

Formulation and evaluation of whey based ready-to-serve therapeutic beverage from *Aloe debrana* juice

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Article history:

Received: 1 March 2021

Received in revised form: 6 April 2021

Accepted: 13 June 2021

Available Online: 18 July 2021

Keywords:

Aloe debrana juice,
Therapeutic beverages,
Whey,
Sensory analysis,
Starter culture bacteria

DOI:

[https://doi.org/10.26656/fr.2017.5\(4\).109](https://doi.org/10.26656/fr.2017.5(4).109)

Abstract

The research was conducted with the aim to formulate and evaluate whey-based ready-to-serve (RTS) therapeutic beverage from *Aloe debrana* juice and determine the phytochemical composition and sensory acceptability of the formulated therapeutic beverage. The whey and *Aloe debrana* juice were prepared in three blending ratios including WA1 (70:30), WA2 (80:20), and WA3 (90:10) respectively; and control (100% whey) with the addition of ingredients such as: sugar 15 g/100 mL, vanilla 0.1 g/100 mL, starter culture of *Streptococcus thermophiles* and *Lactobacillus bulgaricus* (0.02 g/100 mL). The four formulations were generated by Central composite design (CCD). The formulated raw materials range 70% to 90% whey, 10% to 30% *Aloe debrana* juice, and control (100% whey). Design expert 7.0 software was used to formulate the two components. Therapeutic beverages were fermented for 9 hrs at 37°C and 120 rpm using shaker incubator. Mineral contents (ppm) of the formulated beverage ranged from 375 to 573.01, 1244.02 to 1491.03, 460.01 to 742, and 93.02 to 107.0 of sodium, potassium, calcium, and magnesium, respectively. Phytochemical composition (mg/100 g) of the formulated beverages and *Aloe debrana* juice ranged from 43.20 to 147.56, and 4.98 to 11.17 for phytic acid and tannin respectively. The phytic acid and tannin content of *Aloe debrana* juice was (467.6 mg/100 g) and (130.1 mg/100 g), respectively. The therapeutic beverages were stored at room temperature for nine days without any change in sensory attributes. Sensory quality evaluation results showed that sample containing 70% whey and 30% *Aloe debrana* juice (WA1) scored the highest in overall acceptability 4.15, 3.7, 3.2, and 3.03 for the first, third, sixth, and ninth days of room storage temperature, respectively. Based on sensory quality evaluation parameters, therapeutic beverage containing 70% Whey and 30% *Aloe debrana* juice blend ratio revealed sensory acceptable for therapeutic beverage production. Therefore, it can be concluded that *Aloe debrana* juice can be incorporated with whey up to 30% to produce organoleptically acceptable therapeutic beverages.

1. Introduction

Today's busy consumer gets the advantage of convenience and portability from ready-to-drink therapeutic beverages. Ready-to-drink whey beverages are a good source of whey protein and are preferred because of their excellent nutritional qualities, bland flavour, ease of digestibility, and unique functionality in the beverage system (Chavan *et al.*, 2015). Whey is one

of the dairy by-products obtained from the dairy industry producing cheese and it is highly nutritious (Yalcin *et al.*, 1994). The proteins obtained from whey are especially rich in essential amino acids such as tryptophan, which helps enhance brain serotonin levels (Delgado-Andrade *et al.*, 2006).

The total whey production capacity of the world accounted for 187 million metric tons in 2008 where

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only 3.2 million (1.7%) was used by different food industries for manufacturing value-added products like whey powder, whey isolates and whey protein concentrates. In 2009 the utilization rate was increased to 6.3% and sales of 1.03 million sales of energy drinks are increasing than any other food items introduced in the U.S. market (Miller, 2009). According to Smithers *et al.* (1996), the world produces more than 80×10^9 L of whey which is disposed of in rivers and lakes. The annual milk production capacity of Ethiopia is 242,564 (HL), cheese 185 (ton) and whey 1665 (ton) (Rijkers *et al.*, 2010). Even if, Ethiopia has more than 45 small and medium scale dairy processing industries, most of the whey is underutilized (Personal communication). Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO and WHO, 2012). Probiotic bacteria are known to provide health-promoting as well as nutritional benefits such as faster relief from diarrhoea, prevention against lactose intolerance, minimizing the risk of cholesterol and prevention of urogenital infection (Anukam *et al.*, 2006).

Many researchers have developed whey-based beverages by blending whey with different fruit juices. Dhamsaniya and Varshney (2013) have prepared beverages by mixing processed banana juice, *Mentha arvenensis* extract, sugar powder and whey at different proportions. Shukla *et al.* (2013) formulated probiotic beverages from whey and pineapple juice. Sasi (2015) formulated probiotic beverages by blending whey with *Aloe vera* juice using probiotic culture of (*Bifidobacterium bifidus*) with the addition of sugar level 15% and fermentation time of 5, 9, 13, 17, 21 and 24 hrs. Sakhale *et al.* (2012) developed ready-to-serve (RTS) beverages from whey and mango CV. Kesar. Boghani *et al.* (2012) has prepared ready-to-serve beverage (RTS) by blending papaya and *Aloe vera* juice.

Aloe vera (*Aloe barbadensis* Miller) belongs to Lileacea family and traditionally it has been used as contemporary folk remedy (Virdi, 2009). The present dietary scenario necessitates the incorporation of novel ingredients in commonly consumed foods rather than developing new food products (Aleem Zakir *et al.*, 2012). *Aloe debrana* and *Aloe trichosantha* are the two species which grows usually on gentle slopes between 2000 and 2700 meters above sea level in Shoa, Wollo, Gojam floristic regions. Its flowering season is between December to February (Demissew and Nordal, 2010). In this research, *Aloe debrana* was selected for research material. *Aloe* juice is rich in bioactive compounds which serve as an antiulcer, antiseptic, antibacterial, anti-inflammatory, antioxidant, antidiabetic and anticancer agent. The bioactive compounds of *Aloe* juice are used as

antidiabetic, antiulcer, antiseptic, antibacterial, anti-inflammatory, antioxidant and anticancer agents and it is also, effective in treating gastrointestinal problems, radiation injury, stomach ailments, dysentery, in the treatment of skin diseases and diarrhoea (Rabe and Van Staden, 1997). Therefore, based on these facts, the investigation was to formulate and evaluate the phytochemical composition and sensory acceptability of the therapeutic beverages prepared using whey and *Aloe debrana* juice.

2. Materials and methods

2.1 Source of materials, sample collection, transportation and preparation

The experiment for the formulation of whey-based beverages was conducted at Addis Ababa University College of Natural Science, Institute of Biotechnology. The preparation of whey, extraction of *Aloe debrana* juice and sensory analysis of the beverages were conducted in Addis Ababa Institute of Technology, School of Chemical and Bio-Engineering (Food Engineering Stream) laboratory. The fresh and matured leaves of *Aloe debrana* leave samples were collected and transported in a refrigerated ice-cold box from Chanco, which is located 40 km from Addis Ababa; the capital city of Ethiopia to the north. It was identified by a botanist at Addis Ababa University College of Natural Resources National Herbarium Department. Skimmed milk was purchased from Sholla/Lame dairy enterprise and transported in a refrigerated ice-cold box to the Food Engineering Laboratory and Holeta Milk Research Centre for further analysis.

2.1.1 Whey processing

Whey preparation was done according to the procedure described by (De, 2001) with minor modification. Skimmed milk (2% fat) was obtained from the Lame dairy processing factory. Stainless steel vessel was used to boil the milk at 80°C for 10 minutes. Acidification of the boiled milk was done after cooling to 72°C by adding 0.2% (2 g per kg of milk) citric acid followed by continuous stirring until the coagulation of milk proteins (casein) taken place. Cooling was done at room temperature to the mixture of whey and casein and then a muslin cloth was used to separate the whey from casein. Glass bottle was used for filling the obtained whey and stored in the refrigerator ($4 \pm 1^\circ\text{C}$) for further use.

2.1.2 *Aloe debrana* juice extraction

Cold extraction method was applied to extract *Aloe debrana* juice and processed into juice as per the method described by (Williams, 1984). *Aloe debrana* juice was

extracted using the traditional hand filleted *Aloe* processing method and processed into juice as per the method reported by (Ramachandra and Rao 2008). The solution of potassium meta-bisulphite (KMS) of 500 ppm was used to dip the freshly harvested *Aloe debrana* leaves and washed thoroughly with tap water and kept for flash cooling to 5°C for juice stabilization. Stainless steel knife was used to extract the juice and leaves were cut vertically into two half and it was allowed to settle for 12 hrs and then homogenized using a mixer grinder. The pH of the *Aloe debrana* juice was adjusted to 3.0 after it was filtered by adding citric acid and browning was controlled while high heat treatment by adding ascorbic acid. Further, it was deaerated, pasteurized, flash cooled and stored. For further use, the obtained *Aloe debrana* juice was stored at refrigerated temperature.

2.1.3 Whey-Aloe debrana juice blended therapeutic beverages product design and development

According to (Sasi, 2015), whey-Aloe vera juice was optimized with different blend ratios, such that sample A (65 whey: 35 *Aloe vera*), sample B (70 Whey: 30 *Aloe vera*), sample C (75 Whey: 25 *Aloe vera*) and sample D (80 whey:20 *Aloe vera*). Based on Sasi's finding, the researcher of this paper has optimized the combination of whey and *Aloe debrana* juice with different blends ratios, such that sample A control (100% whey), Sample B (70 Whey: 30 *Aloe debrana*), sample C (80 Whey: 20 *Aloe debrana*) and sample D (90 Whey: 10 *Aloe debrana*). The beverages incubation period was optimized by inoculating a concentration of starter culture of 0.02 g/100 mL *Streptococcus thermophiles* and *Lactobacillus delbrueckii subsp. Bulgaricus*) to whey and sample with the addition of sugar level 15% and vanilla 0.1 g.

2.2 Mineral content

Liberato et al. (2013) method was used to determine mineral contents Ca, K, Na and Mg using atomic absorption spectrophotometer (AAS) (AA-6800, Shimadzu Japan) was used for mineral contents determination.

2.3 Phytochemical composition analysis

2.3.1 Phytic acid

The liquid *Aloe debrana* juice and therapeutic beverages were lyophilized and solid samples were obtained. Phytic acid analysis was determined by using Latta and Eskin method as modified by (Vaintraub and Lapteva, 1988). Approximately 10 mL of 0.2 N HCl was used to extract 0.5 g of dried sample for 1 hr at an ambient and centrifuged (3000 rpm/30 mins). Phytate estimation was done using the clear supernatant. Wade

reagent of 2 mL was added to 3 mL of supernatant sample solution and then the solution was homogenized and centrifuged at (3000 rpm/10 mins). UV-Vis spectrophotometer (121427, Cambridge England) was used to measure the absorbance at 500 nm. The difference in absorbance of the blank (3 mL of 0.2N HCl + 2 mL of Wade reagent) and that of the assayed sample was used to calculate phytate concentration. A phytic acid standard curve was used to calculate the amount of phytic acid and the result was described as phytic acid in µg/g fresh weight. The slope and intercept were used for calculation which was maintained from a standard curve that was made from absorbance versus concentration.

$$\text{Phytic acid} = \frac{(\text{absorbance} - \text{intercept})3}{(\text{slope} \times \text{density} \times \text{weight of sample} \times 10)} \quad (1)$$

2.3.2 Catechin equivalent

The Catechin content of *Aloe debrana* juice and therapeutic beverages were analysed as follow: The liquid *Aloe debrana* juice and therapeutic beverages were lyophilized and solid samples were obtained. 0.06 g of sample was weighed in a screw cap test tube and 10 mL 1% HCl was added in methanol to the tube containing the sample, lid according to (Maxson and Rooney, 1972). The tube that was kept on a mechanical shaker was centrifuged at 1000 rpm for 5 mins at room temperature. In another test tube, 1 mL supernatant was taken and mixed with 5 mL of vanillin-HCl reagent. The reaction was completed in 20 mins and the absorbance was read at 500 nm using a UV-Vis spectrophotometer (121427, Cambridge England). From the absorbance of the corresponding vanillin contain sample; the absorbance of the blank is subtracted. The curve of (Absorbance vs Catechin) have been used to construct the standard curve and the linear to produce the standard curve portion of the curve were extrapolated.

$$\frac{\text{Tannin in mg}}{100\text{g}} (\text{Catechin equivalent}) = (\text{absorbance density} \times \text{weight of sample} \times 10) \quad (2)$$

2.4 Sensory quality evaluation

The beverage samples were evaluated according to the method described by (Larmond, 1977) for their sensory characteristics namely colour, flavour, body or consistency and overall acceptability were conducted by 10 untrained judges of youngsters of age 20-39 that were drawn from staff and postgraduate students of the Food Engineering Stream. The untrained judges used water for washing their mouth in between tasting and were asked to record their observations on the score sheet based on a 5 points hedonic scale (5 and 1) points showing like very much and dislike very much) respectively. The data were subjected to statistical analysis using SPSS (version 20.0, Microsoft Inc. USA) and comparison of means by Duncan test.

2.5 Experimental design and data analysis

Central composite design (CCD) of design-expert 7.00 software was used to constrain two components. The experimental runs were conducted with two replicates and were randomized completely to minimize error as indicated in Tables 1 and 2. All statistical analyses were performed using SPSS (version 20.0, Microsoft Inc. USA) and comparison of means by Duncan test, and the result was reported as a mean \pm standard error (SE). To evaluate the data, a one-way analysis of variance (ANOVA) was used. To consider the statistically significant difference a p-value of 0.05 or less was used.

Table 1. Experimental design and treatment combinations using central composite design (CCD) for the three formulations.

Formulations	Whey (%)	<i>Aloe debrana</i> juice (%)
WA0 (control)	100	0
WA1	70	30
WA2	80	20
WA3	90	10

Table 2. Complete experimental design matrix of central composite design (CCD)

Variables	Levels of treatments		
	-1	0	1
Whey- <i>Aloe debrana</i> blend proportion (%)	2.3	5.65	9

3. Results and discussion

The production of therapeutic beverages by mixing whey with *Aloe debrana* juice (30%, 20% and 10% individually in each therapeutic beverage formulation) as well as storage time (0, 3 days, 6 days and 9 days) at room storage temperature. Based on the sensory quality evaluation results of the fresh samples of whey-*Aloe debrana* juice blended and control whey beverages were presented for shelf stability analysis for nine days at room storage temperature. Comparisons were drawn between the control (100% whey beverage) and *Aloe debrana* juice blended therapeutic beverages. The control used for comparison in this study was prepared from whey with superior quality as recommended by

other researchers (Gagrani et al., 1987; Shukla et al., 2004). To explain the variation of quality of therapeutic beverage samples at various conditions were presented in the following Tables.

3.1 Mineral composition of raw materials

Table 3 below shows the mineral contents in parts per million (ppm) of the raw materials (whey and *Aloe debrana* juice) used for the formulation of therapeutic beverages in this finding. The mineral contents (ppm) of whey and *Aloe debrana* juice were 595 and 12, 1547 and 1032, 777 and 216, and 104.5 and 149 for sodium, potassium, calcium, and magnesium, respectively. Significant variations of the essential mineral contents between the raw materials were observed. Whey was shown to have a significant ($P < 0.05$) higher amount of Ca (777) as compared to *Aloe debrana* juice (216.5). The whey had more Potassium (1547) and Sodium (595) contents than *Aloe debrana* juice which contained (1032) and (12) respectively. In general, this finding indicated that both raw materials used for the preparation of whey-*Aloe debrana* juice blended therapeutic beverages could be a good source of these essential minerals.

Table 3. Mineral composition of the raw materials (ppm)

Type	Mineral content in (ppm)			
	Na	K	Ca	Mg
Whey	595 ^b	1547 ^b	777 ^b	104.5 ^a
<i>Aloe debrana</i> juice	12 ^a	1032 ^a	216.5 ^a	149 ^b

Values are presented as means of duplicate. Values with the same superscript within the same column are not significantly different ($p > 0.05$).

3.1.1 Mineral composition of formulated therapeutic beverages

The mineral composition of therapeutic beverages is presented in Table 4. The control therapeutic beverage has shown significantly ($P < 0.05$) higher sodium (573 ± 0.02) and Calcium content (742.0 ± 0.00) than *Aloe debrana* blended therapeutic beverages. This could be due to the high concentration of calcium available in milk and milk bi-products in nature. The mineral contents of the formulated therapeutic beverages ranged

Table 4. Mineral composition of formulated therapeutic beverages (ppm)

Sample	Mineral content in (ppm)			
	Na	K	Ca	Mg
WA0 (control)	573.01 ± 0.02^d	1467.5 ± 0.7^c	742.0 ± 0.00^d	95.95 ± 0.07^c
WA1	375.0 ± 0.01^a	1244.02 ± 0.03^a	460.01 ± 0.02^a	93.02 ± 0.02^a
WA2	495.0 ± 0.04^b	1491.03 ± 1.3^d	614.01 ± 0.01^b	107.0 ± 0.00^d
WA3	497.0 ± 0.00^c	1391.01 ± 0.02^b	622.0 ± 0.00^c	95.02 ± 0.03^b

WA0: Control therapeutic beverage prepared from whey only, WA1: therapeutic beverage prepared from whey and 30% (v/v) *Aloe debrana* juice, WA2: therapeutic beverage prepared from whey and 20% (v/v) *Aloe debrana* juice, WA3: therapeutic beverage prepared from whey and 10% (v/v) *Aloe debrana* juice. Values are presented as mean \pm SD. Values with the same superscript within the same column are not significantly different ($p > 0.05$).

from 375.00 to 573.01, 1244.02 to 1491.03, 460.01 to 742.00, and 93.02 to 107.00 for sodium, potassium, calcium, and magnesium respectively. In general, sample coded WA2 was observed with the highest score in potassium (1491 ± 1.3) content and magnesium (107.0 ± 0.00) among *Aloe debrana* juice blended beverages including control beverage whereas, the control beverage exhibited significantly ($P<0.05$) higher sodium (573 ± 0.02) and calcium (742 ± 0.00) content than *Aloe debrana* juice blended therapeutic beverages. The mineral content of this study is in line with the standard set by (CODEX CAC/GL, 1991).

3.2 Phytochemical composition of formulated therapeutic beverages and *Aloe debrana* juice

The *Aloe debrana* juice scored significantly ($P<0.05$) higher phytic acid (467.68 ± 0.01) content than the formulated therapeutic beverages. The Phytochemical composition of the formulated therapeutic beverages (mg/100 g) ranged from 41.75 to 147.56, and from below detection limit (BDL) to 11.17 for phytic acid and tannin respectively. The raw material *Aloe debrana* juice has scored significantly ($p<0.05$) higher content of phytic acid (467.68) and tannin (130.10) than the formulated beverages. From Table 5. it was observed that the phytochemical composition of Whey-*Aloe debrana* juice blended therapeutic beverages were decreasing with decreasing blend proportion of *Aloe debrana* juice.

Table 5. Phytochemical composition of the formulated therapeutic beverages and *Aloe debrana* juice

Sample	Phytic acid (mg/100 g)	Tannin (mg/100 g)
WA1	147.56 ± 0.01^c	4.98 ± 0.01^b
WA2	97.73 ± 0.01^b	11.17 ± 0.00^c
WA3	43.20 ± 0.01^a	BDL
<i>Aloe debrana</i> juice	467.68 ± 0.01^d	130.10 ± 0.01^d

WA0: Control therapeutic beverage prepared from whey only, WA1: therapeutic beverage prepared from whey and 30% (v/v) *Aloe debrana* juice, WA2: therapeutic beverage prepared from whey and 20% (v/v) *Aloe debrana* juice, WA3: therapeutic beverage prepared from whey and 10% (v/v) *Aloe debrana* juice, BDL: Below detection limit. Values are presented as mean \pm SD. Values with the same superscript within the same column are not significantly different ($p>0.05$).

3.3 Sensory quality evaluation

In this study, the aim was to blend *Aloe debrana* juice to the production of whey blended therapeutic beverages should either enhance the sensory characteristics of the therapeutic beverages as compared to the control. The mean score of sensorial attributes colour, consistency, flavour, and overall acceptability was presented in Table 6.

Whey and *Aloe debrana* juice were blended in three different proportions i.e WA1 (70:30), WA2 (80:20), WA3 (90:10) and evaluated for sensory characteristics such as colour, consistency, flavour, and overall acceptability. The mean sensory score for the formulated beverages ranged from 3.50 to 4.20, 3.80 to 4.35, 3.20 to 4.20, and 3.70 to 4.15 for colour, consistency, flavour, and overall acceptability respectively. The control beverage was seen with the highest score in colour and flavour than whey - *Aloe debrana* blended therapeutic beverages. Samples containing *Aloe debrana* juice have scored less in colour as compared to control beverage (100% whey beverage) on the first day of storage; this indicates that the incorporation of *Aloe debrana* juice to whey has negatively influenced the colour of the beverage.

Table 6. Effect of Whey and *Aloe debrana* juice blend on sensory quality of beverages

Sample	Sensory attributes			
	Colour	Consistency	Flavour	Overall acceptability
WA0	4.20 ^c	4.00 ^b	4.20 ^b	4.10 ^b
WA1	4.00 ^c	4.35 ^c	4.00 ^b	4.15 ^b
WA2	3.80 ^b	3.80 ^a	4.20 ^b	3.80 ^a
WA3	3.50 ^a	3.80 ^a	3.20 ^a	3.70 ^a

WA0: Control therapeutic beverage prepared from whey only, WA1: therapeutic beverage prepared from whey and 30% (v/v) *Aloe debrana* juice, WA2: therapeutic beverage prepared from whey and 20% (v/v) *Aloe debrana* juice, WA3: therapeutic beverage prepared from whey and 10% (v/v) *Aloe debrana* juice, BDL: Below detection limit. Values are presented as means of duplicate. Values with the same superscript within the same column are not significantly different ($p>0.05$).

Sample coded WA1 was seen as highest in consistency (4.35) and overall acceptability (4.15) among whey-*Aloe debrana* juice blended beverages and control. The control beverage has scored the highest in flavour among *Aloe debrana* juice blended beverages. This could be due to the high concentration of protein available which could contribute to its flavour shown in Table 6.

3.3.1 Sensory quality evaluation of therapeutic beverages stored at room storage temperature for 9 Days

The control beverage was seen with a higher score in colour at the initial day of storage and declined at a higher rate after 3 days of storage as compared to sample WA1. Sample coded WA1 scored lower in flavour as compared to control on the first day of storage. The effect of blend ratio was clearly observed in the acceptability of *Aloe debrana* juice blended beverages. Therapeutic beverages which contain 30% *Aloe debrana* juice scored higher in overall acceptability 4.15, 3.70,

Table 7. Sensory quality of therapeutic beverages stored at room storage temperature for 9 days

Storage periods at 22°C (day)	Sensory attributes							
	Colour		Consistency		Flavour		Overall acceptability	
	WA0	WA1	WA0	WA1	WA0	WA1	WA0	WA1
0	4.20	4.00	4.00	4.35	4.20	4.00	4.10	4.15
3	3.10	3.40	3.00	3.10	2.90	3.53	3.10	3.70
6	2.80	3.00	2.90	3.10	2.95	2.85	3.00	3.20
9	2.70	2.90	2.70	2.81	2.40	2.61	2.82	3.03

WA0: Control therapeutic beverage prepared from whey only, WA1: therapeutic beverage prepared from whey and 30% (v/v) *Aloe debrana* juice. Values are presented as means of duplicate.

3.20, and 3.03 for first, third, sixth, and ninth days of room temperature storage respectively. Therefore, sample WA1 has scored higher in overall acceptability throughout the storage period than the control beverage. Acceptability of *Aloe debrana* juice blended beverages has shown to decrease with decreasing levels of *Aloe debrana* juice. As shown in Table 7, the scores for overall acceptability gradually declined with increasing storage period; this finding is in agreement with (Shukla et al., 2013) finding who developed probiotic beverages using 65% whey and 35% pineapple juice.

4. Conclusion

In conclusion, the result showed that the incorporation of *Aloe debrana* juice in therapeutic beverages significantly increased the concentration of bioactive compounds. This might be due to the high concentration of phytic acid and tannin compounds in *Aloe debrana* juice. Sample coded with WA1 containing 30% *Aloe debrana* juice scored the highest in phytic acid (147.56 mg/100 g) among *Aloe debrana* juice blended beverages. The therapeutic beverages including control were stored for nine days at room storage temperature without significant change in sensory attributes. The sensory assessments on therapeutic beverages were relatively acceptable by panellists in increasing order of *Aloe debrana* juice blend percentage among *Aloe debrana* juice blended beverages and sample coded WA1 containing 70% whey and 30% *Aloe debrana* juice scored the highest in overall acceptability (4.15). In this study both control and *Aloe debrana* juice blended beverages gave promising results when stored at room storage temperature for nine days. Finally, it can be concluded that whey and *Aloe debrana* juice can be used for the production of good quality and phytochemical compound enriched therapeutic beverages. However further investigation is required on the vitamin profile, medicinal value, essential amino acid profile.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

The authors would like to thank Wollega University, Addis Ababa University, Holeta Agricultural Research Center, Ethiopian Public Health Institute and BGI Beer factory for their support in finance and laboratory facilities for analyses of samples for research.

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