OSMOTIC TREATMENT OF NETTLE LEAVES - OPTIMIZATION OF KINETICS AND ANTIOXIDANT ACTIVITY

Violeta Knežević[1*], Lato Pezo[2], Biljana Lončar[1], Milica Nićetin[1], Vladimir Filipović[1], Stanislava Ž. Gorjanović[2], Desanka Ž. Sužnjević[2]

[1]Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[2]Institute of General and Physical Chemistry, University of Beograd, Studentski trg 12/V, 11000 Beograd, Serbia

[*]e-mail: violetaknezevic320@gmail.com

Abstract

The main objective in this study was to examine the influence of different osmotic agent type, process temperature and immersion time on the mass transfer phenomena during osmotic treatment, as well as the antioxidant activity of nettle leaves after the osmotic treatment.

The presented paper describes an investigation of osmotic treatment of nettle leaves in two different osmotic solutions (sugar beet molasses and ternary solution, prepared using sucrose in the quantity of 1,200 g/kg water, NaCl in the quantity of 350 g/kg water and distilled water), under atmospheric pressure, at temperatures of: 20, 35 and 50 ^{0}C, after: 30, 60 and 90 min. of process. Different kinetics parameters were monitored, such as: water loss - WL and solid gain - SG. Antioxidant activity of osmotically treated nettle leaves in two different osmotic solutions was measured using a novel assay based on mercury reduction antioxidant power (MRAP), based on decrease of cathodic current of Hg(II) reduction. In order to get an insight into agreement between MRAP assay and commonly used spectrophotometric assay, ferric reducing antioxidant power (FRAP) has been included in this study. Different statistical methods (such as Post-hoc Tukey's HSD test at 95% confidence limit, principal component analysis - PCA and cluster analysis - CA) were applied to characterize classify and discriminate the different samples.

The statistically significant increase in MRAP and FRAP was noticed in nettle leaves samples osmotically treated in sugar beet molasses solution, while FRAP and MRAP values were decreased for samples treated in sodium chloride and sucrose solution. Post-hoc Tukey's HSD, statistically significant at $p < 0.05$ level, has been calculated to confirm statistically significant differences between complex samples.

Standard score analysis (SS) was used in optimal process parameters determination. The optimum condition for osmotic treatment of nettle leaves in sugar beet molasses solutions was at temperature of 35 ^{0}C and treatment time of 90 min.

Key words: *Osmotic Treatment, Antioxidant Activity, Nettle Leaves.*

1. Introduction

Stinging nettle (*Urtica dioica* L., *Urticaceae*) as a weed plant widespread in the world, predominantly in wasteland areas with characteristically unpleasant stinging hairs on the stems and leaves. Nettles are grown in mild climate areas, bottom of barriers, ruins and grassy places, between cultivated plants, street, and water runnels [1]. The presence of valuable biologically important compounds such as: proteins, vitamins, phenolic components, macro and micro elements, tannins, flavonoids, sterols, fatty acids, carotenoids and chlorophylls [2], contributes to the utilization of stinging nettle in different ways. Due to the fact that stinging nettle leaves are rich in: flavonoids, chlorophylls and carotenoids and their degradation products, vitamins [3] proteins [4], mineral materials, organic acids, oil and other components, the stinging nettle is of high value in the folk medicine as well as in scientific medicine [2].

As an animal food, stinging nettle leaves are important for increase and improve of body weight and meat quality [5]. Traditionally, in herbal medicine stinging nettle is used as a diuretic agent and for the treatment of rheumatism and arthritis. Nowadays, in form of leaves

and roots extracts, stinging nettle is used as supportive therapy to help relieve rheumatic complaints and seasonal allergy symptoms [6], and in reducing difficulties in urination associated with early stages of benign prostatic hyperplasia [7]. Stinging nettle leaf extract is used in the manufacture of personal care products and pharmaceutical products, like shampoos, toothpaste and creams with certain functionality [8].

People use the root and above ground parts as medicine, in foods, and young stinging nettle leaves are eaten as a cooked vegetable. Stinging nettle above ground parts are applied to the skin for: muscle aches and pains, oily scalp, oily hair, and hair loss (alopecia) [9].

Antimicrobial and antioxidant activities [2], the possibilities for decreasing of cardiovascular risks [9] and investigations of chemo preventive properties [10] of stinging nettle extracts in breast cancer cells are still researched.

Alterations in chemical composition and quantity of components of stinging nettle is related to environmental growth factors such as: temperature, moisture, light, soil type and nutrients. Among others, development stage, harvest term, form and type of organs, as well as the conditions of storage and drying are important [11].

Osmotic treatment (OT) is used as a pretreatment for many processes, to improve nutritional, sensorial and functional properties of food without changing its integrity [12]. OT is a water removal process, based on soaking food (fruit, vegetable, meat and fish) in a hypertonic solution. In comparison to other drying treatments main advantages of OT process are that water is removed in liquid form, at mild temperatures and that osmotic solution can be reused [13]. This technique also is interesting because it provides partial water removal from a food product, with low energy consumption and mild heat treatment [14].

In recent research was shown that sugar beet molasses is highly effective osmotic medium for treatment of fruits [15], vegetables [14] and meat [16]. Molasses contains high amounts of solids (> 80%), of which around 50% is sugar, 30 - 35% non-sugar compounds and 15 - 20% of water. High dry matter content, specific nutrient composition, low costs and energy requirements are the main reasons way sugar beet molasses is such a useful osmotic solution [17]. However, the practical application of molasses in OT in industrial scale is limited due to its high viscosity at lower temperatures. Also, after OT, the great quantity of diluted molasses solution is generated, which is necessary to be re-concentrated, in order to make the process economically acceptable [14].

The objective of presented work was to investigate the effects of osmotic solution type, processing time and temperature on the mass transfer phenomena during osmotic treatment of nettle leaves in sugar beet molasses and aqueous ternary solution. The aim was to determine: water loss (WL) and solid gain (SG), and antioxidant activity (expressed by mercury reduction antioxidant power - MRAP, and commonly used spectrophotometric assay, ferric reducing antioxidant power - FRAP) as a function of the process variables and to find the optimum osmotic treatment conditions. During the osmotic treatment experiments, it was noticed that antioxidant activity of nettle leaves was increased in sugar beet molasses solution, and that the FRAP and MRAP value decreased in ternary solution.

Experimental results were subjected to analysis of variance (ANOVA) to show relations between applied assays. Pattern recognition techniques (principal component analysis - PCA and cluster analysis - CA) were applied on the experimental data (used as descriptors) to characterize and differentiate among the observed samples.

2. Materials and Methods

2.1 Osmotic treatment

Sugar beet molasses, obtained from the sugar factory Pećinci, Serbia with initial dry matter content of 85.04% w/w, was diluted to concentrations of 80% w/w (this solution was marked as S_1). Aqueous ternary osmotic solution was made from sucrose in the quantity of 1,200 g/kg water, NaCl in the quantity of 350 g/kg water and distilled water. This solution (S_2) was diluted with distilled water to concentrations of 60 w/w.

After each sampling time (30, 60 and 90 minutes), nettle leaves samples were taken out from solutions (S_1 and S_2), lightly washed with distilled water, gently blotted with paper to remove excessive water from the surface, and weighted. Dry matter content of the fresh and treated samples was determined by drying the material at 105 ^{0}C for 24 hours in a heat chamber (Instrumentaria Sutjeska, Croatia). All analytical measurements were carried out in accordance to AOAC (2000). This should be written into References under [24]. All experiments were repeated three times, and the obtained results are presented in Table 1.

2.2 Determination of ferric reducing/antioxidant power (FRAP assay)

FRAP assay was carried out according to a standard procedure [18]. FRAP reagent was prepared by mixing acetic buffer, TPTZ and $FeCl_3$ x 6 H_2O (20 mM water solution) at a ratio of 10 : 1 : 1. Briefly, to a volume of 950 μL of FRAP reagent 50 μL of tea extract was added. After 4 min. the absorbance of blue coloration was measured against a blank sample. All measurements were performed in triplicate. Aqueous solutions of $FeSO_4 \times 7H_2O$ (100 - 1000 μM) were used for the calibration and the results are expressed as mmol/L Fe(II).

Table 1. Experimental results of kinetics parameters and antioxidant activity of nettle leaves

Sol.	t	Temp.	WL	SG	FRAP	MRAP
/	/	/	0.00 ± 0.00[a]	0.0 ± 0.00[a]	1.354 ± 0.024[a]	0.015 ± 0.000[a]
Sugar beet molasses	30	20	0.38 ± 0.01[c]	0.12 ± 0.00[b]	1.323 ± 0.028[a]	0.016 ± 0.000[ab]
	30	35	0.41 ± 0.01[d]	0.12 ± 0.00[b]	1.357 ± 0.018[a]	0.017 ± 0.000[cde]
	30	50	0.46 ± 0.01[f]	0.16 ± 0.00[d]	1.327 ± 0.017[a]	0.016 ± 0.000[bcd]
	60	20	0.49 ± 0.01[g]	0.15±0.00[cd]	1.333±0.017[a]	0.016±0.000[bc]
	60	35	0.50±0.01[gh]	0.14±0.00[c]	1.338±0.003[a]	0.017±0.000[cde]
	60	50	0.54±0.01[ij]	0.18±0.00[f]	1.359±0.030[a]	0.017±0.000[cde]
	90	20	0.52±0.01[hi]	0.16±0.00[d]	1.348±0.026[a]	0.017±0.000[cde]
	90	35	0.51 ± 0.01[gh]	0.14 ± 0.00[c]	1.358 ± 0.023[a]	0.017 ± 0.000[de]
	90	50	0.54 ± 0.01[j]	0.19 ± 0.00[f]	1.335 ± 0.011[a]	0.017 ± 0.000[e]
Ternary solution	30	20	0.35 ± 0.00[b]	0.17 ± 0.00[e]	1.109 ± 0.019[f]	0.015 ± 0.000[a]
	30	35	0.34 ± 0.01[b]	0.17 ± 0.00[e]	1.009 ± 0.008[e]	0.015 ± 0.000[a]
	30	50	0.35 ± 0.01[b]	0.20 ± 0.09[g]	1.033 ± 0.018[e]	0.015 ± 0.000[a]
	60	20	0.43 ± 0.01[de]	0.21 ± 0.09[g]	0.926 ± 0.015[d]	0.015 ± 0.000[a]
	60	35	0.41 ± 0.01[d]	0.20 ± 0.09[g]	0.867 ± 0.021[bc]	0.015 ± 0.000[a]
	60	50	0.43 ± 0.00[def]	0.22 ± 0.00[h]	0.885 ± 0.013[cd]	0.015 ± 0.000[a]
	90	20	0.44 ± 0.00[ef]	0.22 ± 0.00[h]	0.876 ± 0.005[bc]	0.015 ± 0.000[a]
	90	35	0.43 ± 0.01[de]	0.20 ± 0.09[g]	0.834 ± 0.016[b]	0.015 ± 0.000[a]
	90	50	0.49 ± 0.01[g]	0.26 ± 0.00[i]	0.711 ± 0.013[a]	0.015 ± 0.000[a]

[a-n] 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.

2.3 Determination of mercury reduction antioxidant power (MRAP assay)

MRAP assay was carried out according to a standard procedure [19]. 20.0 mL of equimolar mixture of 1 103M Hg^{2+} and H_2O_2 (Hydroxoperhydroxomercury(II) complex, HPMC) in CL buffer pH 9.8, or 20.0 mL buffered solution of 1 103 M $HgCl_2$ in CL buffer pH 9.8 were titrated with the 1 102 M solutions of selected AOs. During measurement of AO activity based on the decrease of Hg^{2+} cathodic current, initial concentration of 5 104 M Hg^{2+} in CL buffer pH 9.8 was used to increase sensitivity of the MRAP assay.

2.4 Statistical analysis

The experimental results were expressed by means, standard deviation (SD) for each treatment. Collected data were subjected to ANOVA to explore the effects of process variables. Furthermore, pattern recognition techniques, including PCA and CA were applied successfully to classify and discriminate the different samples. The evaluation of RSM, ANOVA, PCA and CA of the obtained results was performed using Statistica software version 12 (StatSoft Inc. 2012, USA)® [20].

The experimental data used for the analysis were derived using the Box and Behnken's fractional factorial (3 level-2 parameter) design, 2 blocks, according to RSM [21]. The RSM equations describe effects of the test variables on the observed responses, determine test variables interrelationships and represent the combined effect of all test variables in the observed responses.

The following second order polynomial (SOP) model was fitted to the experimental data. Five models of the following form were developed to relate five responses (Y) and two process variables (X), for each of the different osmotic treatments:

$$Y_k^l = \beta_{k0}^l + \sum_{i=1}^{2} \beta_{ki}^l \cdot X_i + \sum_{i=1}^{2} \beta_{kii}^l \cdot X_i^2 + \beta_{k12}^l \cdot X_1 \cdot X_2,$$

$$k = 1 - 5, l = 1 - 2 \tag{1}$$

where: β_{k0}^l, β_{ki}^l, β_{kii}^l, β_{k12}^l are constant regression coefficients; Y_k^l, either: WL, SG, a_w, DM and DPPH, while X_1 is time, and X_2 is temperature. Model describing osmotic treatment in S_1 solution is marked with $l = 1$, while treatment in S_2 is marked with $l = 2$.

3. Results and Discussion

3.1 Principal component analysis (PCA)

Principal component analysis (PCA) is a mathematical procedure used as a central tool in exploratory data

analysis [21]. It is a multivariate technique in which the data are transformed into orthogonal components that are linear combinations of the original variables. PCA is done by eigenvalue decomposition of a data correlation matrix [22]. This transformation is defined in such a way that the first component has the largest possible variance. This analysis is used to achieve maximum separation among clusters of parameters. This approach, evidencing spatial relationship between processing parameters, enabled a differentiation between the different samples in both solutions (S_1 and S_2).

The PCA, applied to the given data set, Table 1, has shown a differentiation between the samples according to used process parameters and is used as a tool in exploratory data analysis to characterize and differentiate neural network input parameters. As can be seen, there is a neat separation of the observed samples, according to used assays. Quality results show that the first two principal components, accounting for 98.60% 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, SG (which contributed 24.7% of total variance, based on correlations) exhibited positive scores according to first principal component, whereas FRAP (42.9%) and MRAP (32.4%) showed a negative score values according to first principal component (Figure 1). WL (which contributed 59.5% of total variance, based on correlations), SG (25.3%) and MRAP (15.0%) showed the negative influence towards the second principal component.

PCA graphics showed quite good discrimination between solutions S_1 and S_2 solutions. Samples treated in sugar beet molasses solution are located at the left side of the graphic, showing increased FRAP and MRAP. Also, it is evident that SG is augmented for samples treated with ternary solution increased with immersing time and temperature.
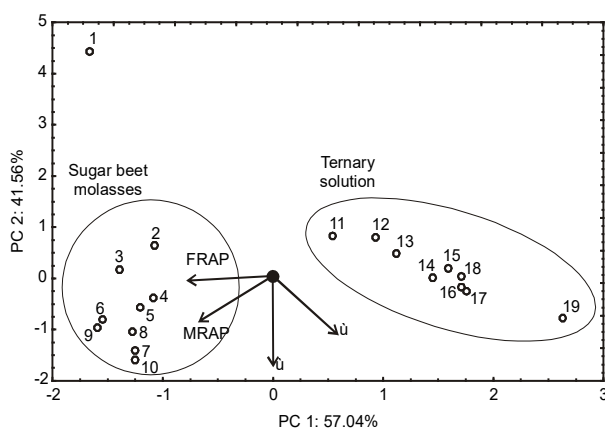

Figure 1. Biplot graphic of nettle leaves osmotic treatment in sugar beet molasses solution and ternary solution

3.2 Cluster analysis (CA)

Figure 2 shows dendrogram of CA for the osmotic treatment of nettle leaves in sugar beet molasses solution and ternary solution. The complete linkage algorithm and city block (Manhattan) distances were used as the measure of proximity among the samples. City block distances (shown on ordinate axis) are measured as the average difference across dimensions of the observed samples. This distance measure yields results similar to the Euclidean distance, but in this measuring technique, the effect of single large differences (outliers) is dampened (since they are not squared). The dendrogram presented in Figure 2 is based on experimental data. The resulting dendrogram showed two main clusters; the first cluster contained samples processed in S_1 solution (2- 10) and samples treated in S_2 solution (11 - 13) , as well as control sample (1), while the second cluster contained samples treated in S_2 solution (14 - 19). The linkage distance (shown on the ordinate axis) between the main clusters was nearly 0.6.
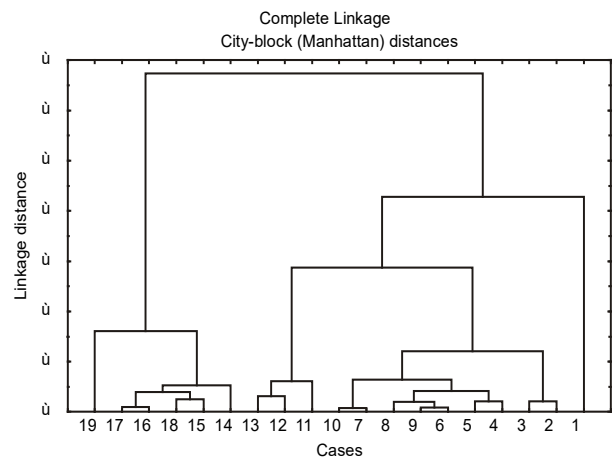

Figure 2. Tree diagram for nettle leaves osmotic treatment in sugar beet molasses solution and ternary solution

3.3 Response surface methodology (RSM)

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 (Table 2).

The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models.

Linear terms of immersion time and solution type were the most influential variables for all process variables calculation. All SOP models had insignificant lack of fit tests, which means that all the models represented the data satisfactorily.

Table 2. ANOVA calculation for nettle leaves osmotic treatment in sugar beet molasses solution and ternary solution

	df	Ternary solution				Sugar beet molasses solution			
		WL	SG	FRAP	MRAP	WL	SG	FRAP	MRAP
t	1	0.090^+	0.018^+	0.137^+	$7.96 \cdot 10^{-8+}$	$3.19 \cdot 10^{-3*}$	$2.84 \cdot 10^{-4*}$	0.0001	$7.48 \cdot 10^{-7*}$
t^2	1	0.028^*	0.006^*	0.010^{**}	$4.63 \cdot 10^{-10}$	$2.05 \cdot 10^{-6+}$	$3.65 \cdot 10^{-6+}$	0.0000	$2.27 \cdot 10^{-9}$
T	1	0.001	0.002	0.007	$1.79 \cdot 10^{-9}$	$3.15 \cdot 10^{-5*}$	$1.11 \cdot 10^{-5*}$	0.0001	$3.27 \cdot 10^{-7*}$
T^2	1	0.000	0.001	0.001	$6.00 \cdot 10^{-10}$	$2.02 \cdot 10^{-4}$	$3.34 \cdot 10^{-6}$	0.0003^{**}	$1.36 \cdot 10^{-7}$
$t \times T$	1	0.000	0.000	0.002	$4.74 \cdot 10^{-9**}$	$7.11 \cdot 10^{-4}$	$2.96 \cdot 10^{-6**}$	0.0001	$8.99 \cdot 10^{-10}$
Error	4	0.007	0.003	0.006	$2.71 \cdot 10^{-9}$	$6.87 \cdot 10^{-4}$	$1.65 \cdot 10^{-5}$	0.0009	$1.52 \cdot 10^{-7}$
r^2		0.961	0.941	0.977	0.985	0.894	0.817	0.556	0.943

$^+$Significant at $p < 0.01$ level, *Significant at $p < 0.05$ level, **Significant at $p < 0.10$ level, 95% confidence limit.

4. Conclusions

- Osmotic treatment of nettle leaves presents some advantages compared with common drying techniques, such as minimizing heat damage to product and reducing energy costs. The use of OT, as a complementary treatment in food processing, particularly prior to drying and freezing operations, reduces energy requirements of these processes.

- Intensive increase in WL (to 0.52%) and the relatively low solid uptake (to 0.16 g/g i.s.w.), was observed after 90 minutes of osmotic process, by using the sugar beet molasses as hypertonic solution.

- Decrease of antioxidant activity was observed during osmotic treatment in ternary solution (FRAP and MRAP values were decreased), while the increase of antioxidant activity was noticed using sugar beet molasses solution.

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5. References

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