (2008) who observed degradation of α_s-casein fractions more in the case of cheese prepared using *L. helveticus* due to enhanced activity by starter and non-starter lactic acid bacteria on decreasing pH.

Conclusions

Among the six strains of *Lactobacillus* screened for adjunct cultures, *L. casei* and *L. plantarum* were selected for preparation of GM-WBC based on salt tolerance, acidification, proteolytic and lipolytic activity. The study suggests the use of *L. casei* as potential adjunct culture in GM-WBC that not only improves the physico-chemical properties but also initiates ripening at early stages. The results are suggestive of formation of degradation products due to onset of ripening which might have some bioactivity.

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RESEARCH ARTICLE

Sensory properties of traditional strained yogurts produced using different ratios of bacterium in yogurt culture

Engin Yaralý[1](✉), Alican Tascýoglu[2] and Sevki Çetiner[3]

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Abstract: The aim of the study is to reveal the effect of different bacterium ratio in yogurt culture on the sensory properties of the product in strained yogurt samples produced in the traditional way. In the study, yogurt production was carried out by using the ratios of 20%+80%, 30%+70% and 50%+50% of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, respectively. Color and appearance, odor, consistency (with a spoon), consistency (in the mouth), taste, flavor and general taste characteristics of the strained yogurts obtained in production were evaluated and scored by the panelists sensorially. In addition, the panelists identified defects in color and appearance, taste and flavor, structure and texture in the strained yogurt samples presented to them as well as providing their personal comments about the products. In the study, the effect of bacterium ratio in strained yogurt samples is statistically significant in terms of all sensory characteristics evaluated. In this sense, strained yogurt samples produced at a bacterium ratio of 50%+50% received the highest scores in terms of preference. In the overall evaluation, the most preferred product by the panelists was strained yogurt samples produced from a bacterium ratio of 50%+50%.

Key words: Bacterium ratio, Sensory properties, Sensory test, Strained yogurt, Yogurt culture

[1]Food Processing Department, Çine Vocational School, Aydin Adnan Menderes University, Aydin, Turkey.
Email: eyarali@adu.edu.tr
[2]Food Processing Department, Çine Vocational School, Aydin Adnan Menderes University, Aydin, Turkey.
Email: alican.tascioglu@adu.edu.tr
[3]Food Processing Department, Çine Vocational School, Aydin Adnan Menderes University, Aydin, Turkey.
Email: scetiner@adu.edu.tr

Engin Yarali (✉)
Food Processing Department, Çine Vocational School, Aydin Adnan Menderes University, Aydin, Turkey
Email: eyarali@adu.edu.tr

Introduction

Yogurt is a fermented dairy product obtained as a result of the symbiotic activity of *L. bulgaricus* and *S. thermophilus* specifically. Yogurt curd is expressed as a heat-induced acid-casein gel (Aryana and Olson, 2017;Kaur et al. 2020). The addition of starter culture to the milk to be processed into yogurt is called inoculation, and the amount of starter culture added is called inoculum amount. In production, starter culture is added after the heat-treated yogurt milk is cooled to the incubation temperature. Starter culture is a microorganism containing selected single or mixed strains that provide the desired sensory, textural and rheological properties in yogurt and provide standard quality characteristics in the final product, and *S.thermophilus* and *L. bulgaricus* mixture culture is used in different proportions in yogurt production. These strains are defined as a ubiquitous family of microbes that can ferment glucose into lactic acid as the major catabolic end product in a specific dairy environment. Generally, the ratio of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in yogurt starter cultures is 1:1 or 1:2, respectively. The different ratios of starter cultures have a significant effect on fermentation time, pH, viscosity and other characteristics of the product. The amount of culture to be added is determined according to the type of culture used and the amount of milk to be processed into yogurt (Alline et al. 2018; Dan et al. 2023; Yang et al. 2017).

Mixed fermentation with the two microorganisms gives the yogurt good textural properties. *S. thermophilus* uses the peptides and free amino acids produced by *L. delbrueckii* subsp. *bulgaricus*, while *L. delbrueckii* subsp. *bulgaricus* uses the pyruvic acid, formic acid, folic acid and long-chain fatty acids produced by *S. thermophilus*. In addition, the production of metabolites such as amino acids and short peptides contributes to the formation of flavour substances in the yogurt (Agagunduz et al. 2015; Dan et al. 2019).

Dairy farm generally first prepare the main culture from commercial culture by multiplying, then the intermediate culture. Finally, the bulk culture (enterprise culture) is prepared and an average of 2-3% of this culture is added to the milk (Kenzheyeva et al. 2022). The lyophilized culture can be used directly without multiplication

since it is frozen at very low temperatures. Therefore, the usage rate is in accordance with the information written on the packaging. The incubation process is one of the most important steps in yogurt production and this process should be terminated when the pH value of the yogurt reaches the isoelectric point of casein. The isoelectric point of casein is pH 4.5-4.6. The water holding capacity of yogurt is optimal pH 4.2-4.6 and therefore incubation in production is terminated at pH 4.5–4.6 (Zimmerman et al. 2020; Yang et al. 2017).

Despite the ease of making yogurt and its many benefits, the shelf life of it is limited. In some cases, its quality may deteriorate within a few days and become inconsumable. Taking into account this situation, various durable yogurts are made in many regions of Turkey and in some other countries, as well. In Turkey, yogurts that have long shelf life are obtained by reducing the water of normal yogurt by a certain amount in various ways (bag yogurt, winter yogurt, dry and tulum yogurt, etc.) (Kirdar and Karaca, 2017; Kirdar, 2022).

The sensory evaluation of any food is a scientific field used to measure, analyze and interpret the human responses to food characteristics perceived through the five senses (sight, taste, odor, touch, and hearing) (Ruiz-Capillas and Herrero, 2021). In addition to the many objective evaluation methods used today, sensory evaluation maintains its importance. Sensory tests are generally used to improve an existing product, to ensure quality in daily production, to develop a new product and in marketing analysis. Yogurt is a preferred food for its sensory properties such as texture, color and appearance, as well as its health benefits. Sensory features of yogurt are determined by numerous variables; milk composition, starter culture, production method, heat treatment, incubation temperature, final incubation pH and cooling process. In yogurt sensory tests, difference and quality-quantity test methods and scales are used. In the sensory analyses of yogurts, panelists evaluate external appearance, consistency with spoon and mouth, odor, taste and flavor, and valuable information about the quality of the yogurt can be obtained (Aktar, 2022). Some investigations have shown that different ratios of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in starter cultures have a critical influence on the resulting aroma of yogurt and are important for determining the overall flavour and sensory properties of the final product (Dan et al. 2017).

The aim of this study is to determine the effect of bacterium ratio in yogurt culture on the sensory properties of the product in strained yogurts produced in the traditional way.

Materials and methods

Bacterial strains

Lyophilized yogurt culture (microMilk S.r.l) used in the study was obtained from the Referans Food Industry and Foreign Trade Limited Company. The culture rate was adjusted in 3 different ways*: 20% Lactobacillus bulgaricus*+80% *Streptecoccus thermophilus*, 30% *Lactobacillus bulgaricus*+70% *Streptecoccus thermophilus* and 50% *Lactobacillus bulgaricus*+50% *Streptecoccus thermophilus*.

Materials

The research was carried out at Aydin Adnan Menderes University, Çine Vocational School, Milk and Products Application Unit. Cow milk, which was used as raw material in the study, was purchased from the factory. The milk, which came to the facility with steel transport containers, was passed through the straining cloth and the metal strainer on the milk intake scale, and its rough cleaning was ensured and the amount was measured in kg. and recorded. pH values (WTW 3310 pH Meter Set 5) were measured by taking a sample of milk from the incoming milk in a glass beaker in an amount representing the batch (Metin and Öztürk, 2017).

Production of strained yogurt

The raw milk was taken to the cooking boiler and heated with steam at 85 ºC in 1 seconds, and the milk that completed the heat treatment was cooled to 48 ºC with a heat exchanger plate in the same boiler. The cooled milk was weighed and divided into 3 batches in equal amounts, taken into separate steel churn and lyophilized yogurt culture was inoculated into the milk. The amount of culture added to the milk in each churn is 6-8 grams per 100 kg of milk. In the study, lyophilized yogurt culture was used and the amount of culture used on the packaging was taken into account (according to the amount of milk to be processed). After the addition of culture, the inoculated milk, which was transferred to the incubation cabinet, was left for incubation. The temperature of the incubation cabinet was set to 45 ºC and the incubation was terminated by pH control. Incubation was terminated when the pH values measured with a pH meter in three separate steel troughs were 4.40. Incubation period was on average 4 hours.

The products that have completed their incubation are taken to the refrigerator operating 0-4 ºC and cooled for 12 hours waiting. The most commonly used method in the production of strained yogurt in Turkey is filtering in a cloth bag. This traditional method is based on the principle of filtering the classic natural yogurt by placing it in cloth bags. The cloth bags used in straining the yogurt have very small pores. According to this traditional method, after cooling, the products were transferred separately to the straining cloths, hung on hooks and naturally filtered for 15 hours. 100% cotton press cloth was used for the filtering process. At the end of the period, pH values were determined by using a pH meter device in strained yogurt samples. In strained

yogurt samples, L*(brightness), a* (redness), b* (yellowness), c (saturation) and H (hue) values were read and recorded using a colorimeter device (3NH TECHNOLOGIES NR200) after calibration. Measurements were made in three iterations and average values were calculated.

Sensory evaluation

In the sensory analysis applied in the study, students studying at Aydin Adnan Menderes University, Department of Food Processing took part and all panelists have successfully completed the course on sensory analysis of foods. There were 14 female students between the ages of 19-25 and 6 male students between the ages of 19-21 in the panel. All the panelists are semi-trained. Before the test, brief information was shared with the panelists about the reason for the difference in the strained yogurt samples, the production method, how the test would be applied and ethical rules of testing. In the study, sensory technique scoring and hedonic scale test were used. Sensory testing was carried out in a controlled environment with standardized lighting and temperature. Three different strained yogurt samples were presented to the panelists at the same time at a temperature of +4-6 ºC, weighing 20 grams each and with transparent plastic containers and different codes.

The panelists evaluated the strained yogurt samples using a 5-point scale (1: poor, 2: moderate, 3: good, 4: very good, 5: excellent) in terms of color and appearance, odor, consistency (with a spoon), consistency (in the mouth), taste, flavor and general taste characteristics. In addition, the hedonic scale was used in the study, and the panelists marked the expressions given to them in the test such as color and appearance (non-homogeneous color and appearance, fat separation, serum separation, gas formation, porosity, presence of foreign matter, unnatural color), taste and flavor (bitter taste, absence of typical yogurt flavor, excessive acid taste, excessive sour taste, buttery taste, metallic taste) and structure and texture (too loose or too firm, too dry, rough, poor gel formation, sticky).

Statistical analysis

The data obtained from the study were analyzed by analysis of variance (ANOVA) using SPSS 18.0 statistical program and statistical difference was determined at $P < 0.05$ level with the help of Tukey-LSD multiple comparison test.

Results and Discussion

The results of the sensory test are presented in Table 1 and Figure 1. In the sensory test applied in the study, the effect of culture bacterium ratio was statistically significant in terms of all sensory characteristics evaluated in strained yogurt samples ($P<0.001$). In terms of color and appearance, odor, consistency (with a spoon), consistency (in the mouth), taste and flavor, the highest scores were obtained by strained yogurt samples produced at a bacterium ratio of 50% + 50%.

The most important difference in sensory tests was determined in the consistency (spoon) evaluation, and the average scores according to the bacterium ratio were 1.30, 2.35 and 3.75, respectively. The highest score (3.85) in terms of general appreciation was found in strained yogurt samples produced at a bacterium ratio of 50%+50% ($P<0.001$). In terms of all sensory characteristics, the total score was calculated as 12.55, 15.75 and 26.00, respectively ($P<0.001$).

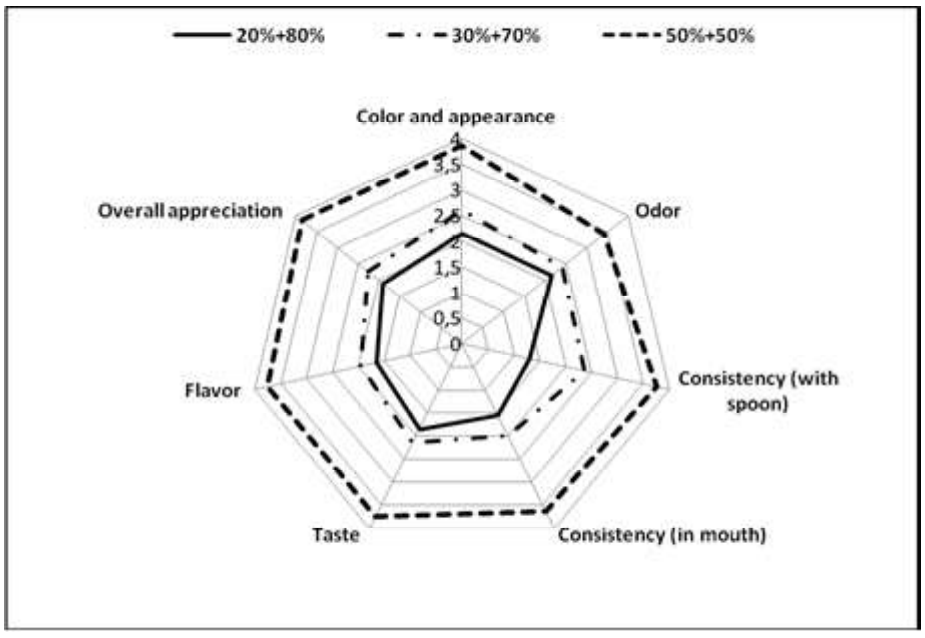

Fig 1. Sensory analysis graph of three different strained yogurt samples

The high score obtained at a bacterium rate of 50%+50% in terms of color and appearance is also directly proportional to the color measurements made with the colorimeter device. In the measurements made with the device, the highest saturation (c) ($P<0.05$) and hue (H) values were determined in the strained yogurt samples produced at a bacterium ratio of 50%+50%. The effect of culture bacterium ratio in terms of L*, a* and b* values is statistically insignificant. The effect of the bacterium ratio is important in terms of pH measurement in strained yogurt samples ($P<0.05$).

In the sensory test, 45% of the panelists detected inhomogeneous color and appearance, 35% of the panelists detected porpourity, 55% of the panelists detected lack of typical yogurt flavor and 35% of the panelists detected excessive sour taste defects in strained yogurt samples produced at a bacterium rate of 20%+80%. In strained yogurt samples produced at a bacterium ratio of 30%+70%, 35% of the panelists identified porosity, 45% of the panelists identified lack of typical yogurt flavor, and 55% of the panelists identified excessively sour taste defects. In strained yogurt samples produced at a bacterium rate of 50%+50%, the defect rate detected in terms of all features is at a very low level.

In addition, in the comments of the panelists who participated in the test, it was stated that the strained yogurt samples produced at a bacterium rate of 20%+80% were very watery, porous, very sour, bitter taste and rough, while for the strained yogurt samples produced at a bacterium rate of 30%+70%, it was stated that they had a liquid structure, sour and acid taste, acceptable flavor and dense consistency. Strained yogurt samples produced at a bacterium ratio of 50%+50% were evaluated as the most appreciated product by the panelists, and expressions of dense consistency, good taste, delicious and fluidity were included for this product.

The optimum growth temperature of *L. bulgaricus* found in yogurt culture is 40-50 ºC and this bacterium has the ability to ferment glucose, fructose, galactose and lactose into lactic acid (Wang et al 2021). The optimum growth temperature for *S. thermophilus* is 40–45 ºC, and this bacterium is capable of fermenting glucose, fructose, lactose, and sucrose (Huang et al. 2024). Yogurt fermentation consists of two stages. In the first stage, *L. bulgaricus*, which has proteolytic activity, stimulates the growth of *S. thermophilus* by releasing essential amino acids from casein (Çakmakoglu et al. 2023). In this stage, *L. bulgaricus* grows more slowly. Due to the high lactic acid concentration, the growth of *S. thermophilus* slows down at the end of the first stage. The second stage begins when *S. thermophilus* produces sufficient amounts of formic acid, which stimulates the growth of *L. bulgaricus*, and through this symbiotic effect, the desired final acidity of the yogurt is achieved (Liu et al. 2016;Singh and Mandal, 2019; Yu et al. 2021).

Table 1 The effect of different bacterium ratios on sensory properties, pH and color parameters of strained yogurt

	20%+80% Bacterium Rate	30%+70% Bacterium Rate	50%+50% Bacterium Rate	SHO	P
Color and appearance (avg.)	2.15^{b}	2.60^{b}	3.85^{a}	0.201	<0.001
Odor (avg.)	2.15^{b}	2.40^{b}	3.40^{a}	0.197	<0.001
Consistency (with spoon) (avg.)	1.30^{abc}	2.35^{abc}	3.75^{abc}	0.179	<0.001
Consistency (in mouth) (avg.)	1.55^{b}	2.00^{b}	3.65^{a}	0.184	<0.001
Taste (avg.)	1.85^{b}	2.15^{b}	3.75^{a}	0.189	<0.001
Flavor (avg.)	1.65^{b}	1.95^{b}	3.75^{a}	0.199	<0.001
Overall appreciation (avg.)	1.90^{b}	2.25^{b}	3.85^{a}	0.168	<0.001
Total score (avg.)	12.55^{abc}	15.75^{abc}	26.00^{abc}	0.901	<0.001
Strained yogurt end product pH	4.07^{a}	3.94^{b}	3.96^{b}	0.030	0.038
L* (brightness)	99.49	99.88	99.99	0.242	0.083
a* (redness)	-2.87	-3.74	-5.04	0.850	0.122
b* (jaundice)	8.92	12.71	13,43	1.370	0.058
c (saturation)	9.31^{b}	13.27^{a}	14.62^{a}	1.470	0.043
H (tint)	107.54	106.90	110.05	3.660	0.565

SHO: Standard mean of error; P: Significance; a-c: Differences between features bearing different letters on the same line are significant ($P<0.05$)

Complex biochemical reactions resulting from the activity of *L. bulgaricus* and *S. thermophilus* bacteria used in starter culture are highly effective on the sensory properties of yogurt. Appearance and color are one of the most important criteria in determining the sensory properties of yogurts. In a good quality yogurt, the appearance should be bright, the color should be white and homogeneous, and the consistency should be dark. There should be no serum separation in yogurt, there should be no gas formation and mold. In sensory tests, the dispersibility of yogurt between the tongue and the palate, whether it is plump or not, and its uniformity are among the other details emphasized. In this sense, a yogurt with a good consistency should be homogeneous and dense, and should not show a fragmented and water-released structure (Yang et al. 2017). Yogurt has a unique odor. The natural odor of yogurt, free from foreign odors, plays an important role in the evaluation of the panelists in sensory analysis. The taste parameter is another important criterion affecting the quality of yogurt and the milk from which it is obtained (Barba et al. 2017; Matejčeková et al. 2019).

Our enjoyment of yogurt is governed by our perception of aroma, taste and texture. Amongst these, flavour has the greatest effect on consumer acceptance and preference. In general, in addition to lactic acid, pyruvic acid, butyric acid, succinic acid, acetic acid, propionic acid, acetaldehyde, acetone, acetoin and a small amount of diacetyl are effective in the aroma and flavor formation of yogurt (Liu et al 2022). Among these, acetaldehyde is reported to be responsible for the typical yogurt flavor (Farag et al. 2022). When a single strain of *L. bulgaricus* or *S. thermophilus* is used in yogurt production, lactic acid and acetaldehyde production is lower compared to that in a mixed culture. *L. bulgaricus* is more capable in producing both acid and acetaldehyde, n-pentaldehyde, and 2-heptanone compared to *S. thermophilus* (Tian et al. 2019).

The viscosity of yogurt is also affected by the type of starter culture used in production (Silva et al. 2017). In studies investigating the effect of strains of *L. bulgaricus* and *S. thermophilus*, it has been reported that these bacteria produce exopolysaccharides that help increase viscosity (Tiwari et al. 2021).

In terms of sensory analysis, in many studies conducted on both fresh yogurt and strained yogurts, the product type, production method, starter cultures used in yogurt production, sensory test method, scoring and other scales used and the characteristics of the panelists participating in the test make a difference on the results. Variations in sensory evaluation results arise due to the use of sensory organs as a tool. The transformation of these tests into an objective evaluation, not a subjective evaluation, can only be achieved by careful planning of the tests and optimizing the conditions. In this study, the increases in taste, odor, flavor and consistency scores due to the increase in *L. bulgaricus* ratio are consistent with the effect of culture on sensory characteristics stated in the literature.

In a study, different ratios of *L. delbrueckii* subsp. *bulgaricus* IMAU20312 and *S. thermophilus* IMAU80809 (1:1, 1:10, 1:100, 1:1000, 1:2000) and commercial yogurt culture were used for yogurt production. The sensory panel was comprised of 15 highly experienced and screened judges who were familiar with dairy products. Colour, taste, aroma and texture of the samples were graded using the 100 point intensity scale according to the Chinese dairy industry guideline. The colours of the yogurts were determined using a ten point scale. Taste and aroma were determined between a range of 0–40, and texture was measured on a 50 point scale. In a reslut, the scores for the 1:100 bacterium ratio were higher than the other four samples and closer to the commercial control (Dan et al. 2023).

In a study, as a starter, *Lactobacillus delbrueckii* ssp. *bulgaricus* (M58) and *Streptococcus thermophilus* (S10) were used and found that the use of the M58 and S10 combination yielded synergistic benefits, positively impacting the physical, chemical, and sensory attributes of the final product (Yang et al. 2017). In another study, three starter combinations were used for the yogurt fermentation; traditional yogurt starter cultures (YF-L904, Chr. Hansen Co., Ltd., Denmark) and two co-cultures of *L. plantarum*. Twenty four panelists were selected, including 12 men and 12 women, aged between 20 and 30. Each sample was rated using a 9 point hybrid hedonic scale. The sensory assessment of yogurt used seven categories, namely milk flavor, typical yogurt flavor, sour milk, vinegar, scream flavor, off-odor, and overall acceptability. In a result, the "typical yogurt flavor," "cream flavor," and "overall acceptability" of the samples co-fermented with *L. plantarum* were stronger than those of the control. Furthermore, there were no significant differences in the other three properties ("off-odor," "vinegar," and "sour milk") of the three yogurt samples, indicating that the use of these strains did not produce excessive negative aroma compounds during fermentation (Tian et al. 2019).

A research was conducted at Akdeniz University, Department of Food Engineering to determine the physicochemical and sensory properties of yogurts. Danisco Yo-Flex 410 commercial culture was used as starter culture in this study. A 15 point hedonic scale was used in sensory analyses and 40 panelists took part. According to the sensory results, bag yogurt scored an average of 10.3 for visual consistency, an average of 11.1 for color, an average of 10.3 for consistency on a spoon, an average of 6.2 for sourness, and an average of 10.6 for overall acceptability (Aktar, 2022).

Although the results of this study are consistent with the results of the study given above, there are some differences. The reasons for these differences can be said to be the type and amount of culture used, the yogurt production method, the sensory test

method applied, the differences between the panelists and the sensory test conditions.

Conclusion

Yogurt is a product with very rich values in terms of nutritional value. Among dairy products, yogurt, which is highly preferred both in our country and around the world, is easy to digest and regulates the digestive system, strengthens the immune system and is easily consumed by people who are sensitive to lactose (intolerant). Sensory properties are characteristics determined by human senses that lead the consumer to accept or reject a food. Sensory tests performed on milk and dairy products and therefore on yogurt are an important identification tool and are an increasingly preferred method to reveal the quality of the product. Sensory analyses in yogurt are performed for purposes such as ensuring quality in daily yogurt production, determining differences in products, developing a new product, improving the quality of currently produced products and revealing consumer taste. In sensory tests, the type of yogurt samples to be tested, the type and ratio of starter culture used, the preparation methods of the samples, the selection and training of panelists, the arrangement of the panel area, the test method to be applied and the analyzing of the test results are important.

In the study, strained yogurt samples produced in the traditional way using 3 different bacterium ratios in yogurt culture were subjected to sensory testing. In terms of the sensory characteristics evaluated by the panelists participating in the test, the highest scores were obtained by the strained yogurt samples produced at a bacterium ratio of 50%+50%, and in the sensory evaluation of the defects, some negativities were detected at very low levels in the same strained yogurt samples. The most preferred product by all of the panelists participating in the test was strained yogurt samples produced at this bacterium rate.

The unique tastes and aromas of traditionally produced yogurts are affected by the quality of raw materials, but are largely due to their microflora. The chemical and physical changes caused by microorganisms during the formation of yogurt determine the taste, aroma and structure of yogurt. Therefore, by determining the microflora of the most popular yogurts in the sensory analysis results of traditionally produced yogurts, suitable culture combinations can be used for the production of yogurt suitable for taste. Usually, yogurt starter cultures are comprised of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in a 1:1 or 1:2 ratio, but with the increasing demand for commercial starter cultures in the fermentation industry, the impact of using different ratios of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* on dairy flavour substances has been of increasing interest to the industry. According to the results of the sensory evaluation in this study, it is concluded that it would be correct to use 50% *L. bulgaricus* + 50% *S. thermophilus* bacterium ratio in yogurt production and to plan the production accordingly.

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Conflict of Interest

The authors have not any conflict of interest to declare.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

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RESEARCH ARTICLE

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

Ashvinkumar S Hariyani (✉), Tanmay Hazra, Ankit J Thesiya, and Kunal Kumar Ahuja

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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

College of Dairy Science, Kamdhenu Univeristy, Amreli-Rajkot Highway, Nr. Shedubhar Village, Amreli- 365 601 (Gujarat)

Ashvinkumar S Hariyani(✉)
Dairy Chemistry Department, College of Dairy Science, Amreli Kamdhenu University, Amreli-365601, Gujarat, India
E-mail: ashvin.hariyani@gmail.com , ash@kamdhenuuni.edu.in

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.

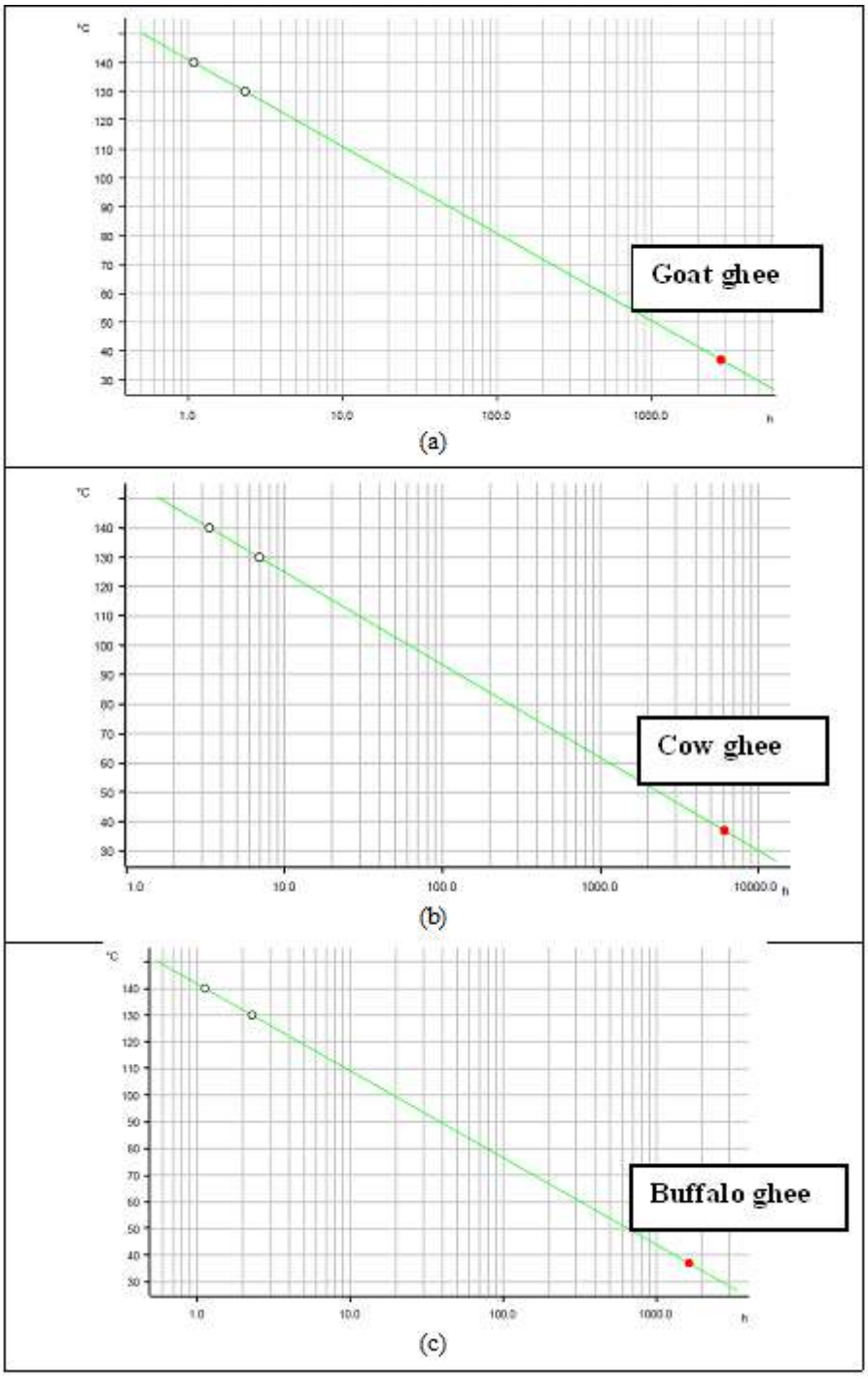

Fig. 1 Extrapolation graph of shelf life calculation of different ghee samples

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

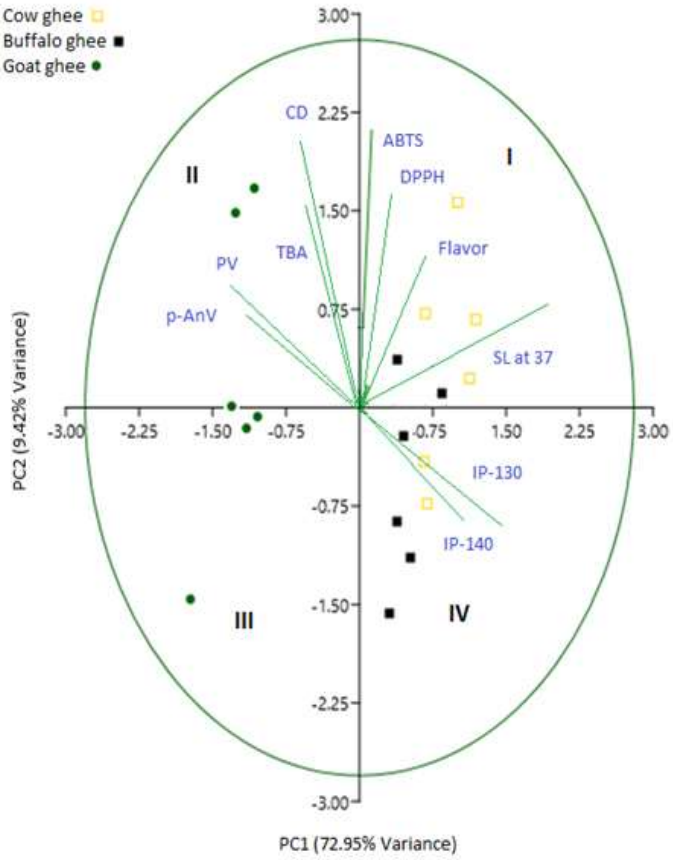

Fig. 2 Biplot and scatter plot of PCA analysis

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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RESEARCH ARTICLE

Preparation and characterization of low-fat and low-sugar lemon grass flavoured herbal lassi

Soma Maji[1](✉), Barsharani Sahoo[2], Binapani Sahu[2], Nihar Ranjan Naik[2], Sushree Madhushmita Behera[3], Monalisa Nanda[3] and Avijit Pradhan[3]

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Abstract: Lassi is an amazing thirst-quenching fermented dairy beverage in India. It has numerous health benefits and therapeutic value in humans. In the present investigation, an attempt was undertaken to prepare a low-fat and low-sugar herbal lassi from double toned milk. For the preparation of lassi, double-toned milk containing 1.5% fats and 9% solids not fat was used. Dahi was prepared using 1% mixed dahi culture. Artificial sweetener (sucralose) @ 0.7% was used as a replacement for sucrose in lassi. Optimization of different concentrations of aqueous lemongrass extract (2%, 3% and 4% v/v) was evaluated by sensory analysis. 3% (v/v) lemon grass added lassi scored the highest overall acceptability. Further chemical analysis viz., pH, acidity, total solids, moisture, fat and protein content was evaluated and compared with control lassi samples. No significant difference was observed between the two samples. Shelf- life analysis of the selected herbal lassi was conducted at refrigerated temperature (5 ± 2°C). The lassi was found stable for 9 days at storage condition (5 ± 2°C, in glass bottle) based on the chemical, microbiological and sensory analysis.

Keywords: Low fat, Low sugar, Lemon grass, Herbal lassi, Shelf life

[1]Department of Dairy Technology, Centurion University of Technology and Management, Paralakhemundi, Odisha, 761211
[2]Department of Zoology, Centurion University of Technology and Management, Bhubaneshwar, Odisha, 752050
[3]Department of Applied Chemistry, Centurion University of Technology and Management, Bhubaneshwar, Odisha, 752050

Soma Maji (✉)
Centurion University of Technology and Management,
Paralakhemundi, Odisha, 761211
Email: somadt08@gmail.com

Introduction

Good health is the need and desire of every individual. A healthy life has become very rare in the current polluted world. The growing concern for health and nutrition among consumers has increased the market potential of different emerging food products day by day. In India around 9% of the total milk produced is converted into fermented milk products (Singh et al. 2006). Fermented milk products are well-known for their nutritional and physiological benefits, which include gastrointestinal infection prevention, serum cholesterol lowering and antimutagenic action and also excellent for lactose-intolerant people (Shiby and Mishra, 2013). Lassi is one of the most popular fermented dairy beverages in India that is prepared by the churning of curd with 1% of mix dahi culture or commercial yogurt culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

In today's health-conscious society, people are increasingly mindful of their well-being and actively seek beverages that promote disease-free living. Lassi incorporated with nourishing herbs emerges as the ultimate combination, offering a delightful and tantalizing drink that not only satisfies the taste buds but also supports a healthy lifestyle. Herbs and spices have long been used as food additives, not just to improve food's organoleptic characteristics, but also to extend shelf life by lowering or eradicating foodborne microorganisms. (Maji et al. 2018, Maji et al. 2023, Miran et al. 2021, Gokhale et al. 2021). Herbs and spices are frequently used as preservatives and have antioxidant, anti-inflammatory, antitumorgenic, anticarcinogenic qualities (Pateiro et al. 2021, Pinto et al. 2021, Bhattacharya et al. 2021). Lemongrass is one of the most refreshing and advantageous herbs that helps to cure different types of diseases. It is a tropical plant and as such will grow best in warm, sunny and humid conditions of the tropic and subtropic. It also helps to prevent the growth of some bacteria and yeast. Lemongrass also contains substances that help to relieve pain and swelling, reduce fever, and improve the level of sugar and cholesterol in the blood. Lemongrass has a high number of antioxidants that help in preventing oxidative stress like cancer, aging, hypertension, memory loss, depression, stroke, asthma etc. Today, the industry is particularly interested in using these herbal bio-actives in several ways that the medical advantages of herbs can be

delivered as carriers through specific meals. Milk and milk products are one of the most significant sources and can be used as carriers for such foods (Sawale et al. 2013). The inclusion of herbal bio-actives in milk products not only allows the industry to meet the growing demands of customers for such foods but also helps to compete with increasingly functional food markets around the world (Paswan et al. 2021). Along with this, the rising prevalence of diabetes has led to an increase in the consumption of low-calorie sweeteners. Sucralose, a low-calorie sweetener has been shown to play a useful role in aiding people lose and maintain weight. Consumers are becoming more health concerned as they become more aware of the negative effects of excess calories, fat and sugar. Hence, considering the medicinal properties of lemongrass and the use of sucralose and double-toned milk in restricting caloric intake, the present research was designed.

Materials and methods

Collection of raw materials:

Collection of milk, sugar and herbs:

Double-toned cow milk (1.5% and SNF was 9.0%) and sugar-free Natura (sucralose) were purchased from the local market of Parlakhemundi, Odisha.

Tender lemongrass leaves were collected from the herbal garden of the University campus.

Collection of starter culture:

Mix dahi culture was collected from the Mini Dairy unit of Centurion University of Technology and Management in Paralakhemundi, Odisha.

Preparation of sugar syrup:

Sugar-free Natura (Artificial Sweetener) was dissolved in distilled water at a ratio of 0.7%. Afterward, the syrup was used for the preparation of lassi.

Preparation of herbal extract:

Fresh tender lemon grasses were washed in tap water and cut into small

pieces using a knife. Afterward, the chopped lemongrass was immersed in boiling water for a brief 30-second period to facilitate hot water washing and was subsequently filtered using a strainer. It was then ground in a mixer grinder. The juice was carefully extracted and filtered using a muslin cloth, ensuring proper pressure was applied. Then the herbal extract was measured in weighing balance as per requirement in herbal lassi preparation.

Preparation of herbal lassi:

Lassi was prepared according to the method used by Maji et al. 2018. Double-toned milk was first heated to 95°C and cooled at 35°C. After that 1% of mix dahi culture (Streptococcus sp., Lactobacillus sp., Lactococcus sp.) was added into the milk and incubated at 35 to 40°C for 6 to 7 hours. The prepared dahi was gently crumbled and combined with the artificial sweetener. Previously prepared lemon grass juice was then added at 3% (v/v) and mixed. The prepared lassi was stored in refrigerated condition (5± 2°C) (Fig 1).

Analytical methods

Chemical analysis:

The prepared lassi was analyzed for the titratable acidity (AOAC, 1995), total solids (IS:12333 1997), pH (using pH meter coupled with glass electrode), moisture content, fat content by Mojonnier method (IS: 10484, 1983), Ash content (IS:1479 1961) and protein content by Kjeldahl method (IDF 20B: 1993).

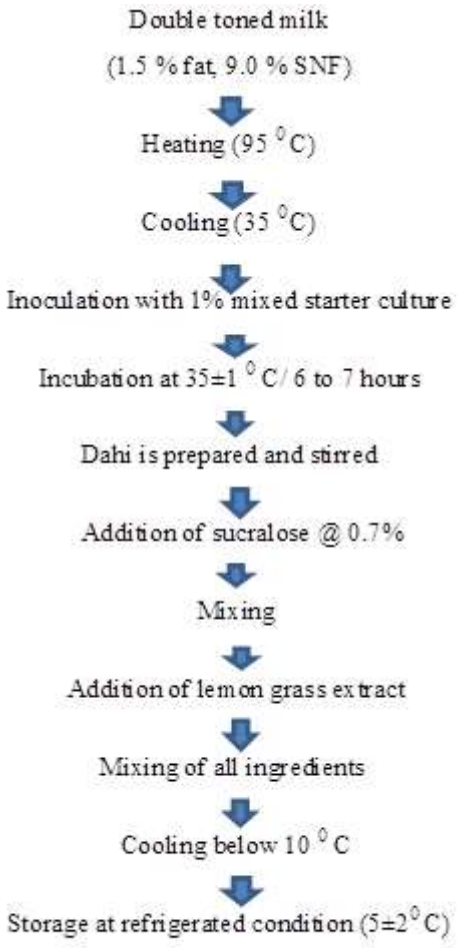

Fig 1: Flow diagram of preparation of lemongrass flavoured lassi

Microbiological analysis:

The Standard Plate Count (SPC), Coliform count, and yeast and mold count in lassi samples were determined following the methods described by FSSAI (2012).

Sensory analysis:

Sensory evaluation of the lemon grass flavoured herbal lassi was done by using a 9-point Hedonic scale as described by the method in Amerine et al. (1965). The samples were judged by a panel of 7 judges in the institution.

Statistical analysis:

Observed data were analyzed for one-way ANOVA using the GraphPad Prism software (version 5.01 for Windows), San Diego, CA, USA. All Data are reported as the mean ± standard error.

Results and Discussions

The optimization of the lemon grass extract for the preparation of flavoured herbal lassi is described below. Different concentrations of lemon grass extract were incorporated into the plain lassi samples. The specific details of each treatment applied are given below.

Treatment Details

T1 - Lassi with no lemon grass extract (Control)

T2 - Addition of lemon grass extract @ 2 percent (v/v) of plain lassi

T3 - Addition of lemon grass extract @ 3 percent (v/v) of plain lassi

T4 - Addition of lemon grass extract @ 4 percent (v/v) of plain lassi

Effect of different levels of lemon grass extract on the sensory quality of lassi:

Different level of lemongrass extract was used to optimize the flavoured lassi. The comparative sensory score of the control lassi and lassi prepared with different levels of lemon grass extract, stored at 5 ± 2°C, are shown in Table 1. The mean score of colour and appearance shows a significant decrease in the case of T2 (2% v/v lemon grass extract added) and T4 (4% v/v lemon grass extract added) compared to T1 (control) and T3 (3% v/v lemon grass extract added). Whereas no significant difference was observed between the T1 and T3 (3% v/v lemon grass extract added) lassi samples. In case of flavour of the herbal lassi, it was observed that the flavour score increased when lemon grass extract was added. A significantly high score was observed in all the treated samples compared to the control i.e. T1. Among all the treated samples, the T3 sample demonstrated the highest

Table 1: Sensory analysis of lemon grass (LG) juice added lassi

Parameters	Level of lemon grass extract (%)			
	T1	T2	T3	T4
Colour and appearance	7.2 ± 0.58^{a}	6.9 ± 0.45^{b}	7.0 ± 0.45^{a}	6.8 ± 0.74^{b}
Flavour	5.1 ± 1.17^{a}	6.3 ± 0.44^{b}	7.8 ± 0.37^{c}	7.06 ± 0.86^{d}
Consistency	5.9 ± 0.64^{a}	6.3 ± 0.62^{b}	6.70 ± 0.70^{c}	6.70 ± 0.70^{c}
Overall acceptability	7.16 ± 0.21^{a}	7.52 ± 0.22^{b}	8.0 ± 0.32^{c}	7.86 ± 0.22^{d}

Values represented as Mean ± SEM, n=7, ^{a,b,c,d}- different superscripts shows significant difference row wise ($p>0.05$)

Table 2: Comparison between chemical composition of control and selected herbal lassi

Parameters (%)	Lassi Samples	
	Control	3% lemon grass added lassi
Acidity	0.72 ± 0.02^{a}	0.74 ± 0.02^{a}
pH	4.79 ± 0.02^{a}	4.77 ± 0.02^{a}
Total Solids	9.05 ± 0.53^{a}	9.12 ± 0.49^{a}
Moisture	90.95 ± 0.54^{a}	90.88 ± 0.48^{a}
Ash	0.69 ± 0.01^{a}	0.71 ± 0.02^{a}
Fat	1.43 ± 0.01^{a}	1.44 ± 0.01^{a}
Sucralose	0.7 ± 0.00^{a}	0.7 ± 0.00^{a}

Values represented as Mean ± SEM, n=7, Same superscript letter in each row represents non significant difference row wise ($p>0.05$)

score in comparison to the others. The consistency profile exhibited a consistent pattern, with higher scores observed in the treated samples as compared to the control sample. The overall acceptability score of control lassi (T1) was found 7.16 ± 0.21. Whereas the scores for the treated lassi samples were 7.52 ± 0.22, 8.0 ± 0.32, and 7.86 ± 0.22 for T2 (2% added), T3 (3% added) and T4 (4% added) respectively. Therefore, it can be concluded that T3 sample (3% v/v lemon grass extract) was the best combination for the preparation of flavoured lassi. The result is comparable with the study of Mule et al. (2018). They used skimmed buffalo milk for the preparation of low-fat lassi. Added with lemon grass extract at a concentration range of 2.5 %, 5%, 7.5% and 10%. Lemon grass distillate at a level of 2% was used for the preparation of dahi and found acceptable for 11 days of refrigerated storage condition (Sutariya and Rao, 2015).

Chemical composition of the selected herbal lassi:

The chemical composition of the control lassi and 3% lemon-grass added lassi was evaluated. The parameters analysed for the lassi were pH, acidity, total solid, moisture, fat and ash content. Table 2 shows the results of the chemical composition of the herbal lassi with a comparison to control lassi.

From table 2, it was observed that the acidity of control lassi and lemon grass added lassi were 0.72 ± 0.02 and 0.74 ± 0.02 percent lactic acid respectively. Whereas the pH of the control and lemon-grass added lassi was 4.79 ± 0.02 and 4.77 ± 0.02 respectively. The results indicated that the addition of lemongrass juice to the lassi did not result in any significant differences in acidity and pH compared to the control lassi in the initial days. These findings are similar to the observations reported by Mule et al. (2018). They found the acidity of control and lemongrass flavoured lassi (2.5% v/v) on the first day was 0.80 and 0.81% of lactic acid. Sutariya and Rao (2015) also found a non-significant difference in pH and acidity of control and flavoured yoghurt samples.

No significant difference was observed in the total solids and moisture content between lemon-grass flavoured lassi and control lassi (Table 2). The total solids content of control and 3% lemon grass added lassi was found 9.05 ± 0.53 and 9.12 ± 0.49 percent, respectively. The moisture content of control and 3% lemon grass juice added lassi was found 90.95 ± 0.54 and 90.88 ± 0.48 percent respectively. The results are in accordance with findings of Mule et al. (2018).

The fat content of control and flavoured lassi showed no significant difference, likely due to the use of double toned milk. The fat content of control and flavoured lassi was found 1.43 ± 0.01 and 1.44 ± 0.01 percent respectively. It was also observed that the addition of lemongrass extract did not affect the fat content in lassi.

The ash content of the control and flavoured lassi was found 0.69 ± 0.01 and 0.71 ± 0.02 percent, respectively. Chaudhari (1959) and Laxminarayan and Shankar (1980), who reported the average ash content of skim milk lassi and plain lassi as 0.70 to 0.75 and 0.7 percent respectively. Mule et al. (2018) observed the ash content of control and lemongrass flavoured lassi was 0.82 and 1.12 % respectively.

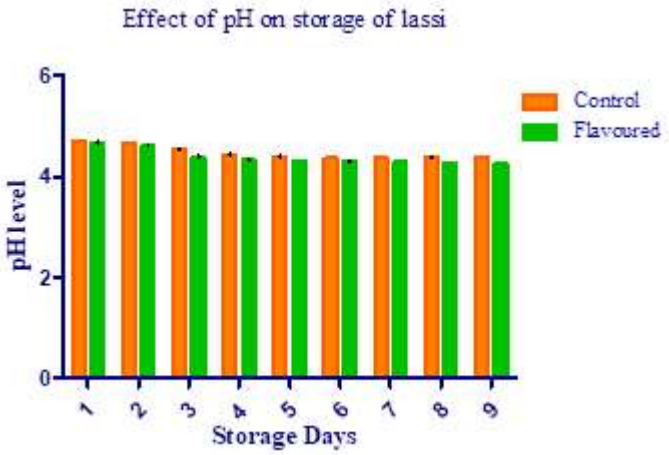

Fig 2: Changes in pH of control and lemon grass flavoured lassi during storage at refrigeration temperature (5±2°C)

Changes in chemical, microbiological and sensory properties of lemon grass flavoured lassi during storage at 5±2°C:

Effect of pH and acidity on the stability of lemongrass flavoured (@ 3% v/v) lassi during storage at refrigeration temperature (5±2°C)

The pH of the control and flavoured lassi was analyzed for a period of 9 days stored in a glass container at refrigerated temperature (5±2°C). It was observed from the results that the pH was decreased with increasing the storage days (Fig 2). In case of the control lassi, the pH on 0th day was observed 4.72 ± 0.01 and on 9th day, it was 4.34 ± 0.01. Whereas, in flavoured lassi, the pH on 0th day was 4.77 ± 0.01 and on 9th day was 4.11± 0.01. The decrease in pH of all lassi samples during storage was due to increasing the microbial load. Therefore, the acidity increases and simultaneously pH decreases. Similar type of result was observed by Kumar et al. (2020). They found a gradual decrease in pH in control (4.53 to 3.91) and flavoured lassi (4.57 to 3.89) in 28 days of storage at 7±1°C.

In case of acidity, it is evident that as the duration of storage increases, there is a corresponding rise in acidity levels. A similar pattern was observed in the case of flavoured lassi samples (Fig 3). It was also observed that the acidity of the flavoured lassi was significantly higher compared to the control lassi. The acidity of control lassi and flavoured lassi on 0th day was found 0.69 ± 0.01 and 0.70 ± 0.01 respectively. Kumar et al. (2020) also did a storage study of herbal honey lassi and observed an increasing trend in the acidity of lassi during a storage period of 28 days at

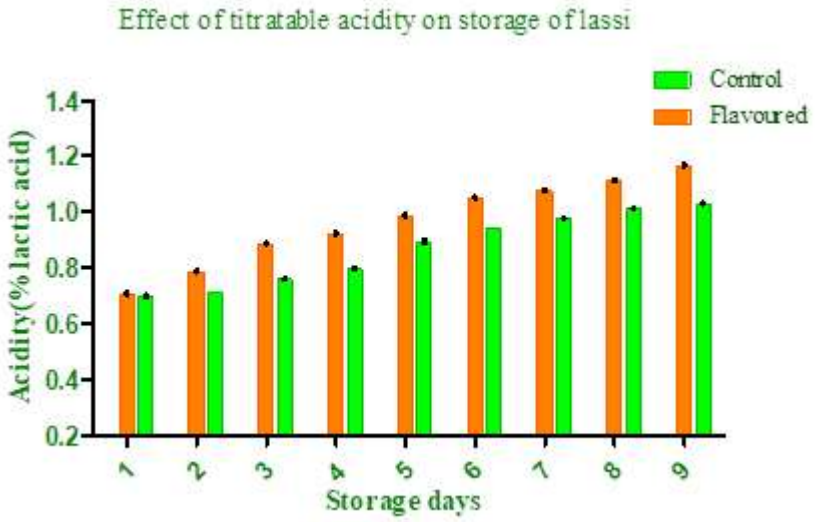

Fig 3: Changes in titratable acidity of control and lemon grass flavoured lassi during storage at refrigeration temperature (5±2°C)

Table 3: Changes in the microbiological quality of control and flavoured lassi during storage at refrigeration temperature (5±2°C)

Parameters	Day 1	Day 3	Day 5	Day 7	Day 9
			Control Lassi		
SPC (cfu/ml)	1.9×10^5	2.4×10^5	2.9×10^5	3.3×10^5	3.9×10^5
Coliform (cfu/ml)	Nil	Nil	Nil	Nil	Nil
Yeast & Mold (cfu/ml)	Nil	Nil	Nil	Nil	Nil
			Lemon grass flavoured Lassi		
SPC (cfu/ml)	0.8×10^5	1.3×10^5	1.8×10^5	2.3×10^5	2.7×10^5
Coliform (cfu/ml)	Nil	Nil	Nil	Nil	Nil
Yeast & Mold (cfu/ml)	Nil	Nil	Nil	Nil	Nil

Values are represented as average of two (n=2) readings. cfu= colony forming unit

7± 1°C. The rate of increase in acidity for control lassi was 0.81 to 1.24% Lactic acid (LA) and herbal honey lassi was 0.77 to 1.38% LA. A similar observation was also observed by Patidar and Prajapati (1998) and Bagal et al. (2007).

Effect of microbial count on the stability of lemongrass flavoured (@ 3% v/v) lassi during storage at refrigeration temperature (5±2°C)

Microbiological analysis of flavoured lassi was done to assess the shelf life of the lassi at refrigeration temperature. The standard plate count, coliform count and yeast and mold count of control and flavoured lassi are given in Table 3. The SPC count of lassi samples was increased with increasing the storage days whereas coliform and yeast and mold count has observed nil throughout the storage period. The results are in accordance with the result of Maji et al. (2018). Where they reported, that in turmeric-flavoured lassi, SPC count increased and coliform and yeast and mold count was nil during storage (7±2°C).

Effect of sensory properties on the stability of lemongrass flavoured (@ 3% v/v) lassi during storage at refrigeration temperature (5±2°C):

Sensory analysis is the best method for figuring out whether a product will be well-liked

by customers or not. The treated lassi samples along with the control lassi were assessed by the four judges from the organization using a "Nine-point Hedonic scale" scorecard. The data obtained for changes in sensory attributes (during storage at 5±2°C) for control lassi and flavoured lassi is presented in Table 4. It was observed that in both the lassi samples, after 7th days onwards the sensory scores were highly affected with increasing the storage days. Comparing the sensory score of both type of lassi, it was observed that the flavour score was significantly higher in case of lemongrass flavoured lassi compared to control one from the day first to the increasing storage days. This is due to the added flavour of fresh lemongrass

Table 4: Sensory analysis of control and flavoured lassi during storage at refrigeration temperature (5±2°C)

Characteristics	Type of Lassi	Storage Days									
		1	2	3	4	5	6	7	8	9	10
Colour	Control	9.0±0.01^{aA}	9.0±0.01^{aA}	9.0±0.01^{aA}	9.0±0.02^{aA}	8.5±0.50^{aA}	8.5±0.50^{aA}	8.0±0.02^{aB}	7.0±0.02^{aB}	6.0±0.01^{aB}	6.0±0.01^{aB}
	Flavoured	9.0±0.01^{aA}	9.0±0.01^{aA}	9.0±0.02^{aA}	9.0±0.02^{aA}	9.0±0.02^{aA}	9.0±0.01^{aA}	8.0±0.01^{aB}	7.0±0.02^{aB}	7.0±0.02^{aB}	6.0±0.01^{aB}
Flavour	Control	7.5±0.50^{aA}	7.5±0.50^{aA}	7.0±0.01^{aA}	7.0±0.01^{aA}	6.5±0.50^{aA}	6.5±0.50^{aA}	6.5±0.50^{aA}	6.0±0.01^{aB}	5.0±0.01^{aB}	5.0±0.01^{aB}
	Flavoured	9.0±0.01^{bA}	9.0±0.02^{bA}	9.0±0.01^{bA}	8.5±0.50^{bA}	8.5±0.50^{bA}	8.0±0.50^{bB}	7.5±0.10^{aB}	7.0±0.50^{bB}	7.0±0.20^{bB}	6.0±0.30^{bB}
Mouthfeel	Control	8.5±0.50^{aA}	8.5±0.50^{aA}	8.5±0.50^{aA}	8.0±0.02^{aA}	8.0±0.02^{aA}	8.0±0.01^{aA}	8.0±0.01^{aA}	7.0±0.01^{aB}	6.0±0.02^{aB}	6.0±0.01^{aB}
	Flavoured	9.0±0.50^{aA}	9.0±0.50^{aA}	8.5±0.01^{aA}	8.5±0.01^{aA}	8.5±0.01^{aA}	8.0±0.02^{aB}	8.0±0.05^{aB}	7.0±0.02^{aB}	7.0±0.01^{aB}	6.5±0.01^{aB}
Consistency	Control	8.0±0.01^{aA}	8.0±0.01^{aA}	8.0±0.01^{aA}	7.5±0.50^{aA}	7.5±0.50^{aA}	7.0±0.01^{aA}	7.0±0.02^{aA}	6.5±0.50^{aB}	6.0±0.01^{aB}	5.0±0.01^{aB}
	Flavoured	8.0±0.02^{aA}	8.0±0.05^{aA}	8.0±0.01^{aA}	8.0±0.01^{aA}	7.5±0.50^{aA}	7.5±0.50^{aA}	7.5±0.50^{aA}	7.0±0.01^{aA}	7.0±0.01^{aB}	6.0±0.02^{aB}
Overall acceptability	Control	7.5±0.02^{aA}	7.5±0.02^{aA}	7.0±0.10^{aA}	6.5±0.50^{aA}	6.5±0.50^{aA}	6.5±0.05^{aA}	5.5±0.05^{aB}	5.0±0.01^{aB}	5.0±0.01^{aB}	4.0±0.01^{aB}
	Flavoured	8.5±0.50^{bA}	8.5±0.50^{bA}	8.0±0.01^{bA}	7.5±0.50^{bA}	7.5±0.50^{bA}	7.5±0.50^{bA}	7.0±0.50^{bA}	6.5±0.50^{bB}	6.5±0.50^{bB}	5.0±0.10^{bB}

Values are represented as mean ± SEM, n=4; $^{a-b}$ different superscript column wise differ significantly between control and flavoured lassi in each characteristics, A-B different superscript row wise differ significantly within control and flavoured lassi ($p>0.05$)

juice in lassi. The mouth feeling of lemongrass flavoured lassi was also higher compared to control but non significantly. The overall acceptability score of lemongrass flavoured lassi was comparatively higher than the control lassi. From the result, it was observed that the lemon-grass flavoured lassi was acceptable for up to 9 days when stored in a closed glass container at 5 ± 2°C. Similarly, the control lassi was found suitable for up to 6 days of storage at similar conditions.

Conclusions

Lassi, a well-known fermented product with therapeutic properties, can be enhanced nutritionally through the addition of various herbs. In this study, a low-fat version of lassi was prepared using double-toned milk, while the use of sucralose instead of sugar resulted in a reduction in calorie content. To improve the flavour, nutritional and functional property of the lassi, different concentrations (2%, 3% and 4% v/v) of lemongrass juice were used for the preparation of the lassi. Through sensory evaluation, it was determined that the lassi containing 3% (v/v) lemon grass juice received the highest preference and was selected as the best option. The final product was analyzed for pH, acidity, total solids, moisture, ash and fat content. No significant difference was observed in the chemical composition of the flavoured lassi with the control lassi. The final product showed a shelf life of 9 days according to chemical, microbiological and sensory evaluation when stored in a closed glass container at refrigeration temperature (5 ± 2°C). Therefore, it can be concluded that the preparation of low-fat, low-sugar lemon-grass flavoured lassi could be efficaciously utilized as a low-calorie fermented product.

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RESEARCH ARTICLE

Antioxidant activity of garden cress seed (*Lepidium sativum)* protein hydrolysate incorporated Kesar flavoured milk

Preetham Gowda HR[1], Ramesh V[2] (✉) and Aneeta Khatak[1]

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Abstract: The present study investigates the application of garden cress protein hydrolysates (GCPH) derived from garden cress protein concentrate (GCPC) using papain (1:25, w/w). The effects of pH (6.0, 6.5, 7.0), temperature (50, 55, 60°C), and hydrolysis time (0, 30, 60, 90, 120, 150, and 180 min) on the degree of hydrolysis (DH) and antioxidant activity were assessed. Optimal conditions (pH 6.5, 55°C, 120 min) yielded a DH of 13.6 ± 1.1% and DPPH radical scavenging activity of 60.03 ± 0.96%. GCPH was incorporated into Kesar flavored milk at 1% and 2% concentrations. The 2% GCPH addition significantly enhanced antioxidant activity to 0.45 ± 0.01 mM Trolox/L, compared to control and 1% GCPH. However, the 2% GCPH imparted a slight bitterness, which was mitigated by increasing the flavor concentration to 3%. These results indicate that GCPH can be effectively used as a functional additive to enhance the antioxidant properties of milk and milk products, providing potential health benefits and improved product quality.

Keywords: Flavoured milk, antioxidant activity, garden cress seed, protein, hydrolysate.

Introduction

Garden cress (*Lepidium sativum*) belonging to *Brassicaceae* family is used as an important medicinal plant in India. The seeds contain 33-54 % carbohydrate, 22-25 % protein and 17-27 % lipids and various biologically active compounds exhibiting various health benefits (Gokavi et al. 2004; Azene et al. 2022). In recent years, there has been an increasing interest in the isolation and modification of the proteins from seeds and legumes to improve the bio-functional properties for its exploitation in food systems (Yadav et al. 2022).

Reactive oxygen species (ROS) produced during oxidative metabolism are considered as a causative factor in several lifestyle-mediated diseases (Hernández-Ledesma et al. 2005). The body has its own defense system to neutralize the free radical oxygen species, oxidative stress occurs when reactive oxygen species exceed the body's antioxidant resistance mechanism (Lobo et al. 2010). The hydrolysates obtained by enzymatic hydrolysis of plant protein has been reported to have antioxidant properties and considered as promising dietary supplement for improvement of antioxidant defense mechanism and a practical approach to reduce the oxidative stress in the body (Daliri et al. 2017; Rizzello et al. 2017; Sarmadi and Ismail, 2010). Hydrolysis of protein from *Bunium persicum Bioss* using alcalase shown to improve the antioxidant properties and metal chelating activity (Shahi et al. 2020). Bagul et al. (2018) reported maximum degree of hydrolysis of 39.49 % and radical scavenging activity of 42.92 % in tamarind protein hydrolysate obtained under optimized hydrolysis conditions with papain-to-protein ratio, hydrolysis time, temperature and pH of 1:5, 3h, 65 °C and 6.0, respectively. In the recent study on hydrolysis of garden cress protein concentrate with alcalase exhibited a broad range of antioxidant activities ranging between 11.18 % to 69.25 % DPPH inhibition and Fe^{2+} chelating activity of 4.32 % to 21.76 %, respectively Mulla and Ahmed (2019). However, no reported literature available on utilization of garden cress protein hydrolysate (GCPH) in beverages. Therefore, the potential of GCPH as an antioxidant ingredient in beverage system needs to be studied for better utilization in functional beverages. Milk-based beverages are a rich source of proteins as well as a high calcium and phosphorus content. They are convenient with good nutritional and easily digestible health foods connected with high protein content. Further, the stability of plant proteins hydrolysate over a wide range of pH makes them suitable for incorporation in healthy beverages. The addition of antioxidant rich GCPH could increase the antioxidant properties and health benefits of flavoured milk.

[1]Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar-125001, Haryana, India
Email: preethamgowda8991@gmail.com
Email: aneetarajkhatak@gjust.org
[2]Department of Dairy Chemistry, G.N. Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar-385506, Gujarat, India
Email: drrameshv@kamdhenuuni.edu.in

Ramesh V (✉)
Department of Dairy Chemistry, G.N. Patel College of Dairy Science, Kamdhenu University, Sardarkrushinagar-385506, Gujarat, India
E-mail: drrameshv@kamdhenuuni.edu.in

Strawberry and chocolate flavoured milk supplemented with 2 % whey protein hydrolysate (flavourzyme, alcalase and corolase) showed an increase in the antioxidant activity (Mann et al. 2014). As a result of the harmful effects of synthetic antioxidants on human health, several workers have studied the production of natural antioxidants from plant proteins for its utilization in food systems (Park et al. 2001; Lourenço et al. 2019). Hence, the present work was carried out to optimize the hydrolysis condition to prepare GCPH with high antioxidant activity and to evaluate the antioxidant potential of Kesar flavoured milk incorporated with GCPH.

Materials and methods

Materials

Garden cress seeds, milk and Kesar flavour were obtained from the local market in Hisar, India. The commercial enzyme papain (e" 30000.0 U/mg) received from NDRI, Karnal, India. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sulphuric acid, sodium hydroxide, hydrochloric acid, copper (II) sulfate, potassium sulfate, ethanol, methyl blue and red, sucrose, Folin phenol reagent were purchased from Sigma-Aldrich, India.

Defatted garden cress seeds powder

Garden cress seeds were defatted by cold press, dried at 55 ± 2 °C for 48 h and ground into a fine powder, then passed through 45-mesh standard sieve and stored in air-sealed plastic bags at 4 °C until further use.

Preparation of protein concentrate

Garden cress protein concentrates (GCPC) were prepared according to the method described by Gaafar et al. (2013), with slight modifications. Briefly, 100 g of defatted garden cress seed flour was dispersed in distilled water (1:20). Suspension was adjusted to pH 9.0 with 1 N NaOH. The mixture was stirred for 60 min at room temperature using a magnetic stirrer and centrifuged (Model: MAX 5200 RPM, Biogen Scientific, Meerut, UP, India) at 5000 rpm for 20 min. The supernatant was collected and subsequently filtered through Whatman No.1 filter paper to eliminate any insoluble components. The filtrate was acidified to 4.5 pH with 1N HCl. The protein precipitate recovered by centrifugation at 5000 rpm for 20 min and washed with water (pH 7.0). The obtained GCPC were dried using freeze dryer (Model: -80 DEG C, Lark, Padi, TN, India) and packed in an air-sealed container and stored at 4 °C until further use.

Estimation of crude protein

Total nitrogen content of GCPC was determined by Kjeldahl AOAC (2006) with some modifications. Briefly, 0.5 g of GCPC was digested with 15 ml nitrogen free concentrated sulphuric acid by using 2.4 g of mixture ($CuSO_4$: K_2SO_4 as 1:4) until the color was transparent greenish and diluted up to 100 ml. Digested sample of 10 ml was taken in distillation flask, 10 ml of 40 % NaOH was added and connected to the distillation unit. The ammonia released was absorbed in 25 ml of 4 % boric acid solution added with mixed indicator (equal volume of 0.1 % methylene blue solution and 0.2 % of methyl red in ethanol). The distillate was titrated against 0.02 N sulphuric acid till the end point of purple colour change was attained. A blank was instantaneously run similar to the sample using 0.5 g nitrogen free sucrose. The total nitrogen was calculated using the formula given below.

$$\text{Total nitrogen (\%)} = \frac{1.4 \times (\text{Sample reading} - \text{Blank reading}) \times N \times 100}{W}$$

Where, W = Sample weight (g), N = Normality of sulphuric acid. The protein content (%) was calculated by multiplying % Nitrogen content by 6.25.

Preparation of protein hydrolysate

The garden cress protein hydrolysates (GCPH) were prepared according to the method with some modifications (Gao et al. 2014; Nwachukwu and Aluko, 2019). The hydrolysis conditions were optimized to obtain hydrolysate with enhanced antioxidant activity from GCPC with 72.61 % protein. The papain to protein ratio was maintained at 1:25 (w/w). In brief, GCPC (5 % w/v) were suspended in distilled water and pH was maintained at 6.0, 6.5, and 7.0 by the addition of 0.1 M HCl or 0.1 NaOH. Thereafter, hydrolysis was performed at 50, 55, and 60 °C. Seven samples were drawn at 0, 30, 60, 90, 120, 150, and 180 min, respectively. At the end of the hydrolysis, the enzyme was inactivated by heating to 90 °C in a hot water bath for 10 min followed by cooling to room temperature, freeze dried and stored at -18 °C. The hydrolyses of protein concentrate was performed in triplicates.

Degree of hydrolysis (DH)

$$\text{DH (\%)} = \frac{100 \times \text{Concentration of soluble protein in TCA (10 \%) mg}}{\text{The total content of protein in mg}}$$

The DH was determined following the method (Hoyle and Merrit, 1994; Sonawane et al. 2017) with modifications. After completion of hydrolysis, added equal volume of 2.0 ml of 20 % Trichloro-acetic acid (TCA) with GCPC solution and hydrolysate, respectively. After incubation at room temperature for 30 min, the mixture was centrifuged at 7000 rpm for 10 min. For measurement of DH, 1 ml of supernatant was added to 5 ml of alkaline reagent followed by addition of 0.25 ml of 1.0 N Folin phenol reagent. The mixture was kept in dark for 10 to 15 min for color development and intensity of the blue

colour was measured at 660 nm using dual-beam spectrophotometer (Model: 2203, Systronics, Ahmedabad, GJ, India). The total soluble protein was obtained by tyrosine standard curve (0.05 - 0.5 mmol/L). The DH was calculated using the formula given below.

Antioxidant activity of GCPH

$$\text{DPPH (\%) Inhibition} = \frac{(\text{Blank reading} - \text{Sample reading}) \times 100}{\text{Blank reading}}$$

Antioxidant activity was measured by scavenging free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Brand-Williams et al. (1995). A stock solution of DPPH (100 mM) was prepared in methanol. The supernatant from TCA precipitated GCPC and GCPH were appropriately diluted with distilled water. Diluted samples (100 µL) were added to 3.9 ml of diluted DPPH solution (1:25 in methanol) and kept in dark condition for 30 min, absorbance was recorded at 517 nm. For blank determination, 100 µL methanol was taken as a replacement for the TCA supernatant and absorbance was measured instantaneously against methanol. The antioxidant activity of the hydrolysate was calculate using the formula given below.

Preparation of Kesar flavoured milk

Freeze dried GCPH prepared under optimal hydrolysis conditions (papain/protein 1:25 w/w) ratio, 6.5 pH, 120 min, 55 °C) was used for the preparation of Kesar flavoured milk. The method of De, 2008 with minor modifications was followed. Homogenized toned milk (3.0 % fat, 8.5 % SNF) was pre-heated to 45 °C. Added 8 % sugar followed by GCPH (1 % and 2 %), 2 % Kesar flavour and mixed. The mixture was filled in a sterilized glass bottle, cap sealed and heated to 88 °C / 7 min, cooled and stored at 5 ± 2 °C. Control Kesar flavoured milk was prepared without addition of the GCPH.

Antioxidant activity of Kesar flavoured milk

Antioxidant activity of Kesar flavoured milk was measured using DPPH scavenging of free radicals by the method of Brand Williams et al. (1995) with modifications. The DPPH solution (100 mM) was prepared and diluted to 1:25 with methanol. The appropriately diluted sample of 100 µL was added to 3.9 ml diluted DPPH solution. The content was mixed and incubated for 30 min under dark condition. The decrease in the absorbance was recorded at 517 nm. For blank determinations, methanol (100 µL) was taken as a replacement of sample and absorbance was measured instantaneously against methanol. The percent inhibition was calculated and Trolox equivalent antioxidant capacity (TEAC) was determined using a standard curve generated by plotting percent inhibition against Trolox concentration (100-1000 µM). The antioxidant activity was expressed in the terms of mM Trolox/L of the flavoured milk.

Sensory evaluation

The sensory analyses of GCPH incorporated Kesar flavoured milk was carried out using 9-point hedonic scale by panel of 21 semi trained judges. The panel members were asked to record scores for flavour, colour, mouth feel, sweetness, and overall acceptability.

Statistical analysis

The data shown in the tables and figures are the mean of at least triplicates. The standard deviation is shown by error bars. Analysis of variance (ANOVA) was used to test for significance, and the comparison of mean was done using the critical difference value at 5 % level of significance. The data was analyzed using Microsoft Excel (Microsoft Office 365).

Results and Discussion

Effect of hydrolysis time on DH and antioxidant activity

The effect of hydrolysis time (0-180 min) on DH and antioxidant activity at 1:25 (w/w) papain/protein ratio, 50°C temperature and 6.5 pH was investigated (Fig. 1a). It was observed that increase in DH and antioxidant activity between hydrolysis time of 0 min to 120 min was significant ($P<0.05$). As the hydrolysis time changed from 120 min to 180 min, no significant ($P>0.05$) changes were noticed in DH and antioxidant activity of the hydrolysate. The hydrolysate showed maximum antioxidant activity of 48.38 % at papain/protein ratio of 1:25 (w/w), 50 °C temperature, 6.5 pH and a hydrolysis time of 120 min. From the results, the hydrolysis time was fixed at 120 min to evaluate the effect of pH and temperature on DH and antioxidant activity. Mahdavi Yekta et al. (2019) observed an increase in antioxidant activity in the quinoa protein hydrolysate as the hydrolysis time increased. According to Ibrahim et al. (2020), the degree of hydrolysis affects the antioxidant activity in defatted chia protein. The optimal DH (14.33 %) and antioxidant activity (75.89 %) of *Chlorella pyrenoidosa* protein were reported by Wang and Zhang (2012).

Effect of pH on DH and antioxidant activity

The effect of pH (6.0, 6.5, 7.0) on the DH and antioxidant activity of hydrolysate at 1:25 (w/w) papain/protein ratio, 120 min, 50 °C temperature were investigated and illustrated in Fig. 1b. A significant difference in DH and antioxidant activity were observed between pH 6.0 to 7.0 ($P<0.05$). At 6.5 pH, found high antioxidant activity of 52.1 % with corresponding DH of 13.8 % as compared to other pH. Furthermore, the pH 6.0, DH and antioxidant activity were 8.8 % and 47.8 %, respectively. Interestingly, DH and antioxidant activity were decreased as the pH raised from 6.5 to 7.0. This could be due that the denaturation of the enzyme structure with loss of activity (Noman et al. 2018). These results were similar with the findings of Islam et al. (2021)

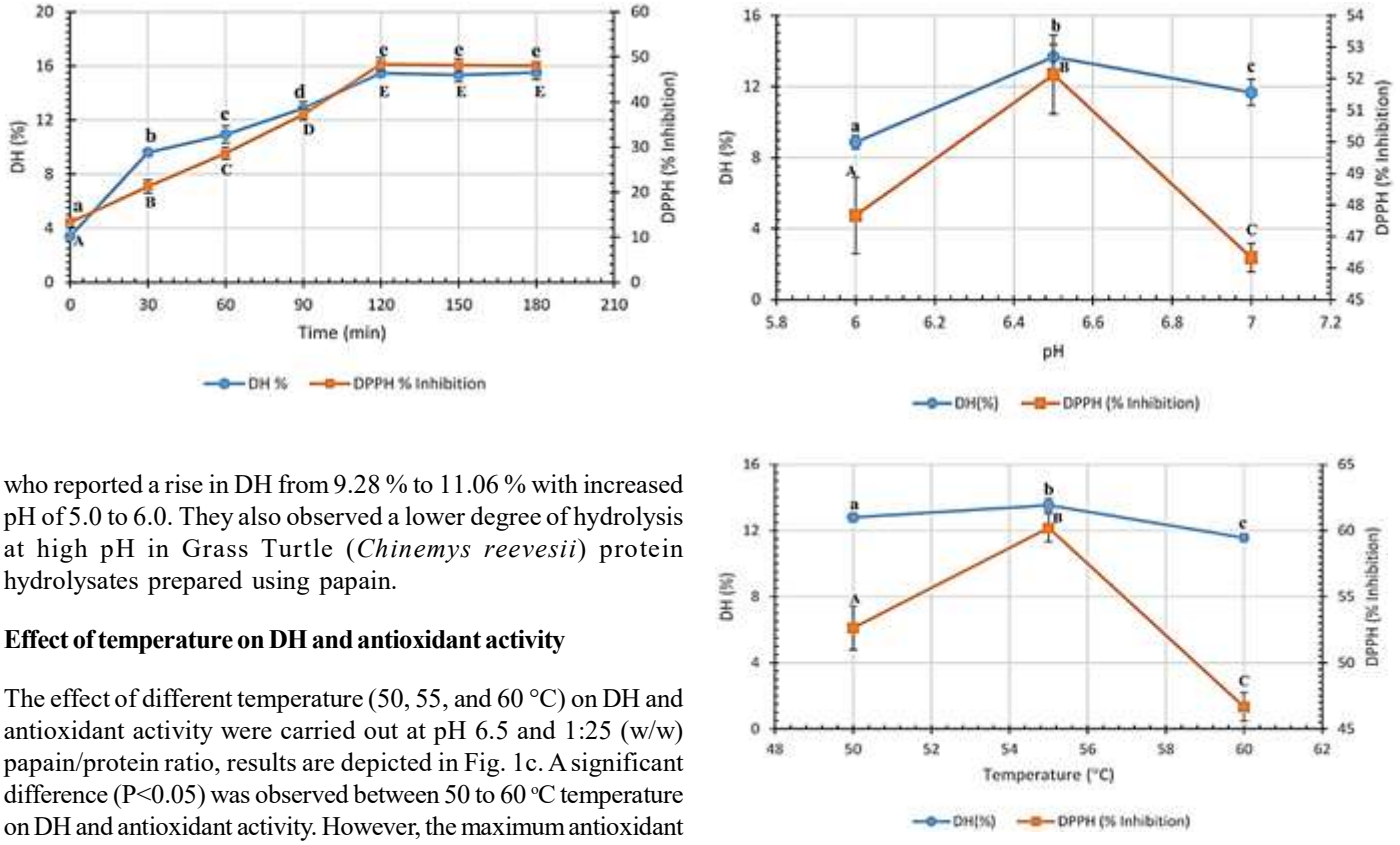

Fig. 1 (a) hydrolysis time, (b) pH, and (c) temperature on the degree of hydrolysis and antioxidant activity of supernatant of hydrolyzed garden cress protein concentrate with papain. Means ± standard deviation (n = 3) with different superscript letters indicate significant differences ($p < 0.05$).

who reported a rise in DH from 9.28 % to 11.06 % with increased pH of 5.0 to 6.0. They also observed a lower degree of hydrolysis at high pH in Grass Turtle (*Chinemys reevesii*) protein hydrolysates prepared using papain.

Effect of temperature on DH and antioxidant activity

The effect of different temperature (50, 55, and 60 °C) on DH and antioxidant activity were carried out at pH 6.5 and 1:25 (w/w) papain/protein ratio, results are depicted in Fig. 1c. A significant difference ($P<0.05$) was observed between 50 to 60 °C temperature on DH and antioxidant activity. However, the maximum antioxidant activity of 60.2 % was found at 55 °C with corresponding DH 13.6 % as compared with other temperatures. The DH and antioxidant activity of 12.8 % and 52.6 % was observed at 50 °C, further decrease in DH and antioxidant activity was observed at 60 °C. Based on these, temperature 55 °C was considered. Mahdavi Yekta et al. (2019) also reported maximum DH and antioxidant activity of quinoa protein hydrolysed with alcalase and pepsin at 55, 50 °C, respectively. Interestingly, they found no correlation between the degree of hydrolysis and antioxidant activity at different temperatures.

Antioxidant activity of GCPH

The GCPH was prepared with optimal hydrolysis conditions (1:25 papain/protein ratio, temperature 55 °C, pH 6.5, and hydrolysis time 120 min). A significant difference ($P<0.05$) in DH and antioxidant activity were observed between hydrolysate and unhydrolyzed GCPC (Table 1). This could be due to that, the cleavage of protein at the site of hydrophobic amino acids at optimal hydrolysis condition. The results obtained in this study are in consistent with the previous reports by Mulla and Ahmed (2019), who reported increased antioxidant activity of 10.69 ± 0.37 % and 64.91 ± 1.55 % with corresponding DH of 3.60 ± 0.30 % and 11.18 ± 0.62 % for unhydrolyzed GCPC and GCPH obtained using Alcalase enzyme. Jamdar et al. (2010) found that the antioxidant activity of peanut protein hydrolysates increased with the degree of hydrolysis. Similarly, Zhidong et al. (2013)

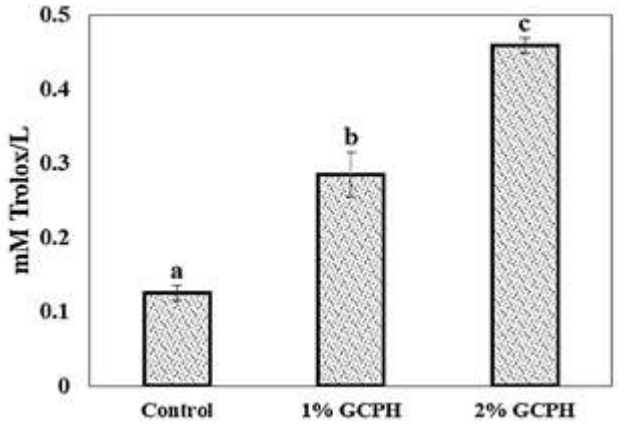

Fig. 2 Antioxidant activity of control, 1 %, and 2 % garden cress protein hydrolysate supplemented Kesar flavoured milk. Means ± standard deviation (n = 8) with different superscript letters indicate significant differences ($p < 0.05$).

Table 1 Degree of hydrolysis and antioxidant activity of garden cress protein and hydrolysate

Sample	Degree of hydrolysis (%)	Antioxidant activity (% Inhibition)
GCPC	2.96 ± 0.23^{a}	10.41 ± 0.52^{c}
GCPH	13.6 ± 1.10^{b}	60.03 ± 0.96^{d}

Values are means ± standard deviation (n = 5) with different superscript letters in the column indicate significant differences ($p<0.05$). GCPC - garden cress protein concentrate; GCPH - garden cress protein hydrolysate.

Table 2 The sensory analysis of garden cress protein hydrolysate supplemented Kesar flavoured milk evaluated using a 9-point hedonic scale (1-9)

Sensory attributes	Control	2 % GCPH (2 % flavour)	2 % GCPH (3 % flavour)
Colour	8.06 ± 0.13^{a}	7.31 ± 0.6^{a}	7.62 ± 0.15^{a}
Mouth feel	7.87 ± 0.11^{a}	6.62 ± 0.14^{b}	7.0 ± 0.09^{ab}
Sweetness	8.0 ± 0.6^{a}	6.66 ± 0.09^{b}	7.27 ± 0.18^{ab}
Flavour	7.56 ± 0.16^{a}	6.81 ± 0.22^{a}	7.35 ± 0.24^{a}
Overall acceptability	7.8 ± 0.23^{a}	6.82 ± 0.26^{a}	7.28 ± 0.12^{a}

Values are means ± standard deviation (n = 21) with different superscript letters in the row indicate significant differences ($p< 0.05$). GCPH - garden cress protein hydrolysate.

observed that whey protein hydrolysates exhibited higher antioxidant activity with increased hydrolysis.

Antioxidant property of Kesar flavoured milk

The antioxidant activity of the kesar flavored milk is shown in (Fig. 2). In order to optimize the addition of GCPH and based on antioxidant activity, the Kesar flavoured milk was prepared by using 1 % and 2 % GCPH with 2 % of Kesar flavour. The addition of GCPH concentration was significant ($P<0.05$) on the antioxidant activity in flavoured milk as compared to control. The antioxidant activities were 0.12 ± 0.01, 0.28 ± 0.03, 0.45 ± 0.01 mM Trolox/L for control, 1 % and 2 % GCPH supplemented flavoured milk, respectively. Mann et al. (2014) also found an increase in antioxidant activity up to 42.10 % and 21.70 %, after supplementation with 2 % of whey protein hydrolysate prepared using corolase and flavourzyme, respectively in strawberry flavoured milk. Similarly, Hajian et al. (2020) also observed an increase in antioxidant activity after addition of 4 % chymotrypsin camel milk hydrolysates in ice cream.

Sensory evaluation

The sensory scores obtained by hedonic scale for Kesar flavoured milk supplemented with different levels of GCPH are presented in Table 2. From the sensory evaluation, it was revealed that 2 % GCPH supplemented Kesar flavoured milk indicated slight bitter taste as compared to 1 % added GCPH. The control flavoured milk was highly acceptable due to less bitter taste. The lower flavour scores for 2 % GCPH added flavoured milk could be due to the generation of hydrophobic peptides at higher degree of hydrolysis, which might have imparted bitter taste to the flavoured milk. Based on the antioxidant activity and sensory evaluation, 2 % GCPH supplemented flavoured milk was selected and the level of flavour was increased from 2 % to 3 %.

A significant ($P<0.05$) difference was observed in colour, mouth feel, sweetness, flavour and over all acceptability. Neverthless, the sensory scores of 2 % GCPH (3 % flavour) was higher as compared to 2 % GCPH (2 % flavour) added flavoured milk, indicating the contribution of addition of higher level of Kesar flavour on sensory scores. Flavored milk supplemented with 2 % GCPH and 3 % Kesar flavor was moderately liked by the sensory panel and was considered optimal on the basis of both sensory and antioxidant activity. Mann et al. (2014) reported that addition 3 % strawberry flavour was sufficient to mask the bitterness in milk beverages incorporated with 2 % flavourzyme and corolase WPHs.

Conclusions

In this study, Kesar flavoured milk was developed with the addition of garden cress protein hydrolysates (GCPH) prepared under optimal hydrolysis conditions (1:25 papain/protein ratio, pH 6.5, and 55°C). The supplementation of 2% GCPH significantly enhanced the antioxidant properties of the Kesar flavoured milk. However, at lower levels of flavor addition, GCPH supplementation negatively impacted sensory scores, imparting a bitter taste to the flavored milk. The results suggest that the sensory quality can be improved by increasing the Kesar flavor concentration to 3% in GCPH-supplemented flavored milk. Therefore, GCPH can be recommended as an antioxidant ingredient to enhance the antioxidant properties of milk and milk products.

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RESEARCH ARTICLE

Bacteriocin production by lactic acid bacteria and their antioxidant property

Trupti K. Vyas[1](✉), Avantika R Patel[1] and K. G. Patel[2]

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Abstract: Lactic acid bacteria (LAB) are well known for their health benefits and hence they have wide application in food industries. These lactic acid bacteria produce bacteriocin, low molecular weight protein, which inhibit the growth of several closely related bacteria. In present study, out of 25 lactic acid bacteria 9 isolates produce higher lactic acid were evaluated for their ability to produce bacteriocin. Their growth pattern suggested that L10 showed highest growth compared to other. These LAB were examined for their antimicrobial activity against pathogens and their results revealed that all the isolates inhibit the growth of *Staphyloccus*, *Enterococcus, Serratia, Micrococcus*, however, unable to inhibit growth of *E. coli, Salmonella, Proteus,* and *Yersinia*. Isolates L1 to L4 shows inhibition of *Listeria monocytogens* while rest of bacteria were unable to show antimicrobial activity against it. Antioxidant activity measured using FRAP assay suggested that isolated L6 (0.236 mM/g) showed highest antioxidant followed L3 and L11 which showed similar antioxidant activity (0.207 mM/g). Bacteriocin was partially purified using ammonium precipitation. Quantification of partially purified bacteriocin revealed that highest production by isolate L3 (32.2 IU) followed by L6 (30.45 IU) and L1 (30.38 IU). Among all the isolates L3 showed higher antioxidant and higher bacteriocin production and hence it was identified as *Enterococcus faecium.* Thus, potential culture can be used for inhibition of pathogenic microbes by its antimicrobial bacteriocin production.

[1]Food Quality Testing Laboratory, N M College of Agriculture, Navsari Agricultural University, Navsari – 396450, Gujarat, India

[2]Department of Soil Science and Agricultural Chemistry, N M College of Agriculture, Navsari Agricultural University, Navsari – 396450, Gujarat, India

(✉) : Email: vyastrupti@hotmail.com

Keywords: Antimicrobial, Antioxidant, Bacteriocin, Fermentation, Lactic acid bacteria

Introduction

Many lactic acid bacteria are extensively used as starter cultures for fermentation processes by the food industry. They provide many health benefits like maintaining a healthy gastrointestinal system, antioxidant effect, anti-inflammatory effect, protection against pathogens, immunomodulatory effect, etc. (George et al. 2018; Saadat et al. 2019; Ayivi et al. 2020). Many LABs have the ability to produce antimicrobial substances like bacteriocin, which hampered the growth of several pathogenic bacteria in the gut and thus maintained a healthy environment in the gastrointestine. Due to their abundant health benefits, many LABs like *Lactococcus lactis, Lactiplantibacillus pentosus, Lactiplantibacillus plantarum, Levilactobacillus brevis,* and *Leuconostoc mesenteroides* are widely used for fermentation in the food industry (Vyas et al. 2017; Mokoena, 2017). They are also used in the preparation of yogurt, cheeses, sauerkraut, fermented milk, fermented meat, fermented cereals, etc. (Choi et al. 2013; Kim et al. 2016).

Bacteriocins are antimicrobial peptides produced by bacteria, notably lactic acid bacteria (LAB), and they impede the growth of closely related bacterial strains. These chemicals are important in microbial ecology because they help bacteria compete for resources and keep their habitats balanced. Bacteriocins differ in structure and mechanism; however, they are broadly classified into classes depending on their properties. Broadly, they are classified into four different classes, viz., lantibiotic, non-lantibiotic, large peptide, and lipid-containing. Lantibiotics comprise unusual amino acids, i.e., lanthionine or methyllanthionine, and nisin is a typical and well-studied example of this class. Non-lantibiotics are smaller, heat-stable peptides, and pediocin and lactocin belong to this class. Class three comprised a larger peptide, which is heat-labile, while class four comprised peptide and lipid. Bacteriocins typically disrupt the bacterial cell membrane, leading to cell lysis. They may bind to specific receptors or interfere with essential cellular processes.

They have important applications in food preservation, where they act as natural preservatives, and in health, where they may contribute to combating infections and promoting gut health. Their efficacy, combined with their generally recognized as safe (GRAS) status, makes them a viable option to chemical preservatives in a variety of industries. Bacteriocins represent a promising area of research with applications in food safety, healthcare, and agriculture. Continued exploration into their mechanisms, production, and applications will likely yield significant benefits in combating microbial resistance and ensuring food safety. Hence, the present study aims on bacteriocin production and its antimicrobial and antioxidant activity by isolated lactic acid bacteria.

Materials and Methods

Lactic acid bacterial culture and their temporal growth

Lactic acid bacteria were isolated from the fermented batter samples of idali, khaman and handavo (Gujarti fermented food) as described previously (Vyas et al. 2017). Out of 25 isolated bacteria, 9 potential lactic acid producing bacteria (culture designated as L1, L2, L3, L4, L5, L6, L8, L10 and L11) were used for bacteriocin production. These bacteria were morphologically characterized by Gram's reaction and found to be Gram positive. For growth pattern, cells were inoculated in De Man–Rogosa–Sharpe (MRS) broth and optical density was monitored upto 96 hrs at 600 nm. Experiment was conducted in triplicate and standard deviation was calculated and expressed as error bar in figure.

Antimicrobial activity

Antimicrobial activity was carried out on Mueller Hinton Agar (MHA) by well diffusion method. The test organisms viz. *Staphylococcus aureus* (ATCC 11632), *Enterococcus faecalis* (ATCC 14506), *Serratia marcescens* (ATCC 14756), *Micrococcus luteus* (ATCC 10240), *Listeria monocytogenes* (ATCC 13932), *E. coli* (ATCC 10536), *Yersinia enterocolitica* (ATCC 23715), *Proteus vulgaris* (ATCC 33420) and *Salmonella poona* (ATCC 4840) were procured from HiMedia, Laboratories Private Limited, Mumbai. All the isolates were grown in nutrient broth for 18 hrs. All these test cultures were spread on Mueller Hinton Agar. A well (7mm diameter) was prepared with sterilized cup borer and 100 µl of supernatant was place in well. Plates were incubated and zone of inhibition was measure after 24 – 48 hrs of incubation.

Antioxidant activity

Antioxidant activity was measured by FRAP assay as method described by Benzie and Strain et al. (1996). Briefly, 5 ml of sample was mixed with 1.5 ml of FRAP reagent and optical density was recorded at 593 nm. After 4 min of incubation optical density was again measured at 593 nm. Change in the optical density after 4 min incubation from initial reading was calculated from the standard curve prepared by $FeSO_4 \cdot 7H_2O$. Experiment was conducted in triplicate and standard deviation was calculated and expressed as error bar in figure.

Protein content

Protein content was measured by Folin and Lowry's method (Lowry et al. 1951). Protein concentration from the sample was extrapolated from the standard graph prepared using bovine serum albumin.

Partial purification of bacteriocin

Partial purification of bacteriocin was carried out by ammonium sulphate precipitation method described by Srinivasan and co-workers (2013). Briefly, isolates were grown in MRS broth for 7 days. Cells were removed by centrifugation at 10,000 rpm for 20 min and supernatant was collected. For precipitation of protein from supernatant, 35 % solid ammonium sulfate was added. The solution was centrifuged, and the precipitate was discarded because it had low bacteriocin activity and the supernatant was used for further precipitation by 75 % ammonium saturation. The precipitates were collected by centrifugation and dissolved in sodium phosphate buffer (pH 6.8). Sample was dialyzed using phosphate buffer (pH 6.8) for 24 hrs. This was used for further bacateriocin estimation and designated as partially purified bacteriocin.

Bacteriocin production

Partially purified bacteriocin was further precipitated with ammonium sulphate and steam sterilized at 120 ºC at 15 psi as method described by Balogu et al. (2017). Quantification was done spectrophotometrically at 450 nm and value was extrapolated from the nisin standard graph and expressed as IU/ml as nisin equivalent. Standard graph of Nisin was prepared as method describe by Papagianni et al. (2006) by adding 0.1g of nisin to solution (10 ml 0.02 N HCl and 0.75% NaCl). Experiment was conducted in triplicate and standard deviation was calculated and expressed as error bar in figure.

Identification of isolate

Potential isolate was identified by morphological and biochemical characteristics. Isolated bacteria were morphologically characterized by Gram's reaction. For identification by biochemical testing, outsourcing service taken and identified using VITEK.

Results and Discussion

Growth of lactic acid bacteria

For growth pattern of LAB, cells were grown in MRS media and it was monitored for 96 hrs. Data revealed that isolated L10 showed higher growth compared to rest of the LAB. Isolated L2,

L6 and L10 showed higher growth at 48 hrs, while rest of the isolates showed higher growth at 72 hrs except isolate L8 which shoed higher growth at 96 hrs of incubation (Figure 1).

Antimicrobial activity

All the lactic acid producing bacteria were tested for their ability inhibit growth of other bacteria by producing antimicrobial agent. Antimicrobial activity was measured by well diffusion assay. Isolates were tested for their antimicrobial activity against *E. coli, Yersinia, Proteus, Salmonella, Staphylococcus, Enterococcus, Serratia, Micrococcus* and *Listeria*. Tested organisms were individually streaked on Mueller Hinton Agar (MHA). All the lactic acid bacteria inhibit the tested bacteria except they were unable to inhibit growth of *E. coli*, *Yersinia*, *Proteus* and *Salmonella* (Table 1, Figure 2). L1, L2, L3 and L4 inhibit the growth of all the tested isolates. L5, L6, L8 and L10 inhibits the growth of all tested organisms except *Listeria* sp. whereas, L11 inhibits three tested organisms except *Staphylococcus* and *Listeria* sp. Among the all tested samples which showed inhibition of tested bacteria, *Entrococcus* showed highest inhibition followed by *Serratia* and *Micrococcus* (Figure 2).

Antioxidant activity

Antioxidant activity of sample was measured using Ferric Reducing Antioxidant Power (FRAP) assay. Highest antioxidant activity was observed in L6 culture (0.236 mM/g), followed by in

Table 1 : Antimicrobial activity (zone of inhibition mm) of the crude bacteriocin by lactic acid bacteria

Tested Culture LAB	*S. aureus*	*E. faecalis*	*S. marcescens*	*M. luteus*	*L. monocytogenes*	*E. coli*	*Y. enterocolitica*	*P. vulgaris*	*S. poona*
L1	13	13	13	12	14	-	-	-	-
L2	14	15	14	13	13	-	-	-	-
L3	13	16	14	11	13	-	-	-	-
L4	14	14	14	12	15	-	-	-	-
L5	13	13	14	13	-	-	-	-	-
L6	12	16	15	12	-	-	-	-	-
L8	13	14	14	13	-	-	-	-	-
L10	14	13	14	13	-	-	-	-	-
L11	-	12	12	12	-	-	-	-	-

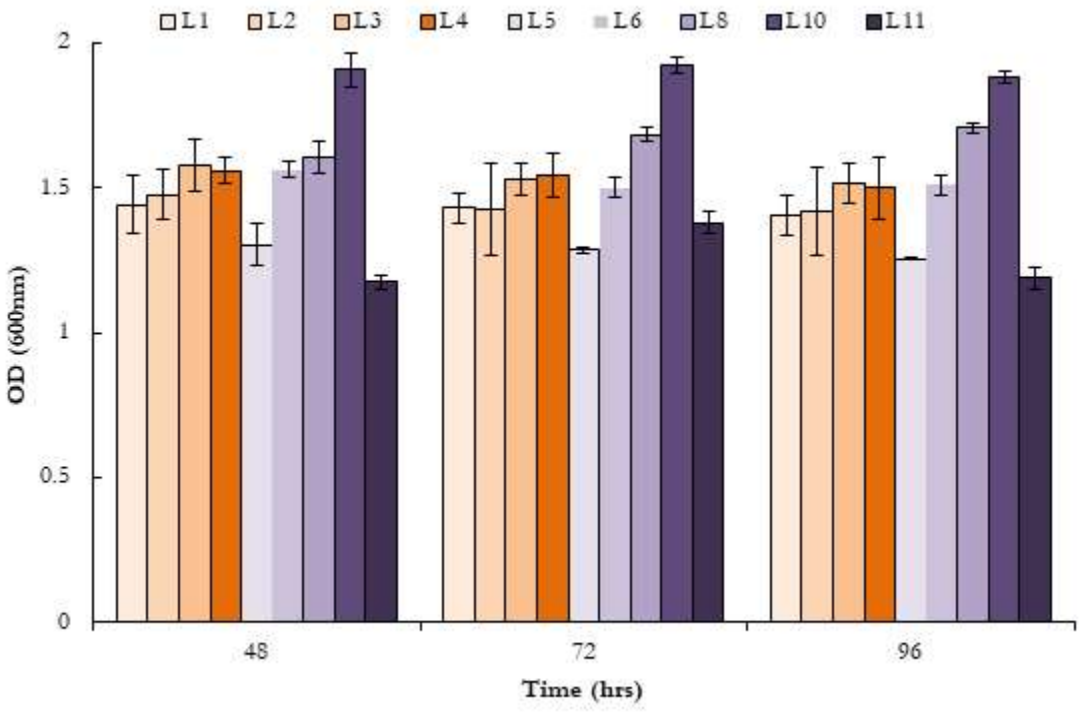

Fig. 1 : Growth of isolated LAB in MRS broth (*Error bar indicates ±Standard deviation

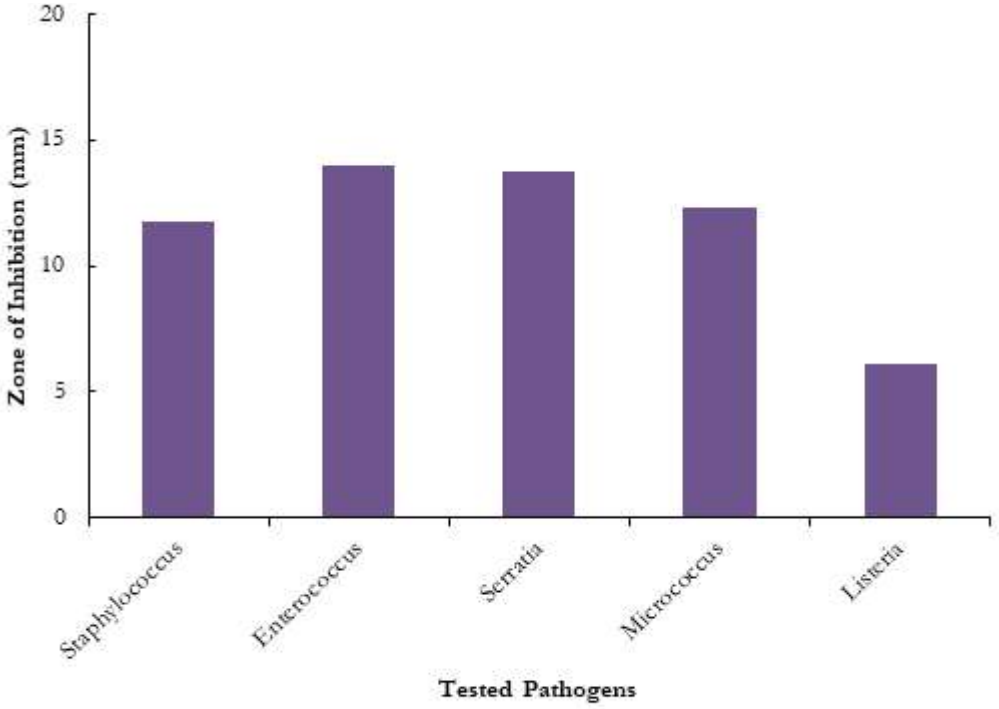

Fig. 2 : Zone of inhibition by lactic acid bacteria against pathogens

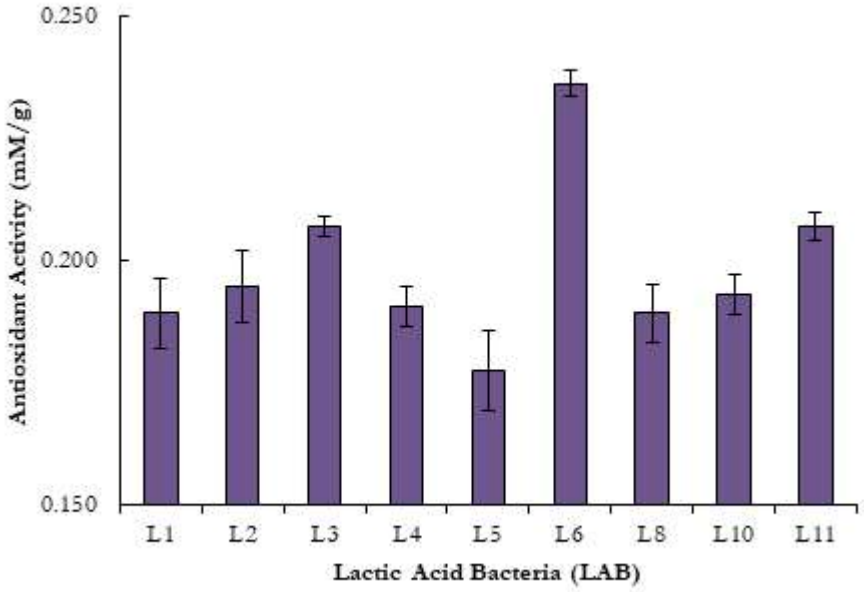

Fig. 3 : Antioxidant activity of crude bacteriocin by FRAP assay (*Error bar indicates ±Standard deviation)

L3 and L11 culture which showed same antioxidant activity (0.207 mM/g) (Figure 3).

Protein content

Lactic acid producing bacteria produced antimicrobial compound, a bacteriocin, which is protein in nature. Hence, protein content was measure by Folin and Lowry's method. L3 showed higher protein content (57.8 µg/ml) followed by L2 (48.2 µg/ml) and L11 (38 µg/ml) (Table 2).

Partial purification of bacteriocin

For extraction of bacteriocin produced by bacterial isolates, protein form the broth was precipitated with 60 - 80 % ammonium sulfate. Precipitated protein was collected and this partially purified protein was used for quantification of bacteriocin.

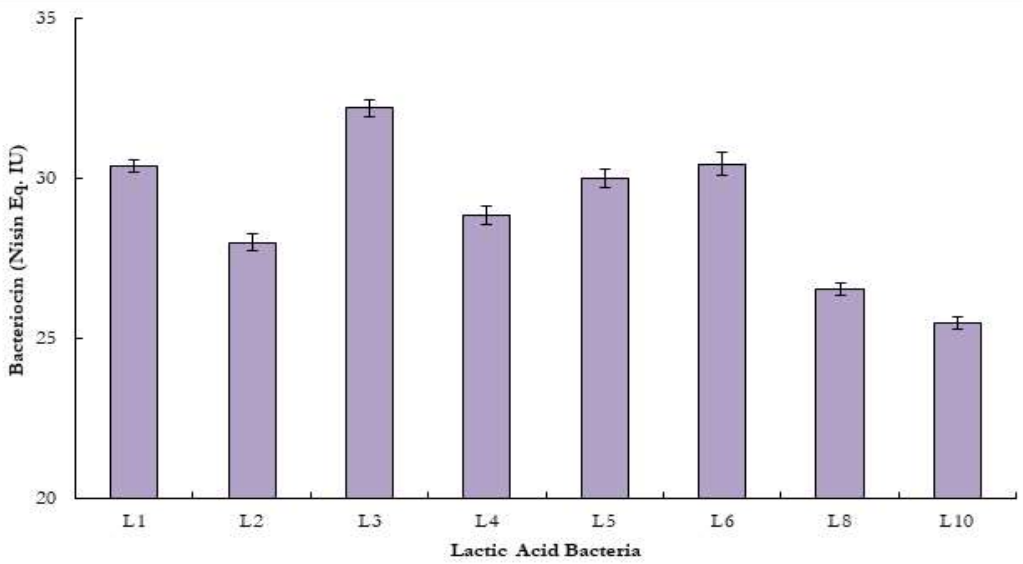

Fig. 4 : Bacteriocin (Nisin Equivalent) production by lactic acid bacteria (*Error bar indicates ±Standard deviation)

Table 2 : Protein content of the crude bacteriocin & partial purified bacteriocin

Bacterial isolates	Crude bacteriocin (µg/ml)	Partially Purified Bacteriocin (µg/ml)
L1	37.4	2.97
L2	48.2	3.19
L3	57.8	3.37
L4	20.3	3.41
L5	24.8	3.75
L6	20.9	3.14
L8	24.3	3.14
L10	17.7	2.17
L11	38.0	3.37

Bacteriocin production

Bacteriocin production was quantified as method described by Balogu et al. (2017) using nisin as standard. All the isolates were found positive Highest production of bacteriocin was observed in culture L3 (32.2 IU) followed by L6 (30.45 IU) and L1 (30.38 IU)(Figure 4).

Identification of isolates

L3 which showed higher protein content and higher nisin production compared to other lactic acid bacteria. Hence, it was morphologically and biochemically characterized. Isolate L3 was identified as *Enterococcus faecium* using VITEK (outsourcing service).

Bacteriocins are antimicrobial peptides produced by bacteria, particularly lactic acid bacteria (LAB), that inhibit the growth of closely related bacterial strains. These substances play a crucial role in microbial ecology by helping bacteria compete for resources and maintain a balance in their environments. Bacteriocins vary in structure and mechanism but are generally categorized into classes based on their characteristics. They have significant applications in food preservation, where they act as natural preservatives, and in health, where they may contribute to combating infections and promoting gut health. Their effectiveness, combined with a generally recognized as safe (GRAS) status, makes them an appealing alternative to chemical preservatives in various industries. The amount of bacteriocin produced by lactic acid bacteria (LAB) can vary widely based on several factors like temperature, pH, nutrient availability, and also on type of strain. Optimization of environmental conditions often enhances yields. *Lactococcus lactis* F44 strain genetically engineered by introducing 17 acid-tolerant genes and 6 lactic acid synthetic genes showed enhanced nisin titers from 2810 IU/ml to 3850, 3979, and 4377 IU/ml by overexpression of hdeAB, ldh, and murG, respectively (Zhang et al. 2016).

In laboratory settings, bacteriocin production is often quantified in terms of activity units (AU) per milliliter or as a concentration measured in micrograms per milliliter (µg/mL). In the present study, higher nisin equivalent production was reported in culture L3 (32.2 IU), followed by L6 (30.45 IU), and L1 (30.38 IU). In

Lactococcus lactis ssp. *lactis* 32 nisin production started in the second week and reached 97 µg/g after four weeks (Hassan et al. 2021). Shimizu and co-worker (1999) reported that *L. lactis,* when grown under anaerobic conditions, produces 7.4 mg/liter of nisin.

Out of the four lactic acid bacteria tested for their antioxidant activity of the DPPH assay, the highest antioxidant activity was reported in *L. brevis* (94.47%), followed by *L. gasseri* (91.29%) at 210 min, while the antioxidant activity of *L. rhamnosus* and *L. plantarum* was 83.41% and 77.53%, respectively, at 210 min (Vougiouklaki et al. 2023). Bacteriocin purified from *Lactococcus lactis* strain CH3 isolated from fermented dairy products showed radical scavenging potential with an EC_{50} value of 12.5 ìg/mL by DPPH assay (Krishnamoorthi et al. 2022). *Enterococcus faecium* GRD AA and *Paenibacillus polymyxa* isolated from toddy and milk showed 85% of DPPH and 85.5% of ABTS radical scavenging activity while 87.6% Fe^{2+} reduction potential (Krishna et al. 2021). Thus, present findings corroborate that *Enterococcus faecium* and other isolated lactic acid bacteria have potential antioxidant activity.

Antimicrobial activity of 28 isolated bacteria against *Escherichia coli*, *Pseudomonas fluorescens*, *L. innocua, Erwinia carotovora, Bacillus cereus*, and *Leuconostoc mesenteroides subsp. mesenteroides* using the agar diffusion bioassay and also against *Penicillium expansum*, *Botrytis cinerea,* and *Monilinia frucitcola* using the microdilution plate method revealed that isolated LABs strongly inhibit all microorganisms tested except *E. coli, Ent. faecium*, *Strep. thermophiles,* and *Lact. casei* (Yang et al. 2012). A similar study carried out by Thuy and co-worker (2024) reported that three strains of LAB *Weissella confusa* CYLB30, *Lactiplantibacillus plantarum* CYLB47, and *Limosilactobacillus fermentum* CYLB55 demonstrated a strong antibacterial effect against *Klebsiella pneumoniae*, *Salmonella enterica* serovar Choleraesuis, *Escherichia coli, Enterococcus faecium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.* Thus, the antimicrobial activity of bacteriocin is well documented and supports the present findings.

Conclusion

In present study, 9 lactic acid bacteria were examined for their bacteriocin production. All the isolates showed antimicrobial effect against *Staphylococcus, Enterococcus, Serratia*, *Micrococcus and Listeri*a, while non-effective against *E. coli, Yersinia, Proteus* and *Salmonella*. They were also possessing antioxidant and have ability to scavenge reactive oxygen species. Isolate L3 showed higher protein content and bacteriocin production hence it was further identified as *Enterococcus faecium*. Thus, *Enterococcus faecium* can be used for inhibition of several plant pathogens by secretion of antimicrobial molecule i.e., bacteriocin.

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RESEARCH ARTICLE

Effect of feeding total mixed ration supplemented with sodium bicarbonate and magnesium oxide on milk yield, milk composition and manure score in early lactating dairy cattle

Kanwarpal Singh Dhillon[1](✉) **and Ravi Prakash Pal**[2]

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Abstract: The study was conducted on 120 crossbred early lactating dairy cows to compare the effect of feeding Total Mixed Ration (TMR) supplemented with or without buffers i.e. sodium bicarbonate ($NaHCO_3$) and magnesium oxide (MgO) on milk yield, milk composition (milk fat percentage, solid not fat) and manure score. Animals were selected from 8 dairy farms in the Hoshiarpur district during the July-August period of 2021 and 2022, they were divided into 3 treatment groups (T1, T2 and T3) at each farm with 5 cows in each group, and 8 replications. During the 60 days trial, each cow in the treatment group T3 was fed a diet consisting of TMR along with a buffer prepared on farm using $NaHCO_3$ and MgO in the ratio of 3:1 and mixed in concentrate ration @ 1kg/ quintal. Each cow in group T2 was fed a diet that contained only TMR. In T1group, no TMR and buffers were fed to the cows, only routinely farmers practiced was followed. The TMR was offered *ad libitum* indoors in T2 and T3 treatment groups at 09:00 and 18:00 h. Milk yield, milk fat percentage, Solid Not Fat (SNF) and manure score were recorded on days 0 and 60 of the 60-day trial. SPSS software was used for statistical data analysis. The results showed that feeding TMR to early lactating dairy cows with or without the addition of dietary $NaHCO_3$ and MgO increased milk yield, milk fat percentage and SNF concentration significantly ($p>0.05$). However, there was no significant ($p>0.05$) difference in milk yield between the T2 and T3 treatment groups. Additionally, cow dung was observed using the consistency and digestion methods. Score 2 was noted in T1 treatment group, whereas Score 3 was noted in the T2 and T3 treatment groups under the consistency method. Using the digestion method, Score 2 was seen in the T1 treatment group, whereas Score 1 was seen in the T2 and T3 treatment groups. Implementing TMR in dairy cattle is efficient and effective, particularly in terms of milk production and milk composition, as well as improving manure score, which is an indicator of feed digestibility.

Keywords: Dairy cattle, TMR, Buffer, Milk yield, Milk composition, Manure score

[1]Krishi Vigyan Kendra, Amritsar, Punjab Agricultural University, Ludhiana, Punjab, India

(✉)E-mail: kanwardhillon55@pau.edu *Tel: +91-9915678787

[2]Dept. of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India.

Introduction

Dairy cattle have been producing more milk over the years, so in an effort to meet the nutritional demands of the cows, the energy density of the ration has also been rising. In order to meet the increased milk production of cross-bred dairy cows in India, feeding practices changed from roughage to a high concentrate diet in the dairy ration. Feeding high concentrate diets results in sub-acute ruminal acidosis (SARA), which lowers the pH of the rumen (Hossain 2020), modifies the rumen microflora (Hashemi and Tavakolinasab 2023), alters rumination (Muthusamy et al. 2021), and lowers the percentage of milk fat (Musa and Pandey 2017). It is generally advised to include buffers in dairy rations when depression of milk fat is an issue (Razzaghi et al. 2020). In most parts of the world, it is common practice to add sodium bicarbonate to the diets of high-yielding dairy cows as a rumen buffer (Neville et al. 2019). Sodium bicarbonate supplementation to the diet raises milk production and milk fat percentage (Musa and Pandey 2017) and stabilizes the pH of the rumen (Kumar et al. 2024). According to various reports (Leno et al. 2017; Tebbe et al. 2018), the efficiency of MgO sources in elevating the ruminal pH and fostering improvements in milk yield and milk fat varies. However, Bach et al. (2018) showed that a magnesium-based product is more effective at neutralizing rumen pH and preserving milk fat content compared to sodium bicarbonate when cows are given an extra 3 kg/d of barley in their diet. Studies have been done to supplement magnesium oxide alone (Neville et al. 2019; Razzaghi et al. 2020; Li et al. 2022), sodium bicarbonate (Musa and Pandey 2017; Musa and Choudhary 2018; Muthusamy et al. 2021), or both in an effort to manipulate the rumen for higher milk production (Bach et al. 2018; Agustinho et al. 2022). A beneficial

effect was demonstrated by adding either 1.5% $NaHCO_3$ alone or in combination with 0.8% MgO to the total ration (40% corn silage and 60% concentrate) during early lactation (Erdman et al. 1980).

Enhancing the performance of dairy cattle is the primary goal of preparing a TMR (Total Mixed Ration). The recent rise in cattle fed TMR has contributed to an increase in milk production worldwide (Schingoethe 2017). Feeding TMR reduces feed selection and sorting, provides adequate nutrition and increases feed utilization efficiency (Mendoza et al. 2016; Beigh et al. 2017; Premarathne and Samarasinghe 2020). As TMR feeding systems are frequently utilized during the non-grazing season, many dairy producers own the necessary equipment and have expertise using them. TMR can be prepared efficiently using various forage species, crop residues and industrial byproducts (Karunanayaka et al. 2022). Additionally, TMR feeding lowered labour costs and can be formulated as per individual animal requirements (Schingoethe 2017). Moreover, it enhanced feed intake, digestibility, and rumen microbial activity, all of which led to higher cow productivity (Karunanayaka et al. 2022). A better milk fatty acid profile can be achieved through the grazing component, and supplementing with TMR could help produce high yields of milk with enhanced protein and fat contents. According to Mohammad et al. (2017) and Cameron et al. (2018), the TMR feeding produced a higher milk yield than regular feeding. Compared to cattle fed TMR, grazing dairy cattle produced less milk (Karunanayaka et al. 2022).

Manure scoring (also called dung or fecal scoring) is a simple method of assessing the condition of a cow's digestive system, ranging from signs of acidosis to excess/insufficient protein in the diet to mycotoxins or disease. According to Musa and Choudhary (2018), this tool aids in assessing the degree of digestibility of cow feed, the appropriateness of water intake, and the nutrient balance of the ration, which includes protein, fiber, and carbohydrates. The ideal manure score fluctuates during lactation due to rumen function changes and dietary modifications (Petrovski et al. 2022). Cows should normally have a manure score of 2.0–2.5 in the first few days of lactation, 2.5–3.0 from 7 to 180 days in milk, and 3.0–3.5 in the last few days of lactation. The mean manure score of early-period dry cows should be 3.0-4.0, while the later dry period should be 2.5-3.5-4.0 (Petrovski et al. 2022). Adverse changes in manure scores may suggest nutritional imbalances in the ration, poor mixing, improper food sorting at the feeding area, or intolerable competition during feeding (Petrovski et al. 2022).

The dairy industry now routinely includes $NaHCO_3$ in diets, either with or without additional MgO. Complex mixtures of buffers, alkalis, and other substances known to influence milk composition or production are also commercially available. Moreover, only few studies available in literature that analyze the effects of TMR combined with $NaHCO_3$ and MgO on milk production performance (Arambel et al. 1988; Bach et al. 2018) and manure scoring in cow. Thus, the goal of this research was to assess how feeding TMR with or without $NaHCO_3$ and MgO supplements affected the performance of early lactation cows.

Materials and Methods

Eight villages were included in the study: Haveli, Motian, Mahilpur from the Mahilpur block; Chak Narial and Jhonjowal from the Garhshankar block; Raipur from the Hoshiarpur-I block; and Mehtiana from the Hoshiarpur-II block of the district Hoshiarpur. Data on milk yield, milk constituent (milk fat percentage, solid not fat) and manure score were collected twice from each animal during the period from July to August, 2021 and 2022. The study area is located in the following geographic coordinate systems: Latitude: 31.3630 ºE, Longitude: 76.0363 ºN; Latitude: 31.2175 ºE, Longitude: 76.1407 ºN; Latitude: 31.510 ºE, Longitude: 75.770 ºN; Latitude: 31.3708 ºN, Longitude: 75.8171 ºE of Mahilpur; Garhshankar; Hoshiarpur-I and Hoshiarpur-II block, respectively, with an average annual rainfall of 630 mm. During the summer, the average daily temperature was 39.3 ºC with a relative humidity of 62.3%. The majority of the dairy farms in the Hoshiarpur district used tie-up stall feeding.

Experimental design

This study uses a Completely Randomized Design (CRD) for its experiment. The study involved the selection of 120 crossbred dairy cows from 8 dairy farms (15 cows from each farm). At each farm, the selected cows were then divided into three treatment groups i.e., T1, T2, and T3 with 5 cows in each group, and 8 replications. All animals were in the early lactation stage and the mean milk production was 14.1±0.16 kg/day/animal in the month prior to the start of the experiment. All cows were kept under identical management conditions to checkmate error due to environment variations.

Experimental diet

During the 60 days trial, each cow in the treatment group T3 was fed a diet consisting of TMR along with buffer made on the farm using a 3:1 ratio of $NaHCO_3$ to MgO and mixed in concentrate ration @1kg/quintal. Each cow in the treatment group T2 was fed a diet that contained only TMR. The cows in the treatment group T1 were not given TMR and buffers; instead, the farmers merely followed their regular practices. The treatment groups T2 and T3 received the TMR indoors, *ad libitum*. All of the animals had access to plenty of fresh, clean water to drink during the trial. Prior to the start of the trials, the research team provided guidance to farmers and farm workers regarding the treatment protocol.

Based on NRC (2001) feeding guidelines, lactating cattle in treatment groups (T2 and T3) were fed a TMR that included roughage and concentrate mixture in a 50:50 ratio, respectively, to meet their nutritional needs (Table 1). On the other hand,

concentrate mixture, green fodder, and dry fodder were fed to the treatment group (T1) and the control groups (T1, T2, and T3) in accordance with routine farmer practices. The composition of used concentrate mixture presented in Table 2. Each day, the TMR was manually mixed separately for every treatment group, containing 63.82% Total Digestible Nutrients (TDN) and 12.41% Crude Protein (CP). The TMR was offered at 09:00 and 18:00 h.

Tests and procedures

With the exception of 10 days for standardization and acceptance of the test ration in accordance with the treatment combinations used on the experimental animals, the trial lasted 60 days. On day 0 (control group) and day 60 (treatment group) of the feeding trial, 200 milliliters of fresh milk were taken twice a day (morning and evening) from each selected animal. Lactoscan was used to analyze each sample for milk components, such as milk fat percentage and SNF. The research team regularly monitored and recorded the milk yield (L/day).

Manure scoring

Manure scoring was also noted during morning milking on days 0 and 60 of the experimental period, following a 10-day adaptation period. Based on a 5-category scale (Table 3), the consistency and digestion of the manure on all the floors was evaluated in

Table 1: Ingredients and nutritive value of TMR (% Dry Matter Basis) fed during the experimental period to the treatment groups

Ingredients	Quantity (%)	
	T2 group	T3 group
Maize Fodder	33.0	33.0
Wheat Straw	17.0	17.0
Ground Yellow Maize	17.0	17.0
Soybean Meal	5.00	5.00
Groundnut Cake	4.50	4.00
Deoiled Mustard Oil Cake	6.40	6.40
Wheat Bran	6.50	6.50
Deoiled Rice bran	5.00	4.50
Molasses	2.50	2.50
Buffer	-	1.00*
Bypass Fat	1.50	1.50
Yeast	0.05	0.05
Methionine	0.05	0.05
Mineral Mixture	1.00	1.00
Common Salt	0.50	0.50
Chemical composition (%)		
Crude Protein	12.68	12.41
Total Digestible Nutrient	64.46	63.82

*Buffer ($NaHCO_3$ and MgO in a 3:1 ratio)

Table 2: Composition of concentrate mixture

Ingredients	Quantity (%)
Ground Yellow Maize	34
Ground Nut Cake	9
Soybean Meal	10
De Oiled Mustard Oil Cake (Expeller Extracted)	12.8
Wheat Bran	13
Deoiled Rice Bran	10
Molasses	5.0
Bypass Fat	3.0
Yeast	0.10
Methionine	0.10
Mineral Mixture	2
Common Salt	1

accordance with Hauge et al. (2012), Musa and Choudhary (2018) and Petrovski et al. (2022).

Statistical analysis

The statistical analysis was performed with Statistical Package for Social Sciences (SPSS) version 20.0 statistical software for Windows (version 16.0; Microsoft). The numerical data of each parameter (milk yield, fat percentage and SNF) was tested for normality. The average of the two values for each parameter was used. Descriptive statistics were calculated for each of the parameters and data were presented as mean and standard error (S.E.) of mean. The following model was used to analyze data on milk yield, milk fat percentage, and SNF using the one-way analysis of variance (ANOVA) technique:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Where, Y_{ij} is each observation (milk yield, fat percentage and SNF); μ represents the total mean; T_i is the effect of i^{th} treatment (i = T1, T2 and T3) and $[_{ij}$ is the residual error.

Tukey's test was used to compare the treatments, and differences were considered as statistically significant at $p>0.05$.

Results and Discussion

Effect on milk yield

The milk yield values for control (day 0) and treatment (day 60) groups are presented in Table 4 as mean and standard error (SE). In T2 and T3 treatment groups, significant ($p>0.05$) increase was observed in milk yield at day 60 of the trial. In contrast to the T3 treatment group, the T2 treatment group exhibits a non-significantly ($p>0.05$) higher milk yield. In the T1 group, there was no significant ($p>0.05$) difference in milk yield at day 0 or day 60 of the experiment. Furthermore, a statistical comparison in Table 4 revealed no discernible difference between the control groups (T1, T2, and T3).

As per the findings of Mohammad et al. (2017) and Cameron et al. (2018), TMR feeding increases milk yield in dairy cows more than normal feeding does. These findings are consistent with the findings of the current study. TMR feeding to dairy cows, on the other hand, was found to have no effect on milk production by Morales-Almaráz et al. (2010). The present study's findings are corroborated by studies conducted by English et al. (1983) and Arambel et al. (1988) which found that adding buffers ($NaHCO_3$ and MgO) to TMR did not significantly alter milk yield in dairy

Table 3: Manure scoring chart

Score	Consistency	Digestion (feeling by hand)
1	This manure is very liquid with the consistency of pra soup. The manure may actually "arc" from the rump of the cow. Excess protein or starch, too much mineral, or lack of fibre , can lead to this score. Excess urea in the hind-gut can create an osmotic gradient drawing water into the manure. Cow with diarrhoea will be in this category.	Manure feels like a creamy emulsion and is homogeneous. There are no visible undigested food particles.
2	Manure appears runny and does not form a distinct pile. It will measure less than 2.5 cm in height and splatters when it hits the ground or concrete. Cows on lush pasture will commonly have this type of manure. Low fibre or a lack of functio nal fibre can also lead to this manure score.	Manure feels like a creamy emulsion and is homogeneous. A few undigested food particles are visible.
3	This is the optical score! The manure has a porridge -like appearance, will stack up 4 to 5cm, have several concentric rings, a small depression or dimple in the middle, make a plopping sound when it hits concrete floors, and it will stick to the toe of your shoe.	Manure doesn't feel homogeneous. Some undigested particles are visible. After squeezing in the han d, some undigested fibres will stick to your fingers.
4	The manure is thicker, will stick to your shoe, and stacks up over 5 cm. Dry cows and older heifers may have this type of manure (this may reflect feeding with low quality forages and/or a shortage of protein). Adding more grain or protein can lower this manure score.	Bigger undigested food particles are clearly visible. A ball of undigested food will remain after squeezing the dung in your hand.
5	This manure appears as firm faecal balls. Feeding a straw based diet or dehydration would contribute to this score. Cows with a digestive blockage may exhibit this score.	Bigger food particles are tangible in manure. Undigested components of the feed ration are clearly recognizable.

Table 4: Effect of treatments on milk parameters in crossbred cows (Mean±S.E.)

Parameter	Control Group (Day 0)			Treatment Group (Day 60)		
	T1 (n=40)	T2 (n=40)	T3 (n=40)	T1 (n=40)	T2 (n=40)	T3 (n=40)
Milk Yield (L/day)	14.2±0.15^{a}	14.1±0.16^{a}	14.0±0.16^{a}	14.1±0.15^{a}	15.7±0.11^{b*}	15.4±0.10^{b*}
Milk Fat (%)	3.79±0.02^{a}	3.81±0.02^{a}	3.81±0.02^{a}	3.80±0.02^{a}	4.24±0.02^{b*}	4.31±0.03^{b*}
Milk SNF (%)	7.84±0.02^{a}	7.86±0.02^{a}	7.84±0.02^{a}	7.81±0.02^{a}	8.02±0.02^{b*}	8.06±0.02^{b*}

Values with similar alphabets as superscripts in a row do not differ significantly ($p>0.05$), separately for control and treatment groups.
*Differ significantly ($p>0.05$) from the respective values of the control group.

cows during the early stages of lactation. According to Strokes et al. (1986), supplementing with buffers ($NaHCO_3$ and MgO) had no effect on the intake of DM, milk yield and milk composition. The present study's findings conflict with those of Erdman et al. (1980) and Teh et al. (1985), who found that feeding dairy cows TMR during the early stages of lactation resulted in a significant increase in milk production.

Effect on milk fat percentage and SNF

The milk fat percentage and SNF values for control (day 0) and treatment (day 60) groups are presented in Table 4 as mean and standard error (S.E.). In this study, on the 60th day of the experimental period, a significant ($p>0.05$) increase in the levels of milk components (milk fat percentage and SNF) was observed in the T2 and T3 treatment groups compared to 0 day of the trial. Nevertheless, compared to the T2 treatment group, the milk fat percentage and SNF of the T3 treatment group are non-significantly ($p>0.05$) higher. Between day 0 and day 60 of the experimental period, there was no discernible change in the milk fat percentage and SNF in the T1 group (Table 4). Additionally, statistical analysis showed that there was no significant ($p>0.05$) difference in milk fat percentage and SNF between the T1, T2, and T3 control groups in Table 4.

Similar results were obtained by Gaafar et al. (2010), who found that cows fed TMR had high levels of milk fat, lactose, solid non-fat, and total solids. However, Teshome et al. (2017) found that feeding pasture or TMR had no effect on the amount of fat, protein, lactose, ash and total solids in milk. Consistent with the current results, studies by English et al. (1983), Stokes et al. (1986), and Arambel et al. (1988) found that feeding dairy cows with similar diets and at a similar stage of lactation, supplementing with $NaHCO_3$ and MgO did not significantly affect the components of milk (milk fat percentage and SNF). In contrast to Erdman's et al. (1982) findings, which showed that adding dietary $NaHCO_3$, MgO, and $NaHCO_3$ plus MgO increased milk fat percentage significantly, the current study's findings show that the combination of $NaHCO_3$ and MgO was more effective than either $NaHCO_3$ or MgO alone. Low milk fat tests were corrected by adding $NaHCO_3$ and MgO, either separately or in combination, according to reports from Erdman et al. (1980).

Effect on manure score

1. Scoring of manure by consistency

The present experiment had observed Score 3 in the treatment groups T2 and T3. When the manure hits the floors, it makes a plopping sound and sticks to the toe of your shoe. It also has a porridge-like appearance, stacks up to 5 cm, and has several concentric rings and a small depression or dimple in the middle (Table 3). In line with the current study's findings, Musa and Choudhary (2018) found Score 3 when $NaHCO_3$ was supplemented through diet. In contrast, Score 2 was noted in the T1 treatment group and the T1, T2, and T3 control groups; in these cases, the manure looks runny and does not form a distinct pile (Table 3). When it hits the ground, it splatters and is less than 2.5 cm tall. Petrovski et al. (2022) state that cattle's overall health, rumen fermentation status, and digestive function can all be determined by observing their feces, which are especially readily acclimated to TMR feeding.

2. Scoring of manure by digestion (feeling by hand)

The current study found that the treatment groups T2 and T3 had Score 1 under this category of manure scoring. The manure has a uniform texture and feels like a creamy emulsion. No visible remnants of undigested food are present (Table 3). Prayitno et al. (2017) supported our findings by showing that digestibility increased and feed intake decreased with reduced chewing time with the TMR. In contrast, Score 2 was noted in the T1 treatment group and the T1, T2, and T3 control groups. Manure is homogenous and has a creamy emulsion-like texture. There are a few visible pieces of undigested food (Table 3). According to Karunanayaka et al. 2022, the feed intake, digestion, and milk production of early lactation cows were impacted by the particle size of the TMR.

Conclusion

The results suggest that TMR feeding, either alone or supplemented with a 1% buffer consisting of $NaHCO_3$ and MgO in a 3:1 ratio significantly ($p>0.05$) increased milk yield, milk fat percentage and SNF content, indicating better milk quality. Additionally, it improves manure score (Score 3 under consistency method and Score 1 under digestion method) which suggests better digestive health or nutrient utilization by dairy cattle. Therefore, feeding TMR without addition of buffers to early lactating dairy cattle producing up to 15 kg of milk could be a more economical option for improving productivity.

Author Contribution Statement

The authors confirm contribution to this paper as follows: Kanwarpal Singh Dhillon coordinated the study's conception and design, as well as the data collection, analysis, and interpretation of results. Ravi Prakash Pal participated in preparing draft manuscript. Both the authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The Director of Extension Education at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana approved this study at the meeting on the vetting of On-Farm Trials of Krishi Vigyan Kendra's (vide no. DEE/2767; dated: 19/03/2021). All pertinent institutional, national, and international guidelines for the care and use of animals were followed.

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Conflicts of Interest

The authors of this article declare that they have no conflicts of interest.

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RESEARCH ARTICLE

Assessing deoiled plants biomass of lemongrass and palmarosa as novel feed resources under *in vitro* conditions

Jannat Saini[1], Sanjay Kumar[2], Rajesh Verma[2], Rajendra Chandra Padalia[2], Debabrata Chanda[2] and Goutam Mondal[1](✉)

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Abstract: The feasibility of newer feed resources must be analyzed for sustainable livestock production addressing food security, climate change, and greenhouse gas emissions concerns. This study aimed to evaluate the nutritional value of lemongrass and palmarosa grass residues after their essential oils are extracted, which are otherwise discarded as wastes and contribute to environmental pollution. Chemical composition, *in vitro* gas production, *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD), and methane production parameters were analyzed for the graded levels of lemongrass and palmarosa grass residues replacing wheat straw up to 50% in the total mixed ration (TMR). *In vitro* total gas production, IVDMD, IVOMD, and methane production did not differ among the graded inclusion levels of lemongrass residues. In case of palmarosa grass residues, IVOMD was highest at the 20% inclusion level however no significant ($p > 0.05$) changes were observed. IVDMD and gas production did not differ among the treatments. Methane share was found to be lowest at the 50% inclusion level as compared to the other treatments but no discernible differences ($p>0.05$) were observed. These results demonstrate that after the extraction of essential oils, the leftover biomass residues or spent grass can potentially be incorporated in the cattle feed, alleviating dry fodder shortages to some extent and may help achieve sustainability in the livestock production system and reduce its environmental impacts.

1Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal, Haryana – 132001, India
2CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, UP – 226015, India

Goutam Mondal(✉)
Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal, Haryana – 132001, India
Email: gmondal1075@gmail.com

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Introduction

Agriculture and animal husbandry are intricately ingrained in human society's cultural, religious, and economic fabric as mixed farming and livestock rearing are fundamental components of rural life (Dagar et al. 2017). Agriculture remains the main source for 70 percent of rural households, with 82 percent of farmers being small-scale operators. On just 2.29% of the world's land area, India caters to approximately 10.7% of the world's livestock population (DADF 2019) and about 17.7% of the human population (UN 2022). These high human and animal populations fight tooth and nail for land resources for food and fodder production. For the last three decades, the area under fodder production has remained stagnant, with only 4-5% of the total cultivated area. Consequently, this caused a shortage of 11.24% green fodder, 23.4% dry fodder, and 28.9% concentrates (Ministry of Agriculture and Farmer Welfare, 2023). Despite these huge shortages, India is the largest milk producer in the world. Total milk production in the country during 2022-23 was 230.6 million tons (DADF 2022). The deoiled biomass was generated (whole plants after essential oil extraction) during the distillation of essential oil from aromatic biomass. Overall it was observed that the average essential oil content in aromatic plants is below 5 % (w/w) which generates a substantial amount of solid biomass of no commercial or environmental friendly use. It has been estimated that annually near about 200,000 tons of deoiled biomass are generated worldwide during extraction of essential oil from essential oil bearing plants (Saha and Basak, 2020).

If strategies like the inclusion of newer feed resources are adopted, there is still room to increase production. The feasibility of newer feed resources must be scrutinized for sustainable livestock production that addresses food security and environmental concerns. Medicinal and aromatic plants (MAPs), such as *Cymbopogon flexuous* (Lemongrass) and *Cymbopogon martinii* (Palmarosa grass), are well-known for their rich tapestry of bioactive compounds such as essential oils, flavonoids and phenolic compounds(Wifek et al. 2016; Hjouji et al. 2024). However, after the essential oils are extracted, the substantial

organic waste is largely discarded and thrown into the water bodies, increasing the biological oxygen demand (BOD). These left biomass residues are often overlooked despite their notable crude fiber and protein content, present considerable opportunities to be incorporated into cattle feed (Manurung et al. 2015). In this context, this research investigated the potential of lemongrass and palmarosa grass residues to be incorporated into cattle feed, alleviating dry fodder shortages to some extent and may help achieve sustainability in the livestock system and reduce its environmental impact. Therefore, this study aimed to evaluate the effects of incorporating these medicinal and aromatic plants residues in cattle diet on gas and methane production, dry matter, and organic matter degradability during *in vitro* fermentation.

Materials and methods

Location of study area and ethical procedure

The experiment was carried out in the Animal Nutrition Division of ICAR-National Dairy Research Institute, Karnal, Haryana – 132 001 with 29.704°N and 76.982°E at an altitude of 245 meters above the main sea level in the Indo-Gangetic alluvial plain. The experimental plan of work was approved by the Institutional Animal Ethics Committee (IAEC) of the Indian Council of Agriculture (ICAR) - National Dairy Research Institute constituted as per article 13 of the CPCSEA rules laid down by the government of India (IAEC Approval No. - 51-IAEC-24-24 dated 03/03/24).

Sample collection and chemical analysis of medicinal and aromatic plants residues

The samples of two important and commercially grown aromatic grass like lemongrass (*Cymbopogon flexuosus*) deoiled biomass (LGR) and palmarosa (*Cymbopogon martinii*) deoiled biomass (PGR) were collected after essential oils extraction through the steam distillation. Steam distillation is carried out by passing dry steam through the aromatic biomass, whereby the steam volatile compounds (secondary metabolites) are volatilized, condensed, and collected in oil receivers. Steam distillation is considered a traditional technology for essential oil extraction (Elyemni et al. 2019). The lemongrass and palmarosa grass residues were dried at 60 °C in a hot air oven for 72 h and ground via a 1mm mesh before the chemical analysis and in vitro assays. The chemical composition of the residues is shown in Table 1.

Chemical and mineral composition of the medicinal and aromatic planta residues

The medicinal and aromatic grass residues received were analyzed for their chemical composition (Goering 1970; Paez et al. 2016).

Table 1 Chemical composition of lemongrass and palmarosa grass residues in comparison to wheat straw

	Lemongrass residues		Palmarosa grass residues		Wheat straw
	L1	L2	P1	P2	
DM %	$98.65^{a} \pm 0.13$	$98.78^{a} \pm 0.14$	$98.78^{a} \pm 0.10$	$98.49^{a} \pm 0.09$	$90.48^{b} \pm 0.41$
CP %	3.64 ± 0.74	3.50 ± 0.18	4.15 ± 0.13	4.72 ± 0.18	2.92 ± 0.09
EE %	$1.88^{ab} \pm 0.04$	$2.06^{a} \pm 0.025$	$2.02^{a} \pm 0.04$	$1.70^{b} \pm 0.05$	$1.91^{ab} \pm 0.09$
NDF %	$79.77^{a} \pm 1.02$	$78.02^{a} \pm 0.74$	$77.30^{a} \pm 0.52$	$77.04^{a} \pm 1.18$	$71.56^{b} \pm 1.07$
ADF %	$39.90^{b} \pm 0.52$	$46.71^{a} \pm 1.12$	$48.66^{a} \pm 1.18$	$47.07^{a} \pm 1.31$	$42.75^{ab} \pm 1.42$
ADL %	$8.45^{bc} \pm 0.44$	$9.61^{b} \pm 0.24$	$12.13^{a} \pm 0.14$	$11.30^{a} \pm 0.41$	$7.49^{c} \pm 0.30$
HC %	$39.86^{a} \pm 0.49$	$31.31^{b} \pm 0.37$	$27.20^{d} \pm 0.66$	$29.97^{bc} \pm 0.33$	$28.81^{cd} \pm 0.48$
Cellulose%	38.31 ± 0.55	37.64 ± 1.32	38.53 ± 1.11	39.92 ± 1.03	38.50 ± 0.62
TA %	3.10 ± 0.43	5.73 ± 1.28	6.75 ± 0.54	6.14 ± 1.19	8.96 ± 0.16

DM = Dry matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; HC = Hemicellulose; TA = Total ash. Different superscripts (a. b, c and d) in a row indicates difference at 5% level of significance.

Table 2 Mineral concentrations in the medicinal and aromatic grass residues

Minerals	Lemongrass Residues	Palmarosa Grass Residues
Ca%	0.80 ± 0.07	0.58 ± 0.09
Mg%	0.35 ± 0.04	0.35 ± 0.03
Cu (mg/kg DM)	7.04 ± 0.35	6.88 ± 0.52
Mn (mg/kg DM)	45.38 ± 2.36	69.05 ± 4.06
Zn (mg/kg DM)	44.48 ± 2.93	37.78 ± 5.88
Na%	Below level of quantification	Below level of quantification
K%	0.66 ± 0.07	1.01 ± 0.04

Each value is the mean of three replicate determinations ± standard error mean.

The mineral profile of the medicinal and aromatic grass residues was analyzed by Atomic Absorption Spectroscopy (Hitachi Model z-5000 with Zeeman correction). The calibration of AAS was done with the working standards prepared from commercially available mineral standard solutions (1000 µg/mL, Merck, Germany). Specific hollow cathode lamps were used for the determination of the minerals, air as oxidant, and acetylene gas as fuel. The mineral composition of the medicinal and aromatic grass residues is presented in Table 2.

Nutritional composition of the experimental treatments

Eleven TMRs were evaluated: (CON) concentrate + maize green + wheat straw, (35:35:30% DM), and the LGR and PGR were included at graded levels replacing wheat straw by up to 50% in the treatment groups during the *in vitro* studies. The nutritional composition of the experimental treatments is presented in Table 3.

Chemical composition of ingredients used for making total mixed ration (TMR)

The total mixed ration was prepared to have forage and concentrate in a 65:35 ratio, with green maize as green fodder, wheat straw as dry fodder, and concentrate mixture. The nutritional composition of various feed ingredients used to formulate TMR for in vitro experiments is shown in Table 4.

In vitro studies

Two/ three sets of *in vitro* trials were conducted to study the rumen fermentation pattern in the substrate having concentrate, green maize, and wheat straw at 35: 35: 30. Lemongrass and palmarosa grass residues were added at inclusion levels of 0, 10, 20, 30, 40 and 50% replacing wheat straw. The trials were conducted in triplicates to estimate parameters viz., total gas production, IVDMD, IVOMD, microbial biomass production, and partitioning factor. These trials were conducted along with respective blanks in triplicates. The substrates used were 200 mg of air-equilibrated samples of TMRs. The incubations were carried out in 100 mL calibrated glass syringes as described by Menke and Steingass (Menke, and Steingass 1988). The substrate was weighed on a plastic boat with a removable stem and was placed into the bottom of the glass syringe without sticking to the sides of the syringe. The piston was lubricated with petroleum jelly and pushed inside the glass syringe. The syringes were incubated in the water bath at a temperature of 39 ± 0.5ºC for 24 hours.

In vitro gas and methane production parameters

In vitro gas production (mL/200 mg substrate) was measured by subtracting the final and initial piston readings during the 24 hours. The piston level was recorded (initial reading) and the syringes were placed in the water bath pre-adjusted at 39 ± 0.5ºC. The syringes were shaken every 30 minutes for the first 2 h from the start of the incubation and thereafter every 2 h up to 6 h of incubation. The total incubation period was 24 hours and the piston level was again recorded (final reading). After 24 h incubation, a suitable aliquot of gas was withdrawn from the tip of the incubation syringe using a gas-tight syringe and analyzed for its methane concentration with the help of a Gas chromatograph (Nucon 5700, India) fitted with a stainless-steel column packed with Porapak-N and Flame Ionization Detector (FID).

In vitro dry matter and organic matter degradability

After incubation of 24 h, fermentation was arrested by chilling at 4ºC, followed by the collection of suitable aliquot of gas for CH_4 estimation; the syringe contents were then transferred to centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 10 minutes and the pellets were used to estimate *in vitro* degradability of dry matter and organic matter. *In vitro* dry matter digestibility (IVDMD) and true organic matter degradability (TOMD) were calculated from the disappearances of dry matter and organic matter. Partitioning factor (PF) and microbial biomass production (MBP) were calculated based on truly degraded organic matter (TDOM) as described by Blummel et al. (1999) and Blummel et al. (2005) respectively.

Statistical Analysis

All the data obtained were subjected to a completely randomized design, and the significance of the differences between the means was determined using Tukey's multiple-range test. The study consisted of eleven treatments with three replications. Differences at $p < 0.05$ were considered statistically significant. All analyses were performed using SAS software, using the following linear statistical model:

$$Yij = \mu + \tau i + \varepsilon ij$$

Where:

Yij= dependent variable (%); μ= average mean; τi= additives effect of treatment i; εij = experiment error.

Results and Discussion

Chemical composition of medicinal and aromatic plants residues

The proximate composition of lemongrass and palmarosa plants residues is presented in Table 1. Dry matter was higher in both the LGR and PGR samples (L1, L2, P1, and P2) as compared to the wheat straw. Neutral detergent fiber and acid detergent lignin were higher in the medicinal and aromatic plants residues than wheat straw ($p > 0.05$). Acid detergent fiber and hemicellulose were found to be variable among the two different samples . However, no discernible differences were between these residues and wheat straw. ($p > 0.05$).

Crude protein and ether extract contents were similar in these medicinal and aromatic plants residues in comparison to wheat straw ($p > 0.05$).

In vitro gas and methane production

No significant differences were observed for total gas production (mL/200 mg DM) among the six graded levels (0%, 10%, 20%, 30%, 40%, 50%) of lemongrass residues replacing wheat straw in the TMR (Table 5). This can be attributed to the proximate composition of lemongrass residues being similar to wheat straw. The total gas production observed among the graded levels varied from 93 to 102 (mL/200mg DM) in different levels of inclusion.

The CH_4 percentage was 13.69 ± 0.36 in the control group of TMR 13.75 ± 0.15 at a 10% inclusion level, followed (13.91 ± 0.34 at 20%). At the 30% inclusion of LGR, a reduction in CH_4% was noted (12.83± 0.84), and minimal fluctuations for 40 and 50% inclusion levels were there, suggesting variable relationship between the inclusion of lemongrass residues and methane percentage of the total gas produced. However, no differences were noted among the treatment groups ($p > 0.05$).

During the *in vitro* studies for the inclusion of PGR, at 0% (CON) inclusion of palmarosa grass residues, the total gas production was varied from 80.39 to 93.06 % mL/ 200 mg at 10% inclusion level, however, with increased inclusion level, the gas production decreased (P>0.05). While some variability was observed, no significant variations were noted ($p > 0.05$) for total gas production during the *in vitro* assay.

In the control group (CON), methane production was 13.69 ± 0.36. It slightly decreased to 12.96 ± 0.32 at the (PGR10) 10% inclusion level, followed by a further decrease in methane production at the (PGR20) 20% inclusion level (11.99 ± 0.26). Increased methane percentage was observed in the further increasing inclusion levels of palmarosa grass residues. At the (PGR50) 50% inclusion level of palmarosa grass residues replacing wheat straw, the methane production decreased to 11.14±0.10%. However, no significant differences were found ($p > 0.05$) among the treatment groups.

In vitro dry matter and organic matter degradability

Table 3: TMR composition of the experimental groups during *in vitro* studies

Treatment	Green maize (g/kg DM)	Concentrate mix (g/kg DM)	Wheat straw (g/kg DM)	Lemongrass residues (g/kg DM)	Palmarosa grass residues (g/kg DM)
CON	350	350	300	-	-
LGR10	350	350	270	30	-
LGR20	350	350	240	60	-
LGR30	350	350	210	90	-
LGR40	350	350	180	120	-
LGR50	350	350	150	150	-
PGR10	350	350	270	-	30
PGR20	350	350	240	-	60
PGR30	350	350	210	-	90
PGR40	350	350	180	-	120
PGR50	350	350	150	-	150

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; **LGR10** – same as Control with 10% of wheat straw replaced by lemongrass residues; **LGR20** - same as Control with 20% of wheat straw replaced by lemongrass residues; **LGR30** - same as Control with 30% of wheat straw replaced by lemongrass residues; **LGR40** - same as Control with 40% of wheat straw replaced by lemongrass residues; **LGR50** - same as Control with 50% of wheat straw replaced by lemongrass residues; **PGR10** – same as Control with 10% of wheat straw replaced by palmarosa grass residues; **PGR20** - same as Control with 20% of wheat straw replaced by palmarosa grass residues; **PGR30** - same as Control with 30% of wheat straw replaced by palmarosa grass residues; **PGR40** - same as Control with 40% of wheat straw replaced by palmarosa grass residues; **PGR50** - same as Control with 50% of wheat straw replaced by palmarosa grass residues.

Table 4: Chemical composition of ingredients used for making total mixed ration (TMR)

Ingredients	CP%	EE%	NDF%	ADF%	TA%
Maize green	7.15 ± 0.26	4.95 ± 0.08	62.09 ± 1.08	36.22 ± 1.33	7.99 ± 0.11
Wheat straw	2.92 ± 0.09	1.91 ± 0.09	71.56 ± 1.07	42.75 ± 1.42	11.31 ± 0.30
Concentrate mix	18.7 ± 0.53	2.95 ± 0.24	31.42 ± 1.23	15.15 ± 0.72	8.96 ± 0.16

Each value is the mean of three replicate determinations ± standard error mean

(CP- crude protein, EE- ether extract, NDF- neutral detergent fiber, ADF- acid detergent fiber, TA- total ash)

There were no differences among treatments in IVDMD values for the inclusion of both the medicinal and aromatic plants residues replacing wheat straw up to 50% (Table7). The values for IVOMD also did not show any discernible differences with the graded inclusion levels of both LGR and PGR ($p < 0.05$) (Tables 7 and 8).

Partitioning factor values ranged from 4.33 (CON) to 5.48 (LGR50) and from 4.33 to 5.54 (CON) to 5.54 ± 0.56 (PGR50). While some variability existed, no significant variations were found among the treatment groups, indicating no potential changes in microbial activity and fermentation efficiency. Likewise, no differences were found in microbial biomass

Table 5 *In vitro* total gas parameters and methane production of experimental treatments (Lemongrass residues)

Attributes	CON	LGR10	LGR20	LGR30	LGR40	LGR50	P-value
Gas (mL/200mg DM)	93.06 ±7.47	101.12 ±11.80	102.12±11.59	100.54 ±8.64	96.58 ±10.12	82.48 ±4.37	0.661
Methane%	13.69 ±0.36	13.75 ± 0.15	13.91 ± 0.34	12.83 ± 0.84	12.80 ± 0.17	12.84 ±0.2	0.232
Methane mL	2.56 ±0.26	2.80 ± 0.33	2.89 ± 0.39	2.67 ± 0.39	2.52 ± 0.29	2.14 ±0.13	0.617

F:C = 65:35

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; LGR10 – same as Control with 10% of wheat straw replaced by lemongrass residues; LGR20 - same as Control with 20% of wheat straw replaced by lemongrass residues; LGR30 - same as Control with 30% of wheat straw replaced by lemongrass residues; LGR40

Table 6.*In vitro* total gas parameters and methane production of experimental treatments (Palmarosa grass residues)

Attributes	CON	PGR10	PGR20	PGR30	PGR40	PGR50	P-value
Gas (mL/200mg DM)	93.06 ± 7.47	89.45 ± 4.74	87.58 ± 6.07	68.59 ± 10.26	92.63 ± 3.60	80.39 ± 3.88	0.150
Methane%	13.69 ± 0.36	12.96 ± 0.32	11.99 ± 0.26	11.94 ± 0.07	12.37 ± 0.88	11.14 ± 0.10	0.065
Methane mL	2.56 ± 0.26	2.20 ± 0.05	2.11 ± 0.17	1.67 ± 0.26	2.29 ± 0.10	1.81 ± 0.94	0.054

F:C = 65:34

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; **PGR10** – same as Control with 10% of wheat straw replaced by palmarosa grass residues; **PGR20** - same as Control with 20% of wheat straw replaced by palmarosa grass residues; **PGR30** - same as Control with 30% of wheat straw replaced by palmarosa grass residues; **PGR40** - same as Control with 40% of wheat straw replaced by palmarosa grass residues; **PGR50** - same as Control with 50% of wheat straw replaced by palmarosa grass residues.

Table 7 *In vitro* dry matter and organic matter degradability, and microbial biomass production of experimental treatments (Lemongrass residues)

Attributes	CON	LGR10	LGR20	LGR30	LGR40	LGR50	P-value
IVDMD	44.55 ± 0.39	45.97 ± 2.44	44.65 ± 4.82	45.11 ± 0.58	51.42 ± 0.81	48.54 ± 2.21	0.336
IVOMD	44.29 ± 0.42	46.80 ± 2.84	46.46 ± 5.23	45.40 ± 0.79	49.65 ± 0.31	49.86 ± 3.26	0.667
Partitioning factor	4.33 ± 0.34	3.65 ± 0.11	4.33 ± 1.05	4.13 ± 0.41	4.73 ± 0.52	5.48 ± 0.56	0.389
Microbial biomass production	38.92 ± 3.19	39.92 ± 8.97	39.19 ± 14.65	38.53 ± 5.32	47.73 ± 4.74	53.99 ± 6.76	0.696

F:C = 65:34

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; **LGR10** – same as Control with 10% of wheat straw replaced by lemongrass residues; **LGR20** - same as Control with 20% of wheat straw replaced by lemongrass residues; **LGR30** - same as Control with 30% of wheat straw replaced by lemongrass residues; **LGR40** - same as Control with 40% of wheat straw replaced by lemongrass residues; **LGR50** - same as Control with 50% of wheat straw replaced by lemongrass residues.

Table 8 *In vitro* dry matter and organic matter degradability, and microbial biomass production of experimental treatments (Palmarosa grass residues)

Attributes	CON	PGR10	PGR20	PGR30	PGR40	PGR50	P-value
IVDMD	44.55 ± 0.39	48.90 ± 1.37	51.59 ± 0.90	45.09 ± 2.70	44.19 ± 0.56	44.21 ± 2.01	0.060
IVOMD	44.29 ± 0.42	51.25 ± 1.80	54.15 ± 0.99	50.43 ± 2.94	50.17 ± 0.07	49.09 ± 2.70	0.053
Partitioning factor	4.33 ± 0.34	5.15 ± 0.01	5.60 ± 0.35	7.07 ± 1.54	4.88 ± 0.13	5.54 ± 0.56	0.238
Microbial biomass production	38.92 ± 3.19	53.22 ± 1.3	59.46 ± 2.80	61.87 ± 9.65	49.92 ± 0.79	53.73 ± 6.34	0.108

F:C = 65:34

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; **PGR10** – same as Control with 10% of wheat straw replaced by palmarosa grass residues; **PGR20** - same as Control with 20% of wheat straw replaced by palmarosa grass residues; **PGR30** - same as Control with 30% of wheat straw replaced by palmarosa grass residues; **PGR40** - same as Control with 40% of wheat straw replaced by palmarosa grass residues; **PGR50** - same as Control with 50% of wheat straw replaced by palmarosa grass residues.

production among the treatments due to the inclusion of both the lemongrass and palmarosa grass residues ($p > 0.05$).

In vitro gas and methane production

Low *in vitro* gas production parameters observed in the LGR40 and LGR50 groups could be partly explained by the higher acid detergent lignin on ruminal fermentation. The higher indigestible lignin contents could have resulted in more unavailable carbohydrates causing reduced fermentative efficiency (Chesson 1988). However, the inclusion of lemongrass residues was not as much; the effect *on in vitro* gas production did not present any discernible difference. Similarly, when palmarosa grass residues replaced wheat straw, in vitro gas production did not vary with the graded inclusion levels. The findings of the current study are in agreement with those of Fidriyanto et al. (2021) who reported similar gas production when the lemongrass residues replaced 50% of the paddy straw.

The essential oils in the medicinal and aromatic plants are responsible for the reduction in methane emissions but after these essential oils are extracted via steam distillation method, the left biomass residues have inadequate ether content, which did not present any methane reductions in the current investigation. These results align with the findings of Manurung et al. (2015) and Fidriyanto et al. (2021) who found no significant differences on lemongrass waste substitution ($p>0.05$).

In Vitro degradability parameters of dry matter and organic matter

In vitro DMD presented no differences on the inclusion of these medicinal and aromatic plants (MAPs) residues replacing wheat straw at graded levels up to 50%. This may be attributed to the similar chemical composition of these MAPs residues and wheat straw and comparatively their lesser incorporation in the total mixed ration. Similar findings were reported for *in vitro* OMD on replacing wheat straw with lemongrass and palmarosa grass residues. These results comply with those of Fidriyanto et al. (2021) investigation findings.

The partitioning factor was found to be similar among all treatment groups of the lemongrass and palmarosa grass residues, indicating no potential changes in microbial activity and fermentative efficiency. The microbial biomass production also presented no discernible differences suggesting no potential changes in microbial growth and activity during the *in vitro* assays.

Conclusions

The results revealed that the inclusion of lemongrass and palmarosa grass residues in the replacement of wheat straw as a forage source did not alter the degradability parameters in terms of dry and organic matter, *in vitro* gas, and methane production. The partitioning factor and microbial biomass production were similar in the treatment groups. The results obtained in this experiment indicate that both these unconventional forage sources can be incorporated into ruminant diets. However, further research and *in vivo* trials are required to validate the effectiveness of the inclusion.

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RESEARCH ARTICLE

An Exploratory study on Existing Bovine Breeding and Management Practices in Jharkhand State

Amandeep Ranjan(✉)[1], Gopal Sankhla[1], Sujeet Kumar Jha[2], Sanjit Maiti[1], Mukesh Bhakat[3] and Pawan Singh[4]

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Abstract: Breeding is an important consideration in the economics of dairy farming. When regular breeding and calving is absent and not done at the appropriate time, cattle rearing will not be profitable. The contemporary study was conducted in three districts of Jharkhand to know the existing bovine breeding and management practices. The data was collected from 204 respondents (180 farmers and 24 service professionals) with the help of pre structured interview schedule. It was found in the study that the majority of the respondents from all the three districts i.e., 80 percent from Ranchi, 63.33 percent from Saraikela-Kharsawan and 58.33 percent from Ramgarh agreed that they serve their animals through artificial insemination. Overall, 92.22 percent of the respondents identified heat in their cattle through bellowing and in buffalo 73.68 percent of the respondents identified heat by observing frequent urination. Animals were checked twice by 67.78 percent of the respondents and 66.46 percent of the respondents inseminate their animals in between 12 hours to 16 hours after the onset of heat. Missing heat was considered as a sign of pregnancy by 92.22 percent of farmers. Only 11.11 percent of the respondents diagnosed pregnancy through rectal palpation with the help of professionals. Animals were served after six months of calving by 57.59 percent of respondents. It was found from the study that the milk productivity of animals was low for most of the respondents (48.89%). The low productivity of the animals might be due to dearth of technical knowledge about breeding, feeding, management and health care practices. Thus, it is recommended to improve the knowledge of the respondents about scientific practices of dairy farming via launching need based, suitable and appropriate extension programmes like animal fairs, field days, on campus and off campus training programmes, and animal health programmes through various agencies.

Keywords: Artificial Insemination, Breeding, Heat detection, Feeding, Drying off

Introduction

Dairying is an efficient instrument to develop rural societies, to generate employment persistent income and it provides assurance against various odds (Prasad, 2011). The dairy sector forms the largest component of animal agriculture. To hold the productive resources like land, labour and capital in dairy farming, the contemporary level of productivity of livestock is too low which should be increased to attract further investments. Yet the underprivileged farmer neither have other skills nor have financial assistance to endeavor into any other enterprise, it is important for the dairy farmer to implement scientific practices at their level which would lead to increased productivity at optimum costs. Breeding is an important consideration in the economics of dairy farming. When regular breeding and calving is absent and not done at the appropriate time, cattle rearing will not be profitable. Getting healthy calf every year is the usual goal of every dairy farmer. This is possible only by increasing the reproductive efficiency of the animals. The performance of cattle is the combined result of various factors like breeding and feeding management. Health of animals have vital role in harnessing the expected production potential. Most of the tribal farmers were found following practices of identifying animals in heat (86.56%), pregnancy diagnosis within one to six months (90.50%). For calving, respondents prefer winter months. Yadav *et al*. (2009) observed that a significantly higher percentage (90.00%) of respondents resorted to natural service and only 2.50 percent adopted artificial insemination. Eqbal *et al*. (2013) reported that majority (65.00%) of the tribal dairy respondents had indigenous cattle, 18.33 percent of dairy farmers had cross bred cattle, 10.83 percent of dairy farmers had cross buffalo, 5.84 percent of dairy farmers had both indigenous cattle and buffalo, most (55.83%) of

[1]Division of Agricultural Extension, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India
[2] ICAR- Head Quarter, New Delhi-110012, India
[3]Division of Livestock Production and Management, ICAR-National Dairy Research Institute, Karnal, Haryana-132001, India
[4]Division of Livestock Production and Management, ICAR-National Dairy Research Institute, Karnal, Haryana-132001, India

(✉) Email:amandeepranjan4@gmail.com

the tribal respondents identified heat in animal by bellowing. For pregnancy diagnosis, 57.50 percent of the tribal respondents diagnosed pregnancy by increased belly size. Selective breeding of cattle and buffalo to increase milk production has been going on for a longtime in our country and has made commendable progress in certain areas. Majority of the cattle and buffalo are still not included in the breed improvement programmes and that is why they are low milk producers. To have knowledge about the existing breeding and management practices followed by the farmers in tribal belt is of great importance as it may help in filling the gap between existing practices followed and the recommended scientific practices. Jharkhand state was purposively selected for the study as the cattle population in the state increased from 8.7 million to 11.2 million, which is highest percentage growth (28.16%) in the country as equated with previous Livestock Census (2012) but the productivity was not at par. The total adult female bovine population of Jharkhand is 38,93,000. Out of 34,58,000 cattle population, more than 91 percent of cattle is indigenous due to which milk production is very less as compared to other progressive states like Punjab and Haryana. As a result of low milk production in the state, the per capita availability of milk is also very low in the state i.e.,177 (grams/ day) as compared to other states like Punjab and Haryana with 1181 & 1087 grams per day (NDDB,2020) respectively. As productivity of animals depends upon the combined result of various factors like breeding and feeding management, therefore this study was undertaken to document the existing breeding and management practices being followed by the farmers of the locale.

Materials and methods

Jharkhand state was purposively selected for the study. Based on the coverage of artificial insemination three districts viz. Ranchi, Saraikela-Kharsawan and Ramgarh were purposively selected. From each purposively selected districts, two blocks were selected randomly and from each block, two villages were selected randomly for study. Fifteen farmers and two artificial insemination service professionals were randomly selected from each village for data collection. Therefore, the total number of respondents selected for the study was 204 (180 farmer respondents and 24 Service Professionals). The criteria for selection of the respondents were that each dairy farmer should be rearing at least one milch animal either cow or buffalo and at least once have tried artificial insemination. For knowing about the existing status of breeding and management practices, data was collected from the respondents by personal interview using a well-structured interview schedule. It was developed in consultation with experts and referring relevant literatures and previous works. The data so collected were converted into meaningful findings using appropriate statistical tools like percentage and cumulative square root frequency.

Results and Discussion

Type of breeding

In table 1, it was found that the majority of the respondents from all the three districts i.e., 80 percent from Ranchi, 63.33 percent from Saraikela-Kharsawan and 58.33 percent from Ramgarh agreed that they serve their animals through artificial insemination. Only 8.33 percent of the respondents from Ranchi, 13.33 percent from Saraikela-Kharsawan and 15 percent from Ramgarh bred their cattle and buffalo naturally. Some of the respondents (11.67% from Ranchi, 23.34% from Saraikela-Kharsawan and 26.67% from Ramgarh) agreed that depending on the situation they either bred their animals through artificial insemination or go for natural breeding. The possible reasons for natural breeding might be the unavailability of inseminators on time at the onset of estrous or inability to detect estrous on time. The pooled value showed that 67.22 percent of the respondents bred their animals through artificial insemination followed by 20.56 percent of the respondents who opted either natural or artificial insemination for breeding. Only 12.22 percent of the respondents still breed their animals naturally. Mainly three service providers were working in the area covering the entire area and the inseminations done by lay inseminators were not counted in the published data of Government which shows different reality at field level. The visible benefits of artificial insemination from fellow farmers who are using the artificial insemination services were might be the possible reasons behind the high adoption of artificial insemination.

Type of animal in which Artificial Insemination used

As depicted in table 2, it was observed that overall, 156 (67.98%) out of 178 non-descript cattle were bred through artificial insemination. Out of 54 crossbred, 48 crossbred (88.89%) were served with the help of artificial insemination. Overall, only 15.79 percent of artificial insemination were done in buffaloes. AI is

Table 1: Distribution of respondents according to the type of breeding of their animals

Type of breeding	Ranchi	Saraikela-Kharsawan	Ramgarh	Pooled
	(n=60) Percentage	(n=60) Percentage	(n=60) Percentage	(n=180) Percentage
Natural Breeding	08.33	13.33	15.00	12.22
Either natural or A.I	11.67	23.34	26.67	20.56
Artificial insemination	80.00	63.33	58.33	67.22

more difficult in buffalo compared with cattle due to variable estrous cycles, reduced estrous behaviour, and reproductive seasonality (Neglia *et al.,2020).* Silent heat and inability to detect heat in buffalo might be the reasons for poor percentage of respondents doing artificial insemination in buffaloes.

Identification of heat in cattle

It was observed that overall, 92.22 percent of the respondents (table 3) identified heat in their cattle through bellowing, 58.89 percent of the respondents observed heat through mucus discharge from vagina. Other symptoms like mounting on other animals (42.22%), frequent urination (21.11%), swollen vulva (26.11%), and restlessness (27.22) were also used to identify heat in cattle by the respondents.

Identification of heat in buffalo

The table 4 depicts that overall 73.68 percent of the respondents identified heat in buffalo by observing frequent urination followed by restlessness. Other symptoms like string of mucus (42.11%), bellowing (44.74%), and swollen vulva (52.63%). Doka method (changes in teat morphology) was also observed by 44.74 percent of the respondents as a method of identification of heat in buffalo.

Time of heat detection

Efficient and timely detection of heat by the dairy farmers is must for those who serve their animals through artificial insemination. It was found in the study that majority (in Ranchi 71.67%, in Saraikela-Kharsawan 68.33% and in Ramgarh 67.78%) of the respondents checked their animal twice for heat detection. Pooled value in table 5 shows that 67.78 percent of the respondents checked twice whereas 21.66 percent of the respondents checked

Table 2 Distribution of respondents according to the type of animal in which A.I is done

Animals	Ranchi (n=55)			Saraikela-Kharsawan (n=52)			Ramgarh (n=51)			Pooled (n=158)		
	n	n*	%	n	n*	%	n	n*	%	n	n*	%
Non-Descript cattle	60	48	80.00	59	38	64.41	59	35	59.32	178	156	67.98
Cross Bred	33	30	90.91	11	10	90.91	10	08	80.00	54	48	88.89
Buffalo	17	03	17.65	12	01	08.33	09	02	22.22	38	06	15.79

n* = animal bred through artificial insemination

Table 3 Distribution of respondents according to the symptoms for identification of heat in cattle (multiple responses)

Symptoms	Ranchi (n=60) Percentage	Saraikela-Kharsawan (n=60) Percentage	Ramgarh (n=60) Percentage	Pooled (n=180) Percentage
Bellowing	95.00	90.00	91.67	92.22
Mucus discharge from vagina	46.67	55.00	75.00	58.89
Mounting on other animal	36.67	36.67	53.33	42.22
Frequent urination	21.67	25.00	16.67	21.11
Swollen vulva	30.00	26.67	21.67	26.11
Restlessness	26.67	28.33	26.67	27.22

Table 4 Distribution of respondents according to the symptoms for identification of heat in buffalo (multiple responses)

Symptoms	Ranchi (n=17) Percentage	Saraikela-Kharsawan (n=12) Percentage	Ramgarh (n=9) Percentage	Pooled (n=38) Percentage
String of mucus hanging from vulva	17.65	66.67	55.55	42.11
Frequent urination	58.82	83.33	88.88	73.68
Bellowing	29.41	50.00	55.55	44.74
Doka phenomenon	35.29	58.33	55.55	44.74
Swollen vulva	52.94	50.00	55.55	52.63
Restlessness	47.06	58.33	77.77	57.89

only in morning and only 10.56 percent of the respondents checked their animals in heat in evening.

Time of insemination

The general recommendation has been to breed bovines in the middle to the end of standing heat for optimum fertility. Because the period of estrus may vary from 6 to 24 hours, however, it is difficult to determine when the midpoint is reached. The general guideline for determining insemination time originated in a study by Trimberger (1948), in the form of the AM-PM rule i.e., if cows are first observed in heat in the morning (AM) they should be bred that afternoon (PM); if they are first seen in heat in the late afternoon (PM), they should be bred the next morning (AM). As mentioned in table 6, it was found from the study that, majority of the farmers from all the three districts i.e., Ranchi 81.82 percent of the respondents, Saraikela-Kharsawan 65.38 percent and from Ramgarh 50.98 percent of the respondents were inseminating their animals in between 12-16 hours after the onset of estrous as the chances of conception is more during that period. Only few of the respondents (1.82% in Ranchi, 5.77% in Saraikela-

Table 5: Time of heat detection by the respondents

Time	Ranchi (n=60) Percentage	Saraikela-Kharsawan (n=60) Percentage	Ramgarh (n=60) Percentage	Pooled (n=180) Percentage
Twice (Morning & Evening)	71.67	68.33	66.67	67.78
Morning	18.33	20.00	23.33	21.66
Evening	10.00	11.67	10.00	10.56

Table 6: Time of insemination after the onset of oestrus sign

Time	Ranchi (n=55) Percentage	Saraikela-Kharsawan (n=52) Percentage	Ramgarh (n=51) Percentage	Pooled (n=158) Percentage
Between 12-16 hrs	81.82	65.38	50.98	66.46
Within 12 hrs	16.36	28.85	39.22	27.84
As soon as heat is detected in animal	01.82	05.77	09.80	05.70

Table 7: Distribution of respondents according to the method of pregnancy diagnosis (multiple responses)

Symptoms	Ranchi (n=60) Percentage	Saraikela-Kharsawan (n=60) Percentage	Ramgarh (n=60) Percentage	Pooled (n=180) Percentage
Missing heat	96.67	90.00	90.00	92.22
Swelling of udder	31.67	53.33	55.00	46.67
Increased abdomen size	55.00	40.00	46.67	47.22
Rectal palpation	11.67	08.37	13.33	11.11

Table 8: Distribution of respondents according to the care before and after parturition (multiple responses)

Practice	Ranchi (n=60) Percentage	Saraikela-Kharsawan (n=60) Percentage	Ramgarh (n=60) Percentage	Pooled (n=180) Percentage
Feeding an extra amount of concentrate	73.33	48.33	38.33	53.33
Not Feeding an extra amount of concentrate	26.67	51.67	61.67	46.67
Drying off of pregnant animals	58.33	55.00	36.67	50.00
Not Drying off of pregnant animals	41.67	45.00	63.33	50.00
Giving lukewarm water	56.67	63.33	55.00	58.33
Feeding Kadha	60.00	70.00	60.00	63.33
Feeding Ajwain	13.33	15.00	15.00	14.44
Timely first milking	65.00	55.00	51.67	57.22

Table 9: Distribution of respondents according to the time of next service after calving

Time	Ranchi	Saraikela-Kharsawan	Ramgarh	Pooled
	(n=55) Percentage	(n=52) Percentage	(n=51) Percentage	(n=158) Percentage
After 2-3 months	23.64	28.85	09.80	20.89
After 6 months	56.36	53.85	62.75	57.59
After cessation of milk	20.00	17.30	27.45	21.52

Kharsawan and 9.80% in Ramgarh) inseminate their animals as soon as the heat detected in the animal. The pooled value shows that overall 66.46 percent of the respondents inseminate in between 12 hours to 16 hours after the onset of heat. This agrees fairly well with the AM-PM rule, which would result in insemination at the optimum time suggested by the Trimberger study of approximately 13 to 18 hours prior to ovulation.

Pregnancy diagnosis

It was found in the study that majority of the respondents (88.33% from Ranchi, 91.63% from Saraikela-Kharsawan and 86.67% from Ramgarh) performed pregnancy diagnosis by self. Overall, 88.89 percent of the respondents performed pregnancy diagnosis by self, followed by 11.11 percent of the respondents who consulted professionals for pregnancy diagnosis (Table 7). Lack of availability of service during pregnancy, fear of injury during rectal palpation might be the reason for self-diagnosis of pregnancy by farmer. It was found from the study that missing heat was the sign of pregnancy by 92.22 percent of the respondents. The others signs were increased abdomen size (47.22%), and swelling of udder (46.67%). Only 11.11 percent of the respondents diagnosed pregnancy through rectal palpation with the help of professionals.

Health care practices of Dairy animals

Concentrate feeding

Animals during the last trimester of their pregnancy should be given extra care. They should not be taken away for browsing in the field to avoid exhaustion. Pregnant animals should be provided adequate and suitable amount of ration for proper foetal development. It was found in the study that 73.33 percent of the respondents from Ranchi, 48.33 percent from Saraikela-Kharsawan and 38.33 percent of the respondents from Ramgarh feed extra amount of concentrate during the last three months of the pregnancy (Table 8). Overall, 53.33 percent of the respondents feed extra concentrate to their animals during the last trimester of pregnancy followed by 46.67 percent of the respondents who did not feed their pregnant animals with extra concentrate.

Drying off of pregnant animals

Dry period characterizes the stretch of optimum time in which rest is given to pregnant animals. It is crucial for the success of unborn calf, upcoming lactation and further reproduction performances of the animal. It cab be observed from the table 8 that majority of the respondents from Ranchi (58.33%) and Saraikela-Kharsawan (55%) and 36.67% of the respondents from Ramgarh dried off their pregnant animal. Overall, half of the respondents dried off their animals whereas rest did not dry off their animals. Overall 44.44 percent of the respondents followed incomplete milking to dry off their animals followed by increasing milking interval (33.34%) and by reducing concentrate feeding (22.22%).

Care after parturition

Calving is a natural course of action and generally do not require any human assistance. However, close observation is necessary to avoid any complications. After parturition, 63.33 percent of the respondents were feeding Kadha to their animals. Lukewarm water was provided by 58.33 percent of the farmers and 14.44 percent of the respondents feeded their animals Ajwain to provide relieve from pain. Timely first milking was done by 57.22 percent of the respondents (table 8).

Next service after calving

It was observed from table 9 that majority of the respondents (57.59%) (56.36% from Ranchi, 53.85% from Saraikela-Kharsawan, 62.75% from Ramgarh) served their animals after six months of calving followed by 21.52 percent of the respondents who served their animal after cessation of milk and 20.89 percent of the respondents served their animals after 2-3 months of calving.

Conclusions

Bovine breeding and management practices play a pivotal role in shaping food production, animal welfare, and economic outcomes. These practices aim to enhance desirable traits in cattle populations, such as milk yield, disease resistance, and reproductive efficiency. Through selective breeding, genetic traits can be improved over generations, leading to more productive and resilient cattle. Improved genetics and management practices can lead to higher yields, lower production costs, and increased profitability for farmers and the livestock industry. The study highlighted that majority of farmers were using artificial insemination as a method of breeding due to its perceived benefit. Advances in technology, such as genomic selection and data

analytics, have revolutionized bovine breeding. These tools allow for more precise selection of desirable traits and faster genetic progress. Implementing sustainable breeding and management practices can reduce the environmental impact of cattle farming, such as lowering greenhouse gas emissions and minimizing resource use. Ethical management practices, including providing proper nutrition, housing, and medical care, improve the well-being of cattle & align with public expectations for humane treatment and one health approach. It was found in the study that majority of the farmers feed extra concentrate to their animals during the last trimester of pregnancy. Kadha and Ajwain were fed to animals as a care after parturition. The dry period is the vital stage of milch animal's lactation cycle. For optimal animal health and superlative performance in the next lactation, they should have an opportunity to rest and regenerate mammary tissue between lactations. The present study was conducted in only three districts of Jharkhand. The study may be carried out in other districts so that its scope and content would be widened. Also, a comparative study may be conducted between progressive and non-progressive districts of Jharkhand with other progressive states like Haryana and Punjab to know the importance of bovine breeding and management practices. Meeting international breeding and management standards can facilitate the access to global markets for meat and dairy products, benefiting export-oriented economies. The continued research in bovine genetics, breeding technologies, and management practices drives innovation, leading to ongoing improvements in cattle production which can ensure the long-term viability of the livestock industry, safeguarding its contributions to food security and rural economies.

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SHORT COMMUNICATION

Effect of blends of Sorghum (Great millet) and whey protein concentrate on the quality characteristics of *lassi*

Aishwarya R (✉) **and Arunkumar H**

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Abstract: An attempt has been made to address the iron and fibre deficiency in dairy products (*lassi*) by utilizing various levels of germinated sorghum flour and whey protein concentrate (WPC-80). Sorghum is one of the significant members among millets and WPC is one of major by-product of dairy industry. In this study, sorghum flour is utilized at 2, 3 or 4 % to optimize the product. The blended product at the 3 % (T_2) awarded the sensory scores of 7.6 for colour and appearance, 8.0 for body and texture, 7.63 for flavour and 8.01 for an overall acceptability score, which was higher than that of the control group which has scored 7.55, 7.85, 7.51 and 7.50, respectively. Thus, in comparison to the control group (0.00 mg of iron and fibre), T_2 had 0.06 mg of fibre and 0.16 mg of iron. In contrast, WPC blended at 2 % level paired with 3 % sorghum flour obtained significantly (P d" 0.05) higher overall acceptability score of 8.10 compared to control (8.01) and had higher amount of protein 4.28 % than control 2.71 %.

Key words: Sorghum, Whey protein concentrate, Iron, Fiber.

Introduction

India is the largest milk producing country in the world by producing total of 230.6 MT during 2022-23 (BAHS, 2023). A considerable quantity of milk is being utilized in the manufacturing of milk products. The bioavailability of milk nutrients can be improved by process of fermentation. Lactic acid bacteria used in fermentation helps in eliminating toxins and anti-nutritional compounds available in different food formulations (García-Burgos et al. 2020). Fermented milk products got therapeutic properties due to presence of several viable cells.

India is the world's biggest producer of millet and plays a key role in the world's millet output. Among all **Sorghum** (*Sorghum bicolor*) stands second by contributing 26**%** in major millet production in India (APEDA. 2024). In general sorghum is a rich source of fiber and B-complex vitamins. It provides dietary fiber by 48 % of the recommended daily value (Samarth et al. 2018). The essential amino acids present in WPC makes it an important functional ingredient required for human nutrition (Yiðit et al. 2023). The study suggested developing a delicious, protein-rich sorghum-based dairy product (lassi) by adding sorghum and WPC to cow's milk, aiming to enhance both its nutritional and sensory qualities.

Cow milk was procured from Students Experimental Dairy Plant (SEDP) of Dairy Science College, Hebbal, Bengaluru. Sorghum millets are procured from Bb Royal BB Royal Organic Jowar/ Sorghum Millet. Whey protein concentrate (WPC-80) was procured from Asitis, Medizen Labs Pvt. Ltd, Bengaluru. Standard lactic culture was procured from department of dairy microbiology, Dairy Science College, Hebbal, Bengaluru. Madhur pure and hygienic cane sugar was purchased from local store.

Lassi was prepared by following the procedure of Patel et al. 2020 with suitable modifications. The cow milk of 3.0% fat and 8.5% SNF was heated to 40-45° C and filtered by using muslin cloth to remove extraneous matter from milk. The germinated jowar flour was added to milk at 2, 3 or 4% and heat treatment was done to 85°C for 5 minutes to denaturate whey proteins and to ensure food safety. Further the slurry was cooled to 37°C and inoculated with standard lactic culture at 2% level. Then the slurry was incubated for about 8 hrs till the pH attainment of 4.7 to obtain enriched curd. Breaking of coagulum was done and added with pasteurized chilled water at 20%. To that the sugar was added as a sweetening agent at 10%. Further all the ingredients were mixed to get sorghum blended *lassi*.

Based on the sensory attributes, the optimized level of sorghum was further blended with WPC-80 at 1, 2 or 3% by following the

Department of Dairy Technology,
Dairy Science College, Hebbal, Bengaluru-560024,
Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

Aishwarya R (✉)
Email: armpatil05@gmail.com

above-mentioned procedure in order to obtain the nutritionally rich delicious functional *lassi* and packaging was done in PET bottles and stored under refrigeration temperature (7±1° C).

The sensory attributes of *lassi* were determined by using 9-point hedonic scale by semi-trained panellists of the Dairy science college, Hebbal, Bengaluru based on parameters like, colour and appearance, body and texture, flavour and overall acceptability.

The % moisture content of sorghum and wpc-80 blended *lassi* was determined by gravimetric method as per IS: 1479 (part II) 1961. The protein content was determined by estimating the % nitrogen by Micro-Kjeldahl method as recommended in IS: 1479 (part II), 1961 and the % nitrogen was multiplied by 6.38 to find out protein %age in *lassi*. Standard Gerber method was used for determination of fat content as per IS: 1224 (part I), 1977. The fibre content and the iron content of *lassi* was estimated by AOAC Method No. 985. 35 (AOAC, 2005).

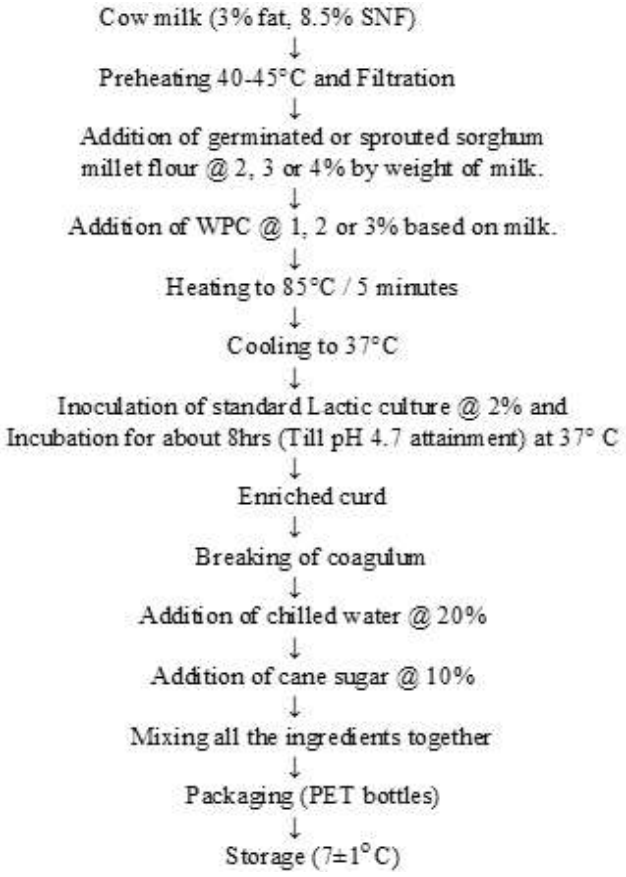

Fig.1 Process flow chart for the manufacture of Sorghum and whey protein concentrate blended *lassi*

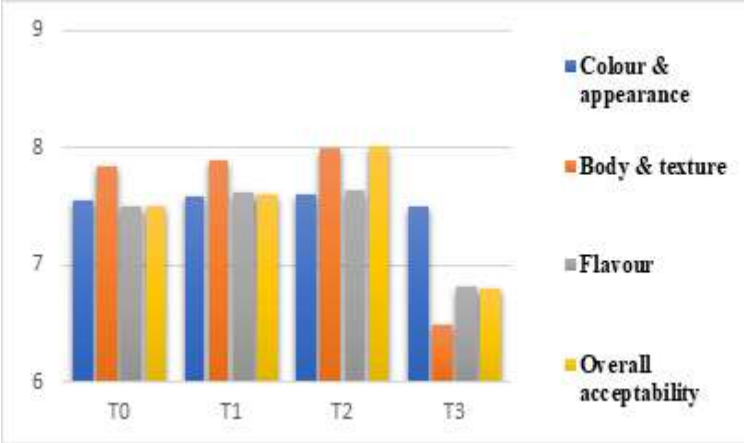

Fig 2: Effect of sorghum on sensory parameters of *lassi*

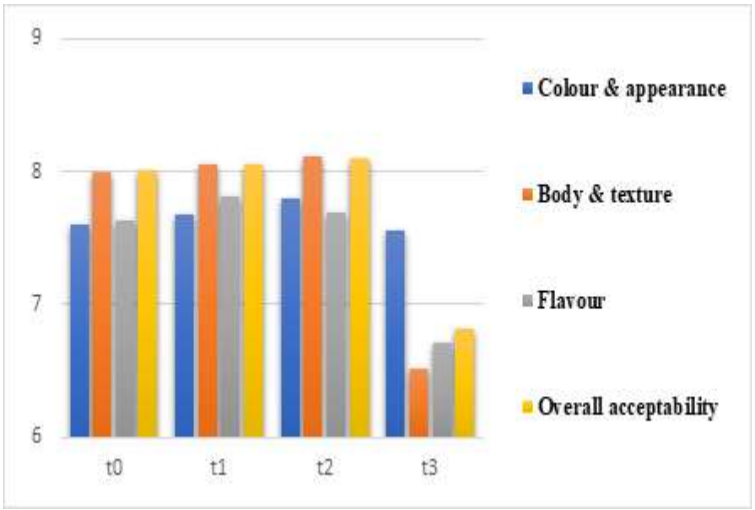

Fig 3: Effect of WPC on sensory parameters of sorghum enriched *lassi*

The data obtained during the research studies was analysed by using R software (R. version 4.0.3) to calculate mean and critical difference to determine significant and non-significant effect of different trails obtained during the study.

The prepared control (T_o) *lassi* and sorghum flour enriched *lassi* at 2% (T_1), 3% (T_2) and 4% (T_3) was analysed for sensory evaluation. For colour and appearance, no significant differences were observed between the treatments (P d" 0.05), with scores ranging from 7.50 for T_3 to 7.60 for T_2. In terms of body and texture, T_2 (3% sorghum flour) scored the highest at 8.00, followed by T_1 (2% sorghum flour) at 7.89. In contrast, T_3 (4% sorghum flour) received the lowest score of 6.50, showing a decline in texture with the higher sorghum flour content. Regarding flavour, T_2 again led with a score of 7.63, while T_3 scored the lowest at 6.81, indicating that higher sorghum flour concentration negatively affected flavour as the panalists observed after taste of sorghum flour beyond 3% level. For overall acceptability, T_2

Table 1: Effect of Sorghum (Great millet) flour on the sensory parameters of *lassi*

Levels of sorghum flour (%)	Colour & appearance	Body & texture	Flavour	Overall acceptability
T_0	7.55	7.85[a]	7.51[a]	7.50[a]
T_1	7.58	7.89[a]	7.62[a]	7.61[a]
T_2	7.60	8.00[a]	7.63[a]	8.01[a]
T_3	7.50	6.50[b]	6.81[b]	6.80[b]
CD ($P \leq 0.05$)	NS	0.45	0.36	0.72

Table 2: Effect of Sorghum (Great millet) flour on the compositional parameters of *lassi*

Levels of sorghum flour (%)	Moisture (%)	Protein (%)	Fat (%)	Fiber (g/100g)	Iron (mg /100g)
T_0	80.90[a]	2.45[b]	2.45	0.00	0.00
T_1	79.16[b]	2.62[ab]	2.43	0.04	0.10
T_2	78.29[c]	2.71[a]	2.42	0.06	0.16
T_3	77.42[d]	2.83[a]	2.40	0.08	0.21
CD ($P \leq 0.05$)	0.36	0.24	NS	NS	NS

Table 3: Effect of WPC on the sensory parameters of Sorghum (Great millet) flour enriched *lassi*

Levels of wpc -80 (%)	Colour & appearance	Body & texture	Flavour	Overall acceptability
t_0	7.60	8.00[a]	7.51[a]	7.50[a]
t_1	7.68	8.05[a]	7.82[a]	7.70[a]
t_2	7.80	8.12[a]	7.69[a]	8.10[a]
t_3	7.55	6.51[b]	6.71[b]	6.82[b]
CD ($P \leq 0.05$)	NS	0.41	0.51	0.72

Table 4: Effect of WPC on the compositional parameters of Sorghum (Great millet) flour enriched *lassi*

Levels of wpc -80 (%)	Moisture (%)	Protein (%)	Fat (%)	Fiber (g/100g)	Iron (mg /100g)
t_0	78.29[a]	2.71[d]	2.42	0.060	0.16
t_1	77.51[b]	3.49[c]	3.11	0.059	0.158
t_2	76.75[c]	4.28[b]	3.12	0.058	0.156
t_3	76.00[d]	4.97[a]	3.13	0.058	0.155
CD ($P \leq 0.05$)	0.21	0.29	NS	NS	NS

received the highest score of 8.01, followed by T_1 with 7.61, while T_3 was the least acceptable by obtaining 6.80. The statistical analysis revealed significant differences in body and texture, flavour, and overall acceptability (P d" 0.05), highlighting that the optimal sorghum flour concentration for the best sensory attributes is around 3%.

By increasing the level of sorghum flour, moisture content of prepared *lassi* was decreased because of increase in total solids content. Sorghum possesses high protein content of 10g/100g (IIMR.2024) hence, the protein content in prepared *lassi* was seen to be increased by increasing the sorghum content. As the milk is devoid of iron and fibre, % of iron and fibre was significantly raised to some extent after enrichment with sorghum flour.

The *lassi* with 3% sorghum flour was kept as a control (t_0) and further enrichment is done by incorporating wpc-80 at 1% (t_1), 2% (t_2) or 3% (t_3) and analysed for sensory evaluation. The organoleptic properties of prepared *lassi* was improved as the brighter color for the wpc-80 incorporated *lassi* was observed by scoring 7.68, 7.8, 7.55 respectively for 1, 2 and 3% than control group which has scored 7.6. Textural properties also were improved by incorporation of wpc upto 2% by raising scores from 8.0 to 8.12 from control to 2% wpc incorporated product. Beyond 2%, the textural scores has been reduced. This may be due to increase in the viscosity (thickness) of the product at 3%. The astringent flavor of wpc was masked by sorghum flour until 2% but beyond that the panalists experienced astringent flavor in the *lassi* as the product was a acidic protein beverage (Carter

et al. 2020). Based on these the obtained overall acceptability scores were 8.01(t_0), 8.05 (t_1), 8.10 (t_2) and 6.82 (t_3). Hence, the panalists optimized *lassi* blended at 2% wpc.

By incorporating the whey protein concentrates, the nutritional value of *lassi* in terms of protein was drastically increased like 2.71(t_0), 3.49(t_1), 4.28(t_2) and 4.97(t_3) along with the fat content. This may be due to the presence of fat content in wpc-80 but there observed the decrease in milk devoid nutrients like fibre and iron.

Conclusions

The developed Sorghum (Great Millet) and Whey Protein Concentrate (WPC-80) blended *lassi* was having high protein content by consuming 200ml of developed *lassi*, a child (4-6 years) can meet around 43% of RDA, an adult male can meet around 14.3 % of RDA and an adult female can meet around 15.5% of RDA of protein. Along with protein the lacuna of iron and fibre in milk products can be significantly fulfilled by blending sorghum and WPC. Intern, enriched *lassi* has great marketing potentiality and contributes for healthy beverage besides consumer satisfaction. Further the scope may exist for the addition of concentrated form of sorghum to raise the nutritional property of the produced beverage.

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