UNIVERSITY OF KRAGUJEVAC

FACULTY OF AGRONOMY ČAČAK

SYMBIOTECH

2nd INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

14–15 March 2024

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

- PROCEEDINGS -

2nd INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

XXIX Savetovanje o biotehnologiji sa međunarodnim učešćem

- PROCEEDINGS -

ORGANIZER AND PUBLISHER

University of Kragujevac, Serbia
Faculty of Agronomy in Čačak

Honorary Committee

Prof. Dr. Marina Pintar, Slovenia; Prof. Dr. Andrej Bončina, Slovenia; Prof. Dr. Branko Kramberger, Slovenia; Prof. Dr. Tomaž Langerholc, Slovenia; Prof. Dr. Ivica Kisić, Croatia; Dr. Kristina Kljak, Croatia; Prof. Dr. Krunoslav Zmaić, Croatia; Prof. Dr. Zvonko Antunović, Croatia; Prof. Dr. Muhamed Brka, B&H; Prof. Dr. Enisa Omanović-Mikličanin, B&H; Prof. Dr. Ivan Ostojić, B&H; Dr. Adrijana Filipović, B&H; Prof. Dr. Božidarka Marković, Montenegro; Prof. Dr. Sanja Radonjić, Montenegro; Prof. Dr. Vjekoslav Tanaskovik, North Macedonia; Prof. Dr. Ivana Janeska-Stamenkoska, North Macedonia; Prof. Dr. Zlatan Kovačević, B&H; Prof. Dr. Željko Vaško, B&H; Prof. Dr. Dragutin Đukić, Serbia; Prof. Dr. Nedeljko Tica, Serbia; Prof. Dr. Branko Ćupina, Serbia; Prof. Dr. Dušan Živković, Serbia; Prof. Dr. Vladan Bogdanović, Serbia; Dr. Darko Jevremović, Serbia; Dr. Marijana Pešaković, Serbia; Prof. Dr. Cosmin Salasan, Romania;

Technical editors

Prof. Dr Vladimir Kurćubić; Prof. Dr Pavle Mašković; Dr Marija Gavrilović; Dušan Marković, BSc

Print-run: 30

Printed by
MEDIGRAF - Čačak, Aleksandra Savića 42, 32000 Čačak

ISBN 978-86-87611-91-7

Year of publication: 2024

DOI: 10.46793/SBT29.67JM

DETERMINATION OF THE CONTENT OF BIOACTIVE COMPONENTS IN DIFFERENT EXTRACTS OF CELERY LEAVES (*Apium graveolens* L.)

Jelena Mladenović[1], Ivana Vranjevac[1], Dusko Brković[1], Nenad Pavlović[1], Milos Marjanović[1], Jasmina Zdravković[2], Nenad Đurić[3]

Abstract: Plants with active compounds, i.e. those that have medicinal properties have been used since ancient times both for medicinal purposes and for preserving food. The aim of the work is to determine the most optimal extraction method for obtaining the highest yield of vitamin C and organic acids from celery leaves, which could have potential application in the food industry. Three extraction methods were combined, the density of the obtained extracts, the content of vitamin C and the content of organic acids were measured. The correlation of the content of bioactive components and the density of the obtained extracts was monitored.

Keywords: extraction, density, content, celery.

Introduction

Celery (*Apium graveolens* L.) is a biennial herb from the Apiaceae family. The plant consists of an upright, ribbed, branched tree up to 1 m tall, with a tail-like thickened root. The leaves are glossy, pinnately compound, with a long stalk, sitting on the stem. The dark green leaves of wild celery reach up to 80 cm in length. The flower stem bears complex shield inflorescences of inconspicuous green flowers speckled with white. The crown leaves are small, white in color. The fruit is up to 1.5 mm long, with two semicircular mericarps. The whole plant, including the tiny brown seeds, is extremely aromatic, (MacVicar, 2006).

Excellent varieties include: French "Dinan" and Dutch "Amsterdam celery for soup". In addition to the listed varieties, the following are characteristic: Chinese celery (*A. graveolens*) - thin stalks that vary from dark green to white in color. It has a distinctive taste; *A. prostratum* – a glossy-leaved creeper that

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia (jelenamala@kg.ac.rs)

[2] Institute for forage crops, Globoder, Kruševac, Serbia,

[3]Institute for vegetable crops, Karadjordjeva 71st, Smederevska Palanka, Serbia.

grows on the Australian coast. It is used as a spice in traditional Australian dishes.

(a) (b) (c)

Figure 1. Celery varieties: (a) celery (*Apium graveolens var.graveolens)*, (b) celeriac (*Apium graveolens var. rapaceum*) and (c) celery leaf (*Apium graveolens var. secalinum*), https://www.google.com/search?sca_1

Celery is characterized by a high content of essential oil. It is mostly found in fruits (2-3%), herbs (0.1%) and rhizomes (0.1%). The main components of the essential oil are: limonene, selinene, r-cymene, β-terpineol, β-pinene and β-caryophyllene (Lajšić and Grujić-Injac, 1998). It is also rich in vitamins (niacin, pantothenic acid, choline, vitamin C and vitamin E) and minerals (potassium, sodium, calcium, phosphorus and magnesium), and it also contains very small amounts of carbohydrates (most of which are dietary fibers), very little fats and proteins (Lakušić, 1990). The plant also contains coumarins, furocoumarin glycosides, furocoumarins, unidentified alkaloids, flavonoids, etc. (MacVicar, 2006). Important constituents of celery are apin, apigenin and lunularin. The components responsible for the smell and taste of celery are butylphthalide and sedanolide (Milić et all.,2012).

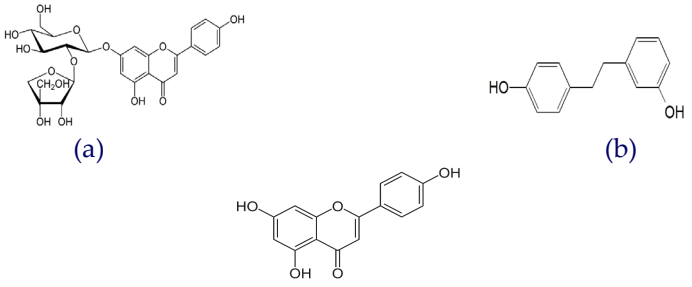


Figure 2. Chemical formula of (a) apin, (b) apigenin and (c) lunularin

It is consumed as a vegetable in many countries around the world. In Europe, hypocotyl is used as a root vegetable. The leaves have a very strong smell and taste, and they are used as herbs in dried form or fresh leaves are used as an addition to soups. In some states, celery is cultivated for its seeds. Celery seed yields a large yield of essential oils used in the perfume industry. Celery seeds are also used to prepare salt with celery, where dried seeds or celery leaves are ground and mixed with salt, (Džamić, 1984).

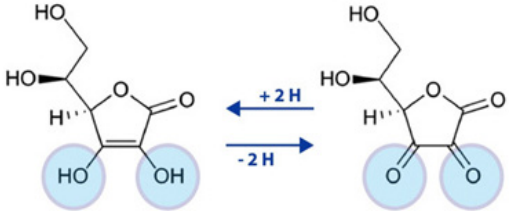
Materials and methods

Celery plant (Apium graveolens L.) was used as material in this final work. Extracts for analysis were obtained from the crushed leaf of the plant.
The moisture content is obtained from the difference in mass before and after drying the tested sample, (Damjanović, 2007).

Infusions are aqueous extractive solutions obtained by pouring boiling water over the plant. They are obtained from plants with a more delicate structure or from plants that contain thermolabile, i.e. easily volatile, active principles.

Maceration is a one-time extraction of a crushed plant sample, and it is performed with a suitable solvent at room temperature. In the preparation of the macerate, a cold solvent is used, which reduces the decomposition of active substances, (Aćamović and Cvijović 2009).

Ultrasonic extraction does not require high temperatures, sometimes using smaller amounts of extractants. This type of extraction allows the penetration of the solvent into the intact cells, which increases the yield in a shorter extraction time. Ultrasonic extraction is performed in an ultrasonic water bath, (Greathead, 2003). For the purposes of this work, the density of extracts obtained by maceration, infusion preparation and ultrasonic extraction was read. The hydrometer is immersed in the tested solution, whereby it sinks more or less depending on the density of the liquid. If the density of the tested solution is higher, the hydrometer sinks less and vice versa. The reading is done by aligning the division on the scale with the liquid level in the container, (Piletić and Miletić, 1989). Quantitative determination of total vitamin C is based on the reversible ability of the oxidation-reduction system ascorbic-dehydroascorbic acid, (Šiler, 2009).



a) b)

Figure 3. a) L-ascorbic acid and b) L-dehydroascorbic acid

For the quantitative determination of vitamin C, the Tillmans method is used, which is based on oxidometric titration during which L-ascorbic acid is oxidized to dehydroascorbic acid with simultaneous reduction of the applied reagent. Titration with 2,6-dichlorophenolindophenol, i.e. Tillmans reagent (TR) is performed in an acidic medium at pH 4-6, (Aćamović and Cvijović 2009).

The oxidized form of the Tillmans reagent solution (which also acts as an indicator) has a dark blue color (at pH 5.2), while in the presence of ascorbic acid, TR changes to its reduced form.

Ascorbic acid content (in mg/10 g extract) = ((V-Vsp) x c x 100)/ Val,

wherein:

V – mean value of TR solution volumes used for the titration of the test sample (ml),

Vsp – mean value of TR solution volumes used for blank titration (ml),

c – titer of TR solution (mg $C_6H_8O_6$/1 mlTP solution) i

Val – volume of an aliquot part of the sample (ml).

The determination of organic acids was carried out by the volumetric neutralization method, where the solution is titrated using a base solution (NaOH) of known concentration, in the presence of the phenolphthalein indicator. Free acidity is expressed in g/100 g of fresh sample through citric acid, which is dominant in the sample.

Free acidity (g/100 g) = (V x K x 100)/G, where:

V – volume in cm^3 of used solution 0.1 mol/dm3 NaOH;

K – coefficient for conversion to a specific organic acid (amount of acid in grams corresponding to 1 cm^3 of 0.1 mol/dm^3 NaOH solution);

G – the amount of the tested sample in grams.

Results and discussion

Moisture content and percentage of dry matter in celery leaves. Analysis was performed in three trials. The mean value was calculated from the obtained values.

Table 1. Moisture content and percentage of dry matter

Mass before drying	Mass after drying	Percentage of dry matter
5.00	0.605	12.10
5.00	0.600	12.00
5.00	0.587	11.74

Table 2. Percentage yield and density of extracts

Celery leaf	Infusion	Macerate	Ultrasonic extraction
Percentage yield,%	8.41	12.15	19.5
Density, g/cm^3	0.37	0.42	0.59
Vitamin C, mg/100g	9.45	11.25	16.0
Content of organic acids,g/100g	0.0608		

The content of dry matter obtained after all three measurements was calculated as their mean value and is 11.95%, and the moisture content was obtained by subtracting the content of dry matter per 100 g of sample for each measurement individually and is expressed as their mean value. Moisture content is 88.05%. Based on the obtained results, it can be concluded that the lowest yield was obtained by the infusion preparation method, followed by maceration, and the highest yield was obtained by ultrasonic extraction, (Greathead, 2003). The results are fully justified considering that ultrasonic extraction is the fastest method and requires lower temperatures compared to maceration, while the preparation of infusion is a suboptimal method, as boiling water is used, which breaks down most of the active substances. Based on the results obtained by measuring the density with a hydrometer, it can be seen that the highest density obtained with ultrasonic extracts is 0.59 g/cm^3, which is correlated with the extraction yield. Based on the obtained results, it can be seen that the ultrasonic extraction method is the most optimal, because the yield was the highest, (Yang, 2010). After the analysis, it was determined that the content of total acids in the sample is 0.0608%, i.e. that 100 g of the sample contains 0.0608 g of organic acids.

Conclusion

After the tests were completed, it was determined that the highest content of vitamin S determined by the ultrasonic extraction method was 16 mg/100 g.

The largest amount of this vitamin was isolated using the ultrasonic extraction method because the temperature in the bath was 40°C, which is lower than the temperature of its decomposition (50-60°C). Boiling water was used for the infusion, and the maceration lasted for 5 days, which probably led to some losses, even though the solvent was at room temperature. When taking into account the fact that the ultrasonic extraction method itself lasts only 30 minutes (the shortest of these three methods) and that the operating temperature is 40°C (lower than the decomposition temperature of vitamin C), it is concluded that the vitamin was quickly extracted and preserved until decomposition.

Acknowledgement

This work was supported by the Ministry of Education, Science and Technological Development Republic of Serbia, project ref, number 451-03-47/2023-01/200088.

References

Aćamović-Đoković G., Cvijović M. (2009). Praktikum iz Organske hemije, Agronomski fakultet, Čačak.

Damjanović B.(2007).Ekstrakcija biakativnih komponenti, Metalurško tehnološki fakultet, Podgorica.

Greathead H. (2003).Plants and plant extracts for improving animal productivity. Proc. Nutr. Soc., 62, 279-290.

Džamić M. (1984). Biohemija, Beograd.

Lajšić S., Grujić-Injac B. (1998). Hemija prirodnih proizvoda, Tehnološki fakultet, Novi Sad.

Lakušić R. (1990). Planinske biljke, Beograd.

Lampe, J.W. (1999). Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies, Am. J. Chin. Nutr., Vol. 70, 475- 490.

MacVicar J. (2006). Ljekovito i začinsko bilje , Naklada Uliks, Rijeka.

Milić J., Primorac M., Savić S. (2012). Farmaceutska tehnologija I, Farmaceutski fakultet, Beograd.

Piletić V. M., Miletić LJ. B. (1989). Organska hemija, Novi Sad, Tehnološki fakultet.

Šiler-Marinković S. (2009). Vitamini, Beograd, Tehnološko-metalurški fakultet.

Yang Y. (2010), Phenolic composition and antioxidant activities of 11 celery cultivars. Journal of Food Science 75(1),C9-C13.