

MINERAL AND BIOACTIVE COMPOUND CONTENT IN PLANT-BASED PROTEIN - ENRICHED PUREES

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Abstract

Adequate, well balanced, diverse diet together with regular physical activity is considered to be the key elements for good health. The intake of macro and microelements has an important role in normal function of our bodies, ensuring enzymatic reactions, nerve impulses and other processes. However overly increased amounts of certain vitamins and minerals in the diet can be toxic. Therefore, the amount of certain nutrients needs to be consumed according to the age, gender and health condition. The aim of the research was to create new plant-based protein-enriched purees with increased protein amount, detect bioactive compound and mineral content and compare the mineral content with the recommended daily mineral intake for Latvians. For this research three recipes of new plant-based purees were created and experimentally made using organically grown ingredients from Latvia. Additional ingredients as whey-protein isolate, cod liver oil and sugar were added. Products were processed using cook-vide. All samples were tested on their mineral compound content (Na, K, Ca, P, Mg, Fe, Zn, Cu, Se), total carotene (TC) and total phenol content (TPC), antiradical activity (DPPH). The obtained data in all samples showed only trace amounts of Se per 100 g of product, on average the content of Na was only 1.2% from recommended daily intake (RDI) for Latvians per 100 g of product. The highest RDI was obtained for Fe in sample Sp1 for men 11.8%, however for women only 7.1% of RDI. Overall the highest content of bioactive compounds was found in sample Sp2.

Keywords: total carotene, total phenols, DPPH, cook-vide

Introduction

The key role of sufficient nutrition is to provide living organisms with quantitatively and qualitatively appropriate nutrients to improve health, well-being and prevent from diseases (Cilla et al., 2018). Micronutrients are components that are needed for us in comparatively small amounts and include vitamins and minerals. Minerals are divided into major (macro) mineral compounds – Ca, Mg, K, Na, Cl, P, S and trace (micro) minerals – I, Zn, Se, Fe, Mn, Cu, Co, Mo, F, Cr, B. These elements can be consumed by balanced nutrition of plant and animal sources (Mohammad et al., 2017).

Increased consumption of fruits and vegetables is recommended in dietary guidelines worldwide and fruits and berries are considered to be rich in nutrients and phytochemicals (Nile, Park, 2014).

Fruit and vegetable biochemical content differs depending on their variety and several environmental factors, however some similarities can be connected among the most widely found specimens of each particular crop.

Agricultural crops as beetroot, carrots and pumpkins are widely grown and used in Latvia. Beetroot (*Beta vulgaris* L.) can be a source of several minerals, for example 100 g of edible root part can contain 77 mg of Na, 16 mg – Ca, 0.79 mg – Fe, 38 mg – P, 305 mg – K, 23 mg of Mg and 0.35 mg of Zn. Beetroot is also a source of highly active pigments – betalains and carotenoids, polyphenols and flavonoids, saponins, but some bioactive compounds found at low levels – glycine, betaine and folate (Chhikara et al., 2019).

Consumption of carrots (*Daucus carota* L.) has increased in recent years due to their recognition as an important source of natural antioxidants, such as β -carotene, an average amount of 5.33 mg per 100 g of fresh carrot. It is also a good source of mineral compounds, where 100 g on average can contain

Ca (34 mg), Fe (0.4 mg), P (25 mg), Na (40 mg), K (240 mg), Mg (9 mg), Cu (0.02 mg), and Zn (0.2 mg) (Sharma et al., 2012).

Pumpkins (*Cucurbitaceae*) have been traditionally eaten both by people and animals and it has several different varieties all with different chemical composition. But overall it is known to be a source of carotenoids, also vitamins like K, several complex B vitamins as well as for minerals – K, P, Mg, Fe and Se (Ozola, Kampuse, 2018).

Jerusalem artichoke (*Helianthus tuberosus*) is an economically important plant with wide use of application both in food production and biofuel production in the world (Yang et al., 2015), however not as widely used in Latvia although grown here as long as potatoes. Overall as a crop Jerusalem artichoke has high yield and wide adaptation to climatic and soil conditions (Yang et al., 2015). The tubers on average contain 80% of water, 15% of carbohydrates and 1 to 2% of protein. Carbohydrates in cells are mostly stored in the form of inulin. Some research shows that boiled Jerusalem artichoke tubers contain 30 mg of Ca, 0.4 mg of Fe, 420 mg of K, 3 mg of Na and also 20.0 μ g of carotenoids (Kays, Nottingham, 2007).

Apples are considered to be of moderate energy and nutritional value among common fruits, however they are consumed in rather large quantities therefore can have a significant contribution (Lee, 2012).

Berries are typically considered to be a good source of several vitamins and bioactive compounds as polyphenols, antioxidants, also minerals and fibres in various concentrations (Nile, Park, 2014).

Strawberries (*Fragaria x ananassa*) are one of the most popular and widely used berry for juicing due to its taste, rich essential nutrients, bioactive compounds, fibre, minerals and phenolic compounds. On average 100 g of strawberries contain 7.68 g of carbohydrate, 2.0 g of fibre and up to 4.89 g of sugar and 58.8 mg of vitamin C.

100 g of fruit can contain K (153 mg), P (24 mg), Ca (16 mg), Mg (13 mg) (Wang et al., 2018). Red raspberries (*Rubus idaeus L.*) are usually grown as perennial crop for their flavour and red colour. Much like previously described berries raspberry contains high levels of phenolic acids, flavonoids and also anthocyanins (Jin et al., 2012). Sea buckthorn (*Hippophae rhamnoides L.*) is also a very common in Europe, Asia and North America. Berries have high contents of vitamin C, E and K, carotenoids, flavanols and sugars, and are used in food industry, medicine, cosmetics (Lukša et al., 2018). But lingonberry is a berry from Nordic countries rich in polyphenols and vitamins C and E (Kivimäki et al., 2013). Some additional mineral composition of lingonberries, apples, pumpkin and red raspberries can be seen in Table 1.

Table 1

Mineral content in lingonberry, raspberry, pumpkin and apple (DTU Fodevareinstituttet, 2019)

Minerals per 100 g of product	Lingon-berry	Rasp-berry	Pump-kin	Apple
Na (mg)	2.0	2.0	2.0	3.0
K (mg)	89.0	228.0	243.0	120.0
Ca (mg)	20.0	19.7	20.1	3.9
P (mg)	16.0	38.0	33.0	17.4
Mg (mg)	9.0	17.0	11.2	4.4
Fe (mg)	0.40	0.55	0.27	0.12
Zn (mg)	0.18	0.34	0.20	0.03
Cu (µg)	0.07	0.11	0.08	0.03
Se (µg)	0.00	0.19	-	0.30

Both bioactive compounds and minerals play an important role to maintain the basic functions of human body. Various bioactive compounds protect the body against diseases and disorders and the damaging effects of free radicals (Nile, Park, 2014), however minerals help to build strong bones and are a part for transmitting nerve impulses among many other functions (Mohammad et al., 2017).

A sufficient nutrition also means to maintain stable energy levels, this is especially important for people with regular increased physical activity such as athletes. However, no matter of what kind of physical activity each of us is doing, the most important thing is to eat different foods that can provide sufficient and regular energy intake of carbohydrates, proteins, fats and microelements (Potgieter, 2013). Products with increased protein content have become very popular with the athlete community. Protein consumption is necessary for muscle protein synthesis thus ensuring positive net muscle protein balance and for athletes engaged in resistance exercise can benefit from this with time by allowing muscle protein accretion and subsequent hypertrophy – growth of tissues or organs (Phillips et al., 2011). For this reason, in the last few years a growing interest in new product market has been noticed for well-balanced products and products, which are high in protein content. And sports nutritionists are more likely to develop individual nutrition plans depending on the needs of a single athlete and following

a plan that athletes should consume diets that provide at least the recommended dietary allowance / adequate intake for all micronutrients (Nutrition and Athletic Performance, 2016).

Traditional cooking can lead to a loss of nutritional compounds and components responsible for flavour due to the required temperature and cooking time. Alternative cooking technologies such as vacuum treatments could help to decrease these losses (Iborra-Bernad et al., 2014; Ozola, Kampuse, 2017). The main advantage of vacuum cooking is the absence of oxygen that allows product cooking under 100 °C that is less harmful for thermolabile compounds (Iborra-Bernad et al., 2014; Ozola, Kampuse, 2017). The most common vacuum treatment is *sous-vide* that allows product cooking inside heat-stable vacuumized pouches. However, in this research a different method, called *cook-vide* or vacuum boiling was used. *Cook-vide* is used to prepare products in hermetically closed cooking kettle by lowering pressure with continuous function of vacuum pump, that decreases the amount of oxygen in kettle and lowers pressure thus allowing to reach product / water boiling point below 100 °C (Iborra-Bernad et al., 2014; Ozola, Kampuse, 2017).

For the purpose of this research the evaluated mineral compound content should ensure a minimum of 10% per compound of recommended daily mineral intake for Latvians per portion (200 g) of created product.

The aim of the research was to create new plant-based protein-enriched purees with increased protein content, detect bioactive compound and mineral content and compare the mineral content with the recommended daily mineral compound intake for Latvians.

Materials and Methods

Three plant-based purees enriched with protein were developed. Most of the plant ingredients were grown organically in Latvia and industrially processed into semi-finished purees, juices or pulp juices. The plant material input in percent for each prepared sample is shown in Table 2. Additional ingredients were used in puree preparation: the main protein source – whey protein isolate in all samples (6%), sugar (1%) in samples Sp1 and Sp2, and cod liver oil (0.5%) in all samples.

Table 2

Plant material input in recipes (%) of protein-enriched plant-based purees

Ingredients	Sample		
	Sp1	Sp2	Sp3
Sea buckthorn pulp juice	5.0	-	-
Apple puree	25.5	12.0	24.5
Carrot puree	15.0	19.0	-
Strawberry pulp juice	13.0	-	13.0
Jerusalem artichoke puree	34.0	-	-
Lingonberry pulp juice	-	16.5	-
Apple juice	-	20.0	-
Red beetroot puree	-	25.0	-
Red beetroot pulp juice	-	-	12.0
Pumpkin puree	-	-	28.0
Red raspberry pulp juice	-	-	16.0

Prepared recipes were vacuum cooked using cook-vide method at 0.06 MPa pressure, with boiling temperature at 79 ± 2 °C for 15 min followed by hot filling in glass jars and pasteurization in hot water bath at 95 ± 2 °C for 20 min. Jars were cooled to room temperature in cold water (7 ± 3 °C) for 1 hour and stored refrigerated till testing.

Samples were tested on their total protein amount and afterwards analysed on their content of total carotenes, total phenols, antiradical activity and mineral content.

Total protein content in samples were detected by Kjeldahl method according to ISO 20483:2013.

The content of *total carotenes (TC)* was detected by spectrophotometric method described by (Полюдек-Фабини, Бейрих, 1981) and analysed using UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK) at 440 nm and expressed as mg 100 g⁻¹ per product (Ozola et al., 2017). *Total phenol content (TPC)* was detected according to the Folin-Ciocalteu method described by (Yu et al., 2003) with modifications, where to 0.5 mL of sample extract 2.5 mL of 0.2 N Folin-Ciocalteu reagent was added, after 5 minutes 2.0 mL of 7.5% NaCO₃ was added and the resulting solution was mixed and left to stand for 30 minutes at 18 ± 1 °C in a dark place (Ozola et al., 2017) and shortly before reading absorption at 760 nm was centrifuged (ELMI Centrifuge CM-6MT, LTF Labortechnik GmbH&Co, Germany) for 2 minutes at 3500 rpm. TPC was analysed using spectrophotometer JENWAY 6300 (Banworld Scientific Ltf., UK) and the obtained data were expressed as mg GAE 100 g⁻¹ per product.

The *antiradical activity (DPPH)* was detected using 2,2-diphenil-1-picrylhydrazyl (DPPH) method described by Yu et al. (2003) with modifications where to 0.5 mL of extracted sample 3.5 mL of freshly made DPPH solution was added. The mixture was mixed and kept in the dark place at 18 ± 2 °C for 30 minutes and centrifuged respectively to TPC detection and the absorbance was measured at 517 nm and the obtained data were expressed as mM TE 100 mL⁻¹ of product (Ozola et al., 2017).

Samples were tested on their content of *minerals* such as Na, K, Ca, P, Mg, Fe, Zn, Cu, Se. The amount of Ca, Mg, Fe, Zn, Cu in tested samples was detected by Institute of Biology of University of Latvia using atomic absorption spectroscopy method, but the amount of K and Na detected by flame photometer Jenway PFP7 (Cole-Parmer, UK) and content of P was detected calorimetrically with ammonium molybdate. The amount of Se in samples was detected by Institute of Food Safety, Animal Health and Environment (BIOR) and their standardised BIOR-T-012-148-2013 method. The amount of minerals was expressed in mg kg⁻¹ per dry sample, but recalculated to mg 100 g⁻¹ per product. Total protein content, TC, TPC and antiradical scavenging activity was measured in three repetitions, but mineral content was determined in single repetition. *Statistical analysis* was done using 'Microsoft Office Excel' 2007 version. Differences between the obtained results on bioactive compound content were analysed

using ANOVA: Single factor analysis with Tukey-Kramer post hoc test. The data is presented as their mean and differences among results were considered to be significant if p value $< \alpha_{0.05}$.

Results and Discussion

The average total protein content in all samples ranged from 6.2 to 6.5 g per 100 g of sample. Sample Sp1 contained 6.5 ± 0.02 g 100 g⁻¹ of product, Sp2 6.2 ± 0.05 g 100 g⁻¹ and sample Sp3 6.5 ± 0.04 g 100 g⁻¹ of product. These amounts are comparable to the expectations during sample preparation, due to the fact that majority of the used ingredient total protein content did not exceed 1 g per 100 g of sample (DTU Fodevareinstituttet, 2019). Also, the obtained protein content was appropriate for this type of product, as the average protein intake for adults should not exceed 10-20% of daily caloric value according to recommended energy and nutrient intakes for Latvian residents. These directions foresee ca. 75 g protein intake for adults on a 2000 kcal diet (*Ieteicamās enerģijas un uzturvielu devas Latvijas iedzīvotājiem*, 2017), in addition to the prepared purees are used as supplements to nutrition.

The obtained data on the prepared sample bioactive compound content showed some differences. Evaluation of the data on total carotene content (Fig. 1.) showed that sample Sp3 had by around 40% lower content of total carotenes than sample Sp2, which is a significant difference according to Tukey-Kramer post hoc test.

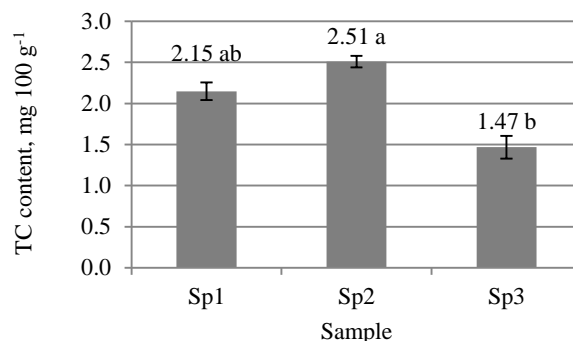


Figure 1. Total carotene content in prepared plant-based purees

Different letters indicate a significant difference in the mean at $p < 0.05$ according to Tukey-Kramer test.

Differences between samples Sp1 and Sp2 were not as significant to be considered relevant in this case. The difference was only 14%. These results are directly influenced by the sample ingredient content, where samples Sp1 and Sp2 contained carrot as the major carotene source and also sea buckthorn in Sp1. The highest content of total phenols was also detected in sample Sp2 (Fig.2), however, contrary to TC content, sample Sp1 had 20% lower TPC, therefore significant differences between these samples were noticed.

Although TC and TPC showed some differences between separate samples, no such data was noticed after antiradical activity evaluation.

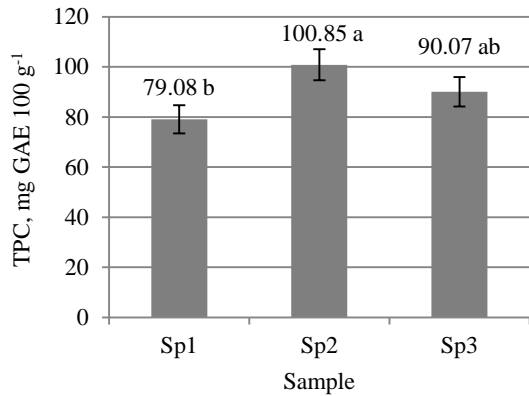


Figure 2. Total phenol content in prepared plant-based purees

Different letters indicate a significant difference in the mean at $p < 0.05$ according to Tukey-Kramer test.

Similarly, to previous observations, sample Sp2 also showed slightly higher antiradical activity (Fig.3) compared to other samples. In comparison a research on high-pressure processed smoothies containing orange juice, papaya juice, melon juice, carrot puree and skimmed milk (Andrés et al., 2016), showed a total carotenoid content of 20.43 ± 0.47 mg 100 mL⁻¹ in thermally processed (80°C, 3 min) samples.

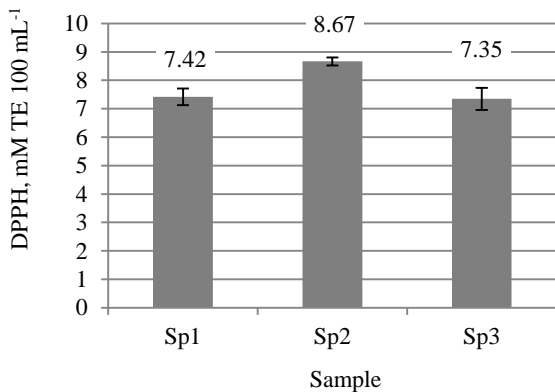


Figure 3. Antiradical activity in prepared plant-based purees

Total phenol content of 45.40 ± 0.70 mg GAE 100 mL⁻¹ and DPPH 53.9 ± 0.7 mM TE 100 mL⁻¹ with a gradual bioactive compound deterioration during 45 day refrigerated storage period was also established (Andrés et al., 2016). Overall, plant-based puree samples showed higher total carotene and antiradical activity levels, but did not exceed total phenol content. These results could suggest that plant-based purees made for this research contain different phenol compounds in rather high concentrations due to variety of berry juices used in the sample preparation. However, they do not have strong antiradical activity, which could shorten the shelf life of these products.

The data from mineral compound content (Table 3) analysis showed only trace amounts of Se (< 0.20 mg kg⁻¹) present, levels were not high enough for precise amount declaration in prepared samples. This information does

coincide with typical sources of Se nutritionally provided by intake of organ meats, cereals and other grains, dairy products, rice, sea food, eggs and other similar products (Selenium, 2018). Other tested mineral compounds were detected in higher and more substantial amounts.

Table 3

Mineral content in experimental samples and average RDI of minerals in Latvia (mg)

Minerals	Sample (mg 100 g ⁻¹)			RDI*
	Sp1	Sp2	Sp3	
K	230.5	180.9	213.1	3500 (m) 3100 (w)
Ca	40.3	36.6	36.6	800
P	34.5	25.6	39.5	600
Mg	18.5	17.1	23.2	350 (m) 280 (w)
Fe	1.1	0.4	0.4	9 (m) 15 (w)
Zn	0.2	0.2	0.2	9 (m) 7 (w)
Cu	0.1	0.1	0.1	0.9
Na	25.4	28.2	21.2	2000

RDI – average values of recommended daily mineral compound intakes for adults, m – adult men, w – adult women

According to the recommended energy and nutrient intakes for Latvian residents (*Ieteicamās enerģijas un uzturvielu devas Latvijā iedzīvotājiem*, 2017) the prepared plant-based protein-enriched purees with increased protein content showed that 100 g of sample Sp1 can provide a potassium daily intake of 7.4% for women and 6.6% for men and 5% of RDI of Ca. Sample Sp1 also showed a comparatively higher amount of iron, which is comparable to 7.1% of RDI for women and 11.8% for men, these amounts are approximately 60% higher than in sample Sp2 and Sp3. Sp1 contains 9.7% of RDI of Cu for both genders. Between the three experimental samples mineral compounds such as P, Mg and Zn were found to be in higher amounts in sample Sp3 than in other samples. The detected amount of Mg in Sp3 could possibly provide 8.3% of RDI for women and 6.6% of nutritional needs for men. This sample also provides the lowest amount of Na, approximately 1.1% of RDI, which is about 24% less than in sample Sp2. Although the evaluation of some bioactive compounds overall showed higher amounts of total carotenes, phenols and antiradical activity in sample Sp2, the content of mineral compounds was relatively low

Conclusions

The chosen ingredients for sample preparation were able to ensure an appropriate amount of some mineral compounds per 200 g serving. However, it would be advisable to search for plant-based products with higher concentrations of selenium or adding a selenium complex to ensure partial coverage of recommended daily intake.

Also, low levels of zinc were detected in all samples, on average ensuring 4% of RDI per serving.

Sample Sp2 containing apple puree, carrot puree, lingonberry pulp juice, apple juice, red beetroot puree, overall had the lowest mineral compound coverage of RDI. The 10% margin per sample serving of mineral compounds was met by content of potassium (K) and copper (Cu) for both men and women, and of magnesium (Mg) for men.

The evaluation of bioactive compound content did not show substantial differences between the samples, slightly larger differences in content of total carotene were detected between samples Sp2 (apple puree, carrot puree, lingonberry pulp juice, apple juice, red beetroot puree) and Sp3 (apple puree, strawberry pulp juice, red beetroot juice, pumpkin puree, red raspberry pulp juice).

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