Research Article

Functional Properties and Metabolic Profile of National Fermented Products of Russia and South Africa

Olga S Savinova[1]*, Anna V Begunova[2], Oluwatosin A.Ijabadeniyi[3], Konstantin V. Moiseenko[1], andTatiana V. Fedorova[1]

[1]A. N. Bach Institute of Biochemistry, Research Center of Biotechnology, Russian Academy of Sciences, Leninsky Ave. 33/2, Moscow, 119071, Russian Federation
[2]Federal State Budgetary Scientific Institution "All-Russian Research Institute of Dairy Industry", Lyusinovskaya str. 35, Moscow, 115093, Russian Federation
[3]Department of Biotechnology and Food Technology, Durban University of Technology, 41/43 M L Sultan Rd, Greyville, Durban, 4001, South Africa
ORCID
Olga S Savinova; 0000-0001-7119-1551

Abstract. Both Russia and South Africa have a long-standing history of fermented milk product consumption. Along with the products widely distributed around the world, such as yoghurts, in each of these countries there are a number of national products. An example of a widely demanded fermented milk product in Russia is Kefir.This productis used not only as a food source in the diet of children and adults, but also in medical institutions, since ithasa positive effect onhuman health when consumed regularly. South Africa is characterized by the consumption of products such as Amasi,which is produced commercially. Its consumption has also been shown to have beneficial effects on the digestive system. In this research, the metabolic profiles(fatty acid composition and volatile compounds) of these fermented milk products were analyzed and these showed significant differences. The results indicated that this metabolite composition reflected the different production protocols and microbial complexity of these dairy products. The functional properties of the studied drinks were also considered.The average content of L-leucine equivalents in Amasi was slightly higher (6.5-8.9mMol×L^{-1}) than in Kefir (4.9-6.7mMol×L^{-1}). Antioxidant and antihypertensive activity of the fermented products correlated with the depth of hydrolysis of the milk proteins. Amasishowed higher antioxidant and antihypertensive activities (600-796μMolTE/ml and 1.3-1.5mg/ml, respectively) than Kefir (246-574μMolTE/ml and 2.0-4.3mg/ml, respectively).

Keywords: fermented products, Kefir, Amasi,metabolic profile, antioxidant potential, antihypertensive properties

Corresponding Author: Olga S Savinova; email: savinova_os@rambler.ru

Dates
Published 13 January 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 8th Scientific and Practical Conference Conference Committee.

1. Introduction

Novadays, the market of fermented products in the world is constantly expanding. Microorganisms used to ferment milk are called probiotic. Interest has increased due to the usefulness and healing properties of such products. They are able to improve

the balance of human intestinal microflora, therefore, products obtained with the use of such microorganisms are considered functional.

Kefir (from the Turkish word “Keyif” which means “good feeling”) is popular beverage in Russia obtained by milk fermentation with a specific type of mesophilic symbiotic starter culture - “kefir grains”, originated inthe Caucasus Mountains. “Kefirgrains” is a symbiotic association of a complex mixture of bacteria and yeasts. According to modern studies, the composition of kefir grains contains from 65 to 80 % of bacteria, mainly of the genera *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Acetobacter*, the remainder is represented by various types of yeasts.Kefir grains, when inoculated into medium such as milk, produce acidified fermented milk that is slightly carbonated and contains small amounts of alcohol. During fermentation, lactic acid, bioactive peptides, exopolysaccharides, antibiotics and numerous bacteriocins are produced [1].Kefir can be produced from whole, semi-skimmed or skimmed pasteurised cow, goat, sheep, camel or buffalo milk. However Kefir from cows’ milk is the most common in Russia and accounts for more than 70 % of all sour-milk products industrially produced.

Recently, scientists from different countries have shown an increased interest in studying the microbial composition and functional properties of kefir, due to the beneficial effect of this drink on human health [1,2]. Regular consumption of Kefir improves digestion, has antibacterial, hypocholesterolemic, antihypertensive, anti-inflammatory effects on the body, and also helps to reduce plasma glucose [1]. It is believed that the functional properties of kefir are mainly due to the presence of biologically active peptides and kefiran, the main soluble exopolysaccharide [3,4]. However, the beneficial effects may also be due to the microbial composition of this fermented milk or secondary metabolites [5].

Amasi (MAAS) is a probiotic fermented raw milk beverage made by the indigenous peoples of some African countries (for example, Zimbabwe, Kenya and South Africa) [6]. For fermentation, raw milk from cattle is used, less often goat milk. Traditionally, this drink is produced by households in different regions, but despite the same name, the bacterial compositions of this drink differ. The quality, consistence and quantity of Amasi are influenced by the seasonal composition, volume and availability of raw milk in each household.In addition, the characteristics of the beverage may change depending on the presence of residual bacteria inside the container used or due to contamination during the milking process.According to the literature, lactic acid bacteria predominate in the starter cultures.In particular, *Lactococcuslactis* strains are found in large numbers. In addition, various species of *Lactobacillus* and *Leuconostoc* are present.Amasi is also produced commercially using *L.lactis* subspecies *lactis* and *L. lactis* subspecies

Figure 1: Indigenous fermented foods:Russian Kefir(A), African Amasi (B).

cremoris[6].It has been proven that consumption of Amasi leads to a reduced risk of developing diarrhea and other digestive disorders.

Despite the recent increase in the number of publications devoted to probiotic microorganisms, information on the metabolic profiles of fermented products obtained with them is limited. This is unfortunate, since it is the spectrum of secondary metabolites formed during the fermentation process that gives the final product its taste, texture, nutritional value and functional properties. Thus, an understanding of the relationship between the metabolic profile and the properties of the fermented product, including its effect on the human body, will contribute to the development of new innovative products, primarily functional and therapeutic purposes.

The aim of this work was to characterize fermented beverages from Russia and Africa (Figure 1) and analyze their properties.

2. MaterialsandMethods

2.1. Metabolomicsanalysis

2.1.1. Fatty Acid Composition Analysis

Fat were extracted from the samples according to Folch method. Chromatographic separation on a MDN-5 column (30m×0.25 mm; Supelco, Japan) was carried out using GC 2010 chromatograph (Shimadzu, Japan) equipped with a mass detector GCMS-QP 2010 in the regime of temperature gradient (exposure 70°C 1 min, then 4°C/min up to 90°C, 10°C/min to 240°C, exposure 4 min 240°C, 15°C/min to 300°C, exposure 3 min 300°C) at the following temperatures: injector, 200°C; interface, 210°C; and detector, 200°C. Helium with a flow of 1.0 mL/min and a flow pressure of 1:20 was used as a

carrier gas. The total analysis time was 32 min. Mass detection was carried out under the TIC registration mode in the mass acquisition range (m/z) from 45 to 400.

2.1.2. VolatileCompounds Analysis

The volatile compounds were extracted by solid phase micro extraction (SPME) and analyzed by gas chromatography coupled to mass spectrometry (GCMS, Shimadzu GS 2010 gas chromatograph directly attached to a GCMS-QP 2010 mass spectrometer, Shimadzu, Japan). SPME extractions were performed using 50/30 mm thick PDMS/DVB (polydimethylsiloxane/divinylbenzene) fibers (Supelco, Bellefonte, PA, USA) and 40 mL flasks fitted with mininert valves (Supelco), that inserted above the gas-phase bottle for extraction for 60 min at 40°C. Desorption was conducted at 250°C for 3 min. The Optima-1 column (25m x 0.25 mm, Supelco, Japan) was initially held 2 min at 70°C, then heated to 90°C at a rate of 4°C/min, to 160°C at a rate of 10°C/min, and finally heated to 280°C at a rate of 20°C/min, which was held for 5 min; at the following temperatures: injector, 230°C; interface, 205°C; and detector, 200°C. Helium with a flow rate of 0.7 mL/min and a flow pressure of 1:20 was used as a carrier gas. The total analysis time was 25 min. Mass detection was carried out under the TIC registration mode in the mass acquisition range (m/z) from 45 to 450.

Volatile compounds were identified by comparing their experimental spectra with those from the National Institute of Standards and Technology (NIST/EPA/NIH Mass Spectral Database (NIST 11), USA).

2.2. Functional analysis

2.2.1. Proteolytic Activity Assay

The proteolytic activity was quantified by the measurement of the amount of released amino groups in supernatants of samples using 2,4,6-trinitrobenzenesulfonic acid solution (TNBS, Sigma-Aldrich, St. Louis, MO, USA) method [7] and D340 was measured using Synergy 2 microplate photometer–fluorometer (BioTek, Winooski, VT, USA). A calibration curve was prepared using L-leucine (L-Leu) as standard (range 0.1–2.0 mmol/L). The results were expressed as mmol/L of L-Leu equivalents.

2.2.2. Antioxidant Activity Assay

The *in vitro* antioxidant activity in samples was determined by the Oxygen Radical Absorbance Capacityfluorescence method (ORAC) [8] using a Synergy 2 microplate photometer–fluorometer. The peroxyl radical was generated directly in the reaction medium during the thermal decomposition of the azo compound 2,20-azobis (2-methylpropionamidine) dihydrochloride (AAPH, Sigma-Aldrich, St. Louis, MO, USA), initiated by incubation at 37 ˚C for 10 min according to [9]. The antioxidant activity was expressed as the amount of Trolox (Sigma-Aldrich, St. Louis, MO, USA) molar equivalents (TE, μM).

2.2.3. ACEI Assay

Angiotensin-converting enzymeinhibitory activity (ACEI) in samples was determined by their ability to inhibit angiotensin I-converting enzyme (Sigma-Aldrich, St. Louis, MO, USA). o-Aminobenzoyl-Phe-Arg-Lys(dinitrophenyl)-Pro (Sigma-Aldrich, St. Louis, MO, USA) was used as a substrate with internal fluorescence quenching [10]. The 96-well, black, nonbinding polypropylene microplates (Greiner Bio One, Germany) were used.The measurements were carried out on a Synergy 2 microplate photometer–fluorometer. The concentrationIC_{50} was determined at which ACE activity decreased by 50%. IC50 was expressed as (mg of protein)/mL.

3. Result and Discussion

For the study, we used Kefir and Amasi purchased in supermarkets in Russia and Africa, respectively. Three samples from different manufacturers were selected for the analysis of each product. A description of the products indicated on the packaging is given in Table 1.

3.1. Characterization of fermented products

The process of fat lipolysis plays an important role in the formation of the fermented dairy products aroma. During this process di- and monoglycerides, volatile fatty acids (acetic, butyric, caproic, valerianic, etc.) and fatty acid derived compounds (aldehydes, ketones, methyl ketones, esters, alcohols) are formed. In the course of fermentation this process is carried out mainly by lipolytic enzymes produced by lactic acid bacteria

Table 1: Description of traditional fermentedmilk products in Russia and South Africa, presented on the labels of the studied commercial samples

Labeling Information		
Product name, fat content, pH, manufacturer	Nutritional Information per 100 g/ml of product	Ingredients
Kefir		
«Ruzskyi Kefir» (3.2-4.0%), pH 4.45, JSC “RUZKE MILK”, Russia	Fat – 3.8 g;Protein – 3.0 g;Carbohydrate – 4.0 g.Energy – from 238 to 268 kJ / from 57 to 64 kcal.	Whole milk, culture on kefir grains. The amount of yeast at the end of the shelf life is at least 10^4CFU/g. The number of lactic acid microorganisms is not less than 10^7CFU/g.
Kefir «Asenievskayaferma» (3.2%), pH 4.4, Agricultural production cooperative ”Agricultural artel (collective farm) ”Pervomaisky ”, Russia	Fat – 3.2g; Protein – 3.0g; Carbohydrate – 4.0g. Energy – 238 kJ / 57 kKal	Normalized milk, culture on kefir grains. The number of lactic acid producing microorganisms is not less than 10^7CFU/g. The amount of yeast at the end of the shelf life is at least 10^4CFU/g.
Kefir «Molochnayakultura» (3.5- 4.5%), 4.39,“Milk Culture” LLC, Russia	Fat – 4.1g; Protein – 3.0g; Carbohydrate – 4.0g. Energy –250-290 kJ / 60-70 kKal;	Whole milk, culture on kefir grains. The number of lactic acid producing microorganisms is not less than 1×10^7CFU/g. The amount of yeast at the end of the shelf life is at least 1×10^4CFU/g.
Amasi		
«Full cream MAAS PASTEURISED» (3.7%), pH 4.51, Pick n Pay Ltd. «PnP», South Africa	Protein – 3.3g; Carbohydrate – 5.0gof which total sugar – 3.3g Total fat – 3.7g of which saturated fat – 2.8g; trans fat – < 0.1g; monounsaturated fat – 0.7g; polyunsaturated fat – 0.1g Dietary Fibre – <0.5g; Total sodium – 42mg; Calcium – 115mg. Energy – 270 kJ	Full Cream milk, Starter Culture.
Full cream MAAS «AMASI OTHANDO» (3.3%), pH 4.49, Clover S.A. (PTY) LTD, South Africa	Protein – 3.3g; Glycaemic Carbohydrate – 4.0g of which total sugar – 3.7g Total fat – 3.3g of which saturated fat – 2.3g Dietary fibre – <0.5g; Total sodium – 41mg; Calcium – 128mg. Energy – 241 kJ	Milk and/or Recombined Milk, Culture.
Full cream MAAS «INKOMAZI Rich and Creamy» (3.4%), pH 4.37, Manufactured for Danone Southern Africa (PTY) LTD, South Africa	Protein – 3.2g; Glycaemic Carbohydrate – 5.0g of which total sugar – 4.8g Total fat – 3.4g of which saturated fat – 1.9g Dietary fibre – 0.0g; Total sodium – 47mg; Calcium – 120.0mg. Energy – 265 kJ	Full fat milk, MAAS cultures.

(LAB). We analyzed the composition of fatty acids (FA) and volatile components (VOC) in the test samples. It was shown that the metabolic profiles did not differ among Kefirs from different manufacturers. Also concerns the samples of Amasi.

3.2. Fatty Acid Analysis

According to the results of the FA compositionanalysis, Kefir samples demonstrated the most diverse set of FAs – 36 compounds, and only 22 compounds were identified in the Amasi samples (Tables 1,2).

The analysis revealed that the dominant FAs in all samples are palmitic (C16:0) – 24-26 % (of the total FAs), oleic (C18:1ω9) – 20-23 %, and stearic (C18:0) – 12-16 %. All this FAs are dominant in the composition of fat of cow's milk.In Amasi the amounts of polyunsaturated fatty acids and linoleic acid (C18:2–6) were 2 times lower in comparison to Kefir. Moreover, transisomers of linoleic acid (C18:2–6), such as 10-trans and 12-cis-linoleic acid that were found in Kefir, but were absent in Amasi.

In the composition of Amasi, a transisomer of oleic acid (9-trans-octadecenoic or elaidic acid) was found – 5.47 % of the total. Milk with a fat content of 2.4 – 4.0% may contain trans isomers from 3.9 to 5.1% of the sum of all fatty acids. Synthesized in a rumen of ruminants - the main sources of TFA of natural origin - trans fats are present in natural dairy products in small amounts. The predominance of certain TFA in ruminant fats depends on the ratio of various unsaturated fatty acids in their diet. However, in contrast to chemical hydrogenation, which leads to a random mixture of isomers, double bonds formed in ruminant fats with the participation of cellulolytic enzymes of the rumen are located in specific positions, and their profile is determined by the preferential diet of the animal: feed or feed concentrate. In the fatty acid composition of ruminant milk fats, transisomers of hexadecenoic (C16: 1), octadecenoic (C18:1) and eicosenoic acids (C20:1) were identified with a quantitative predominance of C18:1 isomers. C16: 1 transisomers have a double bond at positions 3 to 15 with a predominance of 9-trans-hexadecenoic acid; C18:1 transisomers have a double bond at positions 4 to 17 with a predominance of 10-trans -, - octadecenoic acid; C20: 1 transisomers have a double bond at positions from 6 to 17 with a predominance of 13-trans, 15-trans and 16-trans-eicosenoic acids.

In contrast to Amasi the FA composition of Kefir included short-chain fatty acids and their derivatives. Kefir contained butyric, lactic, valerianic, 2-hydroxy-3-methylbutyric, 2-hydroxy-3-methyl valerianic and phenylactic acids. These acids are metabolites of microorganisms included in starter cultures. Phenyllactic acid found in Kefir is one of the metabolites of phenylalanine metabolism and can be formed in the cells of lactic acid bacteria from p-hydroxyphenylpyruvate. Phenyllactic acid exhibits antibiotic activity against gram-positive and gram-negative bacteria, and also acts on a wide range of microscopic fungi [11]. This metabolite is formed by some lactobacilli and propionic acid bacteria.

TABLE 2: Fatty acid profiles of fermented milk products

Compound	Designation	Content, (% of total FA)
Kefir		
Butanoicacid	C4:0	1.8
Lacticacid	$CH_3CH(OH)COOH$	0.49
Pentanoicacid	C5:0	0.04
Butyricacid, 2-hydroxy-3-methyl	2OH3MeC4:0	0.03
Hexanoicacid	C6:0	1.76
Hexanoicacid, 2-hydroxy	2OHC6:0	0.08
Pentanoicacid, 2-hydroxy-3-methyl	2OH3MeC5:0	0.06
Heptanoicacid	C7:0	0.05
1-Hexanol, 2-ethyl	$CH_3(CH_2)_3CH(C_2H_5)CH_2OH$	0.05
Benzoicacid	C_6H_5COOH	0.21
Octanoicacid	C8:0	1.45
Nonanoicacid	C9:0	0.07
Hexanedioicacid	$HOOC(CH_2)_4COOH$	0.04
4-Decenoic acid	C10:1	0.38
Decanoicacid	C10:0	3.6
Phenyllacticacid		0.04
Undecanoicacid	C11:0	0.13
Dodecanoicacid	C12:0	4.37
Tridecanoicacid	C13:0	0.16
Tridecanoicacid, 12-methyl	12MeC13:0	0.09
9-Tetradecenoic acid	C14:1ω5	1.2
Tetradecanoicacid	C14:0	10.39
Tridecanoicacid, 12-methyl	12MeC13:0	0.17
Tetradecanoicacid, 12-methyl	12MeC14:0	0.45
Pentadecanoicacid	C15:0	1.49
9-Hexadecenoic acid	C16:1ω7	1.9
Hexadecanoicacid	C16:0	24.86
Hexadecanoicacid, 15-methyl	15MeC16:0	0.45
Heptadecanoicacid	C17:0	0.62
9,12-Octadecadienoic acid	C18:2ω6	4.01
9-Octadecenoic acid	C18:1ω9	20.28
12-Octadecenoic acid	C18:1ω9	6.01
Octadecanoicacid	C18:0	11.8
10 trans,12 cis -Octadecadienoicacid	C18:2ω6	1.05
5,8,11,14-Eicosatetraenoic acid	C20:4ω6	0.19
8,11,14-Eicosatrienoic acid	C20:3ω6	0.23

Table 2: Table Continued.

Amasi		
Caproicacid	C6:0	1.8
Benzoicacid	C_6H_5COOH	0.36
Caprylicacid	C8:0	1.48
Decenoic acid	C10:1	0.35
Capricacid	C10:0	3.99
Undecanoicacid	C11:0	0.08
Dodecanoicacid	C12:0	0.07
Lauric acid	C12:0	3.99
Tridecanoicacid	C13:0	0.09
Myristoleicacid	C14:1ω5	0.85
Myristic acid	C14:0	10.34
Tetradecanoic acid, 12-methyl	12MeC14:0	0.59
Pentadecanoicacid	C15:0	1.22
Palmitoleic acid	C16:1ω7	1.28
Palmitic acid	C16:0	25.57
Margaric acid	C17:0	0.52
Hexadecanoicacid, 14-methyl	14MeC16:0	0.57
Hexadecanoicacid, 15-methyl	15MeC16:0	0.62
Linoleic acid	C18:2ω6	2.03
Oleic acid	C18:1ω9	22.96
Elaidic acid	C18:1ω9	5.47
Stearic acid	C18:0	15.77

Table 3: Total Fatty Acid Ratio in Fermented Dairy Products

Content,%	Kefir	Amasi
Unsaturatedfattyacids(UFA)	64.8	67.1
Monounsaturatedfattyacids(MUFA)	29.8	30.9
Polyunsaturatedfattyacids(PUFA)	5.4	2.0

Benzoic acid was found in the samples of both products, but its content was different: Kefir - 0.21% of the total amount of all FAs; and in Amasi - 0.36%.

Hydroxy derivatives of fatty acids, 2-hydroxyhexanoic acid, were also found in kefir. ICD strains that form 2-hydroxyhexanoic and other hydroxy derivatives of fatty acids are described in the literature; all of them exhibit antibiotic activity against a wide range of yeasts and molds [11].

In Kefir the proportion of long chain fatty acids to the total amount of FA is higher than in Amashi. It is known that they are associated with increased adhesion of probiotic cultures to the mucous membrane of the intestinal wall. Also in Kefir identified polyunsaturated polyenoic acids of composition C20:cis-8,11,14-eicosatrienoic (dihomo-γ-linolenic) and cis-5,8,11,14-eicosatetraenoic (arachidonic). They are not only structural components of lipids of cell membranes, lipoprotein complexes of the brain and spinal cord, heart, liver and other organs, but also the precursors of a number of their biologically important metabolites - prostaglandins, cyclopentenones, prostacyclins, thromboxanes, leukotrienes, lipoxins, hepoxilins.

3.2.1. Volatile Flavor Compounds Analysis

The spectrum of the volatile organic compounds (VOCs) detected in fermented milk productsis presented in Tables4 and 5.

The main group of identified VOCs in the Amasi was ketones, and in kefir - alcohols. The acid content in both fermented milk products was approximately the same and amounted to about 31 % of the sum of all VOCs of the sample. Carboxylic acid compounds are the main components of most dairy products. These compounds are formed as a result of microbial fermentation of milk. Of the odorous acids, caproic (hexanoic) and caprylic (octanoic) acids were found in booth product samples. Hexanoic acid is known to give a dairy, “rancid” taste to dairy products, and octanoic acid can give a “soapy” taste [12]. Acetic acid in the volatile fraction was found only in Kefir samples in this study. Acetic acid is an important compound that can impart a pungent ”vinegar” taste to dairy products [13] and is commonly found during milk fermentation by *Lactobacillus delbrueckii* subsp. *bulgaricus*[14].

An analysis of the Amasi VOC profile showed that the dominant compound in the ketone group is acetoin (64% of the total VOC), the simplest representative of acyloins (α-hydroxyketones, α-hydroxyketones, ketols), one of the products of butanediol fermentation of lactic acid bacteria. Acetoin in air gradually oxidizes to diacetyl, which has the smell of butter. When fermenting milk with *Lb. delbrueckii* subsp. *bulgaricu*s, as in the case of Amasi, the main group of VOCs were ketones, including acetoin[14].

Analysis of the Kefir VOC profile showed that isoamyl alcohol (36% of the sum of all VOCs) and ethanol (16% of the sum of all VOCs) prevailed in the alcohol group. Isoamyl alcohol, ethanol, and acetic acid produce yeast during the metabolism of sugars, which are part of kefir starter culture (kefir grains).

Table 4: Volatile compounds profile detected in fermented products.

Compound	OdorDescription	Content, (% of total VC)
Kefir		
Ethanol	alcoholic	16.4
Aceticacid	cidervinegar, pungent	14.1
3-Methyl-1-butanol (Isoamylalcohol)	pungentcharacteristicodc	35.8
2,3-Butanediol (Diacetyl)	strongbuttery, creamy	9.9
n-Hexanol	pleasant odor in small quantities	1.1
Hexylmethylketone	reminds the applesodor	0.7
2,7-Dimethyl-4,5-octanediol	–	4.3
Hexanoic (caproic) acid (C6:0)	“goatsmell”odor	8.9
Octanoic (caprylic) acid (C8:0)	fatty-acid, dry	7.2
Decanoicacid (C10:0)	withoutodor	1.6
Amasi		
Acetoin (α-hydroxyketone)	buttery, yogurt, cream	64.4
3-Hydroxypropanoic acid	sour	16.9
Hexanoic (caproic) acid (C6:0)	“goatsmell”odor	6.6
1-Decene	–	1.9
Octanoic (caprylic) acid (C8:0)	fatty-acid, dry	3.4
Decanoic acid (C10:0)	without odor	0.6
Hexadecanoic acid (C16:0)	without odor	0.9
1-Hexadecanol	without odor	2.8
Octadecanoic acid (stearic acid)	without odor	2.5

Table 5: Total VOCs Ratio in Fermented Dairy Products

Content,%	Kefir	Amasi
Acids	31.8	30.9
Alcohols (*Alcohols+Alkens)	67.5	4.7*
Amides	-	-
Esters	-	-
Ketones	0.7	64.4

3.3. The Functional Properties Analysis

The values of antioxidant and ACE inhibitory activity in fermented milk products correlated with the depth of hydrolysis of milk proteins, which depends on the proteolytic activity of starter cultures and the time of fermentation of the product(Table. 6).

TABLE 6: Ranges of values Antioxidant and ACEI activities for the national fermented products of Russia and Africa from different manufacturers.

Product	pH	ORAC, μMolTE/ml	ACEI(IC_{50}), mg/ml	L-Leu eq., $mMol \times L^{-1}$
Kefir	4.39-4.48	246-574	2.0-4.3	4.9-6.7
Amasi	4.37-4.51	600-796	1.3-1.5	6.5-8.9

As shown by broader screening of products from different manufacturers, both type products have a rather wide range of antioxidant activity values, which is apparently due to differences in the production process at different enterprises. However, on average, Amasi has a higher antioxidant activity than Kefir. The range of ACE inhibitory activity values for Kefir was wider than for Amasi. The value of ACE inhibitory activity for Amasi was also higher.

4. Conclusion

Natural fermentation is the best way of extending milk shelf-life and also enhancing its flavor and the nutritional benefits. Both investigated products are probiotic due to their antioxidant and antihypertensive activities.In addition, they contain a fairly wide range of bioactive compoundsthat determine the taste and aroma characteristics of products, which differ significantly. First, the presence of yeast in kefir determines the presence of alcohol, which gives this drink a characteristic alcoholic odor. In addition, acetic acid and isoamyl alcohol found in the VOC profile of Kefir in a sufficiently large amount give the drink a sour-pungent hue. The vital activity of yeast and streptococci as part of kefir grains causes gas formation, which affects the Kefir consistency.Amasi, on the other hand, has a softer, creamier and buttery aroma due to the presence of a large amount of acetoin.

Thus, the production technology and composition of microorganisms has a key influence on the characteristics of the final fermented product, along with the quality of raw materials.A more detailed study of starting consortiamicroorganisms, the isolation of new probiotic microorganisms with unique properties and the study of the properties of fermented milk products opens up great opportunities for creating microbial compositions in order to expand the market for fermented milk products with specified probiotic and organoleptic properties.

5. Funding

The reported study was funded by RFBR and NRF according to the research project № 19-54-60002

References

[1] Rosa DD, Dias MMS, Grzes´kowiak LM et al. Milk kefir: Nutritional, microbiological and health benefits. Nutrition Research Reviews. 2017;30:82-96. doi: 10.1017/S0954422416000275

[2] Prado MR, Blandón LM, Vandenberghe LPS et al. Milk kefir: Composition, microbial cultures, biological activities, and related products.Frontiers in Microbiology. 2015;6. 1177-1177 doi:10.3389/fmicb.2015.01177

[3] Santos A, Mauro S, Sanchez A, Torres JM, Marquina D. The antimicrobial propertiesof different strains of *Lactobacillus* spp.isolated from Kefir. Systematic and Applied Microbiology. 2003;26:434–437. doi: 10.1078/072320203322497464

[4] Rodrigues KL, Caputo LRG, Carvalho JCT, Evangelista J, Schneedorf JM. Antimicrobial and healing activity of kefir and kefiran extract.International Journal of Antimicrobial Agents. 2003;25:404–408. doi:10.1016/j.ijantimicag.2004.09.020

[5] Nielsen B, Gurakan GC, Unlu G. Kefir: A multifaceted fermented dairy product. Probiotics Antimicrobial Proteins. 2014;6:123-135. doi: 10.1007/s12602-014-9168-0

[6] Osvik RD, Sperstad S, Breines E et al. (2013).Bacterial diversity of Amasi, a South African fermented milk product, determined by clone library and denaturing gradient gel electrophoresis analysis.African Journal of Microbiology Research. 2013;7(32):4146-4158.doi: 10.5897/AJMR12.2317

[7] Adler-Nissen, J.Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid.Journal of Agricultural and Food Chemistry. 1979;27(6):1256–1979.doi:10.1021/jf60226a042

[8] Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry. 2001;49:4619–4626. doi:10.1021/jf010586o

[9] Prior RL, Hoang H, Gu L et al. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples.Journal of Agricultural and Food Chemistry. 2003;51:3273–3279. doi:10.1021/jf0262256

[10] Araujo MC, Melo RL, Cesari MH, Juliano MA, Juliano L, Carmona AK. Peptidase specificity characterization of C- and N-Terminal catalytic sites of angiotensin I-converting enzyme.Biochemistry. 2000;39:8519–8525. doi:10.1021/bi9928905

[11] Stoyanova LG, Ustyugova EA, Netrusov AI. Antibacterial metabolites of lactic acid bacteria: Their diversity and properties. Applied Biochemistry and Microbiology. 2012;48(3), 229-243

[12] Condursoa C, Verzeraa A, Romeoa V, Ziinoa M, Conteb F. (2008). Solidphasemicroextraction and gas chromatography mass spectrometry analysis of dairy product volatiles for the determination of shelf-life. International Dairy Journal. 2008;18:819–825. doi: 10.1016/j.idairyj.2007.12.005

[13] ChengHF. Volatile flavor compounds in yogurt: A review. Critical Reviews in Food Science and Nutrition. 2010;50:938–950. doi: 10.1080/10408390903044081

[14] Dan T, Ren W, Liu Y et al. (2019) Volatile flavor compounds profile and fermentation characteristics of milk fermented by *Lactobacillusdelbrueckii* subsp. b*ulgaricus*.Frontiers in Microbiology. 2019.10. 2183-2185.doi:10.3389/fmicb.2019.02183