Determination of Some Organic and Inorganic Nutritional Constituents in Beta Vulgaris Roots Prepared in Different Methods

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Article Info:

Received May 2023
Accepted June 2023
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DOI:

Abstract:

The nutritional value of the roots of Iraqi Beta vulgaris plant that have been prepared using several conventional techniques will be evaluated in the current research. One of the best and most important nutritional sources of iron, nitrate of nitrogen, magnesium, potassium, ascorbic acid, and folic acid is the juice of beta vulgaris roots. Spectrophotometric techniques like UV-Visible, HPLC, and AAS have been used to estimate the levels of some organic and inorganic nutritional constituentsand determine the level of the nitrate ion using an ion selective electrode technique in the prepared beta vulgaris root juice. The concentration of these organic and inorganic components varies depending on how the Beta vulgaris juice is prepared. The roots of Beta vulgaris have been processed in four different ways.

Ascorbic acid content is greatest in the prepared sample with sodium chloride (4.124 mg in 200 mL), while the sample treated with acetic acid has a high concentration of Betalains (10.96 mg in 200 mL) and folic acid (5.736 mg in 200 mL).

The potassium (552 mg in 200 mL) and magnesium (54 mg in 200 mL) concentrations in juice from the acetic acid-treated sample were relatively higher than those obtained from other methods, whereas the prepared sample with NaCl has a high concentration of NO_3^- (616 mg in 200 mL) and the prepared sample with cool water has a high concentration of iron (0.75 mg in 200 mL).

Keywords: Beta vulgaris roots; Organic; Inorganic; Nutritional constituents, Betalains; Atomic Absorption Spectroscopy; Ion Selective Electrode.

تقدير بعض المكونات الغذائية العضوية وغير العضوية في جذور البنجر والمعدة بطرق مختلفة

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الخلاصة:

في البحث الحالي سيتم تقييم القيمة الغذائية لجذور نبات البيتا فولغاريس او (البنجر) الشائع في العراق والذي تم تحضيره باستخدام العديد من التقنيات التقليدية. يعتبر عصير البنجرمن أفضل وأهم المصادر الغذائية للحديد ونترات النيتروجين والمغنيسيوم والبوتاسيوم وحمض الأسكوربيك وحمض الفوليك. تم استخدام تقنيات قياس الطيف الضوئي مثل مطيافية الاشعة فوق البنفسجية-المرئية و تقنية الكروماتوغرافيا السائلة فائقة الاداء اضافة الى تقنية الامتصاص الذري لتقدير مستويات بعض المكونات الغذائية العضوية وغير العضوية ، بالإضافة إلى تحديد مستوى أيون النترات باستخدام تقنية القطب الانتقائي الأيوني في عصير جذور البنجرالمحضر. يختلف تراكيز هذه المكونات العضوية وغير العضوية اعتمادا

على كيفية تحضير عصير البنجر. اذ تمت معاملة جذور البنجر بأربع طرائق مختلفة. يكون محتوى حمض الأسكوربيك أكبر في العينة المحضرة في كلوريد الصوديوم (٤,١٢٤ مجم في ٢٠٠ مل) ، بينما تحتوي العينة المعالجة بحمض الأسيتيك على تركيز عال من البيتالين (١٠,٩٦ مجم في ٢٠٠ مل) وحمض الفوليك (٥,٧٣٦ مجم في ٢٠٠ مل). كانت تراكيز البوتاسيوم (٥٥٢ مجم في ٢٠٠ مل) والمغنيسيوم (٥٤ مجم في ٢٠٠ مل) في العصير من العينة المعالجة بحمض الخليك أعلى نسبيا من تلك التي تم الحصول عليها من طرائق أخرى ، في حين أن العينة المحضرة في كلوريد الصوديوم احتوت على تركيز عال من ايونات النترات (٦١٦ مجم في ٢٠٠ مل) والعينة المحضرة بالماء البارد تحتوي على تركيز عال من الحديد (٠,٧٥ مجم في ٢٠٠مل)

الكلمات المفتاحية: جذور البنجر؛ عضوية؛ غير عضوية؛ المكونات الغذائية، بيتالين؛ مطيافية الامتصاص الذري؛ قطب الانتقاء الايوني

Introduction:

Consumers everywhere are searching for natural ways to improve their health and quality of life, and the recent rise of so-called "functional foods" reflects this demand. Fruits and vegetables have been shown to have beneficial health effects in a wide variety of research settings. Liver disease, brain disease, and cardiovascular disease are just some of the age-related chronic illnesses that can be prevented by eating a diet rich in fruits and vegetables. Such a diet may also help prevent cancer, immune system dysfunction, diabetes, obesity, free radicals, constipation, and diverticulosis (the formation of small, easily irritated, pouches inside the colon). Many different phytochemicals, including betalains, fibres, vitamins, minerals, polyphenols, carotenoids, anthocyanins, and others, have been linked to these natural protective effects [1, 2].

Beta vulgaris root (BVR), also known as red beetroot, has recently gained popularity as a functional food due to its potential health benefits and biological activity. Bioactive compounds such as betalains, B vitamin, phenols, folic acid minerals, carotenoids, fibre, and sugars with a low energy value are abundant in BVR. The betalains are water-soluble, nitrogen-containing pigments that range in colour from red-violet (betacyanins) to yellow (betaxanthins). Only ten families in the order Caryophyllales (formerly known as Centrospermae) and a few types of fungi, such as Amanita muscaria, contain the betalains beta-1,3-glucan [3-7]. Sodium, magnesium, iron and calcium are among the important nutrients that many researchers have been analysing and showing their proportions in plants.() [8-9] Given the importance of BVR to one's diet, numerous studies have been conducted that evaluate the nutritional content of BVR [10-14]. Different methods of sample solution preparation have been discussed in many studies in order to obtain an accurate assessment of the nutritional elements and compounds in BVR. After grinding the BVR samples into a coarse powder, Madhu et al. suggested macerating them in one litter of (water 70%: ethanol 30%) for 72 hours. The final extract was heated to remove any remaining solvent before being run through an atomic absorption spectrophotometer to check for the presence of inorganic nutrients like Zn, Pb, Na, Mg, Ni, Fe, K, Cu, and Cr [15]. However, Dos Santos et al. [16] have used the digestion process with a mixture of nitric acid and 30% hydrogen peroxide to extract Mn, Na, P, Sr, Fe, Zn, Cu, Mg, and K, which are then estimated spectrophotometrically using the Inductively Coupled Plasma-Optical Emission method. BVR samples were lyophilized and ground into a powder by Moyo et al., and then analysed in an inductively coupled plasma-mass spectrometer for traces of Ca, P, Na, Zn, Mn, Fe, Cu, and Mg [17]. Maceration in 70% ethanol was also used by Al-Khazraji to prepare the BVR solution, and then the hemopoietic activity was evaluated [18]. In 2019, researchers proposed another study using two different maceration methods. Method A involved macerating BVR slices in 500 ml of 0.2% citric acid, while Method B involved macerating BVR slices in a mixture of 0.2% citric acid and 0.1% ascorbic acid. Following extraction, the extracts from both the A and B methods were concentrated via evaporation and stored at -20 degrees Celsius until they were analysed using the inductively coupled plasma method for the determination of K, Fe, Ca, Se, and P [19].

Recently, Borjan et al. [20] proposed several green isolation techniques, such as supercritical fluid, ultrasound, cold, and soxhlet extraction methods, to prepare BVR extracts and investigate the effects of these methods on the anti-hyperglycemic, anti-inflammatory, and antioxidant activities, in addition to determining the content of betalains. Specifically, for the Soxhlet and ultrasound extractions, they found that water extraction yielded the highest quality extract, while cold extraction yielded slightly less. According to the results of the efficiency study, extract yield was greatest when extraction was performed in a reflux state. This demonstrates that "reflux" hot solvent systems are superior at recovering antioxidant components, resulting in higher extract yields [20].

Because of their versatility and the useful by-products of each preparation method, beets are a basic of Iraqi cuisine. In order to determine the best way to prepare beets with a high nutritional value, the concept of comparing the methods of preparing beets in terms of organic and inorganic food ingredients has emerged. The purpose of this research is to determine the amounts of betalains, folic acid, ascorbic acid, Mg, Fe, and K present in BVR after being prepared in a variety of traditional Iraqi cooking styles.

Materials and Methods

The following products were purchased from Sigma-Aldrich and Merck: Ammonium hydroxide, acetic acid glacial (AA) (99%), folic acid (FA) (97%), L-ascorbic acid (99%), betanin (red beet extract diluted with dextrin), Dilutions were prepared from a stock solution of iron, 1000 mg/L ($Fe(NO_3)_2$ in HNO_3), magnesium stock solution, 1000 mg/L ($Mg(NO_3)_2$ in HNO_3), and potassium stock solution, 1000 mg/L (KNO_3 in HNO_3).

Apparatus and Experimental Conditions

An analytical balance type (Ohaus Adventurer Pro AV264 - Switzerland) was used to weigh the standard chemical materials and collect samples. UV- VIS spectroscopy test was made for the qualitative and quantitative analysis of betanin by a UV-visible spectrophotometer (1650) designed and manufactured in Japan by Shimadzu, and the amounts of betanin contents were determined at 535 nm [21]. FA [22] and ascorbic acid [23] in each sample solution were determined by HPLC Shimadzu DGU-20As (LC-20AD, SPO-20A). The ISE method was created for the quantitative analysis of nitrate ion employing ion selective membrane, which transforms the activity of dissolved ions in each sample solution into an electrical potential [24]. For a flameless analysis, a graphite furnace and hydride generator were added to a Phoenix-986 AA (UK) spectrophotometer to conduct the AAS measurements of Fe, Mg, and K levels in trace amounts under operating conditions at 248.3, 285.2, and 766.5 nm, respectively [25].

Sample collection and solution preparation

All the BVR used in this study was purchased fresh from area stores and farmers' markets (Iraq). Twenty complete and well-functioning BVR samples were taken. They were washed in both regular and deionized water multiple times. The HPLC calibration curves of FA and ascorbic acid are used as a starting point in the BVR's preparation of stock solutions. In order to get a stock solution with a known concentration of 1000 ppm, 0.1 g of FA was dissolved in 100.0 mL of 10% NH_4OH [22]. To prepare 500 ppm of standard stock solution, 0.05 g of ascorbic acid was dissolved in 100 ml of a mixture of (methanol: phosphate buffer (pH=3.0)).

Use UV-VIS Spectroscopy to determine betalains concentrations quantitatively. After diluting the sample solution ten-fold, the concentration of the aqueous stock solution of betalains was spectrophotometrically determined at 535 nm using a molar extinction coefficient = 65.000 $M^{-1}.cm^{-1}$ [26,27]. Diluting a 1000 ppm mother solution of the aforementioned metals with DI water yielded 1-10 ppm of Fe, 10-100 ppm of Mg, and K, respectively, for use as stock solutions. Standard curves were generated by gradually varying the amounts of standard solutions injected using an auto sampler.

Results

The concentration of the betalains in BVR extracted solutions (juices) are determined according to reference [21]. After diluted sample solutions (juices) ten times with deionized water, by UV-VIS spectroscopy, as shown in **Figure 1**

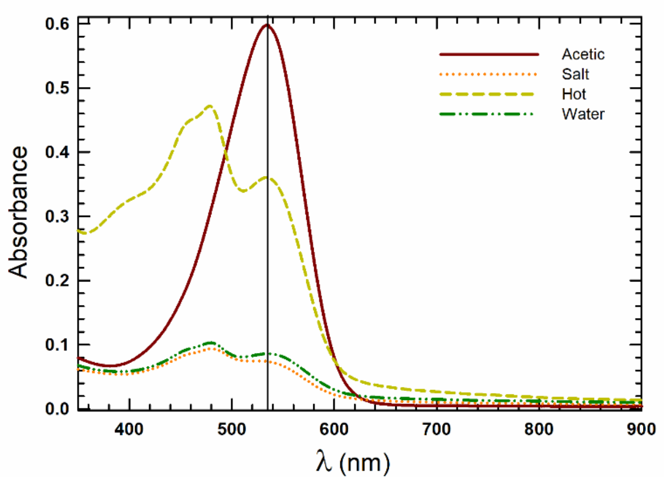

Figure 1. Absorption spectra of the betalains in a beta vulgaris beets extracted solutions by four methods

In accordance with the aforementioned processes, betalains, ascorbic acid, and FA are quantified using the HPLC technique, as indicated in Table 1.

Table 1 Concentration of betalains, ascorbic acid, and Folic acid (ppm) in 200ml of juice.

Method	weight of BVR (gm) (n= 6)	Betalains	Ascorbic acid	Folic acid
I	25.0008	54.80	13.34	28.68
II	25.0024	6.774	20.62	4.049
III	24.9988	33.04	5.860	1.312
IV	25.0012	7.893	3.490	0.97

With the exception of the AA sample (Method I), that exhibits a high acid concentration, all samples can be quantified for nitrate using the ion selective electrodes technique. Also, the concentration of the selected metals in juices are estimated according to the above-mentioned methods I, II, III, and IV and shown in **Table.2.**

Table 2 Concentration of iron, magnesium, potassium, and nitrate ion (ppm) in 200 mL of juice.

Method	weight of BVR (gm) (n= 6)	Fe	Mg	K	NO_3^-
I	25.0008	2.25	270	2760	------
II	25.0024	2.5	160	860	3081
III	24.9988	1.25	240	2640	713
IV	25.0012	3.75	62	160	87

Also, concentrations of the organic and inorganic nutritional constituents, for the four methods, in (mg/200mL) of beta vulgaris beets juice samples were shown in Table.3.

Table 3 Amounts of inorganic and organic nutritional constituents (mg/200mL) of BVR juice samples.

Method	Fe	Mg	K	NO_3^-	Ascorbic acid	Folic acid	Betalains
I	0.45	54	552		2.668	5.736	10.96
II	0.50	32	172	616.2	4.124	0.8098	1.3548
III	0.25	48	528	142.6	1.17	0.2624	6.608
IV	0.75	12.4	32	17.4	0.698	0.194	1.5786

Discussion

UV-VIS spectroscopy was used to determine betalains concentrations in juice samples, while HPLC was used to determine ascorbic acid and FA concentrations. With the exception of the AA sample (Method I), which was unable to be detected due to its excessive acid concentration, each sample's nitrate content was quantified using the ion selective electrode technique. The flameless atomic absorption spectrophotometry method was used to successfully determine the amounts of iron, magnesium, and potassium. The contents of betalains, ascorbic acid, and FA vary depending on the method. As can be seen in Figure 2, the ascorbic acid concentration is highest in the sample prepared with NaCl, while the betalains and FA concentrations are highest in the sample treated with AA. In comparison to other methods, Method I's results showed relatively larger quantities of the components in juice samples. Additionally, The potassium and nitrate concentrations in all of the prepared samples range significantly, with potassium concentrations ranging from (32 mg) in Method IV to (552 mg) in Method I of K, while nitrate concentrations ranging from (17.4 mg) in Method IV to (616.2 mg) in Method II, as shown in Figures 3 (a) and (b).

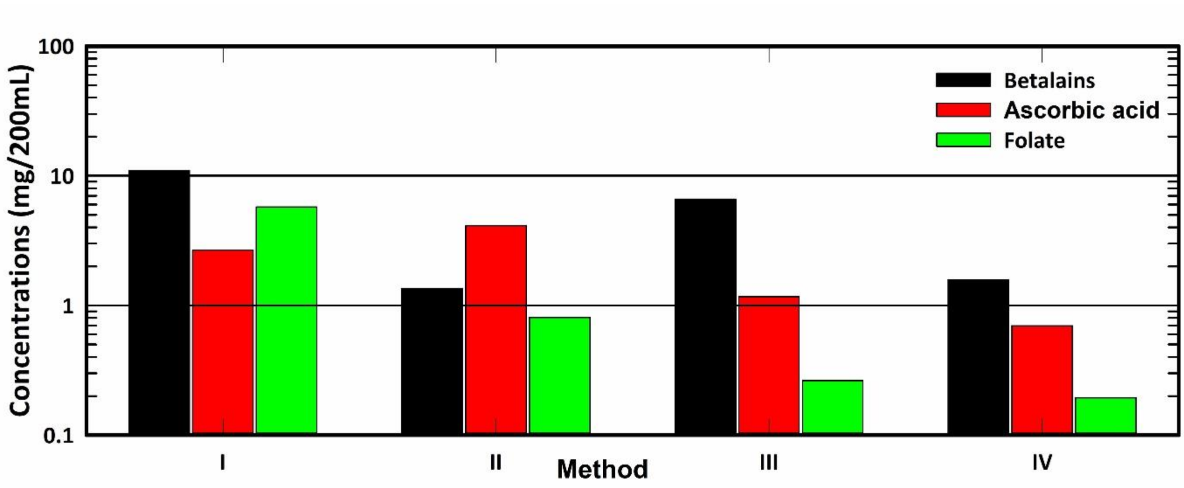

Figure 2: Concentrations of betalains, ascorbic acid, and folic acid (mg/200 mL) in BVR juice

Also, it was evident that the content of magnesium varied across all of the processes, starting at 12 mg in Method IV and ending at 54 mg in Method I (Figure 3c), whereas the iron content is nearly constant (0.25-0.75 mg) for all techniques, as demonstrated in Figure 3d.

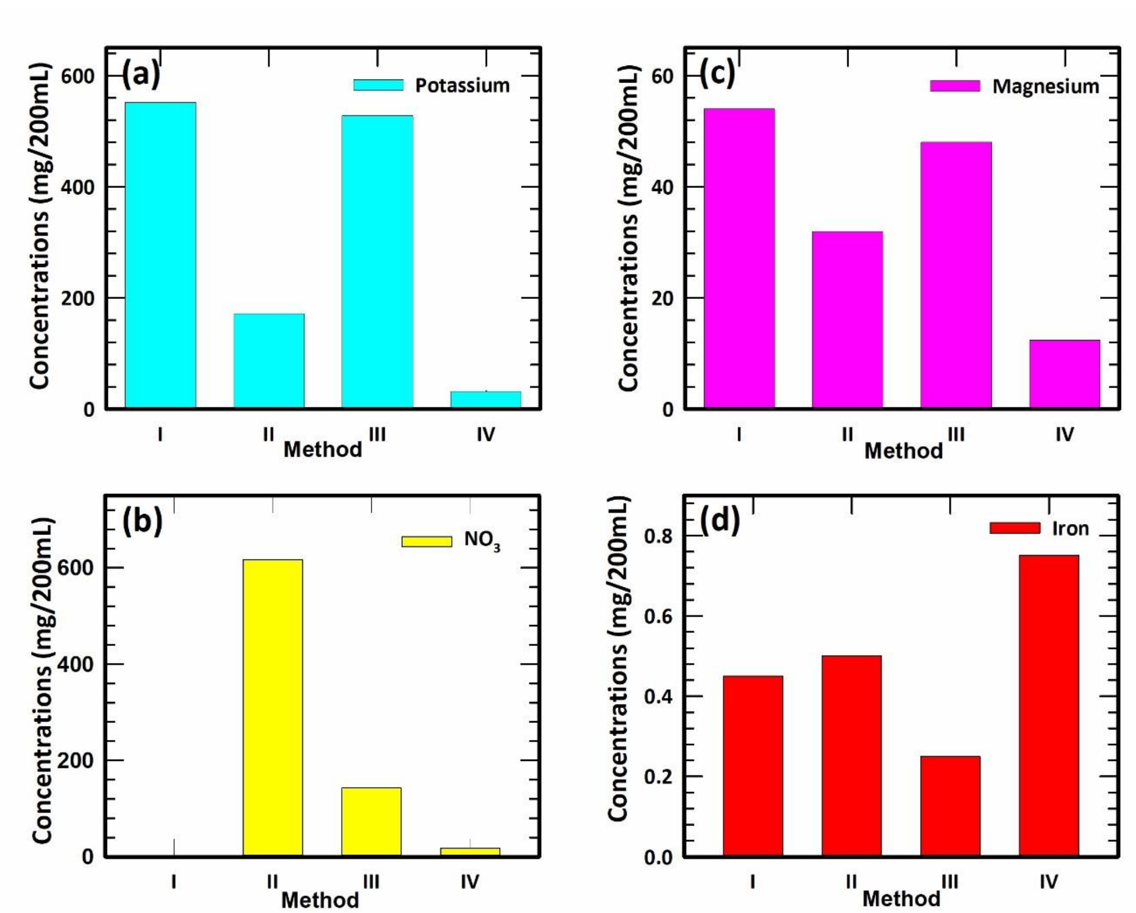

Figure 3: (a) Potassium, (b) Nitrate, (c) Magnesium, and (d) Iron concentration (mg/200 mL) in BVR juice prepared according to the four procedures.

The results of this study show how clinically important each method of preparation is, since the concentrations of nutritional components in the four juice samples were different depending on how they were made. As a result, recommendations can be made regarding whether cooking or eating style is better than others for given conditions. To acquire a good amount of iron, for instance, patients who suffer from lack of iron level in their blood, a single overnight maceration of the BVR in distilled or deionized water is adequate to treat or reduce the effect of iron deficiency. Treatment of the BVR with acetic acid solution (Method I) produces a high FA content. Therefore, it is crucial for pregnant women to eat in order to promote the development of the fetus's spinal cord and brain and to prevent neural tube defects, moreover, consuming it has significant benefits for people who have anemia caused by FA deficiency.

Using a salty water in method II made it possible to get more ascorbic acid and antioxidant activity. In comparison to other approaches, a larger concentration of nitrate was detected in the salty solution since nitrate is known to be crucial for individuals who suffer from angina and high blood pressure, since it is transformed by the body into nitric oxide, which has an antimicrobial activity that aids in the prevention of infections besides being vasodilator. Magnesium is regarded as one of the most significant cofactors in approximately 300 enzyme systems that regulate the function of muscles and neurons, blood pressure, protein synthesis, and blood glucose levels. The human body only requires a very small amount of magnesium, which is present in AA solution (Method I). The final inorganic component is potassium, which enhances the body's water balance and muscle strength, while lowering high blood pressure, stroke risk, anxiety, and stress. In both the AA solution and the hot water solution, K was discovered in high amounts.

The AA method is the most suitable way to employ for a dietary supplement made from BVR, according to the conclusions of this comparative study of the organic and inorganic nutritional constituents in BVR for the four common ways to consume this plant. There are numerous benefits to using this method, including: At room temperature, it can endure for several weeks, not readily contaminated, stable, and contains extremely good amounts of each ingredient that was researched.

Conclusion

Iraq and several of its surrounding nations use four different types of BVR juice. It is regarded as a health enhancer, a disease preventative, and a therapy for specific ailments because of its great nutritional and medicinal value. In order to combat many illnesses including chronic illnesses such as cardiovascular disease, cancer, and diabetes, it is employed as a functional food source. One of the healthiest plant-based foods is BVR juice since it is packed with nutrients including minerals, vitamins, phenols, nitrates, carotenoids, FA, and betalains, among others. High antioxidant activity and stability are demonstrated by BVR juice. As a result, it can be considered as a possible ingredient in the creation of novel and functional meals. When combined in this way, BVR juice can enhance the nutritional content of other foods by working in harmony with them. BVR juice can be used as a standalone product or as an alternative to nitrates as a preservative in food items.

Acknowledgement

The author would like to express his deep gratitude to the college of pharmacy\ Mustansiriyah University for funding and to Assist. Prof. Ali R. Albakaa, Lecturer Nehad K. Abed, and Lecturer Dr. Safaa A. Al-Qaysi for their help and support to achieve this paper.

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