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Research Article

The free amino acid profiling of milk- and cereal-based Kyrgyz ethnic fermented beverages and their contribution to taste formation

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Abstract

The present study aimed to determine the content of 18 free amino acids (FAA), including 10 essential amino acids, in traditional fermented beverages and their umami, sweet and bitter taste potential. The FAAs of traditional dairy and cereal-based fermented beverages were measured using high-performance liquid chromatography (HPLC) with a diode array detector and derivatization with diethyl ethoxymethylenemalonate (DEEMM). FAA content and taste attributes with a focus on umami, sweet and bitter were presented for koumiss, ayran, carbonated ayran, boza, boza with sea buckthorn, and maksym. The ratio of free essential amino acids (EFAA) to nonessential (NEFAA) was in the range of 0.41-1.12 except for ayran (0.28), indicating that each type of beverage has a rich source of their respective essential FAA profile. FAA represented 24.68% of the total protein, with the highest value for koumiss. The respective taste property contribution of bitter FAAs was high in koumiss (51%) and carbonated ayran (56%), whereas sweet-tasting FAAs were dominant in jarma (52%). The distribution of taste-active amino acids in the formation of the corresponding tastes was distinct.

Keywords

taste-active amino acids, HPLC, umami, sweet, bitter

Abbreviations

Ayran CD – carbonated ayran; Boza SB – boza with sea buckthorn; DEEMM – diethyl ethoxymethylenemalonate; FAA – free amino acids; EFAA – essential free amino acids; NEFAA – non-essential free amino acids, ND – not detected;

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Introduction

Long-term food storage involves fermentation to preserve it by creating an acidic environment that inhibits harmful microorganisms while enhancing its quality and flavor (Gemechu 2015). Various cultures have used fermentation techniques worldwide, creating numerous traditional fermented foods and beverages. The fermented beverage types depend on the raw materials used, the fermentation process conditions, and the microorganisms involved. Dairy and non-dairy fermented beverages comprise the two major categories, with dairy being the more traditional type and non-dairy beverages consisting of fruits, vegetables, and grains prevalent in different regions (Marrero et al. 2019).

Koumiss, a fermented dairy drink with nutritional and therapeutic benefits, is consumed by various groups, including Kyrgyzs, Kazakhs, Mongols, Bashkirs, and Yakuts (Danova et al. 2005). There are two methods of Koumiss production: traditional and industrial. The traditional method involves fermenting Koumiss in wooden casks (chelek) or animal skin (saba) vessels, which are cleaned every two to three weeks, greased with butter or fat and smoked with spirea bush branches for taste and aroma (Uzakbaev and Mamyrbaev 2012). The industrial method utilizes pure starter cultures of lactic acid bacteria such as *Lactobacillus bulgaricus*, *Torula spp.*, *Candida spp.*, *Lactobacillus lactis* and *Lactobacillus acidophilus* and lactose-fermenting yeasts (Makwana and Hati 2019). Ayran is another fermented beverage, traditionally made by diluting yogurt with water and adding table salt to enhance the taste. The concentration range should be between 30% to 50% (Erkaya et al. 2015). When produced industrially, ayran can be made in two ways: by adding water to yogurt or by fermenting diluted milk with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* after adjusting the milk's dry matter (Altay et al. 2013). The addition of salt protects the product from microorganisms and affects the finished product's sensory properties. Carbonation can be used to improve palatability (Sarhir et al. 2019).

Fermented cereal beverages like Boza are considered functional foods due to their nutritional and health-promoting properties (Xiong et al. 2022). Boza is produced by fermenting a combination of different cereals with yeast and lactic acid bacteria and is made from millet, wheat, corn, barley, oats, and rice or a combination of two or more cereals (Iskakova et al. 2017a). The cereals are milled into the size of a semolina, boiled with water, and allowed to cool. Prepared wheat malt is added at approximately 15% for starch degradation by α-amylases. After malt addition, it is fermented by adding previously fermented boza, sourdough, or yogurt as a starter culture (~2%). Following fermentation, boza is ready for use (Arici and Daglioglu 2002). Boza with sea buckthorn is produced with the addition of sea buckthorn juice to increase vitamin C content since it retains vitamins for a long time (Zeb 2004).

Maksym, a traditional Kyrgyz fermented beverage, is made by fermenting roasted barley, corn, and millet with yeast and lactic acid bacteria. Wheat flour is roasted with mutton fat or ghee, mixed with the roasted cereals, and cooked with water (1:20) and salt. The cooked mixture is cooled to 25-30℃ and fermented with sourdough or old Maksym (~2%) for 12-14 h. The fermented beverage is cooled to slow fermentation and is ready to drink after cooling (Iskakova et al. 2017 b; Bazhenova et al. 2021).

Jarma is a traditional Kyrgyz drink made like maksym. However, without fermentation. After cooking, yogurt or concentrated yogurt (labneh) is added (10:2), and the drink is ready to consume (Telenbekov and Isirailova 2015). Probiotic cultures are added to enrich jarma. During the fermentation of beverages, proteins in dairy and non-dairy drinks are hydrolyzed by microorganisms, resulting in degradation products like peptides and FAAs. These compounds improve drinks' nutritional, sensory, and palatability properties (Makwana and Hati 2019; Bader et al. 2019). FAA derived from hydrolyzed proteins make them easier to digest and stimulates the symbiotic growth of probiotics that can help normalize bowel function, boost immunity, and reduce the risk of stomach ulcers (Yildiz 2009). When using yeast, the length of time in contact with yeast determines the level of FAA produced. Fermentation is complex because FAAs also serve as a substrate for microorganisms, particularly yeasts. Another source of FAA in beverages with a heterofermentative nature is the hydrolysis of proteins resulting from the autolysis of dead yeast cells (Schmidt et al. 2021). More information about the FAA profile of traditional fermented beverages needs to be published. Some sparse data are available for mare milk fermented with mesophilic LAB, thermophilic LAB, and Korean-type koumiss (Teichert et al. 2021; Lee et al. 2011). However, there is hardly any mention of the FAA content of cereal-based fermented beverages. This research aims to determine the FAA profile of fermented dairy and cereal-based beverages, their umami, sweet, and bitter taste potential, and the contribution of individual FAAs responsible for these flavors. Comparative analysis results are presented for koumiss, ayran, boza, maksym, and jarma.

Materials and Methods

Samples. Industrially produced koumiss, ayran (or ayran), carbonated (CD) ayran, boza, boza with sea buckthorn (boza SB), maksym, and jarma (Fig. 1) from three different production batches were purchased at one-month intervals from a local market in Bishkek, Kyrgyzstan). Since the samples contain live microorganisms, they have a short shelf life (20-60 d), so they were stored in a refrigerator at 2-4℃ until analysis.

Reagents and standards. The amino acids used were L-aspartic acid, L-glutamic acid, DL-serine, L-histidine, glycine, L-threonine, L-arginine, L-alanine, L-tyrosine, L-valine, L-methionine, L-isoleucine, L-leucine, L- phenylalanine, L-ornithine monohydrochloride, L-lysine monohydrochloride (Merck, Darmstadt, Germany), and L-tryptophan (Carl Roth, Karlsruhe, Germany). DEEMM, methanol, boric acid, and sodium hydroxide obtained from Merck were used for the derivatization reactions. HPLC grade acetonitrile (Merck), acetic acid glacial, and sodium acetate (Carlo Erba Reagents, Val-de-Reuil, France) were used for the mobile phase preparation. Water was purified with the PURELAB Option-Q system from Elga Veolia (United Kingdom).

*carbonated ayran; **boza with sea buckthorn

Figure 1. Side- and top-view images of experimental beverages.

Physicochemical analysis. At the time of analysis, all samples were thoroughly mixed to avoid precipitation and to provide a homogeneous sampling. pH, titratable acidity, total solids content, and protein content were determined according to the method proposed by Association of Official Analytical Chemistry AOAC (2000).

The pH of beverages was measured at room temperature using a digital pH meter (Model 220 pH/Conductivity Meter, Denver Instrument, NY, USA).

The titratable acidity was determined according to Method 947.05 (AOAC 2000a).

The content of total solids was determined by a directly forced air oven drying Method 990.20 at 105±1℃ until constant weight (AOAC 2000b).

The Kjeldahl Method 991.22 was used to determine the beverages' protein content using a total nitrogen conversion factor of 6.38 (AOAC 2000c

Sample preparation for amino acid analysis. Well-mixed 2.5 ml of beverage was diluted with purified water in a 10 ml glass flask to determine FAA. This solution was centrifuged at 15000 rpm for 10 min and filtered through filter paper (Rubio-Barroso et al. 2005). Into a 10 ml glass tube, 0.5 ml of the prepared solution, 3 µl of DEEMM, 0.75 ml of methanol, and 1.747 ml of borate buffer (1 M, pH 9.0) was placed for the derivatization procedure, and the cap was screwed. The tube was briefly shaken and placed in an ultrasound bath for 30 min at room temperature (Mazhitova and Kulmyrzaev 2016).

Analytical conditions of HPLC. Derivatized samples were filtered through a 0.45 μm regenerated cellulose membrane filter to 1.5 ml vials and injected into a high-performance liquid chromatography (HPLC) system (Agilent Technologies 1200, US) connected to a Diod Array Detector at 280 nm. Chromatographic separation was performed in a column of C18 (4.6 mm x 250 mm x 5 μm). The column was thermostated at 16℃, and the flow rate was 1.0 ml/min. The injection volume was 20 μl. The mobile phase consisted of acetonitrile and acetate buffer (0.1 M) at pH 6.0 and was used according to the gradient conditions described by Mazhitova and Kulmyrzaev (2016). Identification was made using the retention times obtained from pure compounds. Stock solutions (1000 $\mu g.ml^{-1}$) of 18 amino acid standards were prepared in 0.1 N HCl. Quantifying amino acids was performed using an external standard method with concentrations of 0.01, 0.5, 1.0, 2.0, 3.0, and 4 $\mu g.ml^{-1}$.

Statistical Analysis. Analysis of variance using SPSS version 16.0 (SPSS Inc., Chicago, IL) software was applied for data treatment. The differences between the means of the treatments were compared using Duncan's test at significance levels of $P<0.01$ and $P<0.05$.

Results and Discussion

Physicochemical properties. The highest content of total solids was in boza with sea buckthorn (15.52%), followed by simple boza (13.32%), and the lowest content was in koumiss (5.49%), which was three times less than that in boza SB (Table 1). Adding sea buckthorn juice to boza significantly increases total solids and increases protein content because of high suspended solids and lipoprotein content (Zeb 2004). The total solids of ayran and carbonated ayran were slightly different (P<0.01). Higher total solids and protein contents were observed in jarma than in maksym since jarma is made by introducing yogurt or labneh to unfermented cooled maksym material. Koumiss is a rich source of protein among the studied samples of fermented drinks (2.57%) and corresponds to the literature data (Yildiz 2009). The protein contents of ayran and boza SB were similar. The highest titratable acidity content was found in koumiss, followed by boza and boza SB. The lowest titratable acidity was in the ayran and jarma samples, which can be attributed to the slightly sour taste of these beverages. The pH values of the studied samples were in the range of 3.22-3.95, which indicates a sour taste. Lactic acid, propionic acid, acetic acid, ethanol, and bacteriocins produced by fermentative bacteria and yeasts inhibit the growth of spoilage and pathogenic microorganisms naturally present in foods and preserve and extend the shelf life of foods (Arslan et al. 2015).

Table 1. Physicochemical properties of fermented beverages

Samples	Total Solids, $g.100\ g^{-1}$		Protein content, $g.100\ g^{-1}$		Titratable acidity, °T		pH	
	Mean[1]	SD	Mean	SD	Mean	SD	Mean	SD
Koumiss	5.49[f]	0.17	2.57[a]	0.10	61.85[a]	0.57	3.22[e]	0.02
Ayran	6.24[e]	0.06	1.80[c]	0.01	45.56[d]	1.99`	3.51[c]	0.05
Ayran CD*	6.80[d]	0.13	1.96[b]	0.07	59.26[b]	0.57	3.69[b]	0.06
Boza	13.32[b]	0.26	1.71[d]	0.06	57.04[b]	0.57	3.73[b]	0.04
Boza SB**	15.52[a]	0.26	1.78[c]	0.01	57.41[b]	2.07	3.41[d]	0.06
Maksym	6.90[d]	0.13	0.93[f]	0.02	48.52[c]	3.76	3.27[e]	0.05
Jarma	8.16[c]	0.16	1.54[e]	0.02	43.70[d]	2.07	3.95[a]	0.02

[1]Results of six measurements carried out in duplicate for three independent samples.
Values within column significantly different at $P<0.05$
*carbonated ayran
**boza with sea buckthorn

FAA composition of fermented beverages. The FAA content was determined by HPLC using the external standard method. The calibration curves obtained from pure amino acid compounds at different concentrations (0.01, 0.5, 1, 2, 3, and 4 μg.ml^{-1}) were used for quantification (Table 2). The linear correlation coefficient values obtained from three replicates were higher than 0.9824 except for methionine (0.9586), indicating a good linear relation between the measured peak area as a function of increased analyte concentration. Other analytical parameters related to method validation were published in our previous work (Mazhitova and Kulmyrzaev 2016). Table 3 shows the contents of FAAs in all samples investigated (in units of mg AA.kg^{-1}). Furthermore, the sum of essential free amino acids (EFAA), the sum of non-essential free amino acids (NEFAA), the ratio of EFAA/NEFAA, and the sum of total FAA are listed for each sample. A total of 19 amino acids were detected in this experiment, and not all were determined in the analyzed samples (Table 3), so tryptophan was not detected in ayran and ayran CD. The main amino acid of koumiss (1113.66 mg.kg^{-1}), ayran (41.74 mg.kg^{-1}), boza (24.76 mg.kg^{-1}), and maksym (7.59 mg.kg^{-1}) was glutamic acid, while serine was the most abundant in ayran CD (12.47 mg.kg^{-1}) and boza SD (18.05 mg.kg^{-1}). In koumiss, the detected FAA was approximately dozens of times the amount for the same amino acid seen in other fermented beverages. The concentrations of arginine, alanine, tyrosine, isoleucine, leucine, and lysine were higher in carbonated ayran than in noncarbonated ayran.

Table 2. Analytical parameters related to the determination of FAA and their taste characteristics

Amino acid	Regression	R^2	Taste*
Aspartic acid	y = 212.92x +5.13	0.9974	Umami
Glutamic acid	y = 207.43x + 7.304	0.9995	Umami
Serine	y = 283.25x + 10.84	0.9982	Sweet
Histidine	y = 193.77x -2.52	0.9987	Bitter
Glycine	y = 430.93x + 10.816	0.9987	Sweet
Threonine	y = 254.62x+0.71	0.9991	Sweet
Arginine	y = 168.92x-1.86	0.9993	Bitter
Alanine	y = 330.44x + 5.6951	0.9977	Sweet
Proline	y = 18.59x-1.46	0.9988	Sweet
Tyrosine	y = 147.32x-1.20	0.9991	Neutral
Valine	y = 309.74x + 156.5	0.9898	Bitter
Methionine	y = 106.56x + 25.688	0.9586	Bitter
Cysteine	y = 200.91x + 79.35	0.9824	Neutral
Isoleucine	y = 225.75x-3.24	0.9991	Bitter
Leucine	y = 245.12x - 4.0961	0.9949	Bitter
Tryptophan	y = 125.8x + 2.3259	0.9932	Bitter
Phenylalanine	y = 160.99x-2.10	0.9992	Bitter
Lysine	y = 301.73x-0.58	0.9991	Bitter

*Amino acids taste data are from Kato et al. (1989)

Serine (15.88 mg.kg^{-1}) and proline (36.66 mg.kg^{-1}) were the other main amino acids of ayran. The concentration of alanine for boza (18.02 mg.kg^{-1}) and boza with SB (11.94 mg.kg^{-1}) was also higher. Valine (13.51 mg.kg^{-1}) for boza and serine (18.05 mg.kg^{-1}) for boza with SB were the following most abundant amino acids. Maksym was enriched with alanine (6.40 mg.kg^{-1}), tyrosine (4.48 mg.kg^{-1}), and proline (3.77 mg.kg^{-1}). The major FAAs of the jarma drink were proline (16.60 mg.kg^{-1}), alanine (7.38 mg.kg^{-1}), and aspartic acid (7.29 mg.kg^{-1}). The richest source of essential amino acids (3354.88 mg.kg^{-1}), nonessential amino acids (2988.22 mg. kg^{-1}), and total FAAs (6343.09 mg.kg^{-1}) among the investigated fermented traditional beverages was koumiss. The protein content exceeded ten (sometimes 40 to 90) times higher than that of the other samples, while the protein content of the samples did not differ much. This could be explained by the intensification of the fermentation process involved in koumiss and the microbial diversity of koumiss, in which more than 171 bacterial and 655 yeast isolates were found (Hui and Evranuz 2012; Mu et al. 2012), and the fermentation process in koumiss occurs by the symbiosis of two distinct microorganisms, lactic acid bacteria, and yeast.

The higher content of total amino acids among ayran and carbonated ayran was in noncarbonated ayran. However, the protein content was higher in carbonated ayran. The lowest content of total FAAs was found in maksym (39.15 mg.kg^{-1}), a cereal-based, fermented, and carbonated beverage. The higher total FAA than maksym was in jarma (62.54 mg.kg^{-1}), made from the same cereals but without fermentation and carbonation. It can be concluded that the carbonation of beverages creates unfavorable conditions for lactic acid bacteria and yeast, which play an essential role in the hydrolysis of proteins and the formation of FAAs since fermentation also occurs during storage (Atanasova et al. 2021). The total FAA contents of boza (112.50 mg.kg^{-1}) and boza SB (106.70 mg.kg^{-1}) were similar. The sums of essential FAAs in ayran, ayran CD, boza, and boza SB were similar. The sum of nonessential FAAs was highest in ayran (122.18 mg.kg^{-1}) after koumiss, followed by boza (47.9 mg.kg^{-1}) and boza SB (75.52 mg.kg^{-1}), and lowest in ayran CD (47.92 mg.kg^{-1}), jarma (44.38 mg.kg^{-1}), and maksym (25.96 mg.kg^{-1}).

The ratio of essential FAAs to nonessential FAAs was the highest in koumiss (1.12**)**, followed by ayran CD (1.00) and boza SB (0.57). The lowest ratio was in ayran (0.28), indicating fewer essential FAAs than nonessential ones. Through proteolysis, fermentation generates low-molecular-weight compounds like peptides, amino acids, aldehydes, organic acids, and amines. During koumiss fermentation, approximately 10% of milk proteins are hydrolyzed by generating FAAs (Makwana and Hati 2019). In our results, the content of total FAA was about 24.68% in protein (Table 4). 0.87% of ayran protein consists of FAA. The total FAA content of ayran CD (9.57 mg.100 g^{-1}) was higher than that of maksym (3.92 mg.100 g^{-1}), and jarma (6.25 mg.100 g^{-1}), but the percentages of total protein were the same, 0.48%, 0.42%, and 0.41%, respectively. Approximately 0.2% of the protein of ayran, ayran CD, boza, and boza SB are essential FAAs. Xia et al. reported 100.21mg.100 g^{-1} to 196.53 mg.100 g^{-1} of a total of 17 FAAs detected in koumiss (Xia et al. 2021), while Wurihan et al. revealed 126.53 mg.100 ml^{-1} sum of 14 FAAs, and both results were lower than our findings since 18 amino acids were identified and quantified here, more than in other studies (Wurihan et al. 2019). Irigoyen et al. reported 207.74 mg.100 g^{-1} of FAA in commercial yogurt inoculated with *L. bulgaricus* and *S. thermophilus* as starters (Irigoyen et al. 2012).

Contribution of taste-active amino acids to beverage flavour. Food fermentation is one way to make food's odor and taste more affluent and complex. Proteins themselves do not have much taste; however, during fermentation, proteases from microorganisms or enzymes catalyze the formation of peptides, FAAs, and amino acid derivatives, leading to the development of distinctive tastes such as bitter, umami, sweet, or sour in fermented foods (Zhao et al. 2016). Since beverages were different in terms of their FAA profile, this may contribute to different taste profiles specific to the type of beverage. A rough estimate of the respective FAA taste property contributions is given in Fig. 2.

Table 3. The FAA content of traditional fermented beverages

FAA, mg.kg^{-1*}	Koumiss	Ayran	Ayran CD	Boza	Boza SB	Maksym	Jarma
Aspartic acid	397.13±2.02^{f}	8.95±0.59^{d}	8.08±0.08^{c}	3.08±0.44^{gh}	5.08±0.11^{ef}	1.95±0.17^{ef}	7.29±0.26^{b}
Glutamic acid	1113.66±9.96^{a}	41.74±0.71^{a}	0.72±0.06^{l}	24.76±0.40^{a}	12.94±0.10^{b}	7.59±0.27^{a}	0.90±0.05^{ij}
Serine	421.62±2.64^{d}	15.88±0.46^{c}	12.47±0.10^{a}	5.42±0.15^{f}	18.05±0.58^{a}	2.53±0.17^{e}	5.14±0.18^{c}
Histidine	236.64±3.85^{k}	9.01±0.40^{d}	5.95±0.08^{gh}	3.74±0.07^{g}	5.29±0.88^{ef}	2.03±0.21^{ef}	2.26±0.15^{fg}
Glycine	172.79±2.80^{o}	4.03±0.10^{h}	1.33±0.01^{l}	7.55±0.17^{e}	6.40±0.22^{d}	1.88±0.06^{ef}	2.17±0.11^{fg}
Threonine	323.05±3.35^{g}	5.13±0.29^{f}	3.47±0.15^{j}	3.05±0.29^{gh}	3.94±0.53^{gh}	1.09±0.14^{ghi}	2.01±0.19^{g}
Arginine	75.47±3.82^{p}	3.96±0.08^{h}	5.39±0.11^{h}	2.14±0.31^{hij}	1.93±0.61^{i}	0.88±0.06^{hi}	3.61±0.26^{d}
Alanine	399.28±3.33^{ef}	2.49±0.08$_{i}$	6.35±0.11^{fg}	18.02±0.64^{b}	11.94±0.22^{c}	6.40±0.14^{b}	7.38±0.09^{b}
Proline	261.84±3.05^{j}	36.66±0.67^{b}	7.29±0.22^{de}	11.12±0.41^{d}	6.44±0.64^{d}	3.77±0.03^{d}	16.60±1.46^{a}
Tyrosine	199.05±9.01^{m}	1.88±0.05^{j}	9.11±0.52^{b}	1.98±0.43^{hij}	4.48±0.19^{fg}	4.48±0.01^{c}	2.89±0.42^{e}
Valine	189.24±4.59^{n}	8.65±0.09^{d}	8.21±0.32^{c}	13.51±0.13^{c}	4.10±0.25^{gh}	0.59±0.08^{ij}	3.90±0.02^{d}
Methionine	300.24±11.44^{h}	6.28±0.61^{e}	2.82±0.37^{k}	2.19±0.77^{hi}	0.98±0.33^{j}	1.75±0.18^{fg}	1.43±0.22^{h}
Cysteine	22.58±1.67^{q}	0.60±0.07^{k}	1.19±0.23^{l}	2.11±0.13^{hij}	0.46±0.12^{j}	0.85±0.06^{i}	0.75±0.15^{j}
Isoleucine	282.95±7.45^{i}	4.50±0.11^{g}	7.72±0.59^{cd}	1.14±0.03^{ij}	3.38±0.07^{h}	1.16±0.12^{ghi}	2.63±0.35^{ef}
Leucine	804.42±15.72^{b}	5.24±0.16^{f}	8.86±0.24^{b}	2.39±0.25^{hi}	7.26±0.22^{d}	1.02±0.14^{hi}	3.69±0.23^{d}
Tryptophan	218.84±2.54^{l}	ND	ND	5.51±0.16^{f}	5.47±0.50^{e}	0.09±0.06^{j}	1.27±0.20^{hi}
Phenylalanine	407.99±14.47^{e}	3.86±0.33^{h}	4.21±0.34^{i}	0.89±0.05^{j}	5.30±0.53^{ef}	0.55±0.07^{ij}	0.91±0.28^{ij}
Lysine	627.58±18.21^{c}	2.25±0.48^{ij}	6.85±0.20^{ef}	4.21±0.15^{g}	3.24±0.18^{h}	1.57±0.18^{fgh}	2.27±0.27^{fg}
Sum of EFAA	**3354.88^{A}**	**34.38^{B}**	**47.79^{B}**	**36.97^{B}**	**38.62^{B}**	**13.20^{C}**	**18.16^{C}**
Sum of NEFAA	**2988.22^{A}**	**122.18^{B}**	**47.92^{CD}**	**75.52^{C}**	**68.08^{CD}**	**25.96^{D}**	**44.38^{CD}**
EFAA/NEFAA ratio	**1.12^{A}**	**0.28^{E}**	**1.00^{B}**	**0.49^{CD}**	**0.57^{C}**	**0.51^{CD}**	**0.41^{D}**
Total FAA	**6343.09^{A}**	**156.56^{B}**	**95.70^{C}**	**112.50^{BC}**	**106.70^{BC}**	**39.15^{D}**	**62.54^{CD}**

[*]Results indicate mean values±SD of six measurements (carried out in duplicate for three independent samples)
ND – not detected; Ayran CD – carbonated ayran; Boza SB – boza with sea buckthorn; Sum of EFAA – sum of essential free amino acids; Sum of NEFAA – sum of non-essential free amino acids, FAA – free amino acids.
$^{a-q}$ differences within the amino acids content statistically significant ($P \leq 0.01$)
$^{A-E}$ differences within the samples statistically significant ($P \leq 0.01$)

It characterizes the difference between seven different types of beverages using pie charts based on the average content of FAA (n = 16, excluding the neutral-tasting tyrosine and cysteine) in the analyzed samples of koumiss, ayran, ayran CD, boza, boza SD, maksym and jarma with an emphasis on the basic tastes of umami, sweet and bitter. The diagrams are color-coded according to basic taste (umami = orange; sweet = pink; bitter = blue). It is evident that the relative composition of umami, sweet and bitter FAAs in koumiss and ayran CD is quite similar, with a predominance of bitter taste, and distinctly differs from jarma, in which sweet taste predominates. Ayran and maksym have the same level of bitterness, while the sweetness of boza and ayran is similar. The highest share of sweet taste was found in an unfermented, just yogurt- or labneh-added jarma drink.

The most dominant FAA contribution to the umami taste (Fig. 3 A) koumiss, ayran, boza, boza SB, and maksym originate from glutamic acid (74%, 82%, 89%, 72%, and 80%, respectively).

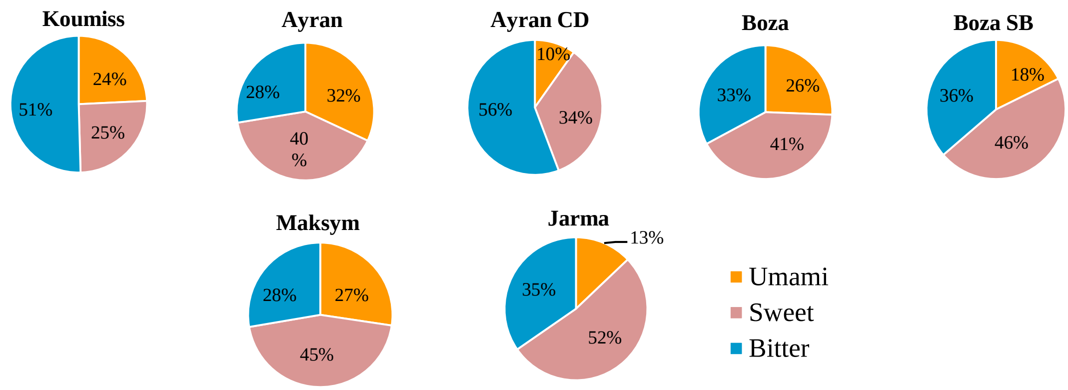

Figure 2. Pie charts based on the average content of FAA (n = 16, tyrosine and cysteine excluded) in analyzed samples. The diagrams are color-coded according to the basic taste attribute of the FAA (orange – umami; pink – sweet; blue – bitter)

In contrast, aspartic acid was more responsible for the umami flavor of ayran CD and jarma (92% and 89%, respectively). Because the umami potential of aspartic acid is much less (8%) than that of glutamic acid, carbonated ayran and jarma drinks do not elicit basal umami (i.e., umami taste by themselves). In most cases, the perceived umami taste of beverages cannot be judged by their free glutamic acid and aspartic acid content alone. Other substances, such as free nucleotides and peptides, including glutathione and disodium succinate, may also affect the taste perception of umami (Schmidt et al. 2021). Thus, the real umami potential of these two beverages depends on whether they are consumed along with other foodstuffs containing free nucleotides. The present study did not analyze free nucleotide content in fermented beverages. Based on the literature, plant-based beverages are only in exceptional cases expected to contain free nucleotides in any appreciable amount; therefore, it can be assumed that they may not be present in the cereal-based fermented beverages studied in this paper. Hence, umami synergy is only when paired with other foodstuffs (Schmidt et al. 2021).

Evident sweet-taste amino acid contributors in koumiss and boza SB are serine (27%) and alanine (25%), respectively. The rest is less than 50%. In ayran, the ratio of the sweet taste of the amino acid proline is about 57%. It is 50% in jarma. In boza and maksym, the sweet taste comes from alanine (40% and 41%) and proline (24% and 24%), respectively. However, in ayran CD, this taste derives from serine (40%) and proline (24%) (Fig. 3 B).

The contribution of bitter taste amino acids of studied beverages differed (Fig. 3 C). The two main FAAs responsible for the bitterness of the analyzed fermented beverages were leucine (26%) and lysine (20%) in koumiss; histidine (21%) and valine (20%) in ayran; valine (16%) and leucine (18%) in ayran CD; valine (38%) and tryptophane (15%) in boza; leucine (20%) and tryptophane (15%) in boza SB; histidine (21%) and methionine (18%) in maksym; and valine (18%) and leucine (17%) in jarma.

Although amino acids can elicit any of the primary tastes, the threshold value of the taste of each amino acid is high (Kato et al. 1989). Some free amino acids in all fermented beverages except for koumiss were lower than their threshold values, although they may not contribute directly to food taste. However, they may have an essential role in making food savory because of the synergistic effect; thus, arginine at a subthreshold concentration significantly enhances salty taste (Zhao et al. 2016), and umami can improve sweet and salty flavors (Fuke and Ueda 1996) and decrease bitter taste (Wolfe et al. 2021).

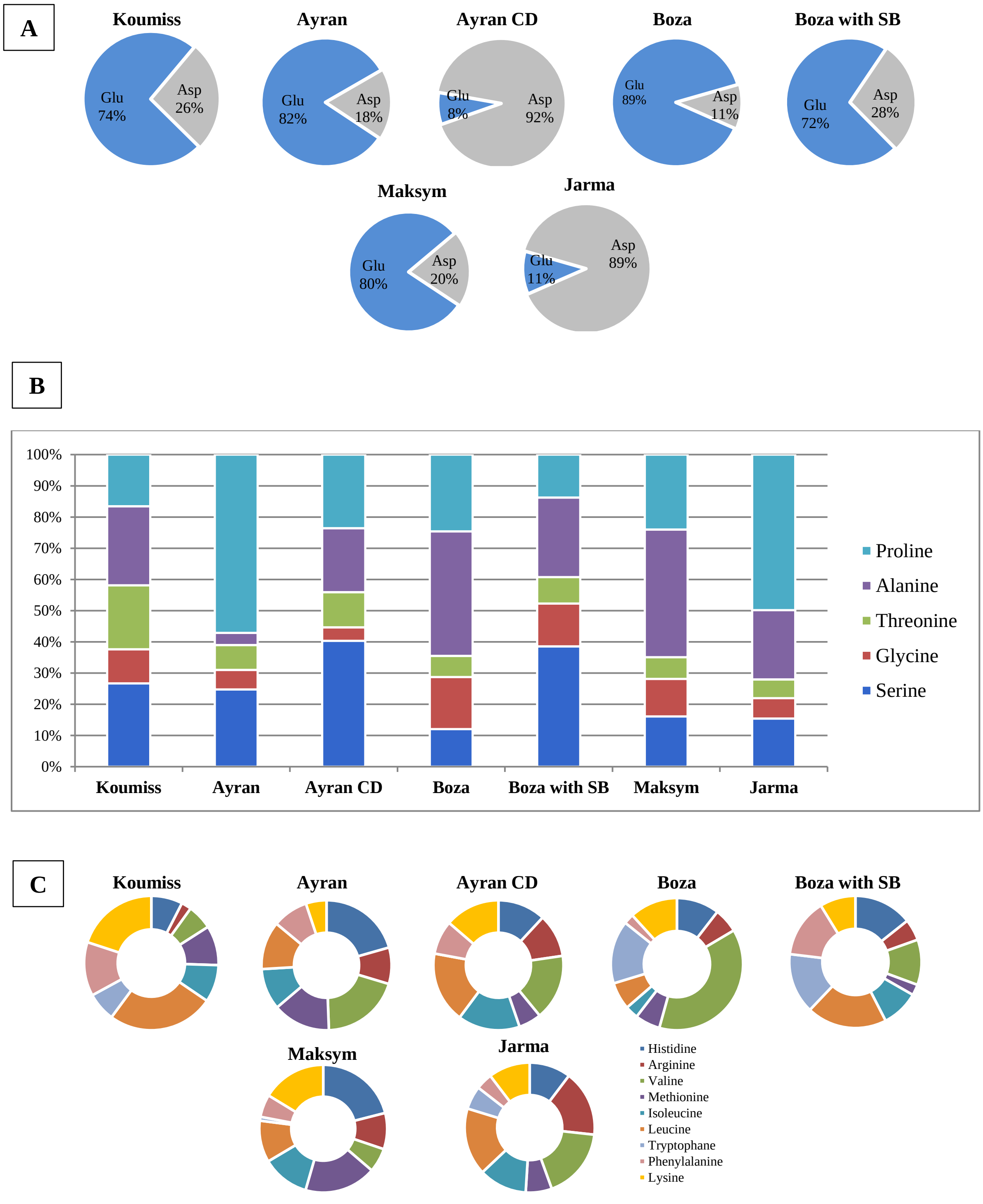

Figure 3. Percentage distributions of (A) umami, (B) sweet and (C) bitter taste FAAs in the analyzed fermented beverages. Asp – aspartic acid; Glu – glutamic acid; Ser – serine; His – histidine; Gly – glycine; Thr – threonine; Arg – arginine; Ala – alanine; Pro – proline; Tyr – tyrosine; Val – valine; Met – methionine; Cys – cysteine; Ile – isoleucine; Leu – leucine; Trp – tryptophan; Phe – phenylalanine; Lys – lysine

Conclusions

To our knowledge, this study is the first to investigate the FAA composition of Kyrgyz fermented beverages and measure these concentrations from ayran, boza, maksym, and jarma. Thus, it provides important information about these traditional drinks that might be interesting for scientific, industrial, and household use. Based on the results obtained, it can be concluded that the amino acid profiles in the studied beverages differed from each other, which caused a difference in taste. The relative ratio of umami, sweet, and bitter amino acids also differed between samples. The concentration of bitter amino acids was the highest in koumiss and ayran CD, and both drinks are carbonated. Ayran varies with the highest umami, while jarma with the highest sweet taste percentages. The carbonation of ayran and the addition of sea buckthorn juice to boza decreased the umami potential of the beverages. Maksym and jarma, made from the same cereals, showed quite different FAA profiles. The lower total FAA content in fermented maksym indicates the low intensity of the fermentation process. However, in ayran, boza, boza SB, and maksym, the content of sweet amino acids was high. The sensory profile of food products is a complex phenomenon in which the concentration of various flavor compounds is only one of the important factors. Therefore, further in-depth organoleptic studies are needed to confirm these predictions, as the interactions of different sensory and gustatory factors influence each other.

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