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AKADÉMIAI KIADÓ

Valorisation of whey for fermented beverage production using functional starter yeast

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ABSTRACT

Indigenous yeast strains *Kluyveromyces marxianus* (MH6), *K. marxianus* (CH1), and *Saccharomyces cerevisiae* (C1) were screened for whey beverage production. *K. marxianus* (MH6) showed significantly higher (P < 0.05) fermentation efficiency (15.2%) as compare to other yeast strains. The conditions optimised for whey fermentation were 16 Brix, pH 5.5, 28 °C, and 72 h without agitation. For fermented whey beverage production, fruits viz., kinnow (Daizy), guava (Allahabad safeda), and mango (Safeda) were blended with whey at different ratios viz., 80:20, 70:30, 60:40, and 50:50. All ratios showed significant differences for biochemical and sensory analysis (P < 0.05), out of which ratios 60:40, 70:30, and 60:40 for whey kinnow, whey mango, and whey guava, respectively, were selected. To enhance the flavour of whey beverage, flavouring agents (cinnamon, cardamom, fennel seeds, and apple essence) were added. A panel of judges assessed all whey beverages on a hedonic scale basis, and cardamom whey guava beverage received the highest score of 8.16. The whey beverages were stored under refrigerated conditions after pasteurisation, and the shelf life was assessed to be 15 days. This study conferred that *K. marxianus* held the potential for fermented whey fruit blend beverages production and these beverages could be an alternative healthy refreshing substitute for synthetic bottled fruit beverages.

KEYWORDS

Kluyveromyces marxianus, whey, fermentation, beverage, sensory

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1. INTRODUCTION

Whey is the by-product of dairy industry formed during coagulation of milk, consisting of lactose as main sugar and residual caseins as protein source that can be valorised through the fermentative action of yeasts (Martini et al., 2021). Since lactose is the only carbohydrate in whey, it does not support the growth of lactose non fermenting yeasts. *Kluyveromyces* species possess galactosidase enzyme encoded by LAC genes to hydrolyse the lactose (Zafar and Owais, 2006; Varela et al., 2017) and hence, this yeast has the potential to ferment whey lactose. Although *Saccharomyces cerevisiae* has been the primary representation in ethanol fermentation investigations, it lacks the ability to ferment lactose, unlike *Kluyveromyces marxianus* (Kokkiligadda et al., 2016). Tofalo et al. (2020) also reviewed the presence of *K. marxianus* along with *S. cerevisiae* and *Pichia* spp. as the predominant yeasts in sour milk, acid curd cheese, fermented coffee, and traditional fermented milk of different regions. *K. marxianus* is a member of the hemiascomycetous group of dairy yeasts, and is the sister species of *Kluyveromyces lactis* (Saini et al., 2017). Several studies documented the phenotypic and genotypic diversity and variability of *K. marxianus* strains of different origin due to its importance in dairy industry (Perpetuini et al., 2018, 2019; Tittarelli et al., 2021). Many fermented foods, alcoholic and non-alcoholic beverages, fermented raw sausages, tequila production, etc., have been produced using whey and yeast strains viz., *K. marxianus*, *K. lactis, Kluyveromyces fragilis,* and *S. cerevisiae* (Ghaly and El-Taweel, 1995; Leite et al., 2000; Satyanarayana and Gotthard, 2017). *K. marxianus,* thus, holds an important role in dairy industry and can be exploited for conversion of whey into a fermented beverage. Production of fermented whey beverage is the best way to utilise the large quantities of whey produced by dairy industries. *K. marxianus* imparts an acceptable flavour and aroma to the product making it highly nutritious and healthy (Gethins et al., 2015). Blending of fruits during production of fermented beverages improves the nutritional quality with addition of essential vitamins and minerals. Hence, in the present research whey was blended with fruits and fermented to produce healthy whey fruit beverage using functional starter yeast.

2. MATERIALS AND METHODS

Whey (acidic) was collected from Department of Livestock products technology Guru Angad Dev Veterinary and Animal Sciences University and from VERKA Milk Plant Ludhiana. Fruits viz., kinnow (Daisy), mango (Safeda) and guava (Allahabad safeda) were procured from the local market in Ludhiana, Punjab, India.

2.1. Yeast isolation and identification of cultures

The three yeast strains used in the present study were isolated from tribal areas of Lahaul and Spiti situated in North Western Himalayan region. CH1 was isolated from churpe (traditional fermented cheese), C1 from curd and MH6 from milk. Sequencing of ITS region was done with the help of commercial sequencing facility Xcelris Labs Ltd., Ahmedabad, India and phylogenetic analyses were carried out in MEGA X software program. The electropherograms of the yeast strains were edited manually and analysed using NCBI BLASTN program (http://www.ncbi.nih.gov/blast) (Altschul et al., 1997).

2.2. Biochemical analysis of whey and fruits

Erma hand refractometer and glass Brix hydrometers were used for measuring the total soluble solids of the whey. Total sugars and reducing sugars were determined by the methods of Dubois et al. (1951) and Miller (1959), respectively. Protein content for whey and fruits was analysed by the method of Lowry et al. (1951) and lactose content was determined according to Lane and Eynon (1923).

2.3. Screening yeast isolates for whey fermentation

Yeast isolates were screened for their fermentation potential by using whey, as a substrate. The yeast inoculum was prepared by inoculating three yeast cultures (CH1, MH6, and C1) into 100 mL GYE broth and keeping on a rotary shaker (100 r.p.m.) at 28 ± 2 °C in a BOD incubator for 24 h. Whey was fermented using 6% yeast inoculum at 28 °C temperature without shaking for 72 h, and parameters viz., total sugars, reducing sugars, protein content, total soluble solids, and lactose content were analysed. After completion of fermentation, ethanol content was measured by the chemical oxidation method of Caputi et al. (1968).

2.4. Standardisation of parameters for fermented whey beverage production

K. marxianus (MH6) was selected on the basis of fermentation ability. Different parameters viz., temperature, degree Brix, agitation, time, and pH were further standardised for whey beverage production.

Whey beverage was fermented at different temperature viz., 24, 28, and 37 °C, pH range from 4.0 to 6.0, different time period (0, 24, 48, 72, and 96 h), different sucrose concentrations (3, 5, 6, and 8%), with (100 r.p.m.) and without shaking. Parameters viz., total sugars, reducing sugars, total soluble solids, and ethanol content were analysed.

2.5. Fermentation of whey after fruit blending

The whey was blended with the respective fruit juices viz., kinnow, guava, and mango at different ratios viz., 80:20, 70:30, 60:40, and 50:50 (whey:fruit) and fermented at standardised parameters.

2.5.1. Extraction of fruit pulp/juices and blending of fruits with whey in different ratios. Fruits were washed, peeled, and their stones were separated. The juice was drained with the help of a muslin cloth and was collected in a vessel followed by pasteurisation at 62 °C for 20 min. Whey was blended with different fruit juices fermented at 28 °C for 72 h. The initial and final (after fermentation) physicochemical parameters viz., total soluble solids, total sugars, reducing sugars, pH, and ethanol content were analysed. The final whey fruit beverage was then pasteurised at 62 °C for 20 min.

2.5.2. Flavouring agents and spices. To enhance the flavour of the whey beverage, apple essence was added by conducting two experiments, wherein 2–3 drops of essence was added before fermentation in the first experiment and after fermentation in the other experiment. The effect of spices (cinnamon, cardamom, and fennel seeds) was tested by conducting three experiments, where small pieces of cinnamon was added during fermentation for 72 h in the first experiment, powdered cardamom in the second experiment, and in third experiment fennel seeds were added during fermentation and parameters viz., total sugars, reducing sugars, total soluble solids, and ethanol content were analysed. After analysing the initial and final parameters, flavoured whey beverage was pasteurised at 62 °C for 20 min (Fig. 1).

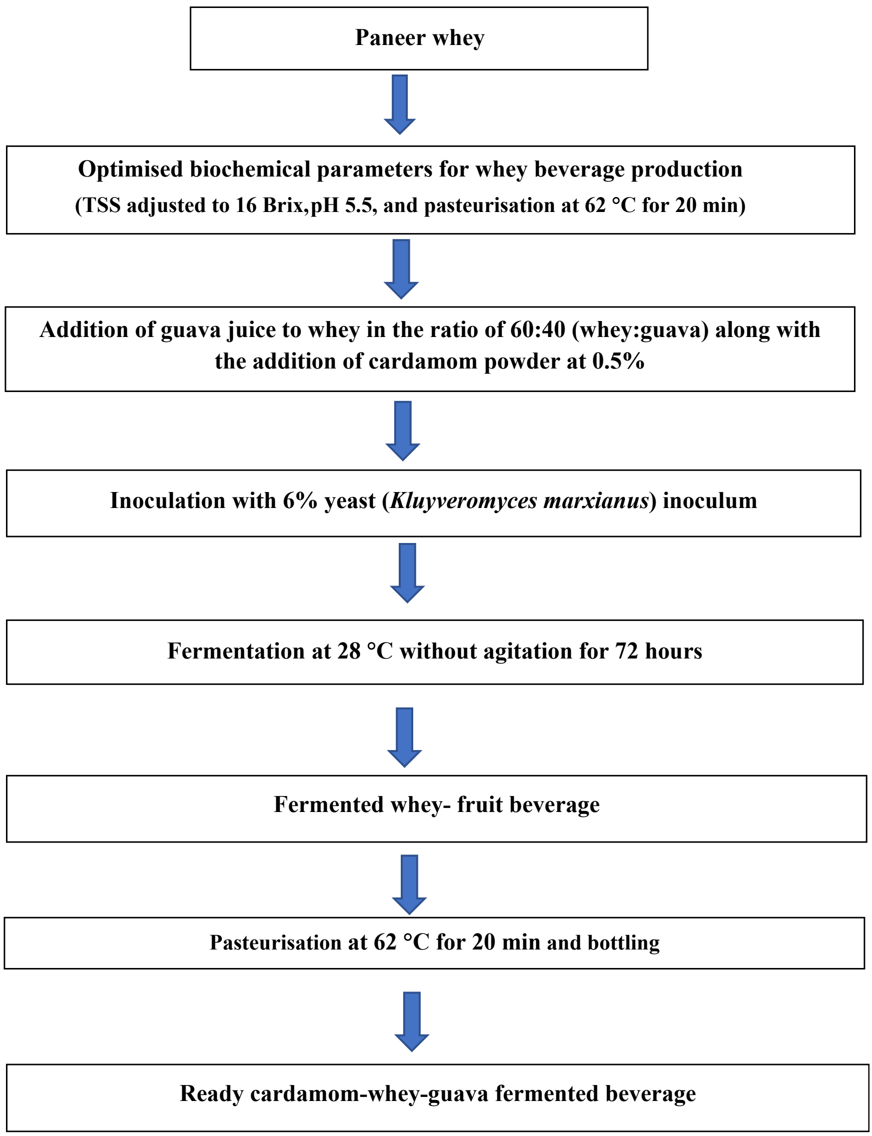

Fig. 1. Flow chart of fermented whey beverage production.

2.6. Sensory analysis

On a nine-point hedonic scale, the product was rated for consumer approval (Amerine et al., 1965). A panel of judges evaluated the sensory profile of a fermented whey beverage based on its appearance, colour, flavour, mouthfeel, and overall acceptability.

2.7. Shelf life studies of the whey beverage

Shelf life studies of the whey beverage was done for 45 days at every 15 d interval, and parameters viz., total sugars, reducing sugars, pH, ethanol content and total soluble solids were analysed and microbial count was determined.

Thorough vortexing of the whey beverage was done followed by pour plating method. Decimal serial dilutions were made, then 1 mL of each dilution was transferred to both nutrient agar (HiMedia) and glucose yeast extract agar to check the bacterial and yeast contamination, respectively. After that, the plates were kept inverted at 30 °C for 24–48 h. Finally, the colonies were counted and the final microbial concentrations were determined.

2.8. Statistical analysis

Statistical analysis of the experimental results was done by One-way ANOVA (analysis of variance).

3. RESULTS AND DISCUSSION

Biochemical characterisation of whey and fruits (mango, guava, and kinnow) are shown in Table 1. Total soluble solids (TSS) was higher ($P < 0.05$) in mango, total soluble sugars was higher ($P < 0.05$) in guava, whereas kinnow showed highest protein content as compared to other fruits used in the study. In our study, paneer whey was used, which is considered as acidic in nature. The pH of the whey was recorded as 5.5. Whey (paneer whey) had a lactose content of 4.33%.

In the study conducted by Jitendra et al. (2017), the biochemical characteristics of guava (Allahabad safeda) were found to be 8 TSS Brix, 6.06% total sugars, and 3.55% reducing sugars, showing quite similar results to our study. Similarly, the biochemical analysis of kinnow showed resemblance with the studies conducted on the same variety by Anonymous (2009).

Table 1. Analysis of whey and fruits

Parameter (%)	pH	Total soluble solids	Total soluble sugars	Reducing sugars	Protein content	Lactose content
Whey	5.5	5	3.85	1.73	0.5	4.33
Mango	6.5	15	7.65	0.88	0.07	–
Guava	6	5	8.55	0.44	0.89	–
Kinnow	5	9	3.07	0.61	0.105	–
CD 5%	0.210	0.230	0.496	0.544	0.134	–

Data are presented as means of three replicates at CD 5%.

3.1. Selection of yeast culture for whey beverage

Internal transcribed spacer (ITS) region sequencing was carried out to identify the yeast strains. After phylogenetic analysis of the sequences, the three yeast strains, viz., MH6, CH1, and C1, were identified as *K. marxianus*, *Kluyveromyces* spp., and *S. cerevisiae,* respectively. *K. marxianus*, is commonly associated with dairy products and one of the most emerging and promising non-*Saccharomyces* yeasts for biotechnological applications. It is able to utilise lactose as sole carbon source due to the presence of LAC12 and LAC4 genes, which encode a lactose permease and a lactose galactosidase, respectively (Tofalo et al., 2014; Varela et al., 2017; Perpetuini et al., 2020). Fermentation of whey was carried out with 6.0% of inoculum. After fermentation, alcohol content was recorded as 1.57% for MH6, which was higher ($P < 0.05$) than for the other yeast strains (C1 and CH1). Total soluble solid, total sugar, and protein contents in the whey fermented by MH6 were lower (Table 2) due to the conversion of lactose into ethanol. Total sugar and lactose contents were higher ($P < 0.05$) in case of *S. cerevisiae* (C1) as it was not able to ferment the lactose present in the whey. After analysis of all parameters, MH6 (*K. marxianus*) was selected for fermentation of whey beverage. One of the phenotypes that distinguish *K. marxianus* and *K. lactis* from *S. cerevisiae* is their ability to utilise lactose. This has important biotechnological applications, including the production of the enzyme galactosidase for the food industry and the remediation of whey by lactose removal (Varela et al., 2017). Current research is also focusing on identification and evolution of pentose sugar transporters of *K. marxianus* for its future functional and biotechnological studies (Donzella et al., 2021).

3.2. Optimisation of parameters for whey beverage production

The optimised parameters (sucrose, temperature, pH, agitation, and fermentation time) for whey beverage production are depicted in Fig. 2 using *K. marxianus* as a starter culture. Because of its frequent isolation from foods, ability to assimilate lactose, thermotolerance, rapid growth, Qualified Presumption of Safety/Generally Regarded as Safe status (QPS/GRAS), and capacity to produce a variety of volatile metabolites with organoleptic properties (Gethins et al., 2015), this yeast is often used for fermentation in the food industry. During optimisation of sucrose level for fermented whey beverage production; fermentation of whey was carried out at different sugar levels, 3%, 5%, 6%, and 8%, making the total soluble solids 12, 16, 18, and 22 °Brix, respectively. Total soluble solids, total sugars, reducing sugars, and alcohol content at 8% sucrose concentration were higher ($P < 0.05$) than for other treatments,

Table 2. Biochemical analysis of different yeast strains after fermentation

Parameter (%)	Total soluble solids	Total sugars	Reducing sugars	Alcohol content	Protein content	Lactose content
MH6	7^{b}	1.16^{c}	0.37^{a}	1.57^{a}	0.57^{c}	1.26^{b}
C1	9^{a}	3.25^{a}	0.31^{b}	0.03^{c}	1.07^{b}	3.89^{a}
CH1	8.9^{a}	2.62^{b}	0.38^{a}	0.28^{b}	2.53^{a}	4.03^{a}
CD 5%	0.257	0.421	0.861	0.323	0.456	0.682

Data are presented as means of three replicates at CD 5%.

$^{a–c}$: Different lowercase letters in the same row mean significant differences ($P < 0.05$).

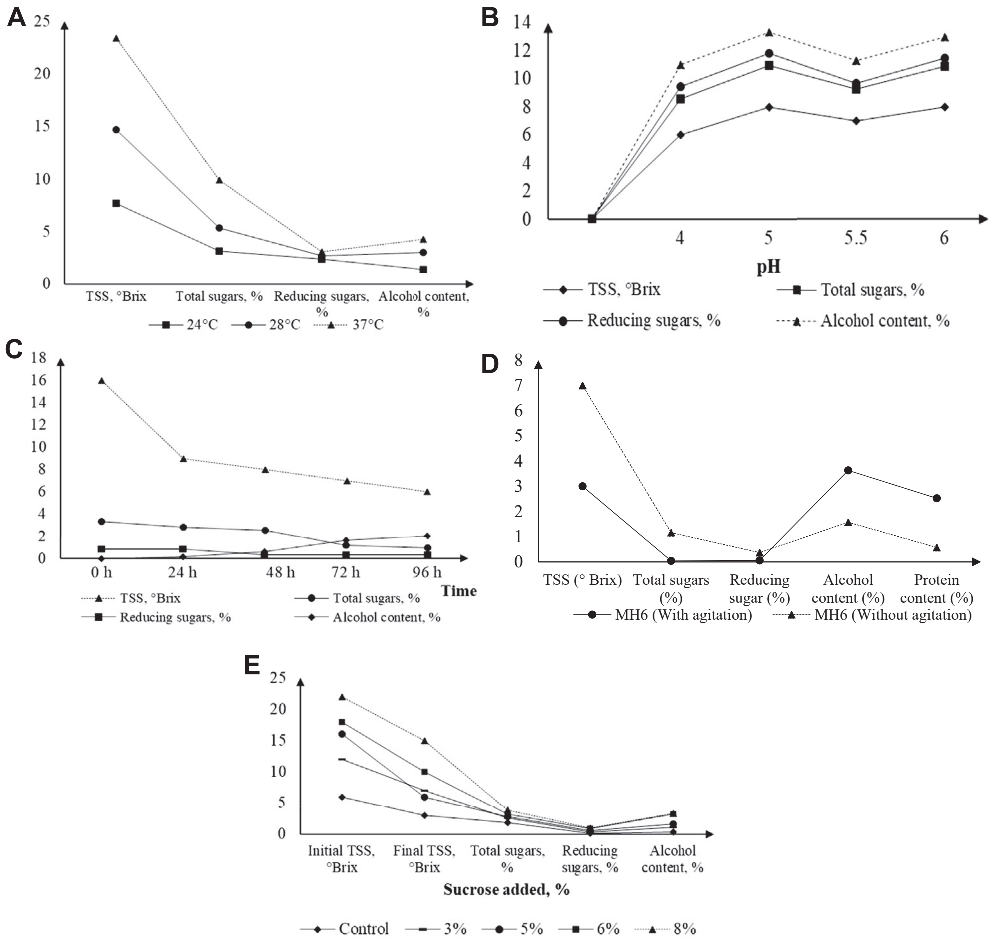

Fig. 2. Optimisation of biochemical parameters for fermentation of whey by *K. marxianus*. A: Total soluble solids, total sugars, reducing sugars, and alcohol contents at different temperatures; B: Total soluble solids, total sugars, reducing sugars, and alcohol contents at different pH values; C: Total soluble solids, total sugars, reducing sugars, and alcohol contents vs. time; D: Total soluble solids, total sugars, reducing sugars, and alcohol contents with and without agitation; E: Optimisation of sucrose addition.

but an off flavour was observed in the whey beverage for this treatment, which may be due to higher alcoholic fermentation as compared to other treatments. The sugar level of 5% was observed to be the most suitable for fermentation, providing good and refreshing flavour. At 28 °C the concentrations of total soluble solids, total sugars, and reducing sugars were lower ($P < 0.05$) than for other treatments, whereas the value of alcohol content (1.62%) was the highest. In a study conducted by Hadiyanto et al. (2014), a temperature of 30 °C was found as optimal for fermentation of cheese whey with *K. marxianus*, which is in concordance with the results of our study. Fermentation at 5.5 pH showed lower ($P < 0.05$) total soluble solids, total sugars, reducing sugars, and alcohol contents than at other pH values (4, 5, and 6). Yamahata et al. (2020) carried out sensory evaluation on fermented demineralised drinks with initial pH values of 5, 6, and 7, among which pH value of 5.0 was found to be most appropriate and showed similarity with our study.

The fermentation was also studied with and without agitation. In case of shaking (100 r.p.m.), the alcohol content was found to be 3.63%, the 0.04% total sugars, 0.56% reducing sugars, and 2.52% protein contents resulted in a buttery foam that gave a sour flavour. However, in case of fermentation without shaking, no foam was observed and contents of 1.57% alcohol, 1.16% total sugars, and 0.37% reducing sugars were recorded. The beverage had a good rather than sour flavour. So, in our study fermentation proceeded without agitation. Whey for fermentation was kept for different time periods (0, 24, 48, 72, and 96 h). The most appropriate time period for whey fermentation was 72 h, resulting in 7 °Brix total soluble solids, 1.16% total sugars, 0.32% reducing sugars, and 1.64% alcohol contents after fermentation. Total soluble solids, total sugars, and reducing sugars contents of 72 h were lower ($P < 0.05$) compared to other prolonged fermentation time periods. However, after 72 h there was a change in colouration and the smell of whey was like a sour lassi, hence, the time was not prolonged further. Therefore, conditions optimised for whey fermentation were 5% sucrose level (16 °Brix), 28 °C temperature, 72 h fermentation time without agitation at pH 5.5. In a study conducted by Longhi et al. (2004), 8.64 g L^{-1} ethanol was produced from whey within 16 h, however, in our study the alcohol production was restricted to 1.64% to enhance the refreshing flavour of the fermented whey beverage with pleasant aroma.

3.3. Blending of whey with fruits and flavouring agents

The whey was blended with fruit juices of kinnow, guava, and mango at different ratios and fermented at the optimised parameters. The biochemical analysis was carried out after fermentation and is depicted in Fig. 3. Total sugars content at 60:40 (whey:guava) ratio was

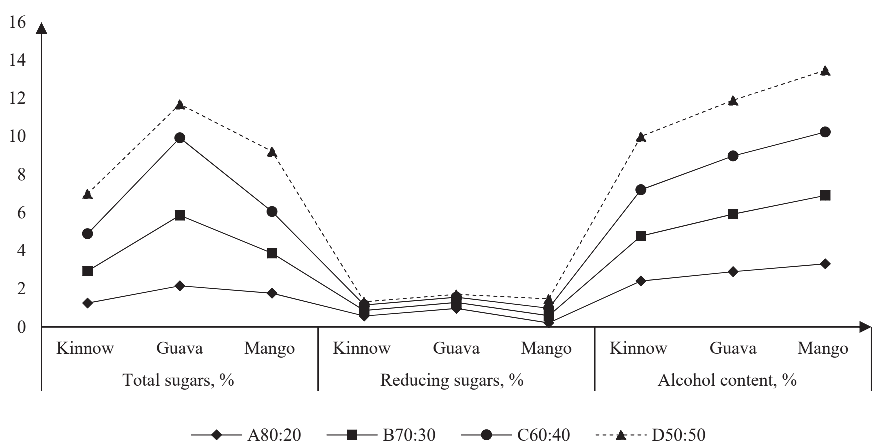

Fig. 3. Biochemical parameters after fermentation of whey-fruit (kinnow, guava, and mango) beverage blended at different ratios by *K. marxianus*

higher ($P < 0.05$) than for other ratios. Reducing sugars content at 80:20 (whey:guava) ratio was higher ($P < 0.05$) compared to other ratios. The alcohol content at the ratio of 50:50 (whey:-kinnow) was found the highest ($P < 0.05$) compared to other ratios with 3.59% (v/v) value. The alcohol content at 60:40 (whey:guava) ratio was found optimal (3.05%) for whey beverage production, as it resulted in good aroma and flavour compared to other ratios. Hence, the ratios 60:40 (whey: kinnow), 70:30 (whey: mango), and 60:40 (whey: guava) were selected.

Flavouring agents were also added to enhance the flavour of whey-fruit blend beverages. In our study, apple essence flavouring agent was used in the fermentation of whey by adding a few drops of apple essence. Similarly, cardamom, cinnamon, and fennel seeds were also added to enhance the flavour. On the basis of the biochemical analysis and sensory evaluation, the whey-guava beverage was the most preferred of all fruit blended beverages. Hence it was selected to blend with the spices. Cardamom, cinnamon, and fennel seeds were added separately to the whey-guava blend that was fermented under optimised conditions. Total soluble solids and alcohol contents were higher in cardamom-whey-guava beverage ($P < 0.05$) compared to other flavouring agents.

3.4. Sensory evaluation

Sensory evaluation of whey beverage was carried out on the basis of its flavour, appearance, mouthfeel, and overall acceptability. In the present study, different combinations of the whey beverages were prepared and evaluated on a nine point hedonic scale (Amerine et al., 1965) by panellists. The codes given to the whey beverages were: WK1 for whey-kinnow beverage, WM1 for whey-mango beverage, WG1 for whey-guava beverage, WA1 for whey-apple essence beverage, EWG1 for cardamom-whey-guava beverage, CWG1 for cinnamon-whey-guava beverage, and FWG1 for fennel seeds-whey-guava beverage. Among the samples EWG1 was the most preferred on the basis of colour, aroma, mouthfeel, and overall acceptability with the highest score of 8.16 (Fig. 4). The colour of the whey-guava beverage was creamy white and whey-kinnow and whey-mango beverages were whitish orange and yellowish, respectively. EWG1 gave a cool mouthfeel with balanced guava flavour compared to other beverages.

In a study conducted by Maya and Ritu (2016), a flavoured herbal-whey-guava beverage was formulated using various treatments of spices, and the most preferred whey-guava beverage was with 6 mL of basil, mint, ginger, *Aloe vera*, and lemon each, on the basis of a hedonic scale. In our study, the cardamom-whey-guava beverage was preferred, as it gave a very pleasant aroma and refreshing mouthfeel to the panellists.

Divya et al. (2014) also formulated a whey-guava beverage with different proportions of whey and guava juice, and the most preferred whey-guava beverage consisted of 67.5% of whey and 20% of guava juice. Similarly, in our study of whey beverage fermentation using functional starter yeast, whey-guava beverage with the essence of cardamom was preferred at an optimal ratio of 60:40, suggesting guava as a ideal fruit to blend with whey for beverage production.

3.5. Shelf-life studies of whey beverages

Shelf life of different whey beverages were studied for 45 d. The whey beverages were stored under refrigerated conditions after pasteurisation. The parameters viz., total sugars, reducing sugars, alcohol content, pH and microbial count were analysed at every 15 d intervals. The shelf

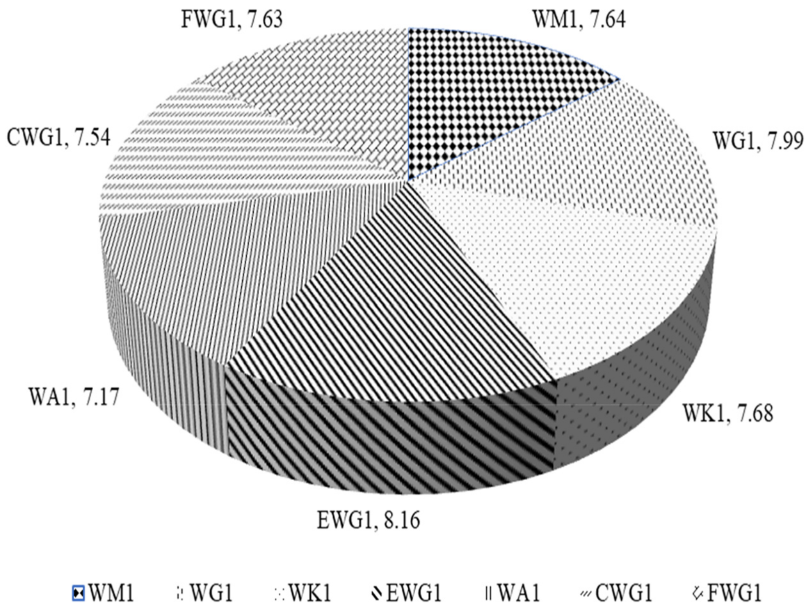

Fig. 4. Mean hedonic scores of different whey beverages. WK1: whey-kinnow; WM1: whey-mango; WG1: whey-guava; WA1: whey-apple essence; EWG1: cardamom-whey-guava; CWG1: cinnamon-whey-guava; FWG1: fennel seeds-whey-guava

life of these whey beverages were estimated to be 15 days depending upon the physicochemical and microbial analyses. After 45 d a change in the colour of the beverages was observed. There were slight variations in the parameters with increasing number of days in the pH, decrease in alcohol content, reduction in the contents of total sugars and reducing sugars followed by a slight decrease in total soluble solids. The present study shows that the beverages can be stored for 15 days without any major change in their biochemical parameters under refrigerated conditions after pasteurisation.

4. CONCLUSIONS

Production of fermented whey beverage is the best way to utilise the large quantities of whey produced during cheese making. The most typical approach for development of whey beverage is blending with fruits. However, fermentation can impart more nutrition to such beverages. Hence, fermented whey-fruit blend beverages can be an alternative healthy substitute for synthetic bottled fruit beverages. Whey fermentation by *K. marxianus* is an efficient method to produce high-value food bioingredients while reducing potential pollution from food waste. In the present research, a functional cardamom-whey-guava beverage was produced by the fermentative action of *K. marxianus,* suggesting it as a potential fermentative yeast for dairy industries.

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ABBREVIATIONS

TSS	Total soluble solids
NCBI	National Center for Biotechnology Information
ITS	Internal Transcribed spacer
GYE	Glucose yeast extract (HiMedia)
BLAST	Basic Local Alignment Search Tool
MEGA X	Molecular Evolutionary Genetics Analysis software
BOD	Biochemical Oxygen Demand

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