

THE EFFECTS OF SOLUTION TYPE TEMPERATURE AND TIME ON ANTIOXIDANT CAPACITY OF OSMOTICALLY DRIED CELERY LEAVES

by

**Milica R. NIČETIN^{a*}, Lato L. PEZO^b, Vladimir S. FILIPOVIĆ^a,
Biljana Lj. LONČAR^a, Jelena S. FILIPOVIĆ^c, Danijela Z. ŠUPUT^a,
and Violeta M. KNEŽEVIĆ^a**

^a Faculty of Technology, University of Novi Sad, Novi Sad, Serbia

^b Institute of General and Physical Chemistry, University of Belgrade, Beograd, Serbia

^c Institute for Food Technology, University of Novi Sad, Novi Sad, Serbia

Original scientific paper

<https://doi.org/10.2298/TSCI191101184N>

Osmotic drying of celery leaves was studied in two osmotic solutions (ternary aqueous solution and sugar beet molasses), at three temperatures (20, 35, and 50 °C), and diverse immersion periods (1, 3, and 5 hours). The aim was to examine the influence of the used hypertonic agent, temperature and immersion time on antioxidant capacity (AOC) and color characteristics of the samples. The AOC of celery leaves was assessed by the spectrophotometric assays (ABTS, FRAP, and DPPH), as well as two direct current polarographic assays, hydroxo perhydroxo mercury (II) complex assay based on the decrease of anodic current of hydroxo perhydroxo mercury (II) complex and mercury reduction antioxidant power assay based on the decrease of a cathodic current of Hg (II) reduction. Total phenolic content was determined by Folin-Ciocalteu assay. The relative antioxidant capacity index, calculated by assigning equal weight to all applied assays was used to achieve a more comprehensive comparison between analyzed samples, as well as applied assays. The obtained results indicated decreases in the AOC of celery leaves during the osmotic treatment in ternary solution, while the AOC was increased in sugar beet molasses solution. According to relative antioxidant capacity index evaluation the most convenient process parameters were temperature of 35 °C and immersion time of 5 hours.

Key words: osmotic treatment, celery leaves, antioxidant capacity, optimization sugar beet molasses

Introduction

As a rich source of phenolic compounds, with strong AOC, vegetables and herbs could possibly supplant the manufactured antioxidant [1]. Celery (*Apium graveolens*) is widely perceived as a medicinal plant and spice, due to its numerous health benefits, such as lowering cholesterol level, prevention of cardiovascular disease, anti-inflammatory, antimicrobial, and anticancer activity [2, 3]. Celery leaves are a good source of minerals, vitamins and pigments, phenols [4]. Many studies have demonstrated that the therapeutic value of celery is related to antioxidants present [5]. Phenolic acids (caffeic, *p*-coumaric and ferulic acid) and flavonoids (apigenin, luteolin, and kaempferol) present in celery leaves possess strong antioxidant ac-

* Corresponding author, e-mail: milican85@live.com

tivities and thereby a possibility to prevent degenerative diseases caused by oxidative stress [4, 6]. Likewise, celery phenolics hinder lipid oxidation, one of the major causes of chemical disintegration of food products, and inhibit various types of oxidizing enzymes. The addition of a natural preservative as celery in the food system, diminish the requirement for synthetic antioxidants and possible health risk related to their use [7, 8].

Various dehydration treatments were applied to preserve this delicate and very perishable vegetable [9]. Traditional drying procedures may be inappropriate, due to the texture degradation, color alteration and nutritional loss [10]. Interestingly, during osmotic drying, which involves soaking a food, in a hypertonic solution, under encompassing or mild modified environment conditions foodstuff is not exposed to high temperatures. Initial nutritional value, functional properties and sensory characteristic of osmotically dried food are minimally changed or improved. Furthermore, osmotic drying is environmentally adequate and energy efficient process [11,12]. The driving force for water evacuation is the concentration gradient between the submerged plant material and the encompassing hypertonic solution [13]. The complex cellular structure of plant tissue acts as a semi-porous membrane, which permits two main counter-current flows: water outflow from the plant tissue into the osmotic solution and the synchronous relocation of solids from solution the tissue [14].

The selection of hypertonic solution relies on the expected water loss and solid gain, and also on the desired nutritional and sensory properties of the final food product. Concentrated sucrose solution, sodium chloride solutions and their combinations are usually used as hypertonic solutions [15, 16]. Recent examinations reported that sugar beet molasses is a very powerful osmotic medium for dehydration of fruits, vegetables and meat. Molasses, the thick, dim syrup is obtained as a byproduct of sugar refining, comprises of fermentable carbohydrates (sucrose, glucose, fructose) and several non-sugar organic materials (betaine and other amino acids; minerals, mainly K vitamins, especially of the B-group, *etc.*) [17, 18]. Few reports also confirmed that molasses is a very good source of residual antioxidant components from the sugar beet (mainly phenolic compounds) and of antioxidant molecules formed during the production process (colored products of Maillard reactions-melanoidins, and products of thermal or alkaline degradation of sugar). Some of specific bioactive components in sugar beet molasses such as syringic acid, ferulic acid, vanillin, hydroxybenzaldehyde, hydroxybenzoic acid, luteolin, kaempferol, caffeoyltartartic acid and feruloyl-arabinose-arabinose have been demonstrated to have high antioxidant properties, so contribute to anti-inflammatory and anti-tumor activities [19-23]. The color of any food product, depending upon the nature and content of hued substances present in food material, may be represented in terms of the CIELAB co-ordinates L^* , a^* , b^* framework [24]. Several scientists have suggested that melanoidins and some other colorants account for the strong antioxidant properties of some foods and beverages [25].

This paper investigates the effects of osmotic solution, immersion time and temperature, on the AOC, total phenolic content (TPC) and colors of celery leaves, to find the optimum conditions for the osmotic drying process. The main objective was to examine the possibility of enhancing antioxidant properties of treated material using sugar beet molasses as osmotic medium.

Materials and methods

Materials

The plant materials used in this examination were celery leaves, obtained from the nearby vegetable store, shortly before utilizing. Fresh celery leaves were cut into small pieces, of measurement around 1×1 cm.

Osmotic treatment

After measuring the initial mass, the samples of celery leaves were submerged in laboratory jars with prepared hypertonic solutions. Two different solutions were used as hypertonic solutions. The first one, S_1 , was aqueous ternary osmotic solution, made from sucrose (1200 g/kg water), NaCl (350 g/kg water) and distilled water. This solution was diluted with distilled water to concentrations of 60 w/w. The second osmotic medium, S_2 , was prepared using sugar beet molasses (obtained from the sugar factory Crvenka, Serbia, with initial dry matter content of 85.04 w/w), diluted to concentrations of 80 w/w. The material to solution ratio of 1 : 20 w/w was used in each experiment. The osmotic treatment process was performed at the three distinct temperatures 20 °C, 35 °C, and 50 °C. After each sampling time (1, 3, and 5 hours) celery leaves samples were taken out from solutions, S_1 and S_2 , lightly washed with distilled water, and gently blotted with paper to remove excessive water from the surface.

Preparation of celery leaves extracts

To prepare the extracts for antioxidant analysis, fresh and osmotically dehydrated celery leaves samples dried at 50 °C in a heat chamber (Instrumentaria Sutjeska, Croatia) until constant weight. Dried samples were finally grounded into a powder, using Universal laboratory mill type WZ-1 (Spolem, ZBPP, Bydgoszcz, Poland). The 2 g of powder for each sample, were extracted with 200 ml of boiled water. After extraction, at room temperature for 10 minutes, obtained aqueous extracts were filtered using Whatmann No. 1 filter paper. The extracts were stored in a refrigerator (4 °C) until further use.

Chemicals for determination of antioxidant capacity

Folin-Ciocalteu reagent, sodium carbonate, sodium acetate trihydrate, acetic acid, hydrochloric acid, (potassium chloride and sodium hydroxide were of analytical grade, acquired from Merck, Darmstadt, Germany). The DPPH was produced by Fluka (Buchs, Switzerland), methanol was obtained from Baker (Deventer, Netherlands). Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), TPTZ (2,4,6-tripyridyl-S-triazine), ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt) as well as gallic acid was purchased from Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany). Hydrogen peroxide 35% (v/v) solution was the medical grade (Belinka, Slovenia). Ethanol 96% (v/v) was obtained from Ada Vrenje (Belgrade, Serbia). Working standard solutions (2.0 mM) were prepared daily in ethanol or water.

Determination of total phenolic content

The content of phenols in the extracts was determined spectrophotometrically according to a modified method described by Gorjanović *et al.* [26] with Folin-Ciocalteu's reagent. Briefly, 0.5 mL of the sample was added into a 50 mL volumetric flask containing 2.5 mL of Folin-Ciocalteu's reagent, 30 mL of distilled water, and 7.5 mL of 20% Na_2CO_3 and filled to the mark with distilled water. After 2 hours, the absorbance of blue coloration was measured at 765 nm against a blank sample. Gallic acid was used as the standard, and the results are expressed as milligrams per litre of gallic acid equivalents (GAE).

Antiradical activity determination by DPPH assay

The antiradical activity of samples against DPPH radical was measured by the method of Brand-Williams *et al.* [27], with some modifications. Briefly, 100 μL of the extract was added to 1.9 mL of 0.094 mM DPPH in methanol up to 2 mL. The free radical scavenging capacity

of the sample was evaluated by measuring the absorbance after 30 min at 517 nm. The AOC was expressed as millimoles per litre Trolox equivalents, using the calibration curve of Trolox (0-1000 μM), a water-soluble vitamin E analogue.

Determination of total reducing power

The FRAP assay was carried out according to the standard procedure previously described [28] with some modification introduced. The FRAP reagent was prepared by mixing acetic buffer, TPTZ, and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM water solution) at a ratio of 10 : 1 : 1. Briefly, to a volume of 950 μL of FRAP reagent was added 50 μL of the extract. After 4 minutes, the absorbance of blue coloration was measured against a blank sample. Aqueous solutions of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (100-1000 μM) were used for the calibration, and the results are expressed as millimoles per litre Fe (II).

Antiradical activity determination by ABTS assay

The Trolox equivalent antioxidant capacity (TEAC) was evaluated by the ABTS radical cation decolorization assay [29]. Stock solutions of ABTS (7 mM) and potassium peroxodisulfate (140 mM) in water were prepared and mixed together to a final concentration of 2.45 mM potassium peroxodisulfate. The mixture was left to react overnight (12-16 hours) in the dark, at room temperature. On the day of analysis, the ABTS radical solution was diluted with ethanol to an absorbance of 0.70 (± 0.02) at 734 nm. All measurements were performed 20 μL of the extract was added to 2.0 mL of the ABTS radical solution, and the absorbance readings were taken after exactly 6 min against the appropriate reagent blank of 20 μL of ethanol instead of the sample. The outcomes obtained from triplicate readings were expressed as Trolox equivalents and derived from a calibration curve determined from this standard (100-1000 μM).

The DC polarographic measurements of AO capacity

Recently developed DC polarographic antioxidant assay, based on the diminishing of anodic current of HydroxoPerhydroxoMercury (II) complex (HPMC) formation in alkaline solutions of hydrogen peroxide at potential of mercury oxidation, [30] and additionally a novel antioxidant test based on the decrease of DC polarographic cathodic restricting current, i_c , of mercury (II) reduction in the presence of antioxidants, *i.e.* mercury reduction antioxidant power (MRAP), were applied in parallel. A reduction of anodic, as well as cathodic i_c , noticed upon gradual addition of antioxidants has been plotted against their volume, v . The slopes of the starting linear part of obtained plots, *i.e.* percentage of i_c decrease per v of samples added, were used as a measure of antioxidant capacity. Polarographic i-E curves were obtained using the Princeton Applied Research 174 Polarographic analyzer and recorded on Houston Instrument Omnigraphic 2000 X-Y recorder. The dropping time of working dropping mercury electrode (with capillary characteristics of $m = 2.5 \text{ mgs}^{-1}$ at the mercury reservoir height of 75 cm) was programmed on time 1 second, while current oscillations were damped with low pass filter of instrument set at 3 seconds. Saturated calomel electrode (SCE) was used as reference and platinum foil as auxiliary electrode. The initial potentials for the both HPMC and MRAP assays were 0.10 V vs. SCE. Potential scan rate was 10 mV/s. Clark and Lubs buffer of pH 9.8, used as supporting electrolyte, and starting solution for HPMC assay were obtained as depicted already by Gorjanović *et al.* [26].

Color co-ordinates

Color attributes of samples were measured instrumentally using a Chroma meter (CR-400, Konica, Minolta, Tokyo, Japan) tri-stimulus colorimeter. The results were expressed in terms of L^* – brightness, from 0 (black) to 100 (white), a^* : greenness/redness, from $-a^*$ (green) to $+a^*$ (red), b^* : blueness/yellowness, from $-b^*$ (blue) to $+b^*$ (yellow). The measurements were observed under constant lighting conditions, at 28 °C, using a white control ($L^* = 98.76$, $a^* = -0.04$, $b^* = 2.01$) [31].

Statistical analysis

All observed samples were checked for variance equality (using Levene’s test) and normal distribution (using Shapiro-Wilk’s test). The exploratory results were presented by means and standard deviations for every treatment. All determinations were performed in triplicate. Collected data were subjected to ANOVA to investigate the impacts of process variables. Principal component analysis (PCA) was applied successfully to characterize and separate the distinctive samples into groups.

The experimental data used for the analysis were obtained using the full factorial (3 level-2 parameter) design, with 2 blocks: time X_1 (1, 3, and 5 hours), temperature X_2 (20, 35, and 50 °C).

The second order polynomial (SOP) model was fitted to the experimental data. Ten models were developed to relate ten responses [12]. The SOP equations describe the effects of factors on the observed responses, test variable interrelationships and represent the combined effect of all factors on the observed responses

The following SOP model was fitted to the experimental data, for each of the different osmotic treatments (OT):

$$Y_k^l = \beta_{k0}^l + \sum_{i=1}^2 \beta_{ki}^l X_i + \sum_{i=1}^2 \beta_{kii}^l X_i^2 + \beta_{k12}^l X_1 X_2, \quad k = 1-8, \quad l = 1-2 \quad (1)$$

where β_{k0}^l , β_{ki}^l , β_{kii}^l , and β_{k12}^l are constant regression coefficients, y_k^l , either: FRAP, ABTS, DPPH, HPMC, MRAP, TPC, L^* , a^* , b^* , and ΔE^* while X_1 is time and X_2 is temperature. A model describing OT in S_1 solution is marked with $l = 1$, while treatment in S_2 is marked with $l = 2$.

The evaluation of ANOVA and PCA of the obtained results was performed using Statistica software version 12 (StatSoft Inc. 2012, USA)® [32].

Results and discussion

According to the outcomes appeared in the tab. 1, the prevalence of sugar beet molasses as an osmotic solution over the ternary solution has been affirmed. It can be seen, that the permanent increase of AOC occurs during the OT process in S_2 solution. In contrast, the lessening of AOC is evident in the OT process in S_1 solution.

The ascent of t and T leads to an increase of AOC in samples dried using S_2 solution and the decrease of those dried in S_1 solution, as evidenced by all applied antioxidant assays. At the highest t (5 hours) and T (50 °C), the maximum increases of AOC values were obtained for S_2 solution: from initial 1.424-1.447 for FRAP assay, from 0.980-1.060 for ABTS assay, from 0.141-0.439 for DPPH assay, from 0.046-0.051 for HPMC assay, from 0.019-0.023 for MRAP assay and from 0.158-0.169 for TPC. On the same conditions, for solution S_1 , the lowest AOC values were obtained: 1.358 for FRAP assay, 0.948 for ABTS assay, 0.390 for DPPH assay, 0.040 for HPMC assay, 0.017 for MRAP assay, and 0.149 for TPC.

Table 1. The AOC and color attributes during OT of celery leaves with ternary and sugar beet molasses solutions

<i>t</i>	<i>T</i>	FRAP	ABTS	DPPH	HPMC	MRAP	TPC	RACI	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	ΔE^*
[h]	[°C]	[mM] Fe(II)	[mM] Trolox	[mM] Trolox	[% ml]	[% ml]	[mgGAEL ⁻¹]					
0	–	1.424 ^{defg}	0.980 ^e	0.414 ^{def}	0.046 ^d	0.019 ^d	0.158 ^{fg}	0.025	16.619 ^f	–8.675 ⁱ	15.018 ^a	24.020 ^c
Ternary solution, <i>S</i> ₁												
1	20	1.401 ^{bcd}	0.979 ^e	0.407 ^{cde}	0.042 ^c	0.017 ^b	0.154 ^{de}	–0.411	17.558 ^g	–9.011 ^h	15.791 ^b	25.275 ^d
1	35	1.424 ^{defg}	0.965 ^{cde}	0.405 ^{cd}	0.042 ^c	0.017 ^b	0.156 ^{ef}	–0.487	18.522 ^h	–9.672 ^g	16.867 ^c	26.853 ^e
1	50	1.427 ^{defgh}	0.955 ^{bcd}	0.404 ^{bc}	0.042 ^c	0.018 ^c	0.153 ^{bcd}	–0.588	18.713 ^h	–10.234 ^f	17.607 ^{de}	27.657 ^f
3	20	1.414 ^{cdef}	0.971 ^{de}	0.394 ^{ab}	0.041 ^b	0.018 ^{bc}	0.154 ^{def}	–0.675	18.420 ^h	–10.159 ^f	16.028 ^b	26.446 ^e
3	35	1.374 ^{ab}	0.944 ^{abc}	0.390 ^a	0.041 ^b	0.017 ^b	0.152 ^{bcd}	–0.991	19.180 ⁱ	–10.472 ^e	16.789 ^c	27.557 ^f
3	50	1.391 ^{abcd}	0.929 ^a	0.386 ^a	0.039 ^{ab}	0.017 ^a	0.147 ^a	–1.170	20.376 ⁱ	–11.234	17.967 ^e	29.397 ^g
5	20	1.383 ^{abc}	0.936 ^{ab}	0.389 ^a	0.039 ^a	0.017 ^a	0.153 ^{cde}	–1.108	19.360	–10.686 ^d	16.525 ^c	27.605 ^f
5	35	1.358 ^a	0.948 ^{abc}	0.390 ^a	0.039 ^a	0.017 ^a	0.150 ^{abc}	–1.336	20.218 ⁱ	–11.599 ^b	17.442 ^d	29.112 ^g
5	50	1.358 ^a	0.948 ^{abc}	0.390 ^a	0.040 ^{ab}	0.017 ^a	0.149 ^{ab}	–1.418	21.362 ^k	–12.410 ^a	17.954 ^e	30.539 ^h
Sugar beet molasses solution, <i>S</i> ₂												
1	20	1.432 ^{efghi}	1.004 ^f	0.4166 ^{ef}	0.046 ^d	0.020 ^f	0.162 ^{hi}	0.400	14.869 ^e	–7.229 ^j	16.685 ^c	23.489 ^b
1	35	1.444 ^{fghij}	1.018 ^{fg}	0.415 ^{def}	0.048 ^e	0.020 ^e	0.161 ^{gh}	0.475	12.219 ^d	–5.642 ⁱ	17.406 ^d	22.002 ^a
1	50	1.455 ^{ghij}	1.026 ^{fg}	0.424 ^{fg}	0.049 ^{ef}	0.020 ^f	0.163 ^{hij}	0.742	10.625 ^c	–4.648 ^m	18.892 ^f	22.167 ^a
3	20	1.438 ^{fghi}	1.029 ^g	0.425 ^{fg}	0.048 ^e	0.021 ^{fg}	0.164 ^{hij}	0.655	12.048 ^d	–6.844 ^k	17.712 ^{de}	22.487 ^a
3	35	1.456 ^{ghij}	1.014 ^{fg}	0.431 ^{gh}	0.049 ^{efg}	0.021 ^h	0.163 ^{hij}	0.912	10.128 ^b	–5.673 ^l	18.804 ^f	22.098 ^a
3	50	1.467 ^{ji}	1.027 ^g	0.434 ^{gh}	0.050 ^{gh}	0.023 ^j	0.166 ^{jk}	1.234	9.045 ^a	–4.115 ⁿ	19.870 ^g	22.216 ^a
5	20	1.459 ^{ghij}	1.013 ^{fg}	0.418 ^f	0.049 ^{fg}	0.021 ^h	0.165 ^{ji}	0.847	10.836 ^c	–5.846 ^l	18.463 ^f	22.191 ^a
5	35	1.463 ^{hij}	1.023 ^{fg}	0.440 ^h	0.050 ^{fg}	0.022 ⁱ	0.169 ^k	1.289	9.028 ^a	–4.319 ⁿ	19.614 ^g	22.019 ^a
5	50	1.477 ^j	1.060 ^h	0.439 ^h	0.051 ^h	0.023 ^j	0.169 ^k	1.605	8.850 ^a	–4.186 ⁿ	20.622 ^h	22.827 ^a

^{a-m} Different letters written in superscript within the same column in the table show significantly different means of observed data (at $p < 0.05$ level). $n = 3$

The relative antioxidant capacity index (RACI) was calculated, with a specific goal to accomplish a more comprehensive examination between analyzed samples, as well as applied assays. The RACI additionally demonstrates an increasing in AOC of samples treated with *S*₂ solution (the positive and increasing RACI scores during OT were obtained), while the samples treated with *S*₁ solution gained negative and decreasing RACI scores during OT. Likewise, with the increase in *t* and *T*, RACI values were higher for *S*₂, but lower for *S*₁.

Clearly, the utilization of sugar beet molasses was accounted for upgrade in FRAP, ABTS, DPPH, HPMC, MRAP, and TPC values. This is consistent with the claim that sugar beet molasses possess strong antioxidant potential, confirmed by Chen *et al.* [21] and Zheng *et al.* [25]. Sugar beet molasses is a rich source of antioxidants [23], and presumably some of them diffuse into the tissue of celery leaves during OT, contributing to the overall AOC of treated leaves.

Table 2. Pearson correlation between TPC, DPPH, FRAP, ABTS, HPMC, and MRAP, RACI and color parameters for osmotic treated celery leaves

	ABTS	DPPH	HPMC	MRAP	TPC	RACI	L^*	a^*	b^*	ΔE^*
Ternary solution, S_1										
FRAP	0.670*	0.710*	0.801 ⁺	0.665**	0.658**	0.879 ⁺	-0.734*	0.748*	-0.331	-0.649
ABTS		0.700*	0.745*	0.864 ⁺	0.938 ⁺	0.899 ⁺	-0.926 ⁺	0.955 ⁺	-0.820 ⁺	-0.944 ⁺
DPPH			0.930 ⁺	0.767*	0.649**	0.877 ⁺	-0.756*	0.766*	-0.408	-0.686*
HPMC				0.848 ⁺	0.709*	0.930 ⁺	-0.789 ⁺	0.809 ⁺	-0.388	-0.708*
MRAP					0.879 ⁺	0.903 ⁺	-0.907 ⁺	0.871 ⁺	-0.719 ⁺	-0.883 ⁺
TPC						0.881 ⁺	-0.983 ⁺	0.973 ⁺	-0.870 ⁺	-0.992 ⁺
RACI							-0.935 ⁺	0.944 ⁺	-0.624**	-0.886 ⁺
L^*								-0.981 ⁺	0.816 ⁺	0.986 ⁺
a^*									-0.766*	-0.966 ⁺
b^*										0.898 ⁺
Sugar beet molasses solution, S_2										
FRAP	0.899 ⁺	0.768 ⁺	0.966 ⁺	0.840 ⁺	0.804 ⁺	0.932 ⁺	-0.928 ⁺	0.892 ⁺	0.968 ⁺	-0.338
ABTS		0.739*	0.906 ⁺	0.816 ⁺	0.832 ⁺	0.918 ⁺	-0.777 ⁺	0.703*	0.882 ⁺	-0.062
DPPH			0.818 ⁺	0.883 ⁺	0.857 ⁺	0.916 ⁺	-0.842 ⁺	0.727*	0.880 ⁺	-0.198
HPMC				0.830 ⁺	0.807 ⁺	0.942 ⁺	-0.959 ⁺	0.890 ⁺	0.969 ⁺	-0.414
MRAP					0.918 ⁺	0.952 ⁺	-0.780*	0.698*	0.881 ⁺	-0.003
TPC						0.937 ⁺	-0.746*	0.667**	0.843 ⁺	-0.006
RACI							-0.892 ⁺	0.808 ⁺	0.964 ⁺	-0.167
L^*								-0.893 ⁺	-0.953 ⁺	0.574
a^*									0.902 ⁺	-0.478
b^*										-0.313

* Correlation statistically significant at $p < 0.01$ level; * Correlation statistically significant at $p < 0.05$ level;
 ** Correlation statistically significant at $p < 0.10$ level; Unmarked correlations are statistically insignificant

Statistically different means in color co-ordinates (L^* , a^* , b^* , and ΔE^*) are evident, contingent upon the sort of used solution, from tab. 1. The data herein recommends that ternary solution generally contributes to the expansion of the lightness value L^* for all samples, corresponding to the increase in temperature and submersion time. The highest augment of L^* value (21.362 from initial 16.619) was noticed after the maximum process parameters, 5 hours and 50 °C. Also, the use of S_1 induces an augment in yellow color parameter, b^* , while the decrement in the green color co-ordinate, a^* , is indicative. The share of a^* parameter is found to have statistically significant difference among all samples. These results indicate that the molasses is responsible for getting darker and more reddish samples. The impact of molasses on darkening is most pronounced after 5 hours of drying, at the 50 °C, when the value of the L^* parameter for celery leaves is reduced to 8.850 compared to the initial value of 16.619. The increasing of b^* parameter is greater during OT with molasses than with ternary solution, from 15.018 to a maximum of 20.622 for molasses and 17.954 for ternary solution. These data confirm that

occurred the diffusion of colored substances (melanoidins and pigments) from molasses into the dehydrated samples during the process. The penetration of color substances is more obvious with the augment of temperature and duration of OT. Obviously, the darker samples with lower L^* values, and greater a^* , and b^* values have higher AOC values, which confirms the fact that some colorants in molasses possess antioxidant potential. According to the report by Filipčev *et al.* [19], melanoidins may be responsible for the strong antioxidant properties of some foods. Good agreement between results of spectrophotometric and polarographic AOC assays as well as color co-ordinates have been confirmed by regression analysis. Pearson correlations between all applied assays on osmotic treated celery leaves are given in tab. 2. The positive correlations between FRAP, HPMC, and RACI assays of samples treated in S_1 solution are obtained, statistically significant at $p < 0.01$ level. The ABTS assay is positively correlated to MRAP, TPC, and RACI assays of samples treated in S_1 solution, as well as to a^* color co-ordinate, and also negatively correlated to L^* and b^* color co-ordinates, statistically significant at $p < 0.01$ level. The DPPH is positively correlated to HPMC and RACI assays, $p < 0.01$ level. The MRAP assay is positively correlated to TPC and RACI, and to a^* color co-ordinate for S_1 solution and negatively correlated to L^* and b^* color co-ordinates, statistically significant at $p < 0.01$ level.

Statistically significant positive correlations (at $p < 0.01$ level) for samples treated in S_2 solution are observed between FRAP assay and ABTS, DPPH, HPMC, MRAP, and TPC assays, RACI, a^* , and b^* color co-ordinates. The FRAP assay is also negatively correlated to L^* color co-ordinate ($p < 0.01$). The ABTS assay is positively correlated to HPMC, MRAP, and TPC assays, RACI, as well as to RACI and b^* color co-ordinate, while the correlation L^* color co-ordinate is negative, statistically significant at $p < 0.01$ level. Strong correlations between DPPH, HPMC, MRAP, and TPC assays, as well as RACI are observed, statistically significant at $p < 0.01$ level. The L^* color co-ordinate is negatively correlated to DPPH and HPMC assays and to a^* and b^* co-ordinates, while b^* color co-ordinate is positively correlated to HPMC, MRAP, TPC and b^* , $p < 0.01$ level.

The TPC is highly correlated with all other applied antioxidant assays, indicating a close relationship between the content of total phenolic compounds and antioxidant capacity of celery leaves. The extent of AOC of treated celery leaves is in accordance with the amount of phenolics present in that leaves. Therefore, total phenolic compounds contribute to the overall AOC, which is consistent with studies by Kim and Choe [1] and Alagarsamy *et al.* [29].

Response surface methodology

The ANOVA showed the significant effects of independent variables to the responses, and to show which of responses were significantly affected by the varying treatment combinations, tab. 3. The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models.

The linear term of solution type (ST) was very important in all SOP models, statistically significant at $p < 0.01$ level. The linear term of t was influential in SOP model for MRAP assay and a^* color co-ordinate calculation ($p < 0.05$). The linear term of T was important in HPMC, a^* and b^* color co-ordinates prediction, statistically significant at $p < 0.01$ level, and also for the prediction of L^* color co-ordinate ($p < 0.05$). The non-linear terms $ST \times t$ and $ST \times T$ were very influential in SOP model for calculation of ABTS, HPMC, MRAP, and TPC assays, and L^* and a^* color co-ordinates, statistically significant at $p < 0.01$ level. The non-linear term $ST \times t$ was influential for the calculation of FRAP and DPPH assays and b^* color co-ordinate (at $p < 0.01$ level). The non-linear term of $ST \times T$ was influential in FRAP and DPPH calculation, while the non-linear term of $t \times T$ was influential for the MRAP and L^* calculation, statistically significant at $p < 0.05$ level.

Table 3. The ANOVA calculation for celery leaves OT in S_1 and S_2 solution

	D_f	FRAP	ABTS	DPPH	HPMC	MRAP	TPC	L^*	a^*	b^*	ΔE^*
ST	1	$1.75 \cdot 10^{-2+}$	$2.27 \cdot 10^{-2+}$	$4.60 \cdot 10^{-3+}$	$2.96 \cdot 10^{-4+}$	$7.13 \cdot 10^{-5+}$	$7.19 \cdot 10^{-4+}$	321.404 ⁺	122.594 ⁺	12.663 ⁺	133.090 ⁺
t	1	$6.19 \cdot 10^{-4**}$	$2.71 \cdot 10^{-6}$	$1.20 \cdot 10^{-6}$	$3.65 \cdot 10^{-7}$	$5.46 \cdot 10^{-7*}$	$8.79 \cdot 10^{-7}$	0.677 ^{**}	0.568 [*]	4.528 ⁺	3.910 ⁺
t^2	1	$3.19 \cdot 10^{-8}$	$1.54 \cdot 10^{-6}$	$9.79 \cdot 10^{-6}$	$1.28 \cdot 10^{-7}$	$2.13 \cdot 10^{-7}$	$1.11 \cdot 10^{-11}$	0.393	0.063	0.006	0.049
T	1	$1.93 \cdot 10^{-4}$	$6.79 \cdot 10^{-6}$	$6.11 \cdot 10^{-5}$	$3.06 \cdot 10^{-6+}$	$4.11 \cdot 10^{-7}$	$2.90 \cdot 10^{-6}$	1.414 [*]	0.724 ⁺	11.423 ⁺	4.453 ⁺
T^2	1	$1.06 \cdot 10^{-4}$	$2.17 \cdot 10^{-6}$	$6.84 \cdot 10^{-6}$	$2.67 \cdot 10^{-8}$	$1.06 \cdot 10^{-7}$	$5.40 \cdot 10^{-7}$	0.335	0.095	0.002	0.252
$ST \times t$	1	$4.08 \cdot 10^{-3+}$	$2.26 \cdot 10^{-3+}$	$6.44 \cdot 10^{-4+}$	$2.02 \cdot 10^{-5+}$	$5.57 \cdot 10^{-6+}$	$7.66 \cdot 10^{-5+}$	19.116 ⁺	6.667 ⁺	1.373 ⁺	5.457 ⁺
$ST \times T$	1	$7.00 \cdot 10^{-4*}$	$1.48 \cdot 10^{-3+}$	$1.84 \cdot 10^{-4*}$	$4.92 \cdot 10^{-6+}$	$2.47 \cdot 10^{-6+}$	$3.77 \cdot 10^{-5+}$	17.150 ⁺	10.068 ⁺	0.149 ^{**}	7.090 ⁺
$t \times T$	1	$3.93 \cdot 10^{-4}$	$1.35 \cdot 10^{-4**}$	$3.16 \cdot 10^{-5}$	$9.91 \cdot 10^{-11}$	$7.28 \cdot 10^{-7*}$	$7.29 \cdot 10^{-7}$	1.205 [*]	0.253 ^{**}	0.024	0.788 [*]
Error	9	$1.10 \cdot 10^{-3}$	$2.66 \cdot 10^{-4}$	$2.75 \cdot 10^{-4}$	$1.67 \cdot 10^{-6}$	$1.27 \cdot 10^{-6}$	$1.16 \cdot 10^{-5}$	1.347	0.594	0.304	0.893
r^2		0.955	0.990	0.953	0.995	0.985	0.986	0.996	0.996	0.990	0.994

⁺ Significant at $p < 0.01$ level, ^{*} Significant at $p < 0.05$, ^{**} Significant at $p < 0.10$, error terms have been found statistically insignificant, d_f - degrees of freedom, ST - solution type, t - time, T - temperature

The residual variance is also shown in tab. 3, where the lack of fit represents other contributions of the higher order terms. A significant lack of fit generally shows that the model failed to represent the data in the experimental domain at which points were not included in the regression [33]. All SOP models had an insignificant lack of fit tests, which means that all the models represented the data satisfactorily.

The coefficient of determination, r^2 , is defined as the ratio of the explained variation the total variation and is explained by its magnitude [34]. It is also the proportion of the variability in the response variable, which is accounted for by the regression analysis. A high r^2 is indicative that the variation was accounted and that the data fitted satisfactorily to the proposed model. The r^2 values for observed variables, both AOC assays and color co-ordinates, were very good and show the good fit of the model to experimental results.

Principal component analysis

The PCA is a mathematical technique utilized as a central tool in exploratory data investigation [35]. It is a multivariate procedure in which the data are transformed into orthogonal components that are linear combinations of the original variables. The PCA is performed by eigenvalue decomposition of a data correlation matrix [36]. This analysis is used to accomplish most extreme partition among clusters (groups) of parameters [37]. This approach, providing spatial relationship between processing parameters, empowered a separation between the different samples in both solutions, S_1 and S_2 .

The PCA, applied to the given data set, tab. 1, has demonstrated a separation between the samples according to the observed process parameters and is used as a tool in exploratory data analysis to characterize and differentiate the observed samples. The neat separation of the observed samples was gained, according to used assays. Quality results show that the first two principal components, accounting for 91.22% of the total variability for solution S_1 and S_2 , can be considered sufficient for data representation. Considering the map of the PCA performed on the data, the negative scores to first principle component calculation was observed by AOC assays (which contributed indistinguishably, somewhere around 10.0% and 10.7% of total variance, based on correlations), TPC (10.6%), RACI (10.9%), and color co-ordinates L^* and a^*

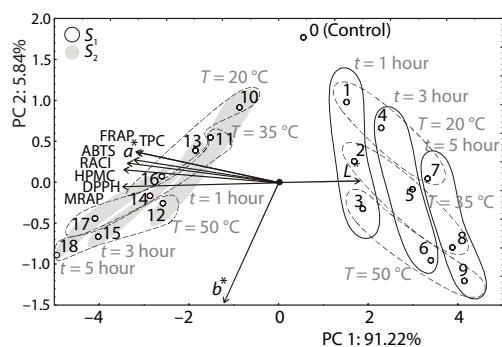


Figure 1. Biplot graphic of celery leaves OT in sugar beet molasses solution and ternary solution

graphic, indicating the increased FRAP, ABTS, DPPH, HPMC, MRAP, and TPC values, contrasted with the control sample, and also to the samples treated in ternary solution. According to fig. 1, samples treated in ternary solution are being brighter throughout the OT, while the samples treated in sugar beet molasses are getting more reddish throughout the process.

Conclusions

The calculated RACI values indicate the pattern of the rising antioxidant activity for samples dried in sugar beet molasses, at higher temperatures and the longer immersion time, while the negative RACI values show the decreasing trend in ternary solution. This discovering support sugar beet molasses as a potential source of antioxidants, which can diffuse during the osmotic drying and enhance the functionality of the final product. It can be presumed that celery leaves osmotically treated in molasses, with extended shelf-life and enhanced antioxidant properties are suitable as food additive or functional food ingredient. In this light, possibility of embedding the sugar beet molasses as a natural antioxidant ingredient in various food formulations, can be considered.

The RSM was used to improve the osmotic drying process, utilizing antioxidant capacity and color attributes of the samples, as the responses, and the solution type, temperature and immersion time as process parameters. Mathematical models for all responses were statistically significant. The chemometric analyses pointed out the beneficial contribution of sugar beet molasses as hypertonic solution. Besides, the use of sugar beet molasses as osmotic agent is economically and environmentally reasonable, because it is a side product of the sugar industry.

Acknowledgment

The Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 451-03-68/2020-14/ 200134

References

- [1] Kim, J., Choe, E., Effects of Selected Herb Extracts on Iron-Catalyzed Lipid Oxidation in Soybean Oil-in-Water Emulsion, *Food Science and Biotechnology*, 25 (2016), 4, pp. 1017-1022
- [2] Al-Asmari, A. K., *et al.*, An Updated Phytopharmacological Review on Medicinal Plant of Arab Region: *Apium Graveolens* Linn, *Pharmacognosy Review*, 11 (2017), 21, pp. 13-18
- [3] Li, M. J., *et al.*, Advances in the Research of Celery, an Important Apiaceae Vegetable Crop, *Critical Reviews in Biotechnology*, 38 (2018), 2, pp. 172-183

(10.7%). The second principal component was influenced adversely by color co-ordinate b^* (92.1%, based on correlations).

The PCA graphically demonstrated very good discrimination between S_1 and S_2 solutions. The impact of processing parameters can be seen in fig. 1, with samples processed with lower immersing time nearer to the centre of the graphic (samples more similar to the control sample). The samples acquired by OT at lower temperature regime are situated in the upper part of the graphic (closer to the control sample). Samples treated in sugar beet molasses solution are located at the left side of the

- [4] Jiang, L., et al., Apigenin from Daily Vegetable Celery Can Accelerate Bone Defects Healing, *Journal of Functional Foods*, 54 (2019), Mar., pp. 412-421
- [5] Dolati, K., et al., Inhibitory Effects of Apium Graveolens on Xanthine Oxidase Activity and Serum Uric acid Levels in Hyperuricemic Mice, *Preventive Nutrition and Food Science*, 23 (2018), 2, pp. 127-133
- [6] Ashoush, Y. A. M., et al., Comparative Study between Celery Leaves and Broccoli Flowers for Their Chemical Composition and Amino Acids as Well as Phenolic and Flavonoid Compounds, *Menoufia Journal of Agricultural Biotechnology*, 2 (2017), 1, pp. 1-13
- [7] Kooti, W., Darei, N., A Review of the Antioxidant Activity of Celery (*Apium Graveolens* L), *Journal of Evidence-Based Complementary & Alternative Medicine*, 22 (2017), 4, pp. 1029-1034
- [8] Minarovičova, L., et al., Qualitative Properties of Pasta Enriched with Celery Root and Sugar Beet by-Products, *Czech Journal of Food Sciences*, 36 (2018), 1, pp. 66-72
- [9] Seifu, M., Tola, Y. B., Effect of Variety and Drying Temperature on Physicochemical Quality, Functional Property, and Sensory Acceptability of Dried Onion Powder, *Food Science & Nutrition*, 6 (2018), 6, pp. 1641-1649
- [10] Gamboa-Santos, J., Companone, K., Application of Osmotic Dehydration and Microwave Drying to Strawberries Coated with Edible Films, *Drying Technology*, 37 (2019), 8, pp. 1002-1012
- [11] Almeida, J. A. R., et al., Effect of Temperature and Sucrose Concentration on the Retention of Polyphenol Compounds and Antioxidant Activity of Osmotically Dehydrated Bananas, *Journal of Food Processing and Preservation*, 39 (2015), 6, pp. 1061-1069
- [12] Filipović, I., et al., The Effects of Technological Parameters on Chicken Meat Osmotic Dehydration Process Efficiency, *Journal of Food Processing and Preservation*, 41 (2017), 1, e13116
- [13] Ničetin, M., et al., The Possibility to Increase Antioxidant Activity of Celery Root during Osmotic Treatment, *Journal of Serbian Chemical Society*, 82 (2017), 3, pp. 253-265
- [14] Mišljenović, N., et al., Optimization of the Osmotic Dehydration of Carrot Cubes in the Sugar Beet Molasses, *Thermal Science*, 16 (2012), 1, pp. 43-52
- [15] Mokhtar, W. M. F. W., et al., Dehydration of Potato Slices Following Brief Dipping in Osmotic Solutions: Effect of Conditions and Understanding the Mechanism of Water Loss, *Drying Technology*, 37 (2019), 7, pp. 885-895
- [16] Ahmed, I., et al., Developments in Osmotic Dehydration Technique for the Preservation of Fruits and Vegetables, *Innovative Food Science & Emerging Technologies*, 34 (2016), Apr., pp. 29-43
- [17] Curčić, B., et al., Osmotic Treatment of Fish in Two Different Solutions-Artificial Neural Network Model, *Journal of Food Processing and Preservation*, 39 (2015), 6, pp. 671-680
- [18] Šuput, D., et al., Effects of Temperature and Immersion Time on Diffusion of Moisture and Minerals during Rehydration of Osmotically Treated Pork Meat Cubes, *Hemjska Industrija*, 69 (2015), 3, pp. 297-304
- [19] Filipčev, B., et al., Sugar Beet Molasses, As an Ingredient to Enhance the Nutritional and Functional Properties of Gluten-Free Cookies, *International Journal of Food Science and Nutrition*, 67 (2016), 3, pp. 249-256
- [20] Chen, M., et al., Antioxidant and in Vitro Anticancer Activities of Phenolics Isolated from Sugar Beet Molasses, *BMC Complementary and Alternative Medicine*, 15 (2015a), 1, pp. 313-321
- [21] Chen, M., et al., Optimisation of Ultrasonic-Assisted Extraction of Phenolic Compounds, Antioxidants, and Anthocyanins from Sugar Beet Molasses, *Food Chemistry*, 172 (2015b), Apr., pp. 543-550
- [22] Guan, Y., et al., Preparation of Antioxidants from Sugarcane Molasses, *Food Chemistry*, 152 (2014), June, pp. 552-557
- [23] Chen, M., et al., The Antibiotic Activity and Mechanisms of Sugar Beet (*Beta vulgaris*) Molasses Polyphenols Against Selected Food-Borne Pathogens, *LWT-Food Science and Technology*, 82 (2017), 1, pp. 354-360
- [24] Blanco, M. E., et al., Colorimetry Technique as a Tool for Determining Release Kinetics and Mass Transfer Parameters of Anthocyanins Encapsulated in W1/O/W2 Double, *International Journal of Food Engineering*, 12 (2016), 7, pp. 615-624
- [25] Zheng, R., et al., Recovery of Phenolics from the Ethanol Extract of Sugarcane (*Saccharum officinarum* L.) Baggasse and Evaluation of the Antioxidant and Antiproliferative Activities, *Industrial Crops and Products*, 107 (2017), Nov., pp. 36-36
- [26] Gorjanović, S., et al., Antioxidant Capacity of Teas and Herbal Infusions: Polarographic Assessment, *Journal of Agricultural and Food Chemistry*, 60 (2012), 38, pp. 9573-9580
- [27] Brand-Williams, W., et al., Use of Free Radical Method to Evaluate Antioxidant Activity, *LWT – Food Science and Technology*, 28 (1995), 1, pp. 25-30

- [28] Ring, J., Chang, M., The FRAP Assay: Allowing Students to Assess the Anti-Oxidizing Ability of Green Tea and More, *Journal of Student Science and Technology*, 8 (1996), 3, pp. 70-76
- [29] Alagarsamy, S., *et al.*, Phytochemical Analysis and Antioxidant Potential of the Crude Extract of Allium Oschaninii Scape, *Oriental Pharmacy and Experimental Medicine*, 18 (2018), 4, pp. 309-316
- [30] Gorjanović, S., *et al.*, Comparative Analysis of Antioxidant Activity of Honey of Different Floral Sources Using Recently Developed Polarographic and Various Spectrophotometric Assays, *Journal of Food Composition and Analysis*, 30 (2013), 1, pp. 13-18
- [31] Babuskin, S., *et al.*, Functional Food Enriched with Marine Microalga Nannochloropsis Oculata as a Source of ω -3 Fatty Acids, *Food Technology & Biotechnology*, 52 (2014), 3, pp. 292-299
- [32] ***, StatSoft Inc. STATISTICA Data Analysis Software System, Tulsa, OK. USA, 2019, Vol. 10
- [33] Mas'ud, F., *et al.*, Optimization of Mango Seed Kernel Oil Extraction Using Response Surface Methodology, Oilseeds and fats, *Crops and Lipids*, 24 (2017), 5, D503
- [34] Filipović, J., *et al.*, The Effects of ω -3 Fatty Acids and Inulin Addition to Spelt Pasta Quality, *LWT-Food Science and Technology*, 63 (2015), 1, pp. 43-51
- [35] Cvetković, B., *et al.*, The Optimization of Traditional Fermentation Process of white Cabbage (in Relation Biogenic Amines and Polyamines Content and Microbiological Profile), *Food Chemistry*, 168 (2015), Feb., pp. 471-477
- [36] Camiz, S., Pillar, V., Identifying the Informational /Signal Dimension in Principal Component Analysis, *Mathematics*, 6 (2018), 11, 269
- [37] Pastor, K., *et al.*, Discriminating Cereal and Pseudocereal Species Using a Binary System of GC-MS Data- A Pattern Recognition Approach, *Journal of the Serbian Chemical Society*, 83 (2018), 3, pp. 317-329