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ORIGINAL PAPER

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THE SIGNIFICANCE OF SAPONINS IN SHAPING THE QUALITY OF FOOD PRODUCTS FROM RED BEET

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ABSTRACT

Background. Food products made from red beet have aroused growing interest among consumers owing to their health qualities. The quality of food products is determined to a large extent by the ingredients they are produced from. The health properties and sensory qualities of a given product are the most important features for consumers when choosing a particular product. The triterpene saponins in red beets constitute a group of compounds which influence the sensory quality of food products from this raw material. However, neither the biological activity of nor the bitter taste caused by red beet saponins has been comprehensively described in the literature. The aim of this research was to determine the influence of saponins on the sensory quality of products which incorporate red beets.
Materials and methods. The analysed food products were juices made from three cultivars of red beet roots. Sensory evaluation of the obtained juices and analytical sensory study of the bitter taste of saponins was carried out. The bitter taste recognition thresholds of saponins isolated from the cultivars under study were used to analyse the taste qualities of the finished products.
Results. The taste qualities of the analysed food products depended on the content of saponins in the final product. As compounds contributing to the bitter taste of red beets, saponins influenced the sensory qualities of the finished food products. In the case of saponins isolated from the analysed beet cultivars, it was found that the values of the defined thresholds of bitter taste recognition were higher than the saponin content in the juices obtained from these cultivars. As a result, the bitter features of juice are not detected due to the low content of saponins in them. This analysis was confirmed by the sensory evaluation of the juices, during which a team of selected evaluators indicated that sweet was the dominant flavour of the juices from Forono, Modana and Tytus cultivars, and that there was no clear sensation of its bitter features.
Conclusion. Based on the determined thresholds of bitter taste recognition in saponins isolated from different beet cultivars (minimum concentration causing the sensation of bitter taste in red beet) and the comparison with the total saponin content in the final product, one can conclude whether or not a given product demonstrates bitter features. The application potential of the red beet cultivars under study was indicated as regards their suitability for use in the production of foodstuffs.

Keywords: quality of products, sensory analysis, bitter taste, red beet

INTRODUCTION

Food products made from red beet have aroused growing interest among consumers owing to their health qualities. Red beet is a kind of raw material recognized as a production speciality in Polish agriculture.

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In Poland, there are currently 24 cultivars of red beet registered in COBORU (Research Centre for Cultivar Testing; http://www.coboru.gov.pl/Polska/Rejestr/rejestr_KR.aspx).

Cultivars of this raw material differ in the qualitative and quantitative composition of the natural substances contained within them, which include betalain pigments, polyphenol compounds and triterpene saponins. Bioactive substances slow the aging process and reduce the risk of disease, including those of civilization, namely diabetes, obesity and cancer (Czapski and Górecka, 2014). Moreover, the bioactive substances in red beet demonstrate numerous beneficial properties for the body, such as anti-inflammatory, antiviral, antibacterial and antioxidant properties (Clifford et al., 2015; Gandia-Herrero et al., 2016; Khan and Giridhar, 2015; Kumar and Brooks, 2018; Sawicki et al., 2016; Sheila et al., 2017; Slimen et al., 2017) as well as anti-cancer effects (Das et al., 2013; Kapadia et al., 2013).

The health properties of red beets result from the content of biologically active substances contained within them; mainly betalain pigments and polyphenol compounds (Carrillo et al., 2019; Koubaier et al., 2014; Wruss et al., 2015; Sawicki and Wiczkowski, 2018; Sentkowska and Pyrzyńska, 2018).

Another group of natural compounds found in red beets is triterpene saponins, the biological activity of which has not been described in the literature so far, with the exception of a few scientific reports written by the authors of this paper (Mikołajczyk-Bator, 2015; Mikołajczyk-Bator and Czyżniejewski, 2016).

In red beets the presence of triterpene saponins has been confirmed both in the roots and leaves (Mroczek et al., 2009a; 2009b; 2012; 2019). According to Mroczek et al. (2009b), 8 saponins have been found in chard roots and 2 other saponins in red beets (Mroczek et al., 2009b; 2012; 2019). Other research has tentatively described the identification of 44 triterpene saponins, including as many as 10 groups of isomers present in red beets (Mikołajczyk-Bator et al., 2016).

The data available in the literature demonstrates that saponins stemming from plants other than red beets are compounds with a wide spectrum of activities, including medicinal and other applications. Saponin compounds are known not only for their antioxidative, but also their antimicrobial, anti-carcinogenic, anti-inflammatory, anti-allergic, anti-arthritic and cardioprotective activities, though which of these they exhibit depends on their type and concentration (Cuéllar et al., 1997; Hierro et al., 2018; Killeen et al., 1998; Kirk et al., 2004; Konishi et al., 1998; Oloyede et al., 2017; Olugbemiro et al., 2017; Podolak et al., 2010; Sparg et al., 2004).

In recent years, as far as the consumption model is concerned, consumer preferences have undergone a change in favour of increased consumption of plant products and decreased consumption of animal products. On the food market, an even broader variety of products demonstrating health properties has appeared. These products include juices obtained from red beets which have high antioxidant properties, ranging from 10.2 to 26.3 μmicromole of Trolox/ml, depending on the cultivar of red beets (Czapski et al., 2009). As well as fresh, one-day-old juice from red beets, there are pasteurized juices available on the food market, which demonstrate antioxidant properties which are just as high as in freshly pressed juices.

Nowadays, influence on health is an attribute of food which, apart from sensory quality and price, has the strongest impact on the purchasing behaviour of consumers in economically developed countries (Czapski and Górecka, 2014).

The quality of food products is determined to a large degree by their ingredients. Sensory quality is a significant factor when it comes to food selection and consumption. Sensory quality is a component of the general quality of a food product and constitutes a group of characteristics of a food product which are perceived by consumers through all their senses and which are assessed comprehensively (Kudełka, 2007). Food quality is a complex term, mainly because of the differences between evaluations given by consumers and specialists. A consumer associates the quality of food products with sensory evaluation and such characteristics as taste, appearance and smell.

The triterpene saponins in red beets constitute a group of natural compounds which have an influence on the sensation of the bitter features of food products obtained from this raw material (Mikołajczyk-Bator, 2018; Mikołajczyk-Bator and Kikut-Ligaj, 2016). Cultivars of red beet differ in terms of their saponin profile and content, which may affect the different health and sensory qualities of finished food products.

The aim of this research was to determine the influence of saponins on the sensory quality of products obtained from red beets. Food products included juices made from three cultivars of red beets produced at laboratory scale. The analysed juices underwent sensory evaluation by means of a descriptive method which provides detailed information on the sensory characteristics of the assessed raw material. The bitter taste recognition thresholds of saponins isolated from the cultivars under study were also indicated, which were then used to analyse the taste of the finished products.

MATERIALS AND METHODS

Red beets

Fresh roots of red beet from 'Forono', 'Chrobry' and 'Tytus' cultivars constituted the testing material. The roots of red beet were supplied by the *Spójnia* Plant Breeding, Seed Production and Horticultural Station in Nochowo, Poland.

Products from red beet

The products from red beets were juices produced at a laboratory scale. The juice was obtained from whole red beet roots after washing with running water. The juice was extracted by means of a juicer (Hurom, Korea) and then centrifuged (10,000×*g*/15 min). Freshly pressed juice underwent sensory evaluation.

Isolation of red beet saponins

Isolation of triterpene saponin mixture from 3 cultivars of red beet roots was conducted in a multi-stage process as described previously (Mikołajczyk-Bator et al., 2016). In the first stage, purified roots of red beet were lyophilized then ground and defatted in a Soxhlet extractor within 18 hours. The defatted plant material was primed with 80% methanol and multiple extractions were conducted for 6 hours at 20°C. The obtained extract was separated from the plant tissues by means of filtration and concentrated in a high-speed evaporator. The dense raw syrup obtained at this stage was purified with the application of column chromatography on LiChroprep RP 40–63 µm from Merck. In this way, a purified saponin fraction was initially obtained. This fraction underwent the final stage of purification by means of extraction to solid phase SPE on Sep-Pak C-18 (cartridges 5 g) from Waters. First, the saponin fraction was extracted on the Lichroprep RP 40–63 µm bed. Than the saponin fraction was concentrated and than was placed on Sep-Pak C18 cartridges Waters, which was later eluted with 80% methanol. This method enabled relatively easy purification and simultaneous deproteinization of the eluate. The cartridge was washed in the following sequence: with water, with 40% methanol and with 80% MeOH (methanol). In the eluate, the following fractions surfaced consecutively: yellow fractions washed with water (sugars), purple (betacyans) and later yellow fractions (betaxanthin), then fractions including both sugars and betalains washed with 40% MeOH, followed by saponin fractions washed with 80% methanol. The fraction washed with 80% methanol including the saponin mixture was concentrated on a vacuum evaporator and was also centrifuged (10,000×*g*/15 min). All fractions, including the purified saponin mixtures, were analysed in parallel with the application of two chromatography methods, including HPLC-MS. The details related to the identification methods of beets are described in an article written by Mikołajczyk-Bator et al. (2016).

Analytical sensory study of the bitter taste of saponin

An analytical sensory study was conducted of the bitter taste peculiar to isolated saponin mixtures.

Selected evaluators. The team of evaluators was subject to tests which verified the sensory sensitivity of the candidates in accordance with PN-ISO 3972:2016-07 norm, referring to taste and PN-EN ISO 4120:2007 (the triangle method). On the basis of the triangle method, 16 people were chosen to make up the team of evaluators (selected evaluators). The team of evaluators was composed of twelve women and four men aged between 21 and 40 and demonstrating good sensory sensitivity.

During the next stage, in order to determine the taste recognition threshold and intensity of the evaluators' sense of taste, a method connected with the concept of threshold values was applied (the dilution factor method) (Baryłko-Pikielna and Matuszewska, 2009). This method is based on the concept of the threshold value recognition. In the methods concerning the determination of threshold values, there is the detection threshold (minimum intensity of a stimulus which is necessary for the appearance of sensation, for instance a barely perceptible taste which is not water, but is very difficult to identify) and the recognition threshold (minimum taste intensity, for example a weak bitter taste). To determine the intensity of bitterness, several aqueous solutions of caffeine were prepared with gradually increasing concentrations: 0.15, 0.17, 0.19, 0.21, 0.23, 0.25, 0.27 and 0.29 g/L, which were subject to evaluation and which were ranked according to their concentrations. The threshold concentration for amounts of caffeine was 0.195 g/L, in accordance with the PN-ISO 3972:2016-07 norm. As a result, 14 people were selected for further sensory analysis tests.

Sensory analysis of saponin taste. Among the studied cultivars of beets, triterpene saponins were isolated and a sensory analysis of their bitter taste was conducted and compared with the standard of the bitter taste of caffeine. For the saponin mixtures obtained from three beet cultivars, bitter taste recognition thresholds were determined. The team of evaluators conducted the evaluation of four different concentrations of *aqueous solutions* of saponins in order to determine the recognition threshold, namely the least intensely perceptible bitter concentration of *aqueous solutions* of saponin mixtures, the taste of which was compared with the bitter taste standard: *aqueous solutions* of caffeine demonstrating a threshold concentration amounting to 0.195 g/L in accordance with the PN-ISO 3972:2016-07 norm.

Aqueous solutions of saponins were prepared by dilution of the initial concentration of 0.4% of saponin mixture extract obtained separately for each red beet cultivar.

The saponin mixtures isolated from beets which demonstrated an initial concentration of 0.4% were diluted in still water pH 6.7 with a low mineral content 420 mg /L to obtain four different concentrations ranging from 0.020 to 0.145 g/L of saponin mixture of the three analysed cultivars of red beet roots. The four different analysed aqueous solutions of saponins differ in the intensity of their bitter taste sensation (Table 1).

The evaluation of bitter taste intensity was conducted with the application of a structured linear scale which was divided into equal sections with seven standard units with indicated threshold descriptions (upper edge – undetectable, lower edge – very intense). The intensity of the taste notes was measured from 0 (bitter taste – perceptible) to 7 (very intense bitter taste) (Fig. 2). Sensory analyses were conducted by means of the sip and spit technique (Frank et al., 2008).

Sensory evaluation of products from red beets

The sensory evaluation of the obtained juices was conducted by means of a sheet drafted with the application of the taste profiling method (Baryłko-Pikielna and Matuszewska, 2009). The evaluated taste attributes were bitterness and amount of aftertaste, as well as astringent, sweet, beet or earthy flavours. The intensity of taste notes of the analysed products was evaluated by means of a 10-scale sheet (from 0 to 9 points). Sensory evaluation was conducted by a 14-person panel of trained and qualified people demonstrating verified sensory sensitivity (PN-EN ISO 8586:2014-03). All sensory evaluations were conducted in four independent repetitions at 7-day intervals.

Statistical analysis

Statistical analysis was carried out by means of Excel (Microsoft). In order to compare average values, a variance analysis was conducted (ANOVA).

Table 1. Content of triterpene saponins in selected aqueous solutions of saponin mixtures subject to sensory analysis

No. concentration	Sensory analysis	Content of triterpene saponins, g/L*			Standard caffeine solution g/L*
		Forono	Modana	Tytus	
1	not bitter	0.030	0.025	0.020	–
2	not bitter	0.070	0.065	0.060	–
3	slightly bitter	0.120	0.115	0.110	–
4	bitter	0.145	0.140	0.130	0.25

*Water solution of the saponin mixture crude extract or caffeine prepared for the sensory analysis.

The significance of the differences between the average values was determined using the Tukey HSD (*honestly significant difference*) post-hoc test with the significance level α = 0.05.

Average values were presented providing a standard deviation. In order to develop the results of the sensory evaluations, one factor variance analysis was applied altogether with verification of hypotheses with the application of significance level α = 0.05. The following hypotheses were verified: the analysed juices from 3 cultivars differ in their taste attributes. The analysed aqueous solutions of saponins differ in the intensity of their bitter taste sensation.

RESULTS AND DISCUSSION

Sensory analyses of red beet juice

As a result of the conducted sensory evaluations of juices from three cultivars of beets obtained at a laboratory scale, it was found that juice from the Forono cultivar had the highest notes for bitter and beet tastes. At the same time, the astringent taste of this juice was minimally perceived. The intensity of sweetness was evaluated at 5.5 points, and the beet taste at 5 points. This juice obtained 1.4 points and 1.6 points for bitter taste and bitter aftertaste respectively. Low notes in sensory evaluations were ascribed to such attributes as astringent taste (2.6 points) and earthy taste (2.0 points).

However, juices from the Modana and Tytus cultivars had a slightly higher perception of bitterness than juice from the Forono cultivar, but also remained at a low level. The bitterness of these juices was sensed at 2.4 points (Modana juice) and 2.3 points (Tytus juice) on the 10-degree scale (Fig. 1). The situation was similar in the case of bitter aftertaste, which was not a dominant feature in any of the evaluated juices. The bitter aftertaste in these juices was sensed at 2.6 points and 2.5 points for the Modana and Tytus cultivars respectively. By contrast, astringent taste sensation was the biggest in the Tytus cultivar (3.5 points). Astringent notes were the smallest for a Modana cultivar beet and was 1.9 points. The sensation of a sweet taste in the Modana cultivar juice was 5.3 points, however, in the case of the Tytus cultivar, the intensity of the sweet taste was significantly lower and amounted to 3.5 points.

In the case of both analysed cultivars, the intensity of the beet taste was evaluated at a medium level, amounting to 4.2 points for the juice from the Tytus cultivar and 4.9 points for the juice from the Modana cultivar. The differences in red beet taste were not statistically significant in the juices from Forono and Modana cultivars (Fig. 1).

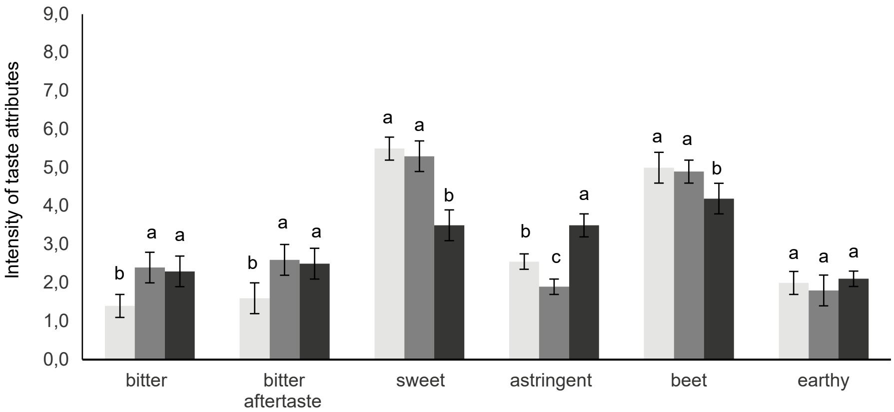

Fig. 1. Sensory quality profilogram of juices obtained from different red beet cultivars. The scale of sensory evaluation from 0 to 9 (10-point scale): a–c – average values of the analysed taste attributes differ significantly depending on the type of juice assessed (Tukey's test, $p < 0.05$)

It was found that all juices obtained slightly higher notes for bitter aftertaste than for bitter taste. However, these differences were not statistically important. The bitter features, although practically not perceived in these juices, result from the triterpene saponin content in the analysed cultivars. Previous studies done by this author proved that the bitter taste of red beets correlates with the total saponin content in red beets and depends on the analysed cultivar and the part of the raw material (Mikołajczyk-Bator, 2018).

The total triterpene saponin content in the analysed juices from the Forono beet cultivar amounted to 0.0435 ±0.004 g/L, while in the case of the Tytus cultivar, it was at a lower level and amounted to 0.0597 ±0.020 g/L. However, it was found that the highest content of saponins amounted to 0.0767 ±0.005 g/L occurred in the juice from the Modana cultivar (Mikołajczyk-Bator, 2018). The juice from the Modana cultivar has a higher triterpene saponin content in comparison with the juice from the Forono and Tytus cultivars.

Analytical sensory study of the bitter taste of saponin

A bitter taste is generally caused by different groups of chemical compounds, including the triterpene saponins present in beets (Mikołajczyk-Bator and Kikut-Ligaj, 2016). Based on the conducted control test of a saponin sample (aqueous extract), the selected evaluators determined the kind of taste quality among different taste attributes – sweetness, bitterness, astringency or sourness, demonstrated by the saponin extract. In the test, aqueous extracts from saponins obtained from three analysed beet cultivars – Forono, Modana and Tytus – were evaluated.

The results of the control test demonstrated that saponin extract causes a strong bitter taste. The next step included a sensory analysis of bitter taste intensity in the saponin mixtures obtained from three analysed beet cultivars, which was compared with the level of caffeine bitter concentration using the multiple comparison method (Baryłko-Pikielna and Matuszewska, 2009). This method consisted of assigning caffeine as the reference solution for the bitterness of the analysed saponin samples prepared in four different concentrations and finding a saponin sample which is the most similar to the abovementioned reference (Table 1, Fig. 2). The saponin samples consisting of aqueous solutions of saponin mixtures which demonstrated the least perceptible (threshold) bitter concentrations are presented in Table 1. The reference for bitter taste was caffeine prepared at different concentrations 0.15, 0.17, 0.19, 0.21, 0.23, 0.25, 0.27 and 0.29 g /L.

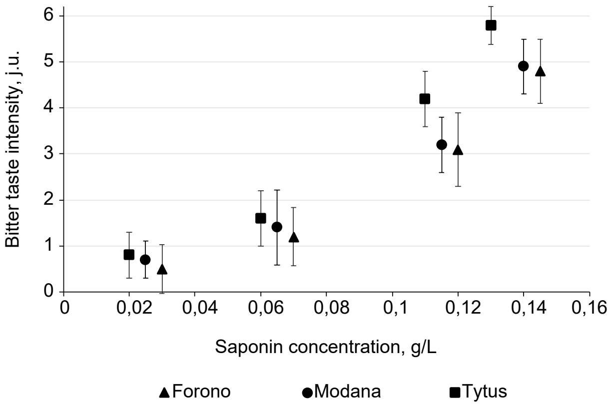

Fig. 2. Sensory analysis of bitter taste intensity of aqueous solutions of saponin mixtures isolated form different red beet cultivars

Among the four prepared concentrations of saponin mixture under study, the selected evaluators indicated the lowest concentration of bitter taste which could be identified, which was labelled the bitter taste recognition threshold. The team of evaluators recognized the minimum intensity of bitter taste in the aqueous solutions of the saponin mixtures, indicating that it is a “weak bitter taste” but one which is perceptible. The results of the sensory analyses which determined the bitter taste recognition thresholds that were the lowest concentrations of particular saponin mixtures to cause a bitter taste are presented in Table 2.

In the present study, the team of selected evaluators determined the bitter concentration threshold of a caffeine solution to be 0.25 g/L, which was compared with the threshold concentrations of the bitter saponin mixtures from particular analysed beet cultivars. Dawid and Hofmann (2014) applied a caffeine threshold concentration of 0.194 g/L. By contrast, in the Polish norm (PN-ISO 3972:2016-07), caffeine is mentioned as a reference substance for bitter taste recognition and, the recommended concentration for evaluating the ability to recognize a bitter taste amounts to 0.195 g/L. For the caffeine threshold

Table 2. Comparison of triterpene saponin content in red beets with the determined bitter taste recognition thresholds

Cultivar of red beets	Sample	Recognition threshold of bitter taste g/L*	Content of triterpene saponins in fresh juices g/L***	The dominant taste of beet juice
–	caffeine	0.195**	–	–
–	caffeine	0.250	–	–
Forono	triterpene saponins	0.120	0.0435 ±0.004	sweet
Modana	triterpene saponins	0.115	0.0767 ±0.005	sweet
Tytus	triterpene saponins	0.110	0.0597 ±0.020	sweet/astringent

*Threshold concentration of the sample under study causing a bitter taste determined on the basis of analytical sensory studies.
**PN-ISO 3972:2016-07.
***According to Mikołajczyk-Bator (2018) ±standard deviation.

concentration determined in the present study by the team of evaluators and resulting from the Polish norm (PN-ISO 3972:2016-07), the differences are small and come from the sensory sensitivity of the people in the team of selected evaluators.

As a result of the sensory analyses conducted, the selected evaluators determined the threshold concentrations of the bitter saponin mixtures to be 0.120, 0.115 and 0.110 g/L, for the Forono, Modana and Tytus cultivars respectively. The threshold concentrations determined for the particular saponin mixtures caused a bitter taste sensation similar to 0.25 g/L of aqueous caffeine solution (Table 2).

Threshold concentrations were used to analyse the flavour of the finished products, which aimed to determine the intensity of the bitter taste, taking into consideration the different beet cultivars on the basis of their saponin content.

The content of triterpene saponins in 1 L of fresh red beet juice from the Forono cultivar is 0.0435 g (Mikołajczyk-Bator, 2018), while the lowest concentration causing a bitter taste (bitter taste recognition threshold) in saponin mixtures from this cultivar amounted to 0.120 g/L and was 2.8 times higher than the saponin concentration in the juice (Table 2). In sensory evaluations of fresh red beet juice from the Forono cultivar, the selected evaluators indicated that the dominant flavour of the juice was sweet, without a clear sensation of bitterness.

The conducted analysis indicates that the triterpene saponin content in red beet from the Forono cultivar is too low for identification of the bitter taste caused by the saponin in this juice.

A similar analysis was conducted in the case of saponin content in juices obtained from the Modana and Tytus cultivars. In one litre of juice from the Modana cultivar, the saponin content was 0.0767 g (Mikołajczyk-Bator, 2018), however, the threshold concentration of saponins isolated from this cultivar which causes a bitter taste sensation was 0.115 g/L and was 1.5 times higher than the concentration of saponins in the juice from this cultivar. As a result, consumers do not sense a bitter taste in the juice from the Modana cultivar, despite the fact that bitter saponins are present in it.

By contrast, 1 litre of juice obtained from the Tytus cultivar included 0.0597 g of saponins (Mikołajczyk--Bator, 2018). However, the level which determined the bitter taste of the saponin extracts was discovered to be 0.110 g/L and was 1.8 times higher than the saponin content in the juice. The results of the sensory analysis conducted on the juice obtained from this cultivar confirm the lack of any dominant bitter features. The saponin content in this juice is too low to sense any bitterness (below the indicated sensation threshold of a bitter taste in the saponin mixtures).

Despite the presence of saponin in the evaluated juices from the Forono, Modana and Tytus cultivars contributing to the sensation of a red beet bitter taste, their content in the juices is so low that bitter features are not detected during their consumption. These results are confirmed by the conducted sensory analyses in which evaluated juices from the Forono, Modana and Tytus cultivars did not demonstrate a dominant bitter taste.

The analysis of the triterpene saponin content in juices obtained from different cultivars and the bitter taste recognition thresholds is significant from the point of view of a commodity science evaluation of beet root cultivars which are available on the market and their further use in the production of specific food products. The sensory quality of a given product is an important feature which is taken into consideration when consumers choose food products. Despite the presence of bitter compounds such as saponins in the three analysed beet cultivars, their content in the juices is so low that it does not cause the sensation of a bitter taste. Cultivars of these beets can be used in the production of unpasteurised juices, fresh juices or fresh salad mixtures where beets are added because they do not exhibit dominant bitter features.

CONCLUSION

The application potential of the analysed red beet cultivars was demonstrated as regards their usefulness in obtaining food products with those sensory qualities that consumers demand. The Forono, Modana and Tytus cultivars analysed have suitable sensory qualities for the production of freshly pressed, unpasteurised juices owing to the lack of a dominant bitter taste.

Based on the determined thresholds of bitter taste recognition in saponins isolated from different beet cultivars (minimum concentration causing the sensation of a bitter taste in red beet) and the comparison with the total saponin content in the final product, one can conclude whether or now a given product demonstrates bitter features.

It was found that the values of the defined thresholds of bitter taste recognition in the case of saponins isolated from the analysed beet cultivars are higher than the saponin contents in the juices obtained from these cultivars. As a result, the bitter features of the juice are not detected due to the low content of saponins in them. This analysis was confirmed by sensory evaluation of the juices, during which the team of selected evaluators, indicated sweetness as the dominant flavour of the juices from the Forono, Modana and Tytus cultivars, with no clear sensation of bitterness.

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