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Impact of Storage on Probiotic Viability, Nutritional and Sensory Quality of Fermented Soymilk Produced from Different Soybean Varieties

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ABSTRACT

Background: In recent years, there has been an increase in consumer demand for beverages made from fermented soybeans. Unfortunately, the nutritional imbalance and flavour profiles of plant-based milk substitutes frequently prevent people from consuming them. Fermentation can aid plant-based milk substitutes' taste profiles, nutritional characteristics, texture and microbiological safety in order to produce more valued and delicious products without adding additional, frequently viewed as fake, ingredients.

Methods: The implications of probiotic fermentation to improve the nutritive and sensory quality of fermented soymilk are assessed. Three soybean varieties as food grade soybean (Krune), low KTI soybean (V-23) and black soybean (VL-Bhatt 65) are used for the formation of soymilk which further fermented by three probiotic strains as *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and *Weissella confusa*. The fermented soymilk samples were stored for 28th days at 4°C to analyse the changes in chemical composition and sensory quality.

Result: High protein content was found in BA (6.85±0.13 g/100g), while the consumer preferred the LR with best overall ranking (6.25±0.05) in hedonic scale. The viability count of probiotic was highest (9.44±0.17 log cfu/ml) in BA after storage. The study suggested that soymilk with enormous number of biomolecules or pigments showed better viability of probiotic after storage and *L. rhamnosus* fermented soymilk preferred by the consumer over others probiotic fermented soymilk.

Key words: Black soybean, Fermentation, Probiotic, Sensory evaluation, Soymilk.

INTRODUCTION

Soybean is the prominent crop in India which majorly grow in Madhya Pradesh. Soybean contain profound amount of protein, which is a good source for vegetarian peoples. Hence, different soy-based food products as soymilk and tofu are consumed all around the world (Kumar *et al*., 2022; Ahmad *et al*., 2021). Apart from the high-quality protein soybean also possess isoflavones which play key role in therapeutic property of soymilk. For people with lactose intolerance, vegetarians, or people who have a milk allergy, it offers a plentiful and affordable source of nutrients (Maheshwari *et al*., 2021; Sasi *et al*., 2022). Additionally, it offers customers health advantages like hypolipidemic, anticholesterolemic and antiatherogenic qualities, as well as the ability to minimise allergenicity (Sasi *et al*., 2022). On the other hand, because the human intestinal tract lacks the enzyme α-galactosidase, soymilk has unfavourable qualities due to its indigestible oligosaccharides such stachyose and raffinose (Donkor and Shah, 2008). These indigestible carbohydrates can be converted into gas and other unpleasant symptoms by GI-residual bacterial groups (Rodrigues *et al*., 2020). In addition, some anti-nutritional factors also found in soybean as phytic acid and trypsin inhibitor (Kumar *et al*., 2022). Phytic acid impedes the bioavailability of bivalent cations as Ca, Mg, Fe and Zn while trypsin inhibitor affects the proteolytic enzyme trypsin to digest protein (Tangyu *et al*., 2019) (Vadodariya *et al*., 2022).

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Probiotics are defined as living microorganisms that, when given to a host in sufficient amounts, confer health benefits on that host. For example, probiotics can change the balance of the intestinal microflora, stop the growth of pathogenic bacteria, promote healthy digestion and immune function, lessen the impact of allergens, improve lactose intolerance and boost resistance to infection (Zhu *et al*., 2020). Probiotics have been used for decades in dairy products including yoghurt, kefir and cultured buttermilk, which causes the development of acids and the release of aromas (Xia *et al*., 2019). Probiotics contained in plant-based products are becoming more and more prevalent recently.

The fermentation of soy milk with lactic acid bacteria (LAB) was discovered to improve the nutritional and bioactivity values of ingested foods, as well as the quality, nutritive value and sensory aspects of soy milk. The aim of this study is to understand the changes in chemical composition, bacterial viability and sensory profiling of fermented soymilk during 28th days of storage at 4°C.

MATERIALS AND METHODS

Soybean seeds

The experiment was design and executed during March 2020 to July 2022 at the Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi and Division of Food Science and Post-harvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi. Three types of soybean variety were used for the preparation of soymilk. Food grade soybean (krune), Low KTI soybean (V-23) and black soybean (VL-Bhat 65) were procured from Division of Genetics, ICAR-IARI, New Delhi, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand respectively.

Chemicals and bacterial strains

All of the chemicals used for the viability investigation and proximate analysis were purchased from Sigma Aldrich in the USA.

For the fermentation of soymilk, three probiotic bacterial strains-*Lactobacillus rhamnosus* 1136 (*LR*), *Lactobacillus acidophilus* 1132 (*LA*) and *Weissella confusa* 30082b (*WC*)- were obtained from the National Centre for Microbial Resource in Pune, India, the Division of Microbiology at the ICAR-Indian Agricultural Research Institute in New Delhi, India and the RIKEN Bioresource Research Centre, In MRS broth, the acquired bacterial cultures were kept alive.

Preparation of soymilk

Using the hot-water extraction method, soymilk was made from food-grade, low KTI and black soybeans. After being cleaned, soybean seeds were steeped in distilled water all night. The swollen soybean seeds were manually dehulled, crushed in hot water (80°C) at a ratio of 1:10 and then filtered through two layers of cheesecloth to get rid of any insoluble residues (Donkor and Shah, 2008). The extracted soymilk was put in a glass bottle and autoclaved for 15 minutes at 121°C to sanitise it. To be used later, the autoclaved soymilk is bottled, cooled to room temperature and kept at 4°C.

Fermentation of soymilk

All three probiotic strains-*LR*, *LA* and *WC* were multiplied twice in MRS broth at 2% (v/v) each and incubated for 24 hours at 37°C for *LR* and *LA* and 30°C for *WC*. Temperatures of 37°C and 30°C, respectively, are ideal for the unhindered growth of the Lactobacillus and Weissella species. Autoclaved and stored soymilk was independently inoculated (1% v/v means 1 mL bacterial culture in 100 ml soymilk) with three different bacterial strains for soymilk fermentation. The inoculation samples were fermented at 30°C (*WC*) and 37°C (*LR*, *LA*), respectively, for 24 hours. To ascertain the impact of fermentation on storing fermented soymilk at 4°C for 28 days, unfermented samples incubated under the identical experimental conditions were utilised as a reference. Overall methodology adopted for sample preparation represented in Fig 1.

Determination of viability of probiotic after 28 days of storage at 4°C

The plate dilution method with MRS agar was used to determine the viability of all three probiotic strains (Man *et al*., 1960). Each sample of fermented soymilk at 48 hr of fermentation and at 28 days after storage was serially diluted and plated in two separate dishes. The plates were then incubated for 48 hours at 37°C (*LR* and *LA*) and 33°C (*WC*) respectively. To calculate the results, colony forming units per millilitre (cfu/ml) were utilised.

Determination of pH and titratable acidity of fermented soymilk after storage

At 48 hours and 28 days of fermentation, the samples pH was assessed using a pH meter. By utilizing 0.1 N NaOH, titratable acidity was measured as a percentage of lactic acid (%TA). 0.1 N NaOH was used to titrate soymilk that had been diluted 1:1 with distilled water using 3-4 drops of phenolphthalein as an indicator. %TA, or titratable acidity, was calculated using the formula below:

$$\%\ \text{Titratable acidity (\%TA)} = \frac{\text{Volume of 0.1 N NaoH consumed for neutralization} \times 0.09 \times 100}{\text{Volume of soyamilk} \times \text{Specific gravity}}$$

Determination of proximate analysis of fermented soymilk after storage

The proximate analysis executed by using AOAC methods (AOAC 2000). All the analysis proceeds in triplicate. 1 g of samples was used for the nitrogen content estimation by using the Kjedahl method and the protein amount was approximated by multiplying the results by a conversion factor (5.71 for soy). The following equations were used for the calculation of % nitrogen and % of protein content in the soymilk sample.

$$\%\ \text{Nitrogen} = \frac{14 \times (V_S - V_B) \times 0.1}{\text{Weight of sample (mg)}} \times 100$$

$$\%\ \text{Protien} = 5.71 \times \%\ \text{Nitrogen}$$

Here: $(V_S - V_B)$ = consumed volume of 0.1N HCl

Total carbohydrate content determined by difference to obtained total content of 100 g. To determine the moisture content, 5 g of the samples were dried in an oven at 70°C till weight of the sample became constant. Ash content in soymilk samples was determine by AOAC method. 2 g of sample heated or burn out at 550°C in muffle furnace, until completely ashes. The calorie value of soymilk samples was determined by using Atwater conversion factor (protein×4, fat×9, carbohydrate×4) (Santos *et al*., 2019).

Sensory analysis of fermented soymilk after storage

For sensory acceptance tests, a standardised 9-point hedonic scale (1 for extremely dislike; 9 for extremely like) was used (Albuquerque *et al*., 2017). 50 consumers (untrained panellists, consisting of men and women with ages ranging from 25 to 50) from the faculty, including teachers, students, staff and visitors from other places, were recruited based on interest and consumption patterns for soy product on each sampling day (at 48 hr and at 28th days) of soymilk and fermented soymilk.

Statistical analysis

All experiments were performed in triplicate and results were expressed as mean±standard deviation (SD). Statistical analyses were carried out using SPSS 10.0 Statistics software and MV app (Julkowska *et al*., 2019). Tukey's test was used at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Viability of bacterial strain of stored fermented soymilk

Lactobacillus rhamnosus (*LR*), *Lactobacillus acidophilus* (*LA*) and *Wessilla confusa* (*WC*) were significantly remained constant in fermented soymilk after 28th days of storage at 4°C. The viability of probiotic strains after 28th days of storage of fermented soymilk at 4°C was shown in Fig 2. The colony forming unit (CFU) of *L. rhamnosus* fermented food grade soymilk (FR), *L. acidophilus* fermented food grade soymilk (FA) and *W. confusa* fermented food grade soymilk (FW) was 6.1, 6.5 and 5.6 log cfu/ml respectively at 28th days of storage. In the case of low KTI soybean milk fermented with same bacterial strains, the CFU was reported 5.8 log cfu/ml for LR, 6.1 log cfu/ml for LA and 5.2 log cfu/ml for LW. The cfu count in *L. rhamnosus, L. acidophilus and W. confusa* fermented black soymilk as BR, BA and BW were reported 9.23 log cfu/ml, 9.44 log cfu/ml and 8.56 log cfu/ml respectively. Among all three probiotic strains *L. acidophilus* showed better cell viability after storage period at 4°C in comparison to *L. rhamnosus* and *Weissella confusa.* Similarly, among the three soybean varieties, black soybean milk showed better viability of bacterial strains than food grade soybean and low KTI soybean variety. The higher

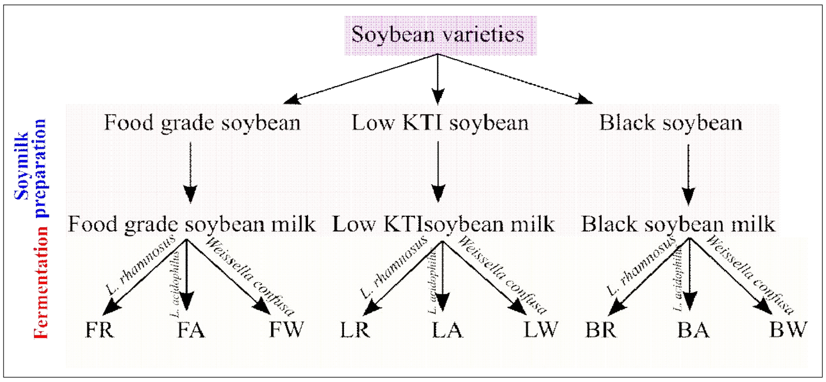

Fig 1: Flow chart indicating the methodology adopted for sample preparation.

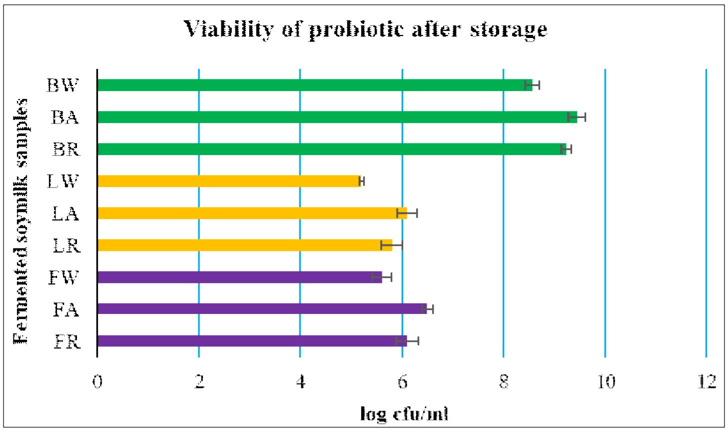

Fig 2: Viability of bacterial strains in fermented soymilk samples after 28th days of storage at 4°C. All the data are expressed as Mean, n = 3.

viability of bacterial strains even after 28th days storage at 4°C indicates that black soymilk harbour plant bioactive compounds as anthocyanin or other phenolic compounds which act as source of hydrocarbon and energy for the growth of bacterial strain during storage (Krishnan *et al*., 2018; Janpaeng *et al*., 2018; Kumar *et al*., 2019; Krishnan *et al*., 2020; Bhartiya *et al*., 2020).

Changes in pH and titratable acidity (%TA) of stored fermented soymilk

During the storage pH of the fermented soymilk decreased while titratable acidity (%TA) increased (Table 1). The pH of the unfermented soymilk from food grade soybean (FC), low KTI soybean (LC) and black soybean (BS) reported as 5.1, 5.3 and 4.8 after storage, while the pH of fermented soymilk samples was reported low in compare to unfermented soymilk. The pH of food-grade soybean milk decreased from 5.1 (FC) to 2.8 (FR), 2.5 (FA) and 2.7 (FW) after 28th days of storage at 4°C. In low KTI soybean milk pH declined during the storage from 5.3 (LC), 3.12 (LR), 2.87 (LA) and 2.91 (LW). The reduction in pH was low in black soybean milk in compare to food grade soybean and low KTI soybean milk. The pH of black soymilk declined from 4.8 (BS) to 3.7 (BR), 3.31 (BA) and 3.62 (BW) after storage.

In reverse to pH, the %TA of the samples enhanced after storage. %TA of unfermented food grade soybean milk (FC) observed 1.41, which increased during storage in fermented soymilk 1.59 (FR), 1.75 (FA) and 1.65 (FW). In low KTI soybean milk % TA observed 0.97 (LC), which increased in fermented sample during storage 1.32 (LR), 1.5 (LA) and 1.41 (LW). %TA of fermented samples of black soymilk raised from 1.94 (BS) to 2.34 (BR), 3.98 (BA) and 3.37 (BW).

The reduction of pH in fermented samples indicates that bacterial strains producing some short chain fatty acids by metabolizing the large hydrocarbon molecules as RFOs (raffinose family oligosaccharides) which led to pH reduction (Nazhand *et al*., 2020) (Sasi *et al*., 2022). Among the three mentioned bacterial strains *L. acidophilus* (*LA*), fermented soymilk samples showed lower value of pH and higher value of %TA in comparisons to remaining probiotic strains.

Changes in chemical composition of stored fermented soymilk

Chemical composition as protein, total fat, carbohydrate, moisture, ash and energy content of non-fermented and fermented soymilk determined by the AOAC methods (AOAC 2000) and the obtained results are shown in Table 1 and Fig 3 (A-D). The protein content in non-fermented soymilk were reported as 1.94 g/100g (FC), 2.26 g/100g (LC) and 2.05 g/100g (BS) which are low in comparison to fermented soymilk (Fig 3A). The higher amount of protein content 6.85 g/100g was found in *L. acidophilus* fermented black soymilk (BA). High bacterial fermentation may cause the enhancement of protein content (Hong *et al*., 2012). The total fat content in non-fermented soymilk were reported as 1.21 g/100 g (FC), 1.87 g/100 g (LC) and 2.53 g/100 g (BS) which are high in comparison to fermented soymilk (Fig 3B). The low amount of total fat 0.7 g/100g was found in *L. acidophilus* fermented low KTI soymilk (LA). The *L. acidophilus* fermented soymilk may be responsible for the substantial decrease in total fat (Lee *et al*., 2015). The higher amount of total carbohydrate 5.41 g/100g was found in *L. acidophilus* fermented food grade soymilk (FA) (Fig 3C) while the high energy value 49.75 Kcal was reported in *W. confusa* fermented black soymilk (BW) (Fig 3D). During the fermentation moisture content in the sample declined while the ash content raised. Hence high amount of moisture content was observed in non-fermented low KTI soymilk sample (LC) was 93.67 g/100 g and low amount of moisture content 88.87 g/100 g was reported in *L. acidophilus* fermented black soymilk sample (BA). Similarly, the high ash content 0.79 g/100 g was reported in *L. acidophilus* fermented black soymilk (BA), while low ash content 0.21 g/100g was observed in non-fermented low KTI soymilk (LC).

Table 1: Changes in titratable acidity, pH and chemical composition in fermented soymilk samples after 28th days of storage at 4°C.

Sample	% TA	pH	Protein (g/100g)	Total fat (g/100g)	Moisture (g/100g)	Ash (g/100g)	Carbohydrate (g/100g)	Energy (Kcal)
FC	1.41±0.042	5.1±0.19	1.94±0.09	1.21±0.04	92.23±2.54	0.25±0.011	4.39±0.14	36.21±0.06
FR	1.59±0.063	2.8±0.15	3.19±0.04	0.98±0.01	90.02±1.62	0.49±0.003	5.36±0.03	43.02±0.16
FA	1.75±0.074	2.5±0.04	4.11±0.11	0.76±0.01	89.22±0.72	0.58±0.001	5.41±0.07	44.92±1.38
FW	1.65±0.018	2.7±0.08	3.52±0.02	0.82±0.03	90.43±0.08	0.55±0.021	4.80±0.19	40.66±0.15
LC	0.97±0.024	5.3±0.04	2.26±0.02	1.87±0.03	93.67±3.12	0.21±0.005	2.02±0.09	33.95±1.47
LR	1.32±0.021	3.12±0.16	3.78±0.01	0.77±0.02	91.21±0.41	0.51±0.012	3.89±0.04	37.61±0.31
LA	1.50±0.001	2.87±0.12	4.10±0.13	0.70±0.02	90.33±2.28	0.59±0.017	4.48±0.07	40.62±1.17
LW	1.41±0.045	2.91±0.17	3.97±0.07	0.75±0.03	91.07±2.54	0.55±0.018	3.88±0.17	38.15±0.86
BS	1.94±0.033	4.8±0.13	2.05±0.03	2.53±0.01	92.77±1.67	0.34±0.013	2.36±0.07	40.41±0.95
BR	2.34±0.063	3.7±0.07	5.71±0.06	1.50±0.03	89.20±3.30	0.75±0.016	3.03±0.11	48.46±1.53
BA	3.98±0.111	3.31±0.07	6.85±0.13	1.20±0.11	88.87±0.48	0.79±0.033	2.45±0.00	48.00±2.03
BW	3.37±0.039	3.62±0.06	6.15±0.21	1.67±0.01	89.11±0.40	0.71±0.002	2.53±0.06	49.75±0.36

(Values are mean±SD (n=3).

Sensory profiling of stored fermented soymilk

Along with the nutritive and health-promoting qualities, fermented products must also have sensory qualities that appeal to consumers. The sensory profiles of stored soymilk samples are shown in Fig 4. The lowest colour value (Fig 4A) was found in non-fermented black soymilk (BS- 3 unit), while the highest colour value was found in non-fermented food grade soymilk (FC-5.8 unit), *W. confusa* fermented food grade soymilk (FW-6.0 unit), non-fermented low KTI soymilk (LC- 5.9 unit), *L. rhamnosus* fermented low KTI soymilk (LR-6.1 unit) and *L. acidophilus* fermented low KTI soymilk (LA-6.23 unit). The high colour value of low KTI soybean milk over food grade soymilk and black soymilk might be due to the presence of lower amount of fat in these samples which responsible for the off-white colour of the product. *L. rhamnosus* fermented food grade soymilk (FR) showed the lowest value (1.1 unit) of mouthfeel (Fig 4B), while the highest value of mouthfeel observed in *L. acidophilus* fermented black soymilk

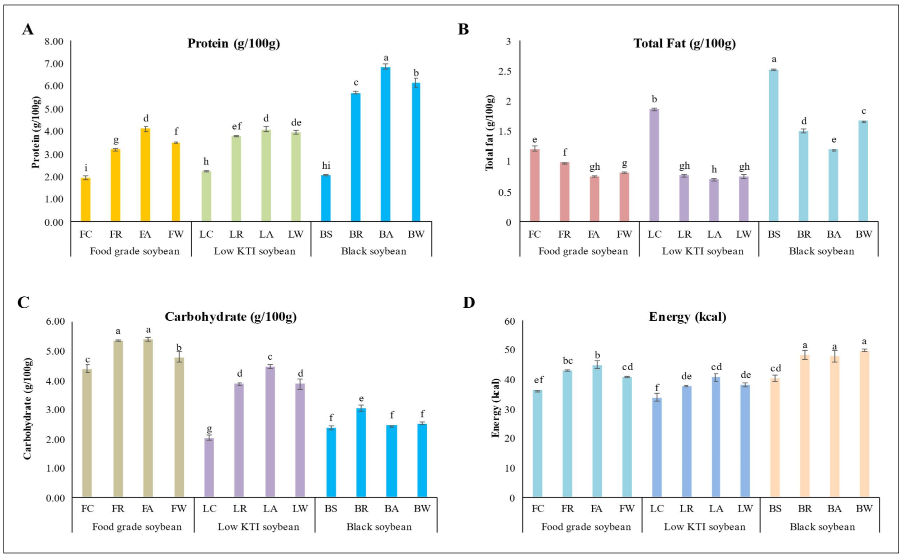

Fig 3: Proximate analysis of stored fermented soymilk. (A) protein, (B) total fat, (C) Carbohydrate and (D) Energy value. All the data are expressed as Mean, n = 3. Different letters indicate that values are statistically significant differences based on two-way ANOVA (P<0.05).

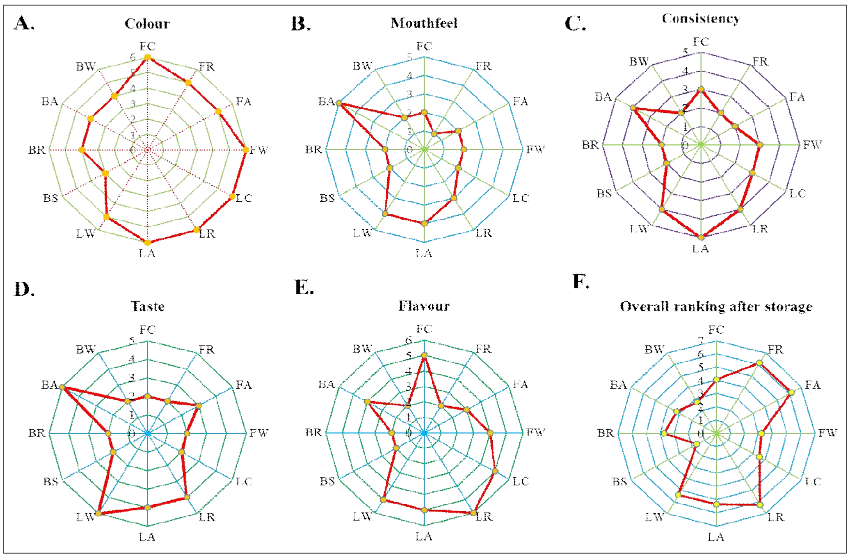

Fig 4: Sensory profiling (scale 1-9) of soymilk samples after 28th days storage at 4°C. (A) colour, (B) Mouthfeel, (C) Consistency, (D) Taste, (E) Flavour and (F) Overall ranking. Hedonic scale: 1 = extremely dislike, 2 = very much dislike, 3 = moderately dislike, 4 = slightly dislike, 5 = neither dislike nor like, 6 = slightly like, 7 = moderately like, 8 = very much like, 9 = extremely like. All the data are expressed as Mean, n = 3.

(BA- 5.2 unit). The higher value of mouthfeel might be due to the presence of excess protein content in the BA sample which precipitate in acidic pH during storage. *L. acidophilus* fermented low KTI soymilk (LA) showed the higher value (5.3 unit) of consistency (Fig 4C), while for the taste point of view *W. confusa* fermented low KTI soymilk (LW) and *L. acidophilus* fermented black soymilk (BA) showed higher value of taste 5.1-unit, 4.9 unit, respectively (Fig 4D). *L. rhamnosus* fermented low KTI soymilk (LR) showed the high flavour value (6.3 unit) (Fig 4E). Higher value of overall acceptance observed in *L. rhamnosus* fermented low KTI soymilk (LR-6.25 unit) followed by *L. acidophilus* fermented food grade soymilk (FA-6.19 unit) (Fig 4F). *L. rhamnosus* secretes exopolysaccharides (EPS), which enhance the texture of fermented products, hence *L. rhamnosus* fermented low KTI soymilk (LR) demonstrated the best consumer approval (Li *et al*., 2014). Additionally, the fragrance of the *L. rhamnosus*-fermented soymilk was pleasant, which encouraged more people to choose the *L. rhamnosus* fermented low KTI soymilk (LR) sample.

CONCLUSION

The research described in this article has demonstrated that during storage of fermented soymilk, probiotic strain, in addition to soybean variety, also affects the pH, titratable acidity, chemical composition and particularly sensory parameters. Black soybean among the three soybean varieties demonstrated higher probiotic activity and improved chemical composition during storage of fermented soymilk. However, consumers appreciated fermented soymilk made from black soybeans less. Therefore, this study may be able to entice researchers to look into blending various pigmented soybean varieties or to separate bioactive ingredients and add them to soymilk for improved overall fermented soymilk performance.

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REFERENCES

Ahamd, D., Jain, S.K., Joshi, M.A., Anand, A., Tomar, B.S., Kumar, S. and Hasan, M. (2021). H_2O_2 as a better index of seed quality and mechanism of cucumber (*Cucumis sativus*) seed deterioration. Indian Journal of Agricultural Sciences. 91(10): 1500-4.

Albuquerque, M.A.C., Bedani, R., LeBlanc, J.G. and Saad, S.M.I. (2017). Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk. International Journal of Food Microbiology. 261: 35-41.

AOAC. (2000). Official Method of Analysis AOAC Intl. 17th ed. Method 947.05. Association of Official Analytical Communities, Arlington, VA, USA.

Bhartiya, A., Aditya, J.P., Pal, R.S., Chandra, N., Kant, L. and Pattanayak, A. (2020). Bhat (black soybean): A traditional legume with high nutritional and nutraceutical properties from NW Himalayan region of India.

Donkor, O.N. and Shah, N.P. (2008). Production of β-glucosidase and hydrolysis of isoflavone phytoestrogens by *Lactobacillus acidophilus, Bifidobacterium lactis* and *Lactobacillus casei* in soymilk. Journal of Food Science. 73(1): M15-M20.

Hong, G., Mandal, P.K., Lim, K. and Lee, C. (2012). Fermentation increases isoflavone aglycone contents in black soybean pulp. Asian Journal of Animal and Veterinary Advances. 7(6): 502-511.

Janpaeng, J., Santivarangkna, C., Suttisansanee, U. and Emsawasd, V. (2018). Effect of Probiotic Fermentation on Bioactivity in Fermented Soy Milk. In The International Conference on Food and Applied Bioscience proceeding book (pp. 209-216). Thailand: Agro-Industry, Chiang Mai University.

Julkowska, M. M., Saade, S., Agarwal, G., Gao, G., Pailles, Y., Morton, M. and Tester, M. (2019). MVApp-multivariate analysis application for streamlined data analysis and curation. Plant Physiology. 180(3): 1261-1276.

Krishnan, V., Gothwal, S., Dahuja, A., Vinutha, T., Singh, B., Jolly, M. and Sachdev, A. (2018). Enhanced nutraceutical potential of gamma irradiated black soybean extracts. Food Chemistry. 245, 246-253.

Krishnan, V., Rani, R., Pushkar, S., Lal, S. K., Srivastava, S., Kumari, S. and Sachdev, A. (2020). Anthocyanin fingerprinting and dynamics in differentially pigmented exotic soybean genotypes using modified HPLC-DAD method. Journal of Food Measurement and Characterization. 14(4): 1966-1975.

Kumar, M., Dahuja, A., Sachdev, A., Kaur, C., Varghese, E., Saha, S. and Sairam, K.V.S.S. (2019). Evaluation of enzyme and microwave-assisted conditions on extraction of anthocyanins and total phenolics from black soybean (*Glycine max* L.) seed coat. International Journal of Biological Macromolecules. 135, 1070-1081.

Kumar, M., Suhag, R., Hasan, M., Dhumal, S., Radha, Pandiselvam, R. and Kennedy, J. F. (2022). Black soybean [*Glycine max* (L.) Merr.]: Paving the way toward new nutraceutical. Critical Reviews in Food Science and Nutrition. 1-27.

Lee, M., Hong, G.E., Zhang, H., Yang, C.Y., Han, K.H., Mandal, P.K. and Lee, C.H. (2015). Production of the isoflavone aglycone and antioxidant activities in black soymilk using fermentation with Streptococcus thermophilus S10. Food Science and Biotechnology. 24(2): 537-544.

Li, C., Li, W., Chen, X., Feng, M., Rui, X., Jiang, M. and Dong, M. (2014). Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains. LWT-Food Science and Technology. 57(2): 477-485.

Maheshwari, C., Garg, N. K., Hassan, M. and Tyagi, A. (2021). An insight: Impact of reduced Rubisco on plant physiology and biochemistry. Indian Journal of Agricultural Sciences. 91(1): 16-20.

Man, J.C. and Rogosa, M., Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. J. Appl Bacterio. 23: 130: 135.

Nazhand, A., Souto, E.B., Lucarini, M., Souto, S.B., Durazzo, A. and Santini, A. (2020). Ready to use therapeutical beverages: Focus on functional beverages containing probiotics, prebiotics and synbiotics. Beverages. 6(2): 26; https://doi.org/10.3390/beverages6020026.

Rodrigues, F.J., Cedran, M.F., Bicas, J.L. and Sato, H.H. (2020). Encapsulated probiotic cells: Relevant techniques, natural sources as encapsulating materials and food applications-A narrative review. Food Research International. 137, 109682.

Santos, D.C., de Oliveira Filho, J.G., Santana, A.C.A., de Freitas, B.S.M., Silva, F.G., Takeuchi, K.P. and Egea, M. B. (2019). Optimization of soymilk fermentation with kefir and the addition of inulin: Physicochemical, Sensory and Technological Characteristics. LWT. 104: 30-37.

Sasi, M., Kumar, S., Hasan, M., Garcia-Gutierrez, E., Kumari, S., Prakash, O. and Dahuja, A. (2022). Current trends in the development of soy-based foods containing probiotics and paving the path for soy-synbiotics. Critical Reviews in Food Science and Nutrition. 1-19.

Tangyu, M., Muller, J., Bolten, C.J. and Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. Applied Microbiology and Biotechnology. 103(23): 9263-9275.

Vadodariya, P., Abuj, B., Karmakar, N., Gudadhe, N., Faldu, P., Narwade, A. and Kanti, D.M. (2022). Comparative biochemical study of different *Lablab purpureus* L. groups under processing. Legume Research-An International Journal. 45(11): 1362-1371. doi: 10.18805/LR-4331.

Xia, X., Dai, Y., Wu, H., Liu, X., Wang, Y., Yin, L. and Zhou, J. (2019). Kombucha fermentation enhances the health-promoting properties of soymilk beverage. Journal of Functional Foods. 62: 103549.

Zhu, Y.Y., Thakur, K., Feng, J.Y., Cai, J.S., Zhang, J.G., Hu, F. and Wei, Z.J. (2020). B-vitamin enriched fermented soymilk: A novel strategy for soy-based functional foods development. Trends in Food Science and Technology. 105: 43-55.