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Evaluation of the biological activity of sugar-free fractionated red beetroot juice

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Abstract: In the case of type II diabetes, the most important preventive and therapeutic effect gives a diet with a minimal amount of easily digestible carbohydrates. Vegetable juices are positioned as healthy food, because of the high content of phenolic and other biologically active compounds. However, due to the high glycemic index, juices are contraindicated in obesity, and diabetes, while juices with a reduced glycemic index, are not available on the market. We have developed a technology for the fractionation of red beetroot juice based on molecular mass using ultrafiltration. The resulting fraction stimulates the absorption of iron, increases blood hemoglobin level, and enhances capillary blood flow more effectively than native juice does. Both effects are important for patients with diabetes because the impaired blood supply to tissues and organs is an important pathogenetic factor in the development of diabetic renal failure, blindness, and gangrene. The sugar content in fractionated beetroot juice is 5–7%, which makes its use in diabetes problematic. The purpose of the study was to develop a technology for removing sugar from fractionated red beetroot juice and assessing the safety of its functional properties. The fractionated native red beetroot juice and fractionated fermented juice were studied. Fermentation was carried out using pre-activated yeast *Saccharomyces cerevisiae*. It was found that after 5-day fermentation, the sugar content in the fermented fractionated juice fell to 0.5–0.7%, while maintaining functional activity.

Key words: rat, chicken, ultrafiltration, sugar removal, fermentation, absorption of iron

Introduction

Diabetes mellitus can be called a disease of civilization. This pathology is becoming more and more global. In 2019, the number of patients in the world reached 463 million, of which 58 million were in Europe (Fuentes-Merlos et al., 2021) In the case of type II diabetes, the most important preventive and therapeutic value is the modification of the chemical composition of the diet, which should contain a minimum amount of easily digestible carbohydrates. Vegetable juices are an essential component of the modern man's diet and, usually, they are positioned as healthy food products. Due to the high content of phenolic and other biologically active compounds, juices contribute to maintaining health. However, due to the high glycemic index, juices are contraindicated in diabetes. The glycemic index, a measure that ranks carbohydrates by their effect on the body's postprandial glycemic response, was introduced at the beginning of 20th century to facilitate glycemic control in diabetic patients (Jenkins et al., 1981). Unfortunately, juices with a reduced glycemic index are not yet available on the market.

A relatively low glycemic index is usual for juices derived from celery, parsley, and spinach. The sugar content of these juices is 0.9–2.0%. In our previous research, it was found, that the sugar content in red beets depends both on growing conditions and on the variety; in beetroot juice it ranges from 3 to 7.5% (Babarykin et al., 2018). We have developed a technology for the fractionation of red beetroot juice based on molecular mass using ultrafiltration. The resulting fraction can stimulate duodenal absorption of iron and increase hemoglobin levels in blood of the animals with iron deficiency anemia as well as to enhance capillary blood flow more effectively than native juice. Both effects are important for patients with diabetes because the impaired blood supply to tissues and organs is an important pathogenetic factor in the development of diabetic renal failure, blindness, and gangrene. The sugar content in fractionated beet juice is 5–7%, which makes its use in diabetes problematic.

As it is known, the most common way to reduce the sugar content in a food product is fermentation. The production of fermented juices is traditional in the countries of Southeast Asia and the Caucasus (Shet, 2017). Fermentation, used as a method of preserving vegetable raw materials in the production of semi-finished and finished food products, has become popular in Europe today. There is a lot of interest in the sector of non-dairy probiotics based on soy, juices, and cereals. Fermentation has been shown to increase the antioxidant activity of apple juice but cause a decrease in total phenols and flavonoids (Li et al., 2019). Similar changes were recorded by the authors during the fermentation of black carrot juice (Toktaş et al., 2018).

The purpose of the study was to develop a technology for removing sugar from fractionated red beetroot juice by fermentation and assessing it's the safety and its functional properties.

Materials and methods

Production of fractionated and fermented red beetroot juices

By household juicer red beetroot juice was squeezed, and centrifugated for 15 min at 5000 g. The obtained juice was tested on the content of iron, sucrose and reducing sugars. Then fresh juice was diluted with distilled water (added 10% of juice volume), fermented for 48 h (at 25 °C) using dried and previously activated culture of *Saccharomyces cerevisiae* (2 $g \cdot l^{-1}$), filtrated via paper and centrifugated for 20 min at 5000 g. The centrifugate was evaporated to the initial juice volume in a vacuum at 40 °C to remove ethanol. Sucrose and reducing sugar concentration dynamics were checked. The next step was the fractionation of the resulting fermented juice according to the previously described method (Babarykin et al., 2018).

The content of total sugar and reducing sugar were determined according to the classical Nelson-Somogyi method (Nelson, 1944). Ascorbic acid concentration in beet juice fraction was determined by 2,6-dichlorindophenol titrimetric AOAC Official Method 967.21 (1968, 2006). Atomic absorption spectrometry *(Perkin-Elmer, Analyst 700)* was used to analyse iron (Fe),calcium (Ca) and zinc (Zn) concentration in samples (Jorhem et al., 2000).

Animals

Chickens. 30-day old Lohmann Brown cockerels have been used in the study. Chickens were housed in cage units with free access to food and water. The animals were fed a full-fed diet containing 208 mg of iron per 1 kg. In an *in vivo* experiment, native red beet juice, fractionated (FRBJ) and fermented fractionated (FFRBJ) ones were studied. The chickens received 0.35 mg of iron (as sulfate water solution) *per os* once, alone, and in combination with 1 ml of the test juice. After 100 minutes, the iron content was determined in blood, duodenal mucosa, liver, and spleen.

The influence of the obtained fractions on the mineral balance of Fe^{2+}, Zn^{2+} and Ca^{2+} in the body of chickens was evaluated. Chickens received orally 0.35 mg of iron and 1 ml of a fraction of native (FRBJ) or fermented beetroot juice (FFRBJ) for 7 days. The balance of trace elements in the body of chickens was assessed by the amount received with feed and excreted with dung during the last 3 days. At the end of experiment cockerels were decapitated in accordance with the recommendations for the euthanasia of experimental animals of the European Convention (Close et al., 1997).

Rats. A similar experiment was carried out using laboratory rats with experimental iron deficiency anemia. The rats of the control group consumed standard laboratory animal food in the form of biscuits, consisting of proteins, carbohydrates, fats, minerals, and vitamins. The content of iron in diet was 270 mg/kg. Other groups received a semisynthetic diet containing 16.3 mg Fe/kg. Test solution, containing 0.35 mg of iron (iron sulfate dissolved in water) alone or in combination with experimental juices (FRBJ) or (FFRBJ) was administered *per os* for 3 days. Rats were euthanized by transcervical dislocation after liver, spleen and blood sampling (Close et al., 1996). The experiments were approved by the local Animal Ethics Committee.

All statistics were performed using the software Statistica 7. Results of body weight and visceral fat mass of rats and biochemical parameters are presented as means ± SE. Multiple group comparison was done using one-way ANOVA and Post-hoc Tukey HSD test. Statistical significance was attributed to $P < 0.05$.

Results and discussion

Table 1 presents the comparative results of the composition of fractionated and fractionated fermented red beetroot juice. Fermentation significantly has reduced the carbohydrate content of red beetroot juice. The fraction obtained from such juice contained 10 times less sucrose than the fraction obtained from natural beet juice.

Table 1. Chemical composition of fractioned and fractioned fermented red beetroot juices

Parameter	Fractionated red beetroot juice (FRBJ)	Fractionated fermented red beetroot juice (FFRBJ)
Sucrose, %	5.00 ± 1.20	0.51 ± 0.04
Reducing sugars, %	2.02 ± 0.51	0.06 ± 0.02
Ascorbic acid, mg/L	0.09 ± 0.15	0.03 ± 0.19
Fe, mg/ml	0.96 ± 0.24	0.77 ± 0.19
Ca, mg/ml	26.2 ± 2.80	20.0 ± 3.10
Zn, mg/ml	1.10 ± 0.27	0.67 ± 0.21

It was observed that the decrease of reducing sugars content by more than 30 times and ascorbic acid – almost three times. The concentration of the studied minerals in the juice after fermentation practically did not change.

It was found that because of 5-day fermentation, the sugar content in the native and fractionated juice fell to 0.5–0.7%. Fractionation as well as fermentation impacted the ability of red beetroot juice to stimulate intestinal absorption of iron and its concentration in the blood. Moreover, the fermented product showed a tendence to increase the level of iron in the blood by reducing the accumulation of the element in the liver (Table 2).

Table 2. The content of iron (Fe) in blood serum and organs of chickens administered *per os* by Fe (0.35 mg) only and simultaneously with fractionated red beetroot juice (FRBR) or fractionated fermented red beetroot (FFRBR) juice

Group	Fe content			
	Liver, mg/g wet w	Spleen, mg/g wet w	Duodenum, mg/g wet w	Blood serum, mg /ml
1. Control	121.3 ± 4.1[a*]	103.4 ± 2.5[a]	17.8 ± 1.2[a]	2.10 ± 0.06[a]
2. + Fe (Fe sulphate buffer solution)	123.3 ± 5.1[a]	102.9 ± 3.5[a]	18.6 ± 2.1[a]	2.29 ± 0.05[b]
3. + Fe +FRBR	126.5 ± 6.3[a]	103.8 ± 2.8[a]	19.6 ± 1.6[a]	2.32 ± 0.07[b]
4. + Fe + FFRBR	116.00 ± 7.1[a]	103.0 ± 3.1[a]	18.5 ± 1.8[a]	2.44 ± 0.11[b]
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$)				

Judging by the content of the studied elements in the dung, *per os* ingested fractionated, and especially fractionated fermented beet juice, stimulated of iron assimilation in the body, without affecting the balance (the ratio between the amount of consumed and excreted with dung) of zinc and calcium (Table 3).

Table 3. Content of mineral elements in dry dung of chickens administered *per os* by Fe (0.35 mg) only and simultaneously with fractionated (FRBR) or fractionated fermented red beetroot (FFRBR) juice

Group	Fe, mg/g	Zn, mg/g	Ca, mg/g
1. Control	833.3 ± 15.3[a, b*]	458.3 ± 11.4[a]	22.03 ± 1.22[a]
2. + Fe (Fe sulphate buffer solution)	866.7 ± 14.5[a]	383.3 ± 15.2[b]	23.75 ± 2.31[a]
3. + Fe +FRBR	800.0 ± 15.8[b]	375.0 ± 20.1[b]	21.17 ± 1.56[a]
4. + Fe + FFRBR	780.0 ± 16.6[b]	366.7 ± 14.9[b]	21.08 ± 1.92[a]
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$)			

In iron-deficient rats, both studied types of beetroot juice contributed to increased assimilation of Fe in the body (Table 4). However, the fermented fractionated juice contributed to a more intensive (22.6%) accumulation of Fe in the liver and an increase of the element concentration in blood.

Table 4. The iron content in blood serum and organs of iron-deficient rats administered *per os* by Fe (0.35 mg) only and simultaneously with fractionated (FRBR) or fractionated fermented red beetroot (FFRBR) juice

Group	Iron content		
	Liver, mg/g	Spleen, mg/g	Blood serum, mg/ml
1. Control (healthy rats)	467.3 ± 19.2[a*]	708.5 ± 25.9[a]	10.5 ± 2.1[a]
2. Iron-deficient rats	61.8 ± 2.20[d]	153.7 ± 4.7[b]	7.7 ± 0.9[b]
3. Iron-deficient rats (+ Fe)	81.6 ± 3.10[c]	153.3 ± 4.5[b]	10.2 ± 1.5[a]
4. Iron-deficient rats (+ Fe + FBRJ)	86.0 ± 4.10[c, b]	158.8 ± 6.9[b]	10.4 ± 0.8[a]
5. Iron-deficient rats (+ Fe + FFBRJ)	105.5 ± 3.51[b]	153.3 ± 5.3[b]	10.8 ± 1.0[a]
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$)			

Based on the data obtained in the study, it is difficult to explain opposite direction of the vectors of changes in the iron content in the liver between chickens and rats under the influence of both types of juice. The issue requires further study. Nevertheless, from the point of view of the possibility to achieve the required outcomes (maintaining the level of iron in the blood in the norm and in conditions of alimentary iron deficiency), the use of fermented fractionated beetroot juice seems justified. This is especially important for those at risk of obesity and diabetes.

The obtained results testify to the prospects of using the fermentation of beetroot and other sugar-containing juices. Fractionation based on molecular weight and the removal of easily digestible sugars significantly expands the target audience of juice consumers and provides new opportunities for health promotion through functional food.

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