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Vitamin C content of different types of lettuce varieties

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Abstract: Lettuce (*Lactuca sativa L.*) is a highly valued vegetable in human nutrition not only for its richness in minerals and vitamins but also for the fact that nowadays it is produced all year round, and consumed fresh so that all the ingredients stay intact. As part of the study of antioxidant features of lettuce, vitamin C (L-ascorbic acid) was determined in several types of lettuce. The research was conducted on lettuce samples of the types butterhead–*Plenti*, oakleaf–*Murai* and *Kibou*, batavia– *Temptation*, and leaf lettuce–*Levistro*. Lettuce samples for determination of the vitamin C content were collected at technological maturity. Using the appropriate mixture of acids, total vitamin C was extracted from the samples, and the content of L-ascorbic acid was determined using the Tillman's method. The highest vitamin C content was determined in lettuce *Levistro* 9.60mg/100g of fresh lettuce, whereas *Murai* contained the lowest amount of vitamin C, 3.50mg/100g.

Key words: vitamin C, lettuce, Tillman's method

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Introduction

Vegetables and fruits are rich sources of antioxidants such as vitamins A, C and E, carotenoids, polyphenolic components, and flavonoids (Diplock et al. 1998, Milivojevic et al. 2010, Mladenovic et al 2011) which prevent the attack of free radicals thus reducing the risk of carcinogenic illnesses. Consumption of antioxidants in food via natural sources is good for the prevention of cardiovascular diseases, especially arteriosclerosis (Hu 2000).

Lettuce (*Lactuca sativa L.*), an annual plant, belongs to the family *Asteraceae* and is a very important leaf vegetable primarily consumed fresh as a salad or in salad mixtures with other kinds of fresh vegetables.

The abundance of different types and varieties of lettuce and its affiliation with yellow-green-red vegetables provides human nutrition with a very significant and high content of biologically important active substances (mineral substances, vitamins, and organic substances) as well as coloured substances from chlorophyll to anthocyanin.

World lettuce production has been constantly increasing over the last years (FAO 2006). For instance, China produces almost half of the world's lettuce output, which amounts to 11 million Mt and is twice the size of the US output (USDA 2005a). At the same time selection work creates new varieties and types of lettuce having improved characteristics. The nutrient and phytochemical content varies depending on lettuce type (Mou 2005, USDA 2005b). The leaf types (Cos and Cutting) have a significant content of ascorbic acid, vitamins A and K, folates, carotenoids and β-carotene, lutein and zeaxanthin. These types are especially rich in vitamin A and β-carotene, the content of which is approximately 15 times larger than the one in the head-forming types of lettuce (Crisphead type) (Cooper 2004).

The role of vitamins and minerals in human health has been clearly established. However, other natural compounds such as flavonoids, tocopherols and carotenoids, etc. are linked to human health only through epidemiological studies (Pavlović et al 2011). Interdependence has been determined between natural ingredients and human health; therefore, researchers have strived to increase the concentration of these complex phytochemical substances in lettuce.

Vitamin C plays multiple roles in the human organism. First of all, it takes part in the formation of collagen, the lack of which causes changes in bones and capillaries. Bones become fragile, joints become swollen and gums are swollen and bleed. A serious disease resulting from this, known as scurvy, is nowadays successfully treated. Vitamin C is known to increase the organism's resistance to viruses and bacterial infections including allergies. Apart from this, it has marked antioxidant characteristics and is one of major antioxidant agents (Padauattu et al. 2003, Foyer 1993, Bieleski et al. 1975) in removing free radicals along with vitamins E and A, and the minerals selenium and zinc. Most animals

synthesize vitamin C, but the human organism does not have this ability, therefore this vitamin has to be taken with food or, if necessary, as a food supplement. Ascorbic acid is an unstable compound, and therefore its preservation in victuals can be a problem (Barry-Ryan and O'Beirne 1999). It is thermally unstable and degrades at 50^0C (Jacobs 1991). Although lettuce is not the richest source of a number of nutrients, including vitamin C (Albrecht 1993), its advantage is that it can be eaten raw, with its nutritive value preserved, as much of it would be lost by cooking.

Material and Methods

Plant Material

Field experiments as part of this study were conducted in the spring cycle of lettuce cultivation in the 2010 growing season. The experiments were performed on vertisol, in the village of Trbušani near Čačak. The lettuce was produced from seedlings planted on black PE foil, at a spacing of 25x25cm. During the production cycle, all standard measures for the cultivation of lettuce on foil were employed.

The study included five different types of lettuce strains: butterhead – *Plenti*, oakleaf – *Murai* and *Kibou*, batavia – *Temptation*, and leaf lettuce – *Levistro*. At technological maturity, lettuce was sampled for laboratory analysis.

Analytical procedures

Extraction of the plant material

Total vitamin C was extracted from the measured quantity of fresh lettuce (100g) by using the mixture of metaphosphoric (HPO_3) and glacial acetic acid (CH_3COOH). The obtained extract was filtrated through filter paper, and an aliquot was taken for titration with Tillman's reagent. If necessary, the aliquot can be diluted with boiled cooled distilled water to obtain around 2 mg of ascorbic acid.

Tillman's method

Quantitative determination of L-ascorbic acid is based on the reversible ability of oxido-reduction system of ascorbic-dehydroascorbic acid. Titration with the reagent 2,6-dichlorophenolindophenol (Tillman's reagent, TR) was performed in an acidic environment with pH of 4-6. The oxidized form of TR (also serving as an indicator) is dark blue at pH of 5.2. In the presence of ascorbic acid, TR changes into its reduced colourless form. At pH of 4.2 TR is red (acidic environment), and when the whole amount of L-ascorbic acid is oxidized, the very next drop of TR colours the examined solution pink.

Preparation of the Tillman's reagent's solution

A total of 0.25 g of 2,6- dichlorophenolindophenol (Na-so, $OC_6H_2NC_6H_4ONa$ x $2H_2O$, M_r = 290,09) was dissolved in 250 cm^3 of warm distilled water containing 0.21 g of $NaHCO_3$. Upon dissolution, the container was filled with up to 1 dm^3 of freshly boiled cooled distilled water. The solution was subsequently filtrated through filter paper and kept in a dark bottle in the refrigerator. The solution was stable for up to four weeks. Before use, the titer of TR was determined according to standard 1mg/cm^3 ascorbic acid solution.

L-ascorbic acid determination procedure

Three parallel titrations were performed for each sample. The determined volume of the lettuce extract (5.00 cm^3 of extract + 5 cm^3 of the mixture of HPO_3 and glacial CH_3COOH) was titrated with Tillman's reagent until it turned into light pink colour that persisted for about five seconds. At the same time a blank was titrated with TR in three parallel determining sessions. The average values of the volumes of three titrations were taken for calculation of the vitamin C content in lettuce.

Results and Discussion

The results on the L-ascorbic acid content in several types of lettuce, expressed in mg/100 g of fresh lettuce, are presented in Table 1 and Figure 1.

Table 1. Vitamin C content in different lettuce types

Lettuce type	*Plenti*-Butterhead	*Murai*	*Temptation*	*Levistro*	*Kibou*
Vitamin C (mg/100 g)	3.85	3.50	4.99	9.60	5.25

The results show dependence of vitamin C content on lettuce type. Some authors (Mou 2005) have also observed this interdependence and reported a higher vitamin C content in leaf lettuce than in head-forming types. This proved to be true in our study as well. Leaf lettuce *Levistro* (9.60mg/100g) and *Kibou* (5,25mg/100g) have a higher content of vitamin C than the head-forming type *Butterhead* (3.85mg/100g). The red-coloured oakleaf *Murai* contains the lowest amount of vitamin C (3.50mg/100g), whereas lettuce batavia-*Temptation* has a higher amount (4.99mg/100g). The lower vitamin C content in the red-leaf lettuce is in agreement with the data of other authors (Still 2007).

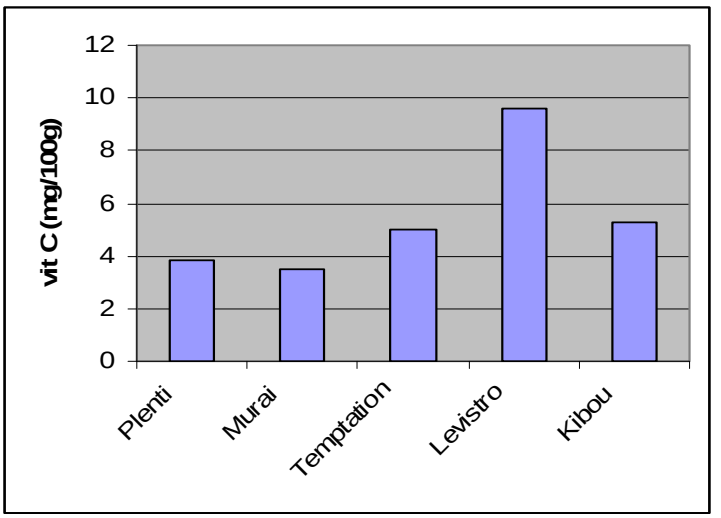

Figure 1 – Vitamin C content in different lettuce types

All lettuce types tested in this study were cultivated on the same type of soil under the same conditions. Therefore, the difference in the vitamin C content was induced by lettuce genotype (Llorach et al. 2008).

Conclusions

The highest content of vitamin C was determined in lettuce *Levistro* (9.60mg/100g), and the lowest in *Murai* (3.50mg/100g) and *Plenti* – butterhead (3.85mg/100g). This study showed that vitamin C content is dependent upon lettuce type, being higher in green-coloured leaf lettuce types, and lower in leaf types of red-leaved lettuce (*Murai*). Lettuce is not rich in vitamin C. However, its advantage is that it is consumed fresh with vitamin C being fully utilized, most notably in terms of the fact that this vitamin is thermally labile and, hence, largely lost during the thermal processing of vegetables and fruits.

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SADRŽAJ VITAMINA C U RAZLIČITIM TIPOVIMA SORTI SALATA

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Rezime

Salata (*Lactuca sativa* L.) je veoma cenjeno povrće u ishrani ljudi ne samo zbog bogatstva mineralima i vitaminima već i činjenice da se u novije vreme proizvodi tokom cele godine, a konzumira u svežem stanju pa svi sastojci ostaju sačuvani. U okviru ispitivanja antioksidativnih osobina salate određen je vitamin C (L-askorbinska kiselina) u nekoliko tipova salate. Istraživanja su vršena na uzorcima salata tipa puterica-*Plenti* (maslenka), hrastov list-*Murai* i *Kibou*, batavija-*Temptation* i lisnata-*Levistro*. Uzorci salate za određivanje sadržaja vitamina C uzeti su u fazi tehnološke zrelosti. Pogodnom smešom kiselina iz uzoraka je ekstrahovan ukupni vitamin C, a sadržaj L-askorbinske kiseline određen je metodom po Tillmans-u. Najveći sadržaj vitamina C nađen je u salati *Levistro* 9,60 mg/100g sveže salate, a najmanje vitamina C sadrži salata *Murai* 3,50 mg/100g.