LEAF VEGETABLES AS SOURCE OF PHYTOCHEMICALS

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Abstract

Different vegetables are considered as sources of human health promoting components. Leaf vegetables are widely used in human diet, they are low in calories and fat, but high in dietary fibres, content of minerals, such as iron and calcium and very high in phytochemicals such as vitamin C, carotenoids, lutein and others. On the one hand phytochemicals are a plant's way of protecting itself. They help shield tender buds and sprouts from predators, the toxic elements, and pollution. On the other hand they have beneficial effect on human health. Genotype along with growing and management conditions can affect the content and the composition of phytochemicals in plants. The aim of research is to determine the content of phytochemicals (chlorophyll a and b, total carotenoids, lutein, vitamin C) in leaf vegetables. The lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), green and violet basil (*Ocinum basilicum*), mustard (*Brassica juncea*) and common nettles young sprouts (*Urtica dioica*) were grown in greenhouse or in an open field. The content of plant pigments were determined spectrophotometrically, the titrimetric method was used for determination of vitamin C content.It was found out that young nettles sprouts and spinach are the good source of chlorophyll, basil (green and violet) contains more than 0.3 mg 100 g^{-1} total carotenoids, but for correcting the deficiency of vitamin C it is useful to use mustard, young nettles sprouts as well as leaves of basil.

Keywords: leaf vegetables, phytochemicals.

Introduction

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fibber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories (Banerjee et al., 2012).

Leaf vegetables came from very wide variety of plants and they are plants with edible leaves. Each of us knows lettuce and spinach, as well as mustard, but also early springtime nettles are valuable source of vitamin C. Green leafy vegetables are popularly used for food, being a rich source of β-carotene, ascorbic acid, minerals and dietary fiber. One of the most popular vegetable is lettuce. Lettuce is cultivated worldwide, and is one the most consumed green leafy vegetables in the raw form for its taste and high nutritive value, being regarded as an important source of phytochemicals, including carotenoids, in the diet (Chang et al., 2013).

Nettles are known as widely used as food in early spring. Stinging nettle young leaves added to soups or salads and dried for winter use (Hojnik et al., 2007).

Spinach contains number of different phytochemicals which have a high nutritional value. It is a rich source of antioxidants, especially high in vitamins A, C, E, K, β-carotene, selenium and omega-3 fatty acids as well as rich in lutein and zeaxanthin. Spinach contains microelements such as potassium, calcium, magnesium, manganese, zinc and others. Spinach extracts have several beneficial effects, such as anticancer, antiaging and protecting of central nervous system (Lomnitski et al., 2003).

Sweet basil is a popular herbal crop grown for fresh or dry leaf or essential oils. The leaves of basil are a good source of vitamin P and ascorbic acid, contain microelements zinc and manganese. It have been found, that basil contains high amount of xantophyll (Calucci et al., 2003).

Mustard, like spinach, contains many phyto-nutrients that have health promotional and disease prevention properties. Mustards are very low in calories and fats, but are supposed to be one of the highest among leafy vegetables, which provide vitamin K. It is a rich source of flavonoids, carotenes, lutein and zea-xanthin. Moreover fresh mustard leaves are an excellent source of vitamin C, several essential minerals such as calcium, iron, magnesium, potassium, zinc, selenium, and manganese (Banerjee et al., 2012).

Knowledge on different phytochemical presence and its content in vegetables is important for an appropriate choice of products according to the physiological needs. Phytochemicals are a large group of plant derived compounds, the plant`s way of protecting itself. In addition they appear to have significant physiological effects in the human body. There are more than thousand known phytochemicals. They are acting as antioxidants, stimulating enzymes, interfering with DNA replication, destroying bacteria, as well as they seem to act to reduce the onset of diseases such as cancer and heart diseases (Krishnaswamy, Raghuramulu, 1998).

The leaves of plants contain number of colour pigments generally falling into two categories chlorophylls and carotenoids. Chlorophylls a and b are the pigments that make plants look green. Chlorophyll is often referred to as the green blood of plants due to the identical molecular structure with hemoglobin with only difference in centre atom (iron or magnesium). This similarity makes chlorophyll so important to our health, it improve digestive, immune and detoxification systems of human body (Kopsell et al., 2005).

Carotenoids are natural pigments that provide the natural yellow, orange or red colours of vegetables and fruits. These colours are a result of the presence of

conjugated double bonds, also providing carotenoids with antioxidant properties. Therefore the interest of carotenoids, which are found in vegetables, is not only due to their provitamin A activity but also to their antioxidant action by scavenging oxygen radicals and reducing oxidative stress in the organism (Rao, Honglei, 2002). They are thought to provide health benefits in decreasing the risk of disease, particularly certain cancers and eye disease. Besides beta-carotene, which is the best-known carotenoid, this group includes alpha-carotene, lycopene, lutein, zeaxanthin and cryptoxanthin. It is known that the largest contribution of vitamin A intake comes from the provitamin A carotenoids in plant food, which may contribute up to 82% of the total vitamin A intake (Van den Berg et al., 2000), therefore the green leafy vegetables are good sources of carotenoids. Carotenoids serve many functions in plants including light harvesting, structure stabilization and excess energy dissipation, they protect plants from free radicals, such as triplet excited chlorophyll (^{3}Chl) and singlet oxygen (^{1}O), produced when light intensity exceeds photosynthetic capacity (Mortensen et al., 2001).

Vitamin C (ascorbic acid) is a major antioxidant in the human body. This water-soluble vitamin is involved in many biological processes. Its biological significance is based on the ability to participate in enzymatic and hydroxylation reactions, participates in the oxidation - reduction processes. Vitamin C promotes the absorption of microelements iron and copper, participates in trace element metabolism, and protects cells from damage caused by free radicals, toxins and environmental pollution. It is directly related to protein exchange. Different fruit, vegetables and berries are rich in vitamin C, especially red pepper, black currents and the content of vitamin C may vary depending on the environmental and stress factors such as light intensity, temperature, humidity conditions, air pollution, etc. (Singh et al., 2012).

The aim of study was to determine the content of phytochemicals in leaf vegetables growing and harvested in Latvia.

Materials and Methods

Investigations were carried out at the Latvian University of Agriculture, Institute of Soil and Plant Sciences and Pure Horticultural Research Centre.

Plant material

Six types of leafy vegetables were selected for analysis. There were lettuce (*Lactuca sativa*) variety 'Rīga', spinach (*Spinacia oleracea*) variety "Matador", green basil (*Ocinum basilicum*) variety 'Genovese', violet basil (*Ocinum basilicum*) variety 'Aromat', leaf mustard (*Brassica juncea*) and common nettles (*Urtica dioica*). Seedlings of lettuce, spinach, basil and mustard were grown in 2 L pots with peat substratum in the polycarbonate greenhouse without artificial illumination. Temperature in green house was maintained between 8 °C (min at night) and 30 °C (max at day time). Vegetation time 2 months.

Nettles were grown in an open field. All samples were harvested in one day, when shoots was 10–15 cm long. Samples were cooled, packed and transported to the laboratory ensuring low temperature. In the same day the samples for biochemical analysis were prepared.

Phytochemical extraction and determination

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used for the absorbance measurements.

The chlorophylls, carotenoides and xantophylls were extracted with ethanol according to the methods described by Kukric et al. (2012) and Chang et al. (2013) with some modifications. For extraction a representative portion of sample (0.1±0.001 g) (mass) was accurately weighted, grinded and quantitavely transmit in a glass test tube. Then ethanol was added till 5 mL to it and the test tubes were held in dark for 15 min with occasional shaking at room temperature and finally centrifuged.

The chlorophylls, carotenoids and xantophylls content were analyzed spectrophotometrically by absorption measurements (A) at 350 to 700 nm with 1 nm interval and calculated according to the following equations:

$$\text{Chlorophyll a (mg g}^{-1}) = \frac{13.7A_{665} - 5.76A_{649}}{mass \cdot 200} \quad (1)$$

$$\text{Chlorophyll b (mg g}^{-1}) = \frac{25.8A_{649} - 7.6A_{665}}{mass \cdot 200} \quad (2)$$

$$\text{Carotenoids (mg g}^{-1}) = \frac{4.7A_{440} - 0.263\ c_{chla+chlb}}{mass \cdot 200} \quad (3)$$

$$\text{Xantophylls (lutein) (mg g}^{-1}) = \frac{11.51A_{480} - 20.61A_{495}}{mass \cdot 200} \quad (4)$$

The content of vitamin C was determined titrimetrically using 2.6- dichlorphenolindophenol (AOAC, 1990). For determination 2±0.001 g of sample was accurately weighted, grinded in porcelain mortar, than quantitatively transfer in 100 mL tubes, added 50 mL of 1% HCl and 5% HPO_3 mixture (v : v=1 : 1) and mix thoroughly. After 30 minutes solution was filtered through a filter paper No. 89th. For determination 10 mL (V_{anal}) of filtrate was titrated with 0.0005 molar solution of 2.6 dichlorphenolindophenol (V_{titr}).

The content of vitamin C was calculating according to the equation:

$$\text{Vitamin C (mg 100 g}^{-1}) = \frac{V_{titr} \cdot 0.044 \cdot V_{total} \cdot 100}{V_{anal} \cdot mass} \quad (5)$$

Experiments were done in 6 replicates and obtained data was elaborated by ANOVA.

Results and Discussion

Genotype along with growing conditions can affect the content and the composition of phytochemicals in

plant. In the present work it was evaluated the content of chlorophylls, total carotenoids, xantophylls (lutein) and vitamin C in the fresh leaf vegetables grown in Latvia. The content of chlorophylls determined in the analyzed samples is characterized in Table 1.

Table 1

Content of chlorophylls in leaf vegetables

Leafy vegetable	Chlorophyll a, mg g^{-1}	Chlorophyll b, mg g^{-1}	Total chlorophylls, mg g^{-1}
Lettuce	0.144±0.012	0.044 ±0.016	0.188±0.015
Spinach	1.043±0.069	0.461 ±0.035	1.503±0.102
Green basil	1.101±0.15	0.330±0.17	1.421±0.05
Violet basil	1.079±0.098	0.360±0.054	1.439±0.152
Mustard	0.687±0.105	0.155±0.026	0.824±0.129
Nettles	1.534±0.26	0.537±0.032	2.070±0.288

Obtained results showed that the richest source of chlorophyll is leaves of young nettle sprouts – the content of total chlorophylls was 10 times higher comparing with lettuce and for 2.5 times higher comparing with leafy mustard. The obtained results confirm the results mentioned in scientific literature (Banerjee et al., 2012, Kukric et al., 2012, Cruz et al, 2012). Hojnik et al. (2007) reported that nettle leaves contain significant amount of chlorophylls till 2.5 mg g^{-1} fresh weight. Beside other varieties of sweet basil, Kosell et al. (2005) characterized green basil variety Genovese. They reported that content of chlorophyll a is in range from 0.758 till 1.794 mg g^{-1}, chlorophyll b – from 0.149 till 0.344 mg g^{-1} depending on growing conditions. These results also confirm with our investigations. Taking into account the benefits of chlorophyll it is recommended to expand the young nettle leaves use as food ingredients, especially in springtime, when the intake of biologically active substances is difficult.

The results of research (Fig.1) showed significantly higher content of carotenoids and xantophylls in young nettle leaves comparing with other analyzed leave vegetables. The content of carotenoids in leaves of nettles was 0.834±0.072 mg g^{-1}, comparatively in leaves of lettuce the content of carotenoids was only 0.072±0.009 mg g^{-1}. For comparison, Kukric et al. (2012) established that content of carotenoids in leaves of young nettles grown in Serbia ranged from 0.216 to 0.323 mg g^{-1}. The content of carotenoids in analyzed samples of spinach, mustard and basil leaves ranged from 0.255 to 0.398 mg g^{-1}.

Similar tendency was observed analyzing the content of lutein. The leave vegetables which are rich in chlorophyll are also a good source of xantophylls. In the current research the content of lutein was determined in six leave vegetables and they can be arranged as follows (starting from vegetable with less lutein content): lettuce<spinach<mustard<basil violet<basil green<nettle.

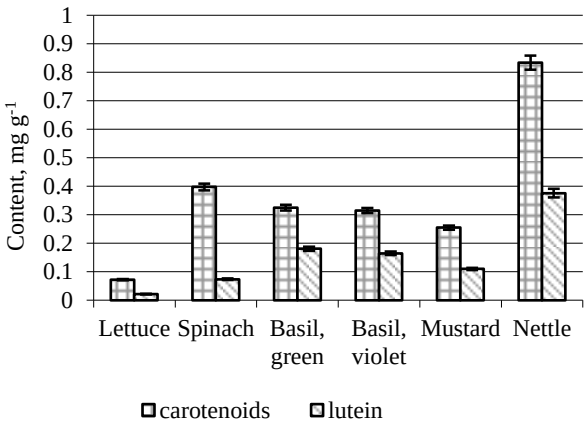

Figure 1. Content of total carotenoids and lutein in leaf vegetables

The obtained experimental results are slightly higher than reported in literature. Krumbein et al. (2005) noted that content of lutein in leaf mustard range from 0.034 till 0.089 mg g^{-1}, but Kopsell et al. (2005) reported that lutein content in sweet basil variety Genovese range from 0.04 till 0.07 mg g^{-1} depending on growing conditions.

Ascorbic acid is considered as one of the most important water soluble vitamins with different important biological functions. Significant amount of vitamin C was determined in mustard and leaves of young nettles sprouts (Fig.2).

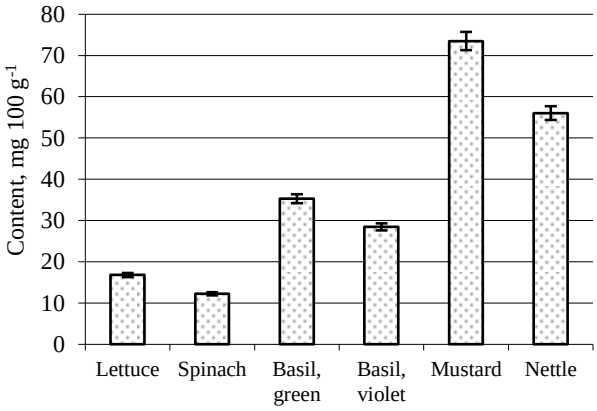

Figure 2. Content of ascorbic acid in leaf vegetables

Ascorbic acid content in leaves can be over a wide range depending on cultivar as well as due to the influence of different factors including environmental and growing conditions. Therefore data in literature regarding ascorbic acid content will be very different. The obtained results confirm with investigations of Staugaitis and Viškelis (2001) done in Lithuania as well as Krumbein et al. (2005) reported that leaves of mustard had relatively high content of ascorbic acid till 89.1 mg 100 g^{-1}. In comparison Banerjee et al. (2012) studies found that content of ascorbic acid in

different mustard varieties range between 12.1 till 19.6 mg 100 g^{-1}.

Summarizing the obtained results the conclusion can be drawn that young nettle sprouts and leaves of mustard are very suitable as sources of human health promoting components due to high content of chlorophyll, carotenoids, lutein and ascorbic acid. For example, increased intake of lutein and β-carotene has been associated with decreased risks of cancer and other chronic deceases, especially age-related eyes diseases (Sommerburg et al., 1999), but sufficient amount of vitamin C ensuring a good health due to it antioxidative properties (Martin, 2003). The Regulations No. 988 of Cabinet of Ministers of Latvia, 2009 September 1 determined that the recommended daily intake for adults is 80 mg of vitamin C, but it will achieve 150 mg (Levine et al., 1999). Unfortunately it should be noted that lettuce which is one of the most popular and consumed green leafy vegetables in the raw form especially in spring time contains less phytochemicals comparing with previous mentioned leafy vegetables. Therefore it will be recommended to popularize and increase the consumption of not so traditional leafy vegetables such as leaf mustard and young sprouts of nettles.

Conclusions

The content of total chlorophylls in leaves of young nettle sprouts was 10 times higher comparing with lettuce and for 2.5 times higher comparing with leafy mustard.

Basil (green and violet) contains more than 0.3 mg 100 g^{-1} of total carotenoids, but the richest source of carotenoids and lutein is young sprouts of nettles.

For correcting the deficiency of vitamin C it is useful to use leaves of mustard, young nettles sprouts and basil.

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