Fermented drinks of HippophaeRhamnoides as a way of innovative development of agriculture in Altai region

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Abstract.One of the technological innovations nowadays is the development of industrial processing of agricultural raw material, manufacturing process leading to lower risks – «Low/No/Reduced» and «Better-for-you». This may be done by light processing of raw materials and use of traditional technologies, i.e. fermentation of craft beverages by natural symbiotic cultures *Oryzomycesindichi* and *Medusomycesgisevi* (kombucha). The recent years saw a worldwide interest to a horticultural crop called *Hippophaerhamnoides* (sea-buckthorn) as it is eco-friendly and healthful. Its fruits have a special smell and taste, are rich in carotene, carotenoids, tocopherols, ascorbic acids, polyphenols. The composition is the reason why it is used for foodstuff, i.e. pastry, dairy products, unclarified juice. Production of beverages fermented by natural symbiotic cultures and local orchard products is a new direction in Russia. The supplies are limited which means that the research is relevant. The aim of the research is to study the possibility of usage of juice of *Hippophaerhamnoides* fruits in the new technology of soft drinks production fermented by *Oryzomycesindichi* and *Medusomycesgisevi*. A single nutritious substrate for *Oryzomycesindichi* and *Medusomycesgisevi* symbiotic cultures cultivation was proved impossible to be produced. Still the possibility to replace polyphenols of the *Medusomycesgisevi* by those of the sea-buckthorn juice must be analyzed. Sea-buckthorn juice added in a culture medium fermented by *Oryzomycesindichi* and *Medusomycesgisevi* symbiotic cultures increases biosynthetic activity of acid-forming bacteria. This tangibly improves organoleptic properties of the fermented soft beverages produced. Besides, the technology of *Oryzomycesindichi* and *Medusomycesgisevi* production presupposes no clarification which makes the process of the *Hippophaerhamnoides* use significantly easier. The research helped to identify that the sea-buckthorn juice used in a must fermented by *Medusomycesgisevi* has many advantages. Namely more comprehensive utilization of carbohydrate, a higher level of reduction of organic acids, and decrease of the time of technological process (24 hours less).

1 Introduction

Sea-buckthorn is a tree and shrubby foodplant used for alimentary, therapeutic, and decorative purposes. The culture differs by the ability to synthesize oil in the pulp and the skin, to be polymorphic, eco-friendly, easy to keep, frost and drought resistant [1, 2]. The results of the work of the Lisavenko Research Institute of Horticulture of Siberia enabled to create industrial plantations of sea-buckthorn in Altai Region. A most significant for the industry feature of the culture is the convenient fruit ripening period – from the mid-August until the end of October for the early and the late ones resp. [3–6].

The foodstuff containing sea-buckthorn is becoming demanded and is treated healthily in many countries. This may be juice, candies, jellies, jams, alcoholic and soft beverages, dairy products [7].

The fruits have a special smell and taste that may be characterized as acid, sour, astringent, and not sweet. The total amount and/or proportion of sugars and acids is a crucial characteristic that regulates the taste of the fruits and ways of their processing [8, 9]. Besides, the sea-buckthorn has a complex composition influencing the production of fermented strong and soft beverages [10–12].

While unclarified products are being produced, i.e. juice or fruit nectar, small parts of the oil join to big flake-like structures and float on beverages, which diminishes its sensorial qualities. Yet the smallest suspended oil drops complicate the filtration of the sea-buckthorn juice to be further clarified [13].

A key innovation in food manufacturing is development of industrial processing of agricultural raw material. The process leads to lower risks – «Low/No/Reduced» and «Better-for-you». Firstly, these directions presuppose a light processing of natural product, first, as modern consumers avoid consuming harshly processed products. Secondly, traditional processes and technologies, such as fermentation, appear to be favored by Russian and foreign producers. They are simpler and more easily accepted by consumers because of the naturalness as compared to “artificial” modern technologies. Thirdly, a popular trend is craft food and beverages. It is a chance for the manufacturers selling “home-made” products by old recipes to enter the market. Craft beverages fermented by natural symbiotic cultures *Oryzomycesindichi* [14–16] and *Medusomycesgisevi* (kombucha [17, 18]) may be ones of the products.

2 Purpose of the study

Beverages of natural symbiotic cultures are quite demanded in the world market, but in Russia they are new, as proved by the limited supply. Thus, the development of scientific bases for fruit raw material use, i.e. for *Hippophae rhamnoides*, in technologies of traditional fermentation of beverages is relevant for production and processing of horticultural crops. So, the aim of the research is to study the possibility to use the juice of *Hippophae rhamnoides* fruits to produce soft drinks fermented by *Oryzomycesindichi* and *Medusomycesgisevi*.

3 Objects of the study

The objects of the study were samples of fermented soft beverages with directly squeezed juice of sea-buckthorn added and *Oryzomycesindichi* and *Medusomycesgisevi* symbiotic cultures used (fig. 1).

Fig. 1.The symbiotic cultures

The directly squeezed sea-buckthorn juice was made of the berries of a variety called “Chuyskaya”. The berries were cut and treated by a pectolytic enzyme Rapidase CR (dosage 20 ml/tof raw material, treatment time 3 hours, temperature 50 °C), then pressed in the expeller. The further organoleptic assessment showed that the juice has a sour- sweet and astringent taste. The smell corresponds to the one of the fresh berries of the variety. The composition is presented in the table 1.

Table 1.Composition of the directly squeezed sea-buckthorn juice (n=3, M±m)

Sample	Mass content, %			
	Organic acids on a malic acid basis	sugars	Dry basis	Polyphenolic substances
Juice	1,18±0,04	8,12±0,08	13,2±0,1	0,125±0,011

The juice-containing substrate was fermented by *Oryzamycesindichi* as follows. The substrates made as formulated were centrifugated to remove suspended particulate matter (SPM). Then they were pasteurized during an hour at 65±2 °C to eliminate foreign microflora affecting the activity of the product and organoleptic, physical and chemical properties of the beverage. First, the culture *Oryzamycesindichi* 5% wt was inserted and, second, the species were thermostated for 5 days in the hot air oven at 25±1 °C to cultivate the substrate. The fermented broth was withdrawn once a day to analyze its physical and chemical properties. The cultivated fermented beverage was separated from the zoogloea through decantation and was further put to refrigerator at 5–7 °C for the next experiments. The zoogloea was constantly washed with distilled water and returned to fermentation.

The juice-containing substrate was fermented by *Medusomycesgisevi* as follows. The substrates made as formulated were centrifugated to remove suspended particulate matter (SPM). Then they were pasteurized during an hour at 65±2 °C to eliminate foreign microflora affecting the activity of the product and organoleptic, physical and chemical properties of the beverage. First, the culture *Medusomycesgisevi* 5% wt was inserted and, second, the species were thermostated for 5 days in the hot air oven at 25±1 °C to cultivate the substrate. The fermented broth was withdrawn once a day to analyze its physical and chemical properties. The cultivated fermented beverage was separated from the zoogloea through decantation and was further put to refrigerator at 5–7 °C for the next experiments. The lower layer of the zoogloea was constantly removed and the zoogloea was washed with distilled water and returned to fermentation.

The control samples were beverages without the sea-buckthorn juice made likewise.

4 Materials and methods

The physical and chemical properties of the sea-buckthorn juice and the samples of the beverages were identified by standard methods. The fermented beverages were titrated with 1 mol of sodium hydroxide as per GOST 6687.4–86 to discover the acid number. The mass content of sugars was determined by the Bertrand copper-reduction method as per GOST 8756.13–87. The mass content of dry basis was found by the aerometric method as per GOST 6687.2–90. The volume concentration of ethyl hydroxide was learnt by the distillation method as per GOST 6687.7–88. The mass of polyphenolic substances was measured by the colorimetry with the Folin–Ciocalteu reagent (FCR) [19]. The absorptivity was detected with the wavelength at 610 nm in the Shimadzu UV-1800 Doble-Beam Scanning Spectrophotometer. Individual organic acids of the samples were analyzed by the capillary electrophoresis (CE) in the system “Kapel’ 105M” (Russia, LLC “Lumex”). The conditions were as follows: sampling at 150 mbar×sec, voltage at minus 20 kv, thermostat temperature at 20°C. The acids were detected by an indirect method of ultraviolet absorption at 254 nm. Organic acids were separated by a buffer solution of 10 mmol of benzoic acid, 9 mmol of diethanolamine, 0,5 mmol of cetrimonium bromide, 0,5 mmol of ethylenediaminetetraacetic acid and capillaries of 75µmol in diameter and 60 cm in length. The collection of data and processing of electropherograms were done in the software “Elphoran” of the “Kapel’ 105M”. The peaks were identified in quality and in quantity using standard calibration solutions.

5 Results and discussion

The literature data showed that composition of culture media for *Oryzomycesindichi* and *Medusomycesgisevi* symbiotic cultures cultivation differs. Thus, the culture *Oryzomycesindichi* needs digestible carbohydrates only to reproduce. The source of nitrogenous matters necessary for the symbiont’s normal vital activity, described in the research [20], was a small quantity of raisin. It may be replaced by fruit and berry juice [21] which would allow to arrange complex industrial processing of *Oryzomycesindichi* using sea-buckthorn juice.

However, the culture *Medusomycesgisevi* requires big quantity of polyphenols, i.a. the ones of the tea liquor, to develop.

Thus, the single nutritious substrate for the *Oryzomycesindichi* and *Medusomycesgisevi* symbiotic cultures cultivation is proved impossible to be produced. Still the possibility to replace polyphenols of the *Medusomycesgisevi* by those of the sea-buckthorn juice must be analyzed.

The table 2 shows the composition of musts for cultivation of the symbiotic cultures for the beverages with the final acidity 5g/dm3 max. The analysis made demonstrates that the acidity of the beverages rise generally to 1,5–1,8 g/dm3 when fermented [20, 21].

Table 2. Composition of samples of must for fermentation of beverages (%)

Raw material	Oryzomycesindichi	Medusomycesgisevi
Directly squeezed sea-buckthorn juice	25,0	25,0
Sucrose	10,0	10,0
Tea liquor*	–	2,25
Drinking water	to 100,0	to 100,0
*Quantity of polyphenols in the strong liquor – 0,215±0,007 %		

An aqueous extract of polyphenols of green tea leaves as a component of the must is added to cultivate the kombucha. This eliminates the unattractive for consumers grayish orange and brown color of the fermented teal leaves. The composition of the must samples made for *Oryzomycesindichi* and *Medusomycesgisevi* cultivation is presented in the table 3.

Table 3.Composition of samples of musts (n=3, M±m)

Sample	Mass content, %		
	Organic acids on a malic acid basis	Sugars	Polyphenolic substances
Oryzomycesindichiand sea-buckthorn juice	0,32±0,02	10,0±0,1	0,031±0,003
Medusomycesgiseviand sea-buckthorn juice	0,31±0,02	10,0±0,1	0,052±0,004

The fig.2 demonstrates the dynamic of organic acids, sugar utilization, and ethyl alcohol accumulated in 5 days in the process of *Oryzomycesindichi* and *Medusomycesgisevi* cultivation.

Fig. 2.Dynamic of organic acids (a), sugar (b), and ethyl alcohol (c) accumulated in 5 days of *Oryzomycesindichi* and *Medusomycesgisevi* cultivation

The fig.2 shows that the fermentation of juice-containing substrates using the symbiotic cultures is likewise. However, utilization of nutritious carbohydrates of the must with *Medusomycesgisevi* cultivation is larger. This results in a more intensive accumulation of ethanol in the medium and in higher concentration of titrated acids. The concentration is due to favorable conditions for reproduction of *Acetobacteraceti* and *Acetobacterxylinum*, prone to biosynthesize ethanoic acid. The results obtained on identification of organic acids in the beverages by the CE method is the proof (fig. 3).

Fig. 3.Typical electropherogram of the beverage with Medusomycesgisevi cultivated for 5 days

Table 4 (results of the CE) demonstrates that the dominant organic acid in the fermented drinks, with the cultures cultivated for 5 days on the substrates, is the malic one. This can be explained by the fact that the malic acid was integrated in the beverage along with the raw material. Its quantity suffered almost no changes during cultivation.The increment in quantity of succinic, lactic, and ethanoic acids can stem from biosynthesis of the microorganisms of the Oryzamycesindichi and Medusomycesgisevi symbiotic cultures.

Table 4.Dynamic of synthesized organic acids in cultivation of the Oryzamycesindichi and Medusomycesgisevi cultures (n=3, M±m)

Organic acid	Concentration of the organic acids during the fermentation, mg/dm ³				
	Day 1	Day 2	Day 3	Day 4	Day 5
Control of Oryzamycesindichi					
Succinic	111,6±0,9	134,7±0,6	167,2±0,4	179,3±0,5	193,2±0,4
Lactic	–	13,6±0,3	24,7±0,2	47,1±0,3	89,3±0,4
Ethanolic	–	–	39,4±0,3	147,9±0,5	354,6±0,6
Oryzamycesindichi with sea-buckthorn juice					
Succinic	118,6±0,8	139,7±0,3	178,2±0,8	192,4±0,3	213,4±0,6
Lactic	–	17,5±0,2	36,9±0,2	67,4±0,2	102,6±0,3
Ethanolic	–	27,9±1,1	47,8±0,8	173,2±1,1	492,4±1,3
Control of Medusomycesgisevi					
Succinic	98,7±0,7	164,3±0,9	204,1±0,9	274,2±1,3	318,3±2,6
Lactic	–	–	–	11,4±1,7	24,7±1,0
Ethanolic	108,2±1,3	179,6±2,4	267,2±2,9	381,9±3,2	505,8±4,6
Medusomycesgisevi with sea-buckthorn juice					
Succinic	93,7±0,5	174,3±1,2	227,3±1,6	264,3±0,9	293,6±2,2
Lactic	–	16,4±0,3	34,7±1,1	72,3±1,0	125,8±2,0
Ethanolic	134,7±2,2	204,7±2,8	316,4±2,4	483,5±3,2	762,3±3,7

The results obtained can confirm that sea-buckthorn juice added in the culture medium, with fermentation of Oryzamycesindichi and Medusomycesgisevi symbiotic cultures, results in biosynthetic activity of acid-forming bacteria. This also effects organoleptic

properties of soft fermented beverages. However, the sharp quantitative increase of synthesized ethanoic acid by *Medusomycesgisevi* leads to organoleptic properties improved within 4 days of fermentation. A steep increase in biosynthesis of organic acids was not registered for fermentation of the control sample and the sample with sea-buckthorn juice by *Oryzamycesindichi*. The duration of fermentation remained the same.

The fig.4 and 5 are profile charts of organoleptic properties of the beverages obtained by fermentation of must samples by the *Oryzamycesindichi* and *Medusomycesgisevi* cultures for 5 days compared to the control data.

Fig. 4.Profile chart of organoleptic properties of cultivated for 5 days *Oryzamycesindichi* with sea-buckthorn

Fig. 5.Profile chart of organoleptic properties of cultivated for 5 days *Medusomycesgisevi* with sea-buckthorn

Hence, sea-buckthorn juice used in the beverage with *Oryzamycesindichi* improves organoleptic properties, making the smell and taste more present and masking the rye flavor, as well as the strong smell of ethanoic acid, present in the control sample. The beverage remains greyish light-yellow without any impurities. Foamability and short-lasting release

of carbon dioxide, while being poured, are the remarkable properties too. The taste is sweet-and-sour, refreshing and nice.

The assessment of organoleptic properties of the control and experimental samples of kombucha with sea-buckthorn showed that the sea-buckthorn juice changed the taste and the smell of the beverage. Kombucha with sea-buckthorn beverage is grayish yellow without any impurities. The taste of the berries is sweet-and-sour, astringent. The smell is complex, comprising fermentation smell, nuances of the variety raw material, and the typical vinegar smell.

6 Conclusions

In the course of research it was stated that *Hippophaerhamnoides* used as raw material to produce soft beverages fermented by traditional symbiotic cultures, i.e. *Oryzomycesindichi* and *Medusomycesgisevi*, was proved to improve organoleptic properties of beverages. However, the technologies of production of *Oryzomycesindichi* and *Medusomycesgisevi* presuppose no clarification which makes the process of *Hippophaerhamnoides* use significantly easier. The research helped to identify that the sea-buckthorn juice used in the must fermented by *Medusomycesgisevi* results in many advantages. They are more comprehensive utilization of carbohydrate, a higher level of re-duction of organic acids, and decrease in time of technological process (24 hours less than for the traditional one).

Thus, the possibility to implement *Hippophaerhamnoides* in technologies of fermentation of soft beverages not traditional for the Russian Federation was studied. Improved organoleptic properties of the samples obtained demonstrate the potential of the suggested craft technologies, with *Oryzomycesindichi* and *Medusomycesgisevi*, should be used for industrial purpose.

References

1. Yu. A. Koshelev, L.D. Ageeva, E.S. Batashov, V.P. Sevodin, E.D. Rozhnov, N.I. Kuleshova, *Sea buckthorn*, 410 (Publishing house of Polzunov Altai State Technical, Biysk, 2015)
2. I.K. Dugarova, G.Ts. Tsybikova, I.T. Alexandrova, *IzvestiyaVuzov. Prikladnaya Khimiyai Biotekhnologiya*, **6 (3)**, 128 (2016)
3. A. Raffo, F. Paoletti, M. Antonelli, *Eur Food Res Technol*, **219**, 360 (2004)
4. S.C. Andersson, M.E. Olsson, E. Johansson, K. Rumpunen, *J. Agric. Food Chem.*, **57 (1)**, 250 (2009)
5. S. Arif, S.D. Ahmed, A.H. Shah, L. Hassan, S.I. Awan, A. Hamid, F. Batool, *Pak. J. Bot.*, **42 (5)**, 3561 (2010)
6. X. Gao, M. Ohlander, N. Jeppsson, L. Björk, V. Trajkovski, *J. Agric. Food Chem.*, **48 (5)**, 148 (2000)
7. E. Christaki, *Food and Public Health*, **2 (3)**, 69 (2012)
8. A. Kårlund, K. Hanhineva, M. Lehtonen, R.O. Karjalainen, M. Sandell, *J. of Agricultural and Food Chemistry*, **63 (3)**, 1010 (2015)
9. O. Laaksonen, L. Mäkilä, R. Tahvonen, H. Kallio, B. Yang, *Food Chemistry*, **138 (4)**, 2421 (2013)

10. B. Negi, G. Dey, World Academy of Science, Engineering and Technology, **30**, 99 (2009)
11. S.L. Wang, L.P. Liu, L.X. Jiao, M.T. Fan, Int. J. of Food Properties, **14**, 776 (2011)
12. S.L. Wang, L.X. Jiao, Y.H. Li, M.T. Fan, Int. J. of Food Properties, **15**, 1381 (2010)
13. R. Arimboor, V.V. Venugopalan, K. Sarinkumar, C. Arumughan, R.C. Sawhney, J. of the Science of Food and Agriculture, **86 (14)**, (2006)
14. J. Stadie, A. Gulitz, M.A. Ehrmann, R.F. Vogel, Food Microbiology, **35 (2)**, 92 (2013)
15. D. Laureys, L.D. Vuyst, Applied and Environmental Microbiology, **80 (8)**, 2564 (2014)
16. M.S. Muneer Alsayadi, A.J. Yaser, M. Belarbi, F.Z. Sabri, J. of Microbiology, Biotechnology and Food Sciences, **2 (6)**, 2444 (2013)
17. I. Vina, P. Semjonovs, R. Linde, I. Denina, J. of Medicinal Food, **17 (2)**, 179 (2014)
18. J.M. Leal, L.V. Suarez, R. Jayabalan, J.H. Oros, A. Escalante-Aburto, J. of Food, **16 (1)**, 390 (2018)
19. A. Blainski, G.C. Lopes, J.C. Palazzo de Mello, Molecules, **18 (6)**, 6852 (2013)
20. Yu.S. Zintsova, E.D. Rozhnov, M.N. Shkolnikova, Technology and the study of merchandise of innovative foodstuffs, **3 (32)**, 37–42 (2015)
21. Yu.S. Zintsova, M.N. Shkolnikova, E.D. Rozhnov, Tovaroved prodovolstvennykh tovarov, **7**, 12 (2015)