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ORIGINAL ARTICLE

INFLUENCE OF TEMPERATURE AND BREWING TIME OF NETTLE *(URTICA DIOICA L.)* INFUSIONS ON VITAMIN C CONTENT

Jolanta Wolska[1], Michał Czop[2], Karolina Jakubczyk[1], Katarzyna Janda[1*]

[1]Pomeranian Medical University in Szczecin, Department of Biochemistry and Human Nutrition, Szczecin, Poland
[2]University of Strathclyde, Naval Architecture, Ocean & Marine Engineering, Glasgow, UK

ABSTRACT

Background. Stinging nettle (*Urtica dioica* L.) can be found in temperate climate zones of Europe, Africa and America Nettle may be a source of nutritional ingredients, mineral salts, vitamins and antioxidants.

Objective. The aim of the study was to determine the effect of temperature and brewing time *Urtica dioica* L. infusions from different parts of this plant on vitamin C (ascorbic acid) content.

Material and methods. Infusions of nettle leaf, stem and root were prepared at room temperature, 50°C, 60°C, 70°C and 80°C for 10 minutes. Leaf infusions were also brewed for 5, 10, 15 and 20 minutes at initial water temperature of 60°C. The amount of vitamin C was determined by the spectrophotometric method.

Results. The best temperature of brewing nettle infusions, in terms of vitamin C concentration, is between 50 °C and 60 °C as it is sufficient to extract the substance, yet not high enough to destroy it.

Conclusions. The optimal time of brewing appeared to be 10 minutes as the prolonged exposure to high temperature appeared to be detrimental for ascorbic acid as well.

Key words: *infusion, leaf, stem, root, Urtica dioica, vitamin C*

STRESZCZENIE

Wprowadzenie. Pokrzywa zwyczajna *(Urtica dioica* L.) występuje w strefie klimatu umiarkowanego Europy, Afryki i Ameryki. Może być źródłem składników odżywczych i mineralnych, witamin i antyoksydantów.

Cel badań. Celem badań było określenie wpływu temperatury i czasu przygotowywania naparów z różnych części pokrzywy zwyczajnej na zawartość witaminy C (kwasu askorbinowego).

Materiał i metody. Napary z liści, łodyg i kłączy były przygotowywane w temperaturze pokojowej, 50°C, 60°C, 70°C i 80°C przez 10 minut. Ponadto napary z liści były przygotowane w temperaturze 60°C przez 5, 10, 15 and 20 minut. Zawartość witaminy C określono spektrofotometrycznie.

Wyniki. Optymalna temperatura przygotowywania naparów z pokrzywy, pod kątem uzyskania maksymalnej zawartości witaminy C, mieści się w przedziale od 50°C do 60°C. Jest wystarczająca do przejścia tego związku do naparu i jednocześnie nie jest na tyle wysoka, aby ją zdegradować.

Wnioski. Optymalnym czasem przygotowywania naparów z pokrzywy celem uzyskania jak największego stężenia witaminy C jest czas 10 minut; przedłużanie tego czasu może prowadzić do degradacji kwasu askorbinowego i zmniejszonej jego zawartości w naparach.

Słowa kluczowe: *napar, liście, łodygi, kłącza, Urtica dioica, pokrzywa zwyczajna, witamina C*

INTRODUCTION

Stinging nettle *(Urtica dioica L.*) can be found in temperate climate zones of Europe, Africa and America. Its leaves and stem are covered with small needles that inject substances such as histamine, formic acid or acetic acid, causing irritation and pain when they come in contact with skin, which serves as a protection mechanism. Stinging nettle is associated by most people with red itching bumps it causes and it is often forgotten that nettle is an extremely important plant for medicine. Stinging nettle has a huge variety of properties that are beneficial for human organisms. It can be used to treat arthritis and joint pain. It is also known to have antiulcer, antimicrobial, antioxidant [10, 15, 18, 25] and analgesic properties [4, 11]. It has

[*]**Corresponding author**: Janda Katarzyna, Department of Biochemistry and Human Nutrition, Pomeranian Medical University in Szczecin, Broniewskiego Street 24, 71-460 Szczecin, Poland, tel. +48 91 4414806, e-mail: katarzyna.janda@pum.edu.pl

also been used as a nutritional tea for anaemic people [17]. Stinging nettle is remarkably rich in vitamins A, C and D along with minerals such as iron, manganese, potassium and calcium and is rich in proteins [3]. Nettle leaves contain vitamin C (270 mg%), carotenoids (50 mg%), vitamins: B, K (200 mg%) and E, coumarins, flavonoids, phenolic acids, tannins, phytoncides, glycoside urticin, organic acids, sterols, chlorophyll (up to 5%), alkaloids and minerals. Moreover, nettles contain minerals (especially iron), vitamin C and pro-vitamin A, that is simply digested in the host's small intestine. Nevertheless, to enjoy all the wonders of nettle it must be first turned into an ingestible form. Besides the aforementioned industrial products nettle can be used in home cooking. It is also quite common to prepare nettle in the form of soup or puree. Nettle leaves can also be used as a food additive to other dishes in a similar way as parsley. In spite of the huge variety of possibilities, the most common method of serving stinging nettle is preparing infusions. Nettle is considered to be a nutritive food. Nettle leaf has a long history as a herbal remedy and nutritious addition to the diet [2].

Vitamin C has the molecular formula $C_6H_8O_6$ and the atomic weight of 176.13. It is a water-soluble vitamin as it is polar due to presence of hydroxyl groups. Its structural formula resembles the one of glucose which it is derived from. It is an excellent reducing agent as it can donate one or two electrons forming semi-dehydroascorbic acid and dehydroascorbic acid, respectively. Vitamin C exists only as a solid and as a pure substance it occurs as colourless powder. It is vulnerable to light and air as well as to high temperature. It decomposes completely at approximately 190°C. Vitamin C is considered as one of the most important water soluble vitamins with different important biological functions [7]. Vitamin C is considered to be one of the most active anti-oxidants, which means that it helps the organism to neutralise free radicals. These are elements with unpaired electrons on the valence shell which makes them very reactive species. They are potent oxidation agents, which makes them immensely dangerous as excessive oxidation leads to cell degradation and cancer. Free radicals may be formed in different ways, including digestion of certain types of food, inhalation of tobacco smoke or as a result of being exposed to radiation.

Vitamin C is one of the most important micronutrients postulated to have a beneficial role in health-promoting effects (antioxidant, biosynthesis of collagen, carnitine and hormones, immune response, iron absorption) [5, 12]. According to American National Institute of Cancer vitamin C can be used to improve cancer treatment, yet it is not recognised as a valid method of fighting the disease [8, 16]. Vitamin C is known to boost the immune system, thus its regular ingestion is helpful in protecting oneself from bacteria and viruses which cause flu and other illnesses. Vitamin C is important for maintenance of immunity and stimulation of the interferon synthesis, thus participating in immune modulating processes [1, 9]. People require vitamin C for collagen formation and tissue repair. It is reversibly oxidized to dehydroascorbic acid in the body, which makes it important to oxidation-reduction reactions. Vitamin C is involved in tyrosine metabolism, carbohydrate metabolism, synthesis of lipids and proteins, conversion of folic acid to folinic acid, iron metabolism and helps to improve resistance to infections and cellular respiration [5, 12]. Moreover, vitamin C enhances wound healing and prevents many other diseases such as scurvy [22]. Plants synthesise this compound in order to protect themselves from oxidising stress appearing as a result of photosynthesis, metabolic processes and external pollutants [22].

The aim of the study was to determine the effect of temperature and brewing time of nettle (*Urtica dioica* L.) infusions on vitamin C content.

MATERIALS AND METHODS

Plant material of *Urtica dioica* L. was taken from the ecological farm "Goat Delicacies" in Marwice located in West Pomeranian Voivodeship in northwestern Poland. The nettle was collected in April before blooming. Directly after picking, the nettle was separated into parts: leaves, stems and roots, placed in a refrigerator and stored at constant temperature of -20°C. All measurements were performed within two weeks from plant collection.

Previously frozen parts of *Urtica dioica* L. were unfrozen and then homogenised. 1g of a sample was transferred to a conical flask to which 100 cm^3 of water at given temperature was added. Flask with infusion has been closed and rotated with a speed of 180 rpm for a chosen time. After brewing the plant parts were separated from infusion through filtration. This work shows how the time and temperature of brewing affect the amount of vitamin C present in infusions of three parts of plant. The presented results were obtained in the tests in which the parts of nettle were brewed for 10 minutes at the following temperatures: 25 °C, 50 °C, 60 °C, 70 °C, 80 °C. Infusions from leaves of nettle were also checked for vitamin C presence at constant brewing temperature 60 °C for the following brewing times: 5, 10, 15 and 20 min.

Determination of vitamin C content was carried out according to ISO 6557-2:1984 [13]. In this method 2,6-dichlorophenoloindophenol (2,6-DCPIP) is added to a sample, reacts with vitamin C and after extraction with xylene its excess is determined spectrophotometrically. Absorbance measurements were taken at 500 nm in a 1 cm quartz cuvettes with xylene as a reference.

Measurements were taken on Agilent 8453 UV-VIS spectrophotometer. In this work following chemicals were used: o-xylene of spectrophotometric grade, 98% (SIGMA-ALDRICH), 2,6-dichlorophenolindophenol sodium salt hydrate (SIGMA-ALDRICH), remaining chemicals were of analytical grade. Concentration of vitamin C was expressed in mg of vitamin C per 100 g of infusion (mg/100 g).

Table 1. Statistical parameters of calibration curve for 2,6-dichlorophenoloindophenol from ChemStation

Parameters	2,6-dichlorophenoloindophenol
Number of standards	10
Equation of calibration curve	V = k1*A
Coefficient k1 [cm^3]	2.3216
SD of k1 [cm^3]	0.0404
SD of calibration curve [cm^3]	0.0680
Correlation coefficient R^2	0.9973

The results were analysed statistically using the Statistica 10 software (StatSoft, Poland). Vitamin C measurement for every type of infusion has been repeated five times. From obtained data the mean value and standard deviation (SD) were calculated. *Pearson* coefficient has been used in order to interpret correlation of calibration curve. In order to assess the statistical significance of *Pearson* coefficient P-value has been calculated alongside the Pearson coefficient. Normal distribution of data has been checked with *Shapiro-Wilk's* test. Statistically significant differences between mean values of obtained results were determined by *Student's* t-test for independent samples. Tests were evaluated at the significance level $\alpha = 0,05$.

RESULTS AND DISCUSSION

Vitamin C is unstable and decomposes during the collection of plant material. It is sensitive to light, heat and pH, therefore some amount of this vitamin decomposes during preparation of infusions. Content of vitamin C in infusions depends on various factors: time and temperature of brewing, phase of development of the plant used for preparation of infusions [6].

In Figure 1 the amount of vitamin C with SD in investigated infusions of nettle parts obtained for constant brewing time (10 min) but at different temperatures is presented.

Statistical analysis (*Student's* t-test) implied that between vitamin C contents in all investigated nettle's parts, for almost all temperatures, there is a significant difference ($p < 0,01$). Only one deviation, for leaf vs. stalk at 70 °C, has been found. There is no statistically significant difference between vitamin C amount in infusions made of nettle's leaves and stalk at 70 °C ($p = 0.2878 > \alpha$). Mean values of vitamin C in infusions of leaf and stalk at 70 °C are almost similar (1.88 mg/100 g and 1.85 mg/100 g, respectively). There is observed an increase in vitamin C content along with the increase of temperature of brewing up to 60 °C and then a decrease at higher temperatures: 70 °C and 80 °C. Infusions prepared from leaves of nettle showed higher amount of vitamin C comparing to infusions from other parts of tested plant. At brewing temperature of 60°C the infusions prepared from leaves contained 3 mg/100 g, whereas the infusions from stalk and root contained 1.9 and 2.2 mg/100 g of vitamin C, respectively. Surprisingly infusions from roots of nettle obtained at brewing temperature from 25°C to 60 °C contained more vitamin C than nettle's stalk. Influence of time of brewing on vitamin C concentration in infusions has been investigated at 60°C for nettle's leaf infusions. Those conditions have been chosen due to the highest content of vitamin C.

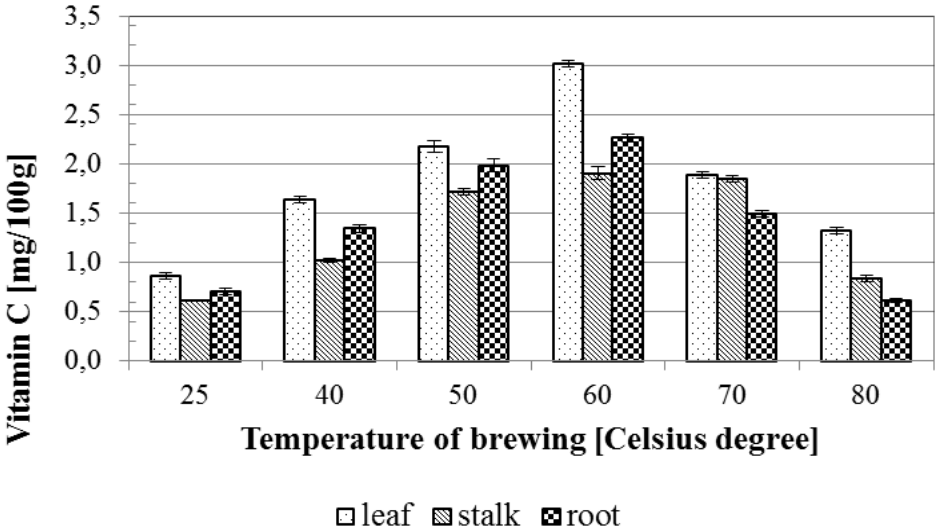

Figure 1. Amount of vitamin C present in infusions from different parts of nettle depending on temperature of brewing

In Figure 2 the results obtained for nettle's leaves infusions at constant temperature (60°C) but for different brewing times are presented.

There is a statistically significant relationship of amount of vitamin C in infusion on time of brewing, which has been confirmed by *Student's* t-test ($p < 0.00001$). It is also clearly visible that 10 minutes is the most efficient time of brewing. The concentration of vitamin C after 10 minutes of brewing is 3 mg/100 g, whereas when the brewing time is extended to 15 and 20 minutes, the amount of vitamin C decreases to 1.3 and 1.0 mg/100 g, respectively. At the same time when the brewing time was 5 minutes the amount of vitamin C in infusion was about 2 mg/100 g.

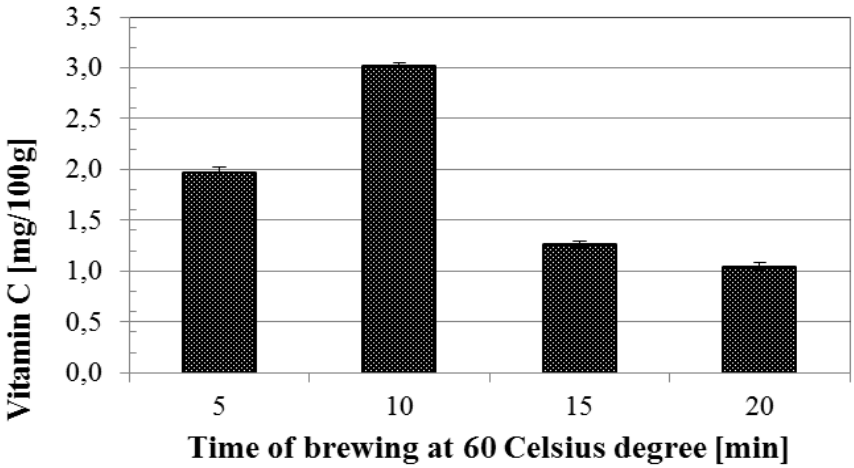

Figure 2. Amount of vitamin C present in nettle's leaves infusions for different time of brewing at constant temperature of 60°C

According to many authors, temperature is one of the main factors that significantly influence the stability of vitamin C in solution [12, 14, 20]. *Njoku* et al. [19] showed that along with the increase of temperature from 20 °C to 80 °C the amount of vitamin C in fruit's juices decreases significantly reaching minimum value at 80 °C. *Suntornsuk* et al. [23] proved, that even storing juices at low temperature of 4°C caused losses in vitamin C concentration. *Skalozubo*va & *Reshetova* [21] determined amount of vitamin C in infusions made of nettles' leaves with iodimetric titration and titration by 2,6-dichlorophenolindophenol sodium salt. Investigated infusions were prepared at 40 °C and possessed small amount of vitamin C – 0.0032 mg%. Our results showed concentration of vitamin C on the level of 1 mg% for 1% infusions made of nettles' leaves. This significant dissimilarity could have been caused by the following differences: chosen method of determination of vitamin C, temperature and procedure of preparation of infusions, source of plant and also degree of fragmentation of nettle.

CONCLUSIONS

In this study it was found that both temperature and time of brewing affects the amount of vitamin C in infusions of leaves, stalk and root of *Urtica dioica* L. Amount of vitamin C present in infusions made of leaves of nettle on average is 1.6 times greater than in infusions from stalks and 1.3 times greater than in infusions from nettle's roots. It appeared that the most effective conditions of brewing, on account of the highest vitamin C concentration in infusion, are 60 °C and 10 min. Based on infusions made from leaves of nettle has been established that while increasing the time of brewing above 10 min, the concentration of vitamin C decreases 2.2 to 2.9 times for 15 and 20 min, respectively. Presented results have proved, that infusions made of different parts of nettle are significant source of vitamin C. Nettles' infusions added to everyday diet, besides pro-health qualities, can be also a valuable replacement of vitamin C for present on the market synthetic L-ascorbic acid.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Akbay P., Basaran A., Undeger U.*: In vitro immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. Phytother Res 2003; 17: 34-37.
2. *Alibas I.*: Energy Consumption and Colour Characteristics of Nettle Leaves during Microwave, Vacuum and Convective Drying. Biosyst Eng 2007; 96: 495–502.
3. *Bisht S., Bhandari S., Bisht N.S.: Urtica dioica* (L): an undervalued, economically important plant. Agricul Sci Res J 2012; 2: 250–252.

4. *Chrubasik S., Enderlein W., Bauer R., Grabner W.:* Evidence for antirheumatic effectiveness of *Herba Urticae dioica* in acute arthritis: a pilot study. Phytomedicine 1997; 4: 105–108.
5. *Davey M.W., Montagu M.V., Inzé D., Sanmartin M., Kanellis A., Smirnoff N., Benzie I.J.J., Strain J.J., Favell D., Fletcher J.,* Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J Sci Food Agr 2000: 80: 825-860.
6. *Dmowski P., Śmiechowska M., Prystupa A.:* Aktywność antyoksydacyjna wybranych naparów *Ilex paraguariensis* dostępnych na rynku Trójmiasta [Antioxidant properties of selected beverages of *Ilex paraguariensis* available on the Tricities market]. Bromat Chem Toksykol 2011: 3: 620-624 (in Polish).
7. *Duma M., Alsina I., Zeipina S., Lepse L., Dubova L.:* Leaf vegetables as source of phytochemicals. Foodbalt. 9th Baltic Conference on Food and Science. Available http://llufb.llu.lv/conference/foodbalt/2014/FoodBalt_Proceedings_2014-262-265.pdf (Accessed 26 August 2015)
8. *Grano A., De Tullio M.C:,* Ascorbic acid as a sensor of oxidative stress and a regulator of gene expression: The Yin and Yang of Vitamin C. Med Hypoth 2007; 69: 953–954.
9. *Guelcin I., Kuefreviogu O.I., Oktay M.: Bueyuekokuroglu M.E.,* Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J Ethnopharmacol 2004; 90: 205- 215.
10. *Gutowska I., Jakubczyk K., Dec K., Baranowska-Bosiacka I., Drozd A., Janda K., Wolska J., Łukomska A., Dębia K., Chlubek D.*: Effect of the extract from nettle (*Urtica dioica* L.) fruit cluster on the synthesis of pro-inflammatory agents in hepatocytes treated with fluoride. Fluoride 2014; 47: 113-122.
11. *Hajhashemi V., Klooshani V.:* Antinociceptive and anti-inflammatory effects of *Urtica dioica* leaf extract in animal models. Avicenna J Phytomed 2013; 3: 193-200.
12. *Hernández Y., Lobo M.G., González M.:* Determination of vitamin C in tropical fruits: a comparative evaluation of methods. Food Chem 2006; 96: 654-664.
13. ISO 6557-2:1984, Fruits, vegetables and derived products. Determination of ascorbic acid content - Part 2: Routine methods
14. *Iwase H.:* Use of an amino acid in the mobile phase for the determination of ascorbic acid in food by high-performance liquid chromatography with electrochemical detection. J Chromatogr A 2000; 881: 317- 326.
15. *Kanter M., Coskun O., Budancamanak M.:* Hepatoprotective effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J Gastroenterol 2005; 11: 6684-6688.
16. *Knowles H.J., Raval R.R., Harris A.L., Ratcliffe P.J:,* Effect of ascorbate on the activity of hypoxia-inducible factors in cancer cells. Cancer Res 2003; 63: 1764–1768.
17. *Meral I., Kanter M.:* Effects of *Nigella sativa* L. and *Urtica dioica* L. on selected mineral status and hematological values in CCl_4-treated rats. Biol Trace Elem Re 2003; 96: 263-270.
18. *Namazi N., Esfanjani A.T., Heshmati J., Bahrami A., Nazemiyeh H.:* A systematic review about effects of aerial portions of *Urtica dioica* (nettle) on some cardiovascular risk factors in diabetes mellitus. Int J Pharm 2012; 8: 306-313.
19. *Njoku P.C., Ayuk A.A., Okoye C.V.:* Temperature effects on vitamin C content in citrus fruits. Pak J Nutr 2011: 10: 1168-1169.
20. *Nováková L., Solich P., Solichová D.:* HPLC methods for simultaneous determination of ascorbic and dehydroascorbic acids. TrAC Trends in Analytical Chemistry 2008; 27: 942 – 958.
21. *Skalozubova T.A., Reshetova V.O.:* Leaves of common nettle (*Urtica dioica* L.) as a source of ascorbic acid (Vitamin C). World Appl Sci J 2013; 28: 250-253.
22. *Smirnoff N.:* The function and metabolism of ascorbic acid in plants. Ann Bot 1996; 78: 661–669.
23. *Stephen R., Utecht T.:* Scurvy identified in the emergency department: a case report. J Emerg Med 2001; 21: 235-237.
24. *Suntornsuk L., Gritsanapun W., Nilkamhank S., Paochom A.:* Quantitation of vitamin C content in herbal juice using direct titration. J Pharm Biomed Anal 2002; 28: 849–855.
25. *Yener Z., Celik I., Ilhan F., Bal R.:* Effects of *Urtica dioica* L. seed on lipid peroxidation, antioxidants and liver pathology in aflatoxin-induced tissue injury in rats. Food Chem Toxicol 2009; 47: 418–424.

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