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THE EFFECT OF OSMOTIC TREATMENT ON CANNABIDIOL (CBD) AND TETRAHYDROCANNABINOL (THC) CONTENT IN INDUSTRIAL HEMP

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Abstract

Two major cannabinoids are present in *Cannabis* plants. The first one is a psychoactive cannabinoid substance (-)-trans-Δ9-tetrahydrocannabinol, well-known as THC, based on which medicinal cannabis is categorized as a prohibited narcotic. The other one is cannabidiol (CBD), presented specifically in hemp. CBD is familiar as a pharmacologically active substance, and its application is becoming increasingly important in medicine. Many studies testify to osmotic treatment's success in removing water from different plant and other materials. Sugar beet molasses has proven to be an efficient osmotic solution in water removal, nutritional enrichment, food safety and quality aspects. The use of a mild temperature regime to preserve plant tissue and nutritional values is one of this process's advantages. This work is concerned with determining the effect of the drying temperature on CBD and THC content after the osmotic treatment (OT) compared to the natural drying at ambient temperature.

Osmotic treatment of three different industrial hemp types (DZ1, DZ2 and DZ3) was performed in sugar beet molasses solution (80%), under atmospheric pressure, for 1.5 hours, at 20, 35 and 50 ^{0}C with the sample to solution ratio 1 : 20. The principal component analysis (PCA) has been used operatively for better visualization and the samples' differentiation. The optimization of OT was performed using artificial neural networks (ANN). The optimization of the output variables was based on the ANN model.

The reduction in CBD and THC content is to a greater extent during osmotic treatment than in natural drying, mainly decreased with increasing solution temperature. However, the positive aspect is a more significant reduction in psychoactive substance in comparison to natural drying. The temperature of 35 ^{0}C and sample DZ2 gave optimal experimental results of 4.266 ± 0.168 and 0.138 ± 0.006 µg/g for CBD and THC, respectfully.

It can be concluded that OT of all hemp samples resulted in a decrease of both CBD and THC content, although the positive aspect is a more significant reduction in psychoactive substance than after natural drying.

Key words*: Osmotic treatment, CBD, THC, Industrial hemp, Sugar beet molasses.*

1. Introduction

Food preservation is nevertheless one of the most applicable and challenging unit operations in the food processing industry [1]. Osmotic treatment is significant food dehydration method with numerous advantages including mild processing temperatures, base waste materials and energy savings [2]. Osmotic mediums should be conscientiously chosen for the type of food materials; the most frequently used ones are binary and ternary glucose or sucrose and salt solutions, sucrose, glycerol, sorbitol, corn syrup, glucose fructooligosaccharides, honey and maple syrup solutions [3, 4].

Sugar beet molasses have shown to be a unique osmotic solution for OT of different food samples, essentially due to high dry matter content (above 80%) and a characteristic chemical composition that provides high osmotic pressure for the drying process [5, 6]. Sugars (mainly sucrose, raffinose, glucose, and fructose) make about 47 - 48% of solids in sugar beet molasses, while non-sugar segment form mineral and trace elements, namely potassium, sodium, calcium, magnesium, iron, and copper, also significant bioactive compounds in particular crude proteins, non-nitrogen substances, vitamin B complex, biotin, etc. [7, 8].

As an annual plant native to Central Asia, industrial hemp has been with humanity for more than 10,000 years since the time of the hunters-gatherers. As a multipurpose plant, it was traditionally used for the production of fiber, for medical and ritual purposes, and hemp grain as a source of human nutrition. Because of its high quality fiber, for a long time, hemp was traditionally grown as an industrial fiber crop [9]. Modern trends of returning to natural raw materials recognize hemp as a source of valuable phytochemicals. Of the over 500 different compounds identified so far, phytocannabinoids are the most important in addition to terpenes. So far, more than 100 phytocannabinoids have been identified, of which cannabidiol (CBD) and tetrahydrocannabinol (THC) are the most important due to their therapeutic effect [10]. Due to its psychoactive effect, THC is currently a limiting factor in the production of industrial hemp in most parts of the world, where its presence in dry herb is l limited to a content of 0.3% and 0.2%, respectively [11].

The artificial neural network (ANN) model is a nonlinear modeling method with secure function approximation potential [12]. The implementation of ANN was in details presented in food drying from aspects of the kinetic model, physicochemical properties and quality analysis, intelligent control design [13]. In recent years, ANN was proved a useful mathematical tool for modelling the drying process dynamic [14].

The aim of the present study was to determine the effect of the drying temperature on CBD and THC content after the osmotic treatment (OT) compared to the natural drying at ambient temperature. For better visualization and the samples' differentiation PCA has been used. The optimization of two response variables (CBD and THC) was based on model developed by ANN.

2. Materials and Methods

The F2 population of narrow leaf hemp (NLH) originating from the cross between *C. sativa* x *C. indica* was grown in 2020 at the locality of Bački Petrovac (45.20.11 N, 19.40.12 E). The technological procedure during cultivation was in terms of GAP [15]. In the phase of physiological maturity of the seed (October 22), 50 cm of the main stalk tops from three randomly selected plants were removed manually. The samples were dried in ambient conditions and after that the herb was manually separated from stalk and seeds and prepared for further analysis. Analysis of cannabinoids was performed after sample homogenization and extraction with absolute ethanol using gas chromatograph equipped with flame ionization detector.

Sugar beet molasses was obtained from the sugar factory Crvenka, Serbia with initial dry matter content of 85.04% w/w, with distilled water it was diluted to concentration 80% w/w. After preparation samples were measured and immersed in hypertonic solution. Sample to solution ratio was 1 : 20 (w/w) which can be considered high enough to neglect the changes of solution concentration during the process. The osmotic dehydration was carried out in laboratory jars under atmospheric pressure at constant solution temperatures of 20, 35 and 50 ^{0}C.The osmotic dehydration was carried out for 1.5h as it was reported by Knežević *et al.*, [16].

The principal component analysis (PCA) has been applied effectively to classify and segregate the different samples. The optimization of OT was performed using artificial neural networks (ANN). The ANN model was developed as described by Pezo *et al.*, [17]. All mathematical analyses were performed using the software package STATISTICA, ver. 10 (StatSoft Inc., Tulsa, USA). The obtained results are shown as the mean value of three mesurement ± standard deviation (SD).

3. Results and Discussion

The results of a *post-hoc* Tukey HSD test, based on which the significance of the differences in the contents of CBD and THC, between the individual hemp samples were assessed, are shown in the Table 1. The values of the CBD and THC content are expressed in µg/g of the initial sample. It was found that most of the samples were statistically significantly different at $p < 0.05$, which proved that the tested samples were sufficiently diverse to approach statistical analysis.

Table 1 shows that the use of sugar beet molasses as an osmotic medium for osmotic treatment of hemp leads to decrease in the both CBD and THC content in all treated samples. It is also observed that the increase of the temperature during osmotic treatment cause reduction in observed substances. Therefore, the samples obtained at temperatures of 50 ^{0}C, have the lowest quantities of CBD and THC. Furthermore,

Table 1. Quantities of CBD and THC after different drying methods of different hemp types

Sample	Drying method	Hemp type	CBD	THC
1	20 ^{0}C OT	DZ1	4.783 ± 0.259^{cde}	0.147 ± 0.003^{bc}
2	20 ^{0}C OT	DZ2	4.267 ± 0.192^{bc}	0.138 ± 0.005^{abc}
3	20 ^{0}C OT	DZ3	8.919 ± 0.313g	0.206 ± 0.009^{f}
4	35 ^{0}C OT	DZ1	5.235 ± 0.138^{def}	0.151 ± 0.007^{cde}
5	35 ^{0}C OT	DZ2	4.266 ± 0.168^{bc}	0.138 ± 0.006^{abc}
6	35 ^{0}C OT	DZ3	8.782 ± 0.231^{g}	0.208 ± 0.012^{f}
7	50 ^{0}C OT	DZ1	3.338 ± 0.074^{a}	0.127 ± 0.006^{ab}
8	50 ^{0}C OT	DZ2	3.474 ± 0.189^{ab}	0.125 ± 0.004^{a}
9	50 ^{0}C OT	DZ3	5.779 ± 0.150^{f}	0.158 ± 0.004^{cde}
10	Natural drying	DZ1	4.585 ± 0.169^{cd}	0.148 ± 0.006^{cd}
11	Natural drying	DZ2	5.878 ± 0.222^{f}	0.168 ± 0.004^{de}
12	Natural drying	DZ3	13.297 ± 0.620^{i}	0.298 ± 0.010^{h}
13	Fresh hemp	DZ1	5.562 ± 0.088^{ef}	0.169 ± 0.003^{e}
14	Fresh hemp	DZ2	4.332 ± 0.091^{c}	0.145 ± 0.006^{abc}
15	Fresh hemp	DZ3	10.766 ± 0.513^{h}	0.254 ± 0.010^{g}

Legend: ^{abcdefghi}Different letters in superscript in table indicate on statistically significant difference between values, at the significance level of $p < 0.05$.

it can be concluded that the using natural drying for hemp dehydration induces a higher content of CBD and THC compared to the osmotic treatment. The presence of psychoactive components in the hemp plant draws on a number of possible issues, mostly regarding public approval [18]. The positive outcome of hemp's osmotic treatment is a more significant THC reduction, differentiating from natural drying. The greatest reduction of THC content (0.125 ± 0.004 µg/g) was observed in DZ2 at 50 ^{0}C.

3.1 PCA

In order to better explain the structure of the exploratory data that would contribute to the comprehension of likenesses and dissimilarities of the hemp samples after drying treatment, PCA was applied and the results are presented in Figure 1.

The first principal component explained 99.68% of the total variance in the experimental data.

The separation between samples could be observed from the PCA graph, where the highest CBD and THC content were noticed for the samples on the right side of the graphic, while the samples with the lower CBD and THC content are grouped on the left side of the graphic. The map of PCA analysis showed that the first principal component described the differentiation among the samples according to CBD and THC content.

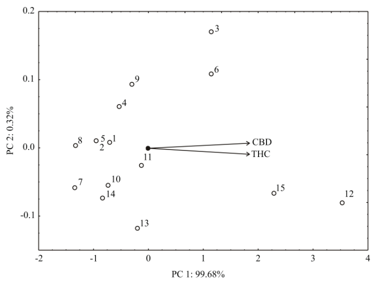

Figure 1. The PCA biplot diagram, illustrating the effect of different temperature regime on CBD and THC content in the hemp samples

3.2 ANN model

A multi-layer perceptron model (MLP), which consisted of three layers (input, hidden, and output) was used for modeling an artificial neural network model (ANN) for prediction of CBD and THC content during the drying process, based on the drying parameters (hemp type, drying method and temperature).

The calculated optimal neural network ANN model, developed to predict the CBD and THC content, showed good fitting properties to experimental data. Based on the ANN performance, it was noticed that the optimal number of neurons in the hidden layer for ANN was: 8 (network MLP 8-8-2) to obtain the highest values of r^2 (during the training cycle r^2 for output variables (CBD and THC content) was: 0.958), Table 2.

Table 2. Artificial neural network model summary (performance and errors), for training, testing, and validation cycles

Network name	Performance			Error			Training algorithm	Error function	Hidden activation	Output activation
	Train.	Test.	Valid.	Train.	Test.	Valid.				
MLP 8-8-2	0.958	0.979	0.947	0.009	0.121	0.065	BFGS -1	SOS	Identity	Tanh

Legend: *Performance term represents the coefficients of determination. While error terms indicate a lack of data for the ANN model.

Table 4 presents the elements of matrix W_1 and vector B_1 (presented in the bias row), and Table 5 presents the elements of matrix W_2 and vector B2 (bias) for the hidden layer, for ANN model, which were calculated using equation explained by Bajic et al. 2020 [19].

Optimization of the ANN outputs was carried out by operating with the results from Tables 4 and 5, applied in equation explained by Voca et al., 2021 [14]. The optimal result was reached at the temperature of 35 ^{0}C for the sample DZ2 and it was 4.266 ± 0.168 and 0.138 ± 0.006 µg/g for CBD and THC, respectfully.

The quality of the ANN model fit was tested in Table 6.

The residual analysis of the developed model was also performed. Skewness estimates the deviation of the distribution from normal symmetry. If the skewness is obviously differing from zero, then the distribution is asymmetrical, although normal distributions are perfectly symmetrical. Kurtosis calculates the "peakedness" of a distribution. If the Kurtosis is obviously differing from zero, then the distribution is either flatter or more peaked than normal; the Kurtosis of the normal distribution is zero. A relatively high r^2 is suggestive that the variation was accounted and that the data fitted acceptably to the presented model [20, 21].

4. Conclusions

- On the basis of these results it can be concluded that OT of all hemp samples resulted in a decrease of both CBD and THC content, although the positive aspect is a more significant reduction in psychoactive substance than after natural drying.
- Temperature of 35 ^{0}C and sample DZ2 gave optimal experimental results of 4.266 ± 0.168 and 0.138 ± 0.006 µg/g for CBD and THC, respectfully.
- The developed artificial neural network model displayed a good fit to experimental data and has possible practical application.

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Table 4. Elements of matrix W_1 and vector B_1 (presented in the bias row), for ANN model

Input variable	Hidden layer							
	1	2	3	4	5	6	7	8
Drying method (20 ^{0}C OT)	-0.10584	-0.2013	-0.12549	0.19781	0.06781	-0.12867	-0.2052	0.42688
Drying method (35 ^{0}C OT)	0.03251	0.11811	0.03135	-0.18199	-0.114	0.13603	0.16804	-0.315
Drying method (50 ^{0}C OT)	0.20381	0.2588	0.17071	-0.41484	-0.17825	0.27785	0.52231	-0.84582
Drying method (natural drying)	-0.08807	-0.10564	0.11426	0.06431	0.05186	-0.0843	-0.08519	0.1919
Drying method (fresh hemp)	-0.07446	-0.09319	0.06767	0.17809	0.08084	-0.05127	-0.15428	0.24342
DZ1 M	0.04007	0.06343	0.09965	-0.1532	-0.06382	0.09459	0.22352	-0.34575
DZ2 M	-0.04713	-0.01533	-0.04891	0.14087	0.0495	-0.09452	-0.16487	0.28435
DZ3 M	0.00255	-0.08247	-0.20661	0.11895	0.04213	-0.06698	-0.05211	0.12232
Bias	0.00079	0.02553	-0.01766	0.04322	-0.05053	-0.00407	-0.03034	0.00049

Table 5. Elements of matrix W_2 and vector B_2 (presented in the bias column), for ANN model

Output variable	1	2	3	4	5	6	7	8	Bias
CBD	0.24255	-0.59172	-0.84491	0.6017	0.27782	-0.5649	-0.79703	0.52909	0.32522
THC	0.17883	-1.52311	-0.44742	0.95605	0.13631	-1.5568	-0.40937	1.09721	0.32824

Table 6. The "goodness of fit" tests for the developed ANN model

Output variable	χ2	RMSE	MPE	r2	Residual analysis				
					Skewness	Kurtoisis	Average	SD	Variance
CBD	3.710	1.790	26.726	0.737	0.737	0.729	1.303	0.177	1.85
THC	0.001	0.034	15.343	0.702	0.702	0.774	0.845	0.003	0.035

5. References

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