Characterization and development of Novel Whey-based Fermented Fruit Beverages

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Abstract

Fermented beverages are developed from fruits or cereals or nuts. The development of the beverage changes with the composition of the ingredient, the inoculum and the fermentation conditions. Milk-based fermented beverages are also available commercially. Whey is one of the waste milk products rich in proteins and can be considered for the development of beverages using fruits with rich medicinal value. The current study aims at developing a whey-based fruit-mixed fermented beverage (WFFB). Blueberry, kokum and pomegranate fruit pulps have been considered to prepare whey-based fermented beverages. The characteristic parameters are assessed prior to and post-development of beverages including organoleptic analysis.

The results indicated concentration and type of the fruit mixture influence the beverage characteristics and richness with phytochemical composition. The organoleptic studies indicated that whey-based plum and pomegranate beverages are in consumer demand. These novel beverages with increased nutritional value, attractive colors, fruity smells, low alcoholic concentration, eco-friendly and best from waste play a significant role for preferred start-ups.

Keywords: Whey, fermented fruit beverages, phytochemicals, proteins.

Introduction

Fermented beverages comprise of alcohol and have been consumed by humans since the neolithic age. These include wines, beers, spirits and other products. Most of these originate from the process of fermentation due to yeast activity or other microbial metabolism. Fermented beverages are majorly classified as wines fermented from various fruits containing fermentable sugars and beers, produced from starch-containing products. Most of cereals undergo enzymatic splitting by malting and mashing before the fermentable sugars become available for the yeasts. Different fruit-based beverages and fermented spirits are developed and their composition and bioactive components are well characterized.[5,6,12,17,21]

Sweet and acid whey was found to be the by-product during the production of cottage cheese and industrial casein with a COD of 68 gm/liter and 35 gm/liter of BOD. Due to the presence of lactose, proteins and mineral salts and high BOD and COD values, whey was considered a powerful pollutant. This industrial waste indicated a potentially negative environmental impact almost 100 times higher than that of domestic sewage[7]. The fermented whey known as Blaand with little alcohol content was produced for centuries by farmers of Scotland, Iceland, the Faroes and Norway for their consumption. Whey is used as a supplement of protein for athletes, gymnasts etc. and can assist in building muscle mass.

The current study is aimed at reusing whey waste and valorizing it to meet the UN Agenda 2030 of transforming the world's requirements, particularly with Goals for Sustainable Development, Responsible Consumption and Production, Industry Innovation and Infrastructure and, Drinking Water and Sanitation. The process of developing whey-based beverages and wines is a better option as these beverages contain relatively low alcohol (10%-11%) and, are free from caffeine. Earlier studies indicated the development of whey-based sports drinks[20] and fruit-based whey drinks [21]. The current study is a unique attempt in the Indian context, aimed at developing a whey-based fermented fruit beverage, an alternative to caffeinated and carbonated beverages.

Material and Methods

Sample collection: Milk samples were tested initially for starch, formalin, caustic ions, maltose, dextrin, alcohol, urea, sugar, salt and sugars adulteration. The samples that were devoid of these adulterant contents were considered for the preparation of acid whey[19]. The quality tests such as the methylene blue reduction test and clot on boiling were also given due consideration before whey preparation[1].

A quality check of the samples was considered as first step while developing the beverage. Collected milk samples were pasteurized. They were homogenized, chilled and standardized to 4.5% fat and 8.5% SNF. The microbial analysis for coliform bacteria, yeasts and molds was performed using standard plate count[19]. The whey was prepared as per standard protocol (Fig. 1).

The second step was the development of beverage and processing. During this process, the whey was pasteurized at 90°C and stored at 5°C. Whey was thawed and filtered using a muslin cloth and reheated at 65-70°C. Fruit extracts of blueberry (*Vaccinium meridionale)*, *kokum (Garcinia indica*) and a mixture of plum (*Prunus cultivar)* and Pomegranate *(Punica granatum)* were considered for fermented beverage preparation.

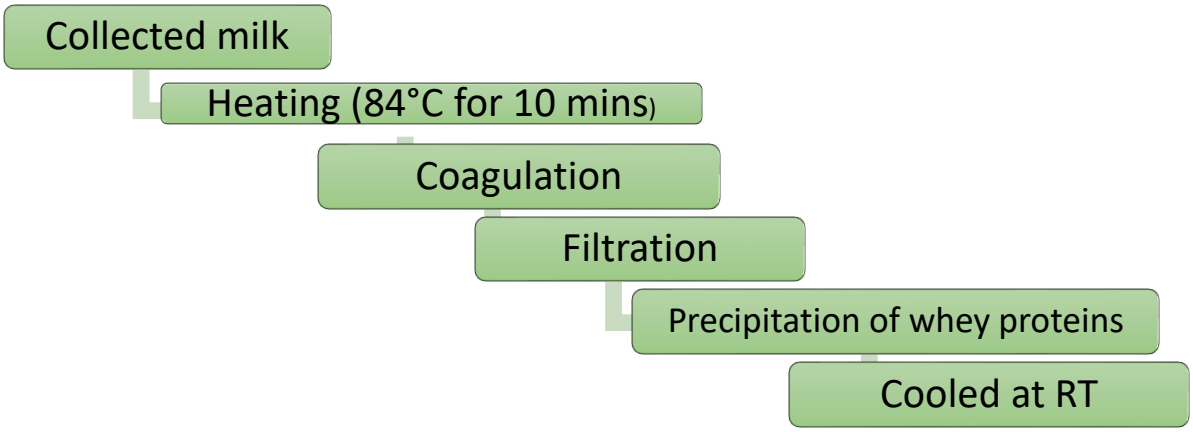

Fig. 1: Steps involved in Whey preparation

10%, 20% and 30% of blueberry, kokum and plum-pomegranate extracts in whey were added with yeast under aseptic conditions in a laminar cabinet. Commercially available dry yeast (*Saccharomyces cerevisiae*) was used for starter culture/ yeast pitching to initiate fermentation.

All the flasks were wrapped using cling wrap and kept for fermentation for 15 -25 days at temperatures ranging between 21°C to 27°C in the dark.

The CO_2 produced during fermentation was released by the disruption of the cling. The odor checks were carried out to assess contamination at an interval of 24 hours. The specific gravity and density of the samples were assessed before and after fermentation. The end of the fermentation process was confirmed by phase separation.

After the decantation, the upper clear layer was collected and, further processed for 15 days at 5°C. The cleared sample was drawn from each flask before the bottling process and subjected to the physico-chemical (Protein, Titrable acidity, Total suspended solids, Brix, Alcohol and organic acids) and phytochemical analysis [9,14]

Physico-Chemical Parameters: Physical parameters such as pH by laboratory pH sensor (Mettler Toledo, USA), density by densitometer (ABS DENS-V Spectro densitometer, India) and specific gravity (ABS-DensV) were assessed before and after the fermentation process.

Qualitative Test of Proteins (Ninhydrin test): 5-6 drops of ninhydrin solution were added to the sample and the mixture was boiled for 5 minutes. The formation of purple color indicates the presence of proteins in the test samples.

Protein Estimation by Pynes's method: 10ml of the sample was added with 5-6 drops of phenolphthalein indicator and 0.4ml saturated potassium oxalate. The mixture was kept for 2-4 minutes without disturbance and titrated against 0.1N NaOH until light pink color appeared. 2ml of neutralized formaldehyde was added and the solution turned to white. This solution was titrated against 0.1N NaOH till faint pink color appeared as an endpoint. Burette reading was recorded and the total protein percentage was calculated using the formula:

Total Protein (%)= Burette reading x 1.7

Titratable Acidity: 10ml of distilled water was added with 1ml phenolphthalein indicator followed by 10ml of the sample. The mixture was titrated against 1N NaOH until faint pink coloration appeared as end point.

Total Suspended Solids (TSS): 2.5ml of the sample was taken in an aluminum pan and was kept in the moisture analyzer and the TSS was estimated.

Brix (Estimation of Residual Sugar): Residual sugar of all the samples was calculated using the formula:

Specific gravity= (1- °Brix)/0.444 or Brix= 1-(Specific gravity X 0.444)

Estimation of Alcohol Content: Alcohol by volume of the samples was determined using the initial and the final specific gravities of the samples using the formula:

Alcohol by Volume (ABV) % = (Initial specific gravity-Final specific gravity) x132.5

Estimation of organic acids: Paper chromatography was performed to assess the presence of organic acids (malic acid, lactic acid and tartaric acid). The occurrence of malolactic fermentation was confirmed based on the Rf values.

Rf= Distance traveled by the solute / Distance traveled by the solvent.

Phytochemical analysis: The presence of tannins, flavonoids, terpenoids, glycosides and reducing sugars of the cleared sample after fermentation was determined following the standard protocols.[14]

Test for flavonoids (Ammonia test): 1 ml of 10% ammonia solution was added to the 2 ml of samples. The yellow color denoted the presence of flavonoids in the given sample.

Test for tannins (Lead acetate Test): 1 ml of 10% lead acetate was mixed with 2 ml of sample. The formation of a white precipitate indicates the presence of tannins.

Test for Carbohydrates/reducing sugars: Benedict's reagent was added to 2ml of samples in each test tube and was boiled until the color changed. Developing brick red, yellow, orange and green color indicates the presence of carbohydrates in the sample.

Test for Terpenoids: 2ml of the sample was added to the mixture of chloroform and conc. solution of H_2SO_4 carefully from the side of the test tube. The formation of reddish-brown coloration at the interface indicates the presence of terpenoids

Test for Glycosides: 2ml of the sample was added to a mixture of 5% $FeCl_3$, concentrated H_2SO_4 and glacial acetic acid. The brown ring denotes the presence of glycosides.

Microbiological Analysis: The sample was also checked for the presence of mold/yeasts using standard assessment techniques such as standard plate count and yeast / mold count.[1]

Standard Plate Count: Serial dilutions of 10^{-1},10^{-2} and 10^{-3} samples were prepared with Ringer solution (1 ml of the sample diluted with 9 ml Ringer solution). 1 ml of each dilution was considered for the standard plate count test using SPC agar[19]. The plates in triplicate were kept for incubation for 24 hours at 37°C and the results were recorded.

Yeast / Mold Count: Serial dilutions of 10^{-1},10^{-2} and 10^{-3} samples were prepared with Ringer solution (1 ml of the sample diluted with 9 ml ringer solution). 1ml of each sample dilution was considered for the test using PDA agar[19]. The plates in triplicate were kept for incubation for 48 hours at 25°C and the results were recorded.

Organoleptic Evaluation: Characteristic features of the fermented beverage positively impact the customer's preference and consumption. The sense organs are considered for the qualitative assessment[16]. Sensory evaluation was carried out for all the samples and the observations were recorded for taste, aroma, clarity and body on a 5-point scale and further assessed by descriptive statistics.

After getting satisfactory results, sodium meta bisulfite was added. The fermented beverage was bottled and sent for packaging/aging (Fig. 2). The interrelationship of physico–chemical parameters of the post-fermentation whey-based beverages was assessed using Pearson correlation (significant at 5% probability). This exercise was made to understand and strategize the process of whey-based fermented beverages development irrespective of the fruit/combination considered.

Results and Discussion

Whey-based beverages are of great commercial and nutritional importance and are viable for industrial production. Numerous studies of whey beverages indicated their food value and nutritional importance and classified them into mixtures of whey, with fruit or (rarely) vegetable juices; dairy-type, 'thick' beverages, thirst-quenching carbonated beverages and alcoholic beverages.[4,5,9]

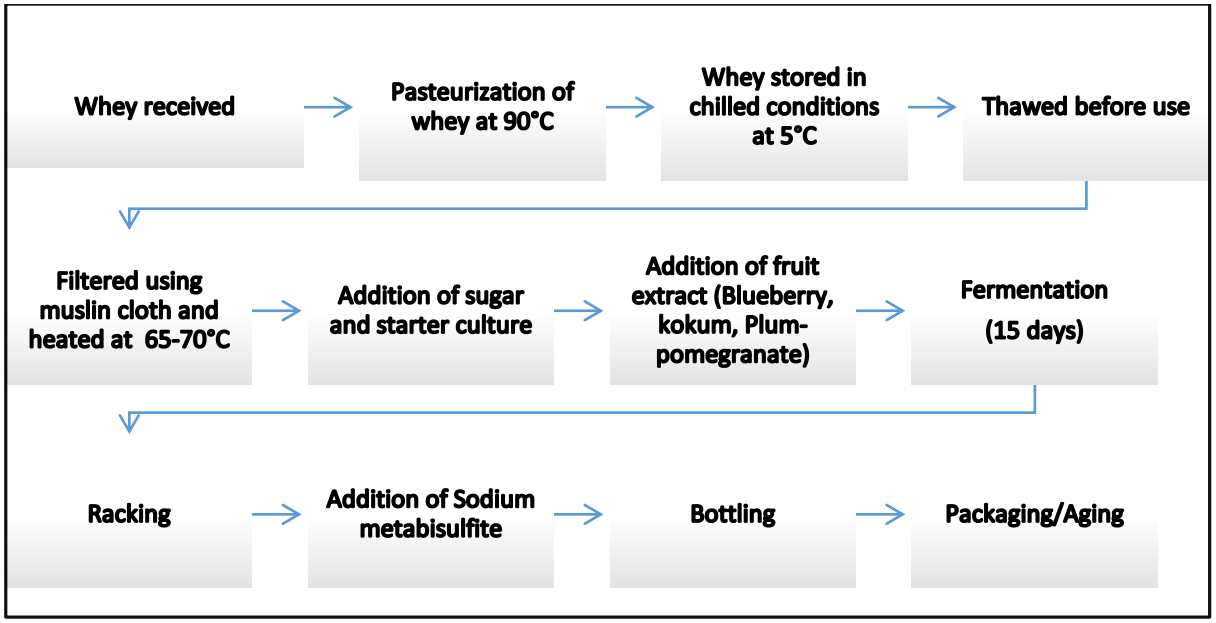

Fig. 2: Steps involved in the development of beverage

The whey-based beverage with the addition of different concentrations of hydrolyzed collagen was found to increase the bioavailability, nutritional value and antioxidant activity of the beverage as well as act as an antimicrobial agent[3]. Similarly, the previous studies indicated different types of whey based fermented beverages prepared with fruit pulp, sugar and *Lactobacillus plantarum* for fermentation[2]. The other types of whey beverages were prepared using either of *Lacidophilus* LA-5 or *Bifidobacterium animalis* spp *lactis* BB-12 bacterial species or both and replaced the fruit with different milk by-products[11]. The recent whey date beverage preparation considered Kefir grains and response surface methodology in the Fermentation procedure[15].

The common yeast *Saccharomyces serviceae* was considered as the microbial fermenter during the current whey-based fruit-fermented beverage preparation. During the current study, the milk samples collected from different locations showed almost similar composition values of fats, temperature and SNF. The fat content values varied from 3.6 to 4.1 % with an average of 3.77 % and the SNF values were almost non-significant as well as temperature (Table 1).

The milk certified with adulterant-free conditions was considered for whey preparation and subsequent fermented fruit beverage processes. The protocol of the whey-based beverage process was initiated soon after the whey was pasteurized. Previous studies have considered mango, pineapple (*Ananus comosus)* as the fruits for the preparation of whey-based beverages[9]. The current fruit-based beverage is unique in its way while considering the choice of fruits such as blueberry (*Vaccinium meridionale*), kokum *(Garcinia indica*), plum (*Prunus cultivar)* and pomogranate (*Punica granatum).* The selection of fruit pulp supplements was based on their medicinal value [8,10,18,22].

Table 1
Heat map depicting the values of fat, SNF and temperature of raw milk samples

Fat	SNF	Temperature
3.9	8.5	5.2
3.7	8.3	5
3.8	8.3	5
3.7	8.3	5
3.7	8.3	5
3.7	8.3	5
3.6	8.4	5.2
3.6	8.4	5.2
3.6	8.4	5
3.5	8.5	5.2
3.7	8.5	5
3.6	8.5	5
3.9	8.7	5.2
3.8	8.6	5
3.9	8.5	5
3.9	8.5	5
3.8	8.5	5
3.9	8.3	5
4	8.6	5
4.1	8.5	5.2

Table 2
Physicochemical parameters of the 3 fermented beverages (Mean ± SE)

Parameters	**WBB**			**WKK**			**WPP**		
Fruit Extract conc.	**10%**	**20%**	**30%**	**10%**	**20%**	**30%**	**10%**	**20%**	**30%**
Specific gravity	0.062	0.061	0.048	0.080	0.067	0.062	0.080	0.067	0.062
Density(g/cm^3)	0.062	0.022	0.017	0.066	0.049	0.035	0.080	0.040	0.010
pH	4.5 ±0.01	4.1 ±0.01	3.2 ±0.01	3 ±0.005	2.8 ±0.01	2.3 ±0.01	2.6 ±0.01	2.9 ±0.001	3.2 ±0.002
Alcohol by volume ABV(%)	8.2 ±0.02	8.1 ±0.01	6.3 ±0.01	8.7 ±0.01	6.5 ±0.02	6.8 ±0.01	10.6 ±0.03	8.9 ±0.03	8.2 ±0.02
Total protein (%)	0.51	1.36	0.34	0.51	0.34	0.17	0.68	0.68	0.85
Titrable acidity (%)	0.8	0.57	0.2	0.6	0.19	0.17	1.2	0.8	0.5
TSS (%)	7.67± 0.01	14.04± 1.02	16.44± 1.03	8.03± 0.01	14.79± 1.01	16.04± 1.23	7.26± 0.01	15.56 ±1.02	15.88 ±0.01
°Brix	15.5± 0.04	15.4 ±0.05	12 ±0.01	16.5 ±0.06	12.33 ±0.01	12.9 ±0.01	20.2 ±1.01	16.9 ±0.05	15.6 ±0.01
Rf	0.63	0.7	0.75	0.84	0.83	0.7	0.79	0.72	0.8

The required ingredients of fruit pulp (10%,20% and 30%), sugar and yeast inoculum were added and kept for fermentation for 15 -25 days at temperatures ranging between 21°C to 27°C in dark and wrapped in cling wrap so that the CO_2 produced during the process can be released. The process was carried out in triplicate for each of the three trials of each variety.

The physico –chemical properties of the post-fermentation whey-based beverages indicated that the overall values varied with increasing fruit sample concentrations though three combinations were different (Table 2). With increasing concentration, each of the combinations indicated decreasing values of specific gravity, density, pH, ABV (Alcohol by volume), titrable acidity and Brix (Residual sugar). On the contrary, the percentage of total suspended solids showed an increase with increasing concentrations of fruit pulp.

A correlation between different parameters was considered during the study. The Pearson Correlation was assessed to characterize the fermentation levels and revealed noticeable changes. The three different combinations followed different patterns and the parameters showed unique and different levels of correlations. The whey-based blueberry (WBB) sample showed a strong + ve correlation between the pH and ABV, density, titratability and specific gravity (>0.80). The total SS and Rf showed a high correlation with each other (>0.95) and a negative correlation (>-0.8) with pH ABV, density, titratability and specific gravity.

In the whey-based kokum fruit (WKK) fermentation, a different correlation pattern was indicated between pH, ABV, titrability, Rf, specific gravity and density (positive correlation > 0.75). TSS showed a –ve correlation not only with Rf (-0.67) but with all other parameters as well. The interrelationship between the parameters considered during whey-based plum and pomegranate (WPP) fermentation showed another unique condition. The pH showed a highly negative correlation with ABV, titrability, specific gravity and density (>-0.97); however, a highly positive relation was noticed between ABV and titrability and density. Unlike the previous two combinations, pH showed a positive correlation with TSS, protein (0.88) and specific gravity and density (0.99) (Fig. 3).

Whey based Blue Berry fermentation(WBB)

	pH	ABV(%)	Protein	Titrable acidity	TSS	Rf	change in Specific gravity
pH							
ABV%	**0.97**						
Protein	0.37	0.59					
Titrable acidity	**1.00**	**0.94**	0.29				
TSS	-0.89	-0.74	0.10	-0.93			
Rf	-0.95	-0.84	-0.06	-0.97	**0.99**		
change in Specific gravity	**0.97**	**1.00**	0.58	**0.95**	-0.75	-0.85	
Density	**0.80**	0.62	-0.26	**0.85**	-0.99	-0.95	0.64

Whey based Kokum fermentation(WKK)

	pH	ABV(%)	Protein	Titrable acidity	TSS	Rf	change in Specific gravity
pH							
ABV%	**0.80**						
Protein	0.97	0.92					
Titrable acidity	**0.75**	**1.00**	0.89				
TSS	-0.81	-1.00	-0.93	-0.99			
Rf	0.98	0.65	0.90	0.59	**-0.67**		
change in Specific gravity	**0.80**	**1.00**	0.92	**1.00**	-1.00	0.65	
Density	**0.96**	0.94	1.00	**0.91**	-0.95	0.87	0.94

Whey based Plum+Pomogranate fermentation(WPP)

	pH	ABV(%)	Protein	Titrable acidity	TSS	Rf	change in Specific gravity
pH							
ABV%	**-0.97**						
Protein	0.87	-0.73					
Titrable acidity	**-1.00**	**0.99**	-0.82				
TSS	0.88	-0.97	0.53	-0.92			
Rf	0.11	0.12	0.60	-0.03	**-0.37**		
change in Specific gravity	**-0.97**	**1.00**	-0.73	**0.99**	-0.97	0.11	
Density	**-1.00**	0.99	-0.82	**1.00**	-0.92	-0.03	0.99

Figure 3: Corel gram of Whey based fruit fermentations (WBB,WKK and WPP) parameters

The composition of good phytochemical compounds in a beverage determines its nutritional value for good health. The whey-based fermented beverages comprising of blueberry (*Vaccinium meridionale*), kokum *(Garcinia indica*), plum (*Prunus cultivar)* and pomegranate (*Punica granatum)* as fruit pulp supplements also increase their medicinal value. Irrespective of the concentration and type of fruit pulp added, all the beverage samples indicated the presence of flavonoids, tannins, terpenoids and glycosides. These phytochemicals have medicinal values as anti-microbial, anti-parasitic, anti-viral, anti-allergic, anti-inflammatory, chemotherapeutic, anti-hyperglycaemic and anti-spasmodic characteristics[13].

Microbiological analysis: Samples represented white, whitish-yellow, off-white and yellow colonies in all the whey-based fermented beverages with circular shapes and smooth waxy textures. The presence of molds was not observed.

Organoleptic Evaluation: The main way to evaluate its organoleptic properties is a sensory evaluation which is a very important tool to assess the acceptability of a beverage by the consumer. This tool plays a major role while deciding the acceptability despite the advanced levels of development of laboratory tools for analyzing the quality of the wine. In WBB samples, 30%WBB samples showed major acceptability by the consumers in terms of clarity, taste and body of the sample than the other two. In WK samples, 20% WK samples showed major acceptability by the consumers in terms of clarity, taste and body of the sample than the 10% and 30%.

In WPP (mix) samples, out of the 3 concentrations, 30% WPP (mix) samples showed more acceptability by the consumers in terms of clarity, taste and body of the sample. In terms of aroma, all the whey wine samples were reported to have a fruity aroma followed by a flowery aroma (Fig. 4).

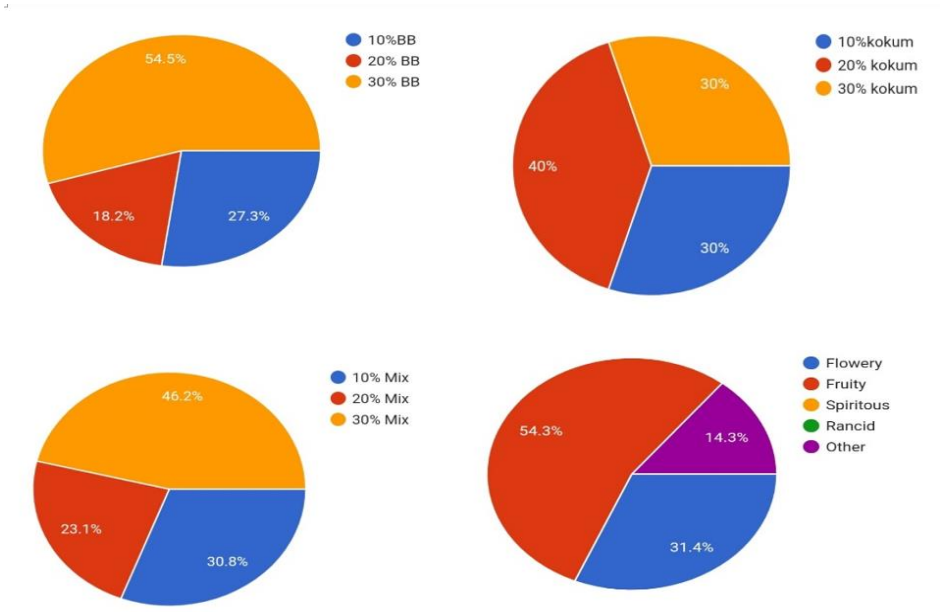

Fig. 4: Organoleptic analyses of the whey-based fermented fruit beverages

Fig. 5: Whey-based fermented Fruit beverage (Blueberry, Kokum and Plum+ Pomegranate)

The fruity aroma in wine is mainly contributed by the various esters of fatty acids that are formed due to the reaction between an organic acid and alcohol during fermentation as mentioned in previous studies. The whey wine samples also had a slightly bitter finish. This bitterness may be due to the polyphenol or tannin content present in the samples (Fig. 5).

Conclusion

The WFFB is a novel beverage that can reduce the wastage of whey which has high nutritional value. The process supports promoting the agenda of UN 20203 as well as a good non-alcoholic beverage that has low alcohol, good nutrition value and is healthy due to phytochemical composition. The attractive colors of the fermented whey-based fruit beverages and the fruity smells and low alcoholic concentration attract the consumers. The prospects for the developed WFFB are bright, economically feasible and satisfy consumers' preferences, good taste, color and smell.

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