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RESPONSE SURFACE METHODOLOGY APPROACH FOR OPTIMIZATION OF FLAX SEED OIL BLEACHING PROCESS

Miroslav Ondrejovič[1,2], Daniela Chmelová[3] , Tibor Maliar[1]*

Address(es): RNDr. Miroslav Ondrejovič, PhD.,
[1]University of SS. Cyril and Methodius, Faculty of Natural Sciences, Department of Biotechnology, Nám. J. Herdu 2, 917 01 Trnava, Slovak Republic, phone number: 033/59 21 460.
[2]Food Research Institute in Bratislava, Department – Biocentrum, Kostolná 5, 900 01 Modra, Slovak Republic.
[3]Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

*Corresponding author: miroslav.ondrejovic@ucm.sk

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ABSTRACT

Flax seed is an important source of omega-3 polyunsaturated fatty acids essential for human physiology. For low oxidation stability, specific taste and concomitant color compounds this oil is poorly applicable as nutraceutical additive. The aim of this study was optimization of flax seed oil bleaching. The optimal conditions for the bleaching process were determined using response surface methodology. A central composite design was used to investigate the effects of three independent variables, namely solid to liquid ratio, temperature and time, to output parameters of the bleaching process such as crude oil color expressed as optical density at 490 nm, acid and peroxide value. Calculated optimal conditions for the bleaching, expressed by the optical density of the oil were as follows: temperature 50°C, bleaching time 77 minutes and solid-liquid ratio 56 g of bleaching agent to 1 liter of oil.

Keywords: Flax seed oil, bleaching, RSM optimization, acid value, peroxide value

INTRODUCTION

Omega-3 polyunsaturated acids (O3PUFA) are essential for many metabolic processes in human physiology. Human body is not able to synthesize O3PUFA within the own metabolic pathways, therefore it is necessary to consume it in food **(Gorjão *et al.*, 2009)**. Although the most known sources of the O3PUFA are marine fishes, it is possible to find these lipids in plenty marine plants (*Phaeodactylum sp.* and *Monodus sp.*) and earth plants as rapeseed, walnut, hazelnut and almond **(Dyerberg *et al.*, 1980; Bell *et al.*, 2003; Marangoni *et al.*, 2007; Gecgel *et al.*, 2011)**. From earth plants, the flax seed (*Linum usitatissimum*) has been found out as an important and rich source of the O3PUFA.
The oil, prepared from this material can be declared as healthy food applicable e. g. in cold kitchen, either as nutraceutic, or food supplement aimed to increase a content of O3PUFA or moderate O3PUFA and O6PUFA ratio towards to suggested value 1:3 **(Luna *et al.*, 2008)**. However, the additives of the crude „virgin“ oil means undesirable changes of the food sensory properties. Moreover, flax seeds oil is known by the highest content of PUFA **(Bozan and Temelli, 2008)**. It is necessary to fill specific advances during the refining process to prevent oxidative and other damage of the fatty acids. Bleaching process is based on the sorption of the colored and other accompanied components as phospholipids, carotenes etc. It seems to be relative quick and safe method of the bleaching process, should be carried out under lower temperature, optimally under inert gas conditions **(Kaynak *et al.*, 2004; Wang and Lin, 2006)**.
The aim of this study was to optimize the earth consumption expressed as solid to liquid ratio (bleaching earth to crude oil), bleaching temperature and time for flax seed oil, taking in account the maintenance color of oil, expressed as optical density at 490 nm and secondarily of the oil quality expressed by acid and peroxide value.

MATERIAL AND METHODS

Material

Flax seeds (*Linum usitatissimum*) of the food quality were purchased from Ekvia, Ltd. (Czech Republic, harvested in year 2009) and pressed on the Uno-SE oil seed screw press (Farmet, Czech Republic). The obtained oil was degummed (75% H_3PO_4 g/kg), neutralized (NaOH 5.2 g/kg) and two times washed (10% v/v). Pretreated oil was stored at 4°C under nitrogen atmosphere. Bleaching earth was acquired from Rudex Ltd. (Slovak Republic).

Experimental design

Three factors, five level experiment was carried out with tested, independent variables- temperature (8, 23, 45, 67 and 82°C), bleaching time (30, 60, 105, 150 and 180 minutes) and solid-liquid ratio (4.5, 16, 33, 50 and 61.5 g/l). Real variables values were transformed into non-dimensional coded form (Table 1). Measured dependent variables were OD 490 nm, acid value, expressed in mg KOH/g oil and finally peroxide value, expressed in mmol of O/kg oil. Experimental data were fit by the polynomial regression of the second order (Eqn 1), and regression coefficients (bi) were calculated.

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{\substack{i=1\\ i<j}}^{k-1} \sum_{j=2}^{k} b_{ij} X_i X_j$$

where X_i are independent variables responsible for response Y and b_i are regression coefficients, describing relations of the measured properties to coded levels of the selected parameters. For computer and statistical processing, the Statgraphics plus software 5.1 (Statpoint technologies, Virginia) was applied. All experiments were carried out as four parallel attempts.

Acid value determination

Acid value was determined according to AOCS Cd 3d-63 method, based upon the titration of the ethanol solution of the oil sample by the 0.1 M solution of potassium hydroxide on the phenolphthalein as indicator (**AOCS, 1989**).

Peroxide value determination.

Peroxide value of flax seed oil samples has been determined immediately after bleaching process, according to the method AOCS Cd 8b-90 (**AOCS, 1989**).

Oil color photometric measurement

All prepared samples were subjected to measurement of the optical density at 490 nm (further as OD 490 nm) aimed to quantify amount of the accompanied color components and thus oil quality.

Statistical analyses

All experiments were carried out as four parallel attempts and statistical analysis was calculated by Statgraphics plus software 5.1.

Table 1 Parameter values: optical density at 490 nm (OD 490 nm), acid value expressed as mg KOH/g oil and peroxide value expressed in mmol O/kg oil

Experiment No.:	Independent variables			Dependent variables		
	Temperature [°C]	Bleaching time [min]	Solid-liquid ratio [g/l]	OD 490 nm	Acid value	Peroxide value
1	67 (1)	150 (1)	16 (-1)	0.618	1.302	1.691
2	67 (1)	60 (-1)	50 (1)	0.157	1.289	0.641
3	23 (-1)	60 (-1)	16 (-1)	1.232	1.487	0.799
4	45 (0)	105 (0)	33 (0)	0.320	1.284	0.391
5	23 (-1)	150 (1)	50 (1)	0.255	1.261	1.350
6	67 (1)	60 (-1)	16 (-1)	0.707	1.452	0.623
7	67 (1)	150 (1)	50 (1)	0.161	1.222	1.575
8	45 (0)	105 (0)	33 (0)	0.333	1.311	0.577
9	23 (-1)	60 (-1)	50 (1)	0.302	1.399	0.789
10	23 (-1)	150 (1)	16 (-1)	1.002	1.439	1.931
11	45 (0)	180 (1.682)	33 (0)	0.62	1.107	0.971
12	82 (1.682)	105 (0)	33 (0)	0.441	1.382	2.257
13	45 (0)	30 (-1.682)	33 (0)	0.422	1.250	0.392
14	45 (0)	105 (0)	33 (0)	0.328	1.303	0.993
15	45 (0)	105 (0)	61.5 (1.682)	0.250	1.326	1.949
16	45 (0)	105 (0)	4.5 (-1.682)	1.213	1.705	1.716
17	8 (-1.682)	105 (0)	33 (0)	0.983	1.339	1.976

RESULTS AND DISCUSSION

Selection of variable range

The research about flax seed oil, aspects and applications are published relatively purely **(Luna *et al.*, 2008; Bozan and Temelli, 2008; Willems *et al.*, 2008; Żuk *et al.*, 2011; Rusinek *et al.* 2012)**. It may be for fact, that flax seed oil still remains forgotten or application could be difficult, therefore bleaching process of flax seed should be researched.

Primary parameter – OD 490 nm as lower as possible was subjected to applied aim to prepare colorless oil as suitable food supplements. Figure 1 presents the course of OD 490 nm on the amount of the bleaching earth in 1 liter of oil, the found relation has exponential course with asymptotic value = 0.050, achieved at 50 g activated bleaching agent per liter of oil. Further increasing of the agent does not lead to higher bleaching efficiency.

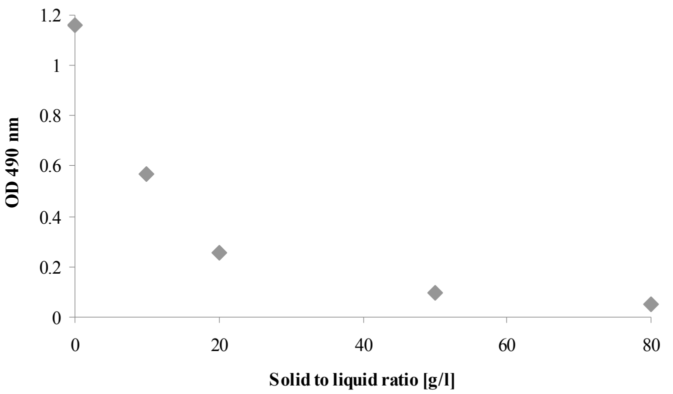

Figure 1 The relation of the optical density at 490 nm (OD 490 nm) of the bleached oil on the amount of the added activated bleaching earth expressed as solid-liquid ratio parameter.

The influence of the bleaching time on the decrease of the parameter OD 490 nm was researched as kinetic experiments at three different temperatures: 20, 50 and 80°C. From the measured data (Figure 2) it is evident, that at 20 and 50°C it was observed decreasing course during first 60 minutes, without significant differences of the set temperature. However, at the highest temperature (80°C), the rapid decreasing of the OD 490 nm with following moderate increasing was found out, probably due to formation of the oxidative degradation products of PUFA which means contribution to the OD.

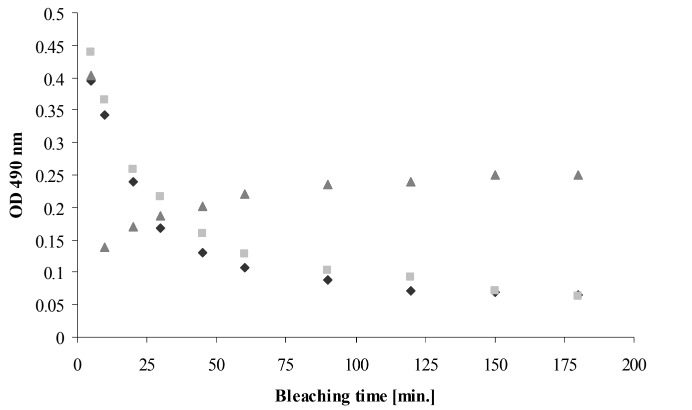

Figure 2 The relation of the optical density at 490 nm (OD 490 nm) of the bleached oil on the bleaching time at various temperatures (♦ - 20, ■ - 50 and ▲ - 80 °C).

Based upon these findings, other experimental values range from 23 – 67 °C for bleaching temperature and 60 – 150 minutes for bleaching time were proposed for.

Bleaching experiment optimization assisted by response surface methodology (RSM)

RSM is used mainly for optimization of the processes of the biodiesel or biofuel production **(Zinatizadeh *et al.*, 2006; Halim *et al.*, 2009; Chou *et al.*, 2010)** less for edible oils **(Škevin *et al.*, 2012; Ajemba *et al.*, 2013)** but bleaching optimization of flax seed oil is not published. Optimal conditions of the bleaching experiment were calculated by the software Statgraphics plus 5.1 by RSM approach. Table 1 presents experimental values of the independent parameters (OD 490 nm, acid value and peroxide value). Based upon the statistical analysis it is possible to declare, that compared dependent variable expressed self-independent relation.

Multiple linear regression

For the purpose of the fitting of the presented results, presented in the Table 1, polynomial regression of the second order (Eqn 1) with regression coefficient $R^2 = 0.964$ for OD 490 nm as parameter 1, $R^2 = 0.853$ for acid value as parameter 2 and finally $R^2 = 0.848$ for peroxide value as parameter 3 was applied.

Regression coefficient analysis

Regression coefficients of the model for OD 490 nm, acid and peroxide value obtained by multiple polynomial regression are presented Table 2. Dependent variables in coded form (Table 1) allows direct interpretation of the effect (linear, quadratic and interaction) of the independent variables to dependent variables and visualization by 3D surface plots (Figure 3) assisted visualization of the statistically important factors (with the asterisk in the Table 2) obtained from statistical analyze.

Table 2 Regression coefficients of the model polynomial regression of the second order for dependent variables – optical density at 490 nm (OD 490 nm), acid value expressed as mg KOH/g oil and peroxide value expressed in mmol O/kg oil

Model parameters		OD 490 nm	Acid value	Peroxide value
Intercept effect		2.979	1.627	2.416
Linear effect	Temperature (A)	-0.037131*	-0.001435	-0.087979
	Bleaching time (B)	-0.006892	0.006332*	0.018822*
	Solid to liquid ratio (C)	-0.059947*	-0.023436*	- 0.070329
Quadratic effect	A x A	0.0002259*	0.00001215	0.0008764*
	B x B	0.0000203	-0.00003532*	-0.0000436
	C x C	0.0004024*	0.00026926*	0.0011169*
Interaction effect	A x B	0.00002424	-0.00000694	0.0000390
	A x C	0.00022393	-0.00001236	0.0001648
	B x C	0.00004598	0.000008987	-0.0001152

* Reggresion coefficient with statistical significance at $p<0.05$

Determination and experimental validation of the optimal conditions

Optimal values of the parameters for bleaching process of the crude flax seed oil by bleaching agent are presented in Table 3. Predicted parameter values were comparable with experimentally measured values at the level of the statistical significance at $p < 0.05$. Achieved results confirm the possibility to predict the course of the bleaching process of crude flax seeds oil by activated bleaching agent by the model under particular experimental conditions.

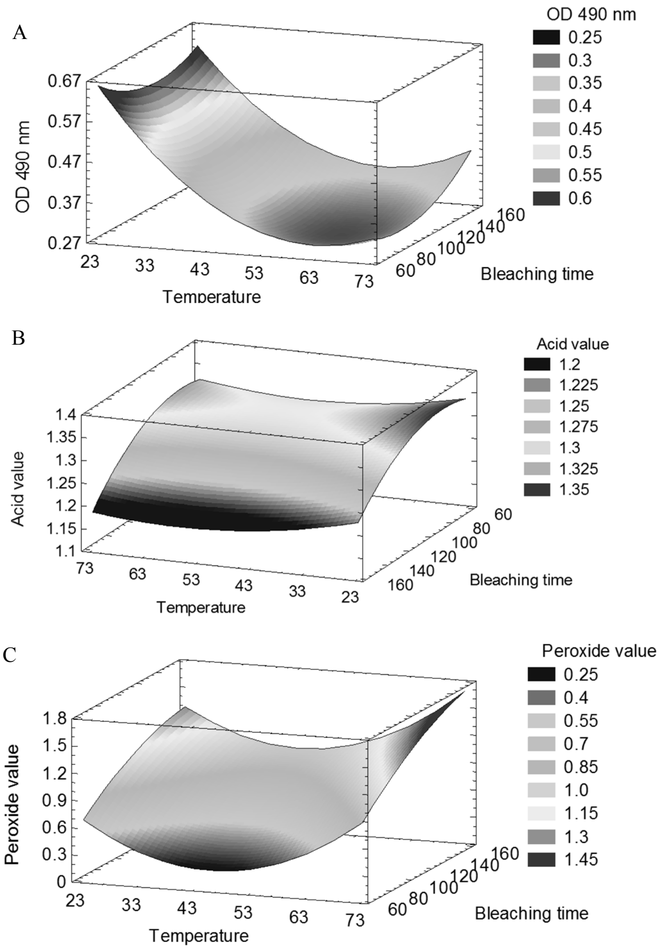

Figure 3 The relation of the dependent variables (optical density at 490 nm, acid value and peroxide value) on the temperature and bleaching time at constant solid to liquid ratio, which were 33 g/L oil; A – optical density at 490 nm (OD 490 nm); B – acid value, expressed as mg KOH/g oil; C – peroxide value expressed in mmol O/kg oil

Table 3 Comparison of predicted and experimental measured values of dependent variables at optimal parameters of the bleaching process of crude flax seeds oil by activated bleaching earth

Parameter	OD 490 nm	Acid value	Peroxide value
Temperature [°C]	50	56	42
Bleaching time [min.]	77	180	40
Solid – liquid ratio [g/l]	56	42	34
Predicted values	0.069	1.062	0.0
Experimental values	0.065	1.108	0.1

Optimal bleaching temperature and contact time of vegetable oil varied from one type of oil to another. Optimum conditions of bleaching process are 95°C and 2.5 h for palm oil, 85°C and 0.25 h for cottonseed oil and 85°C and 0.5 h for maize germ oil **(Nguetnkam *et al.* 2008)**.

CONCLUSION

The method of the designed experiment was successfully applied on optimization of the bleaching process of the crude flax seeds oil by activated bleaching agent. Model polynomial regression of the second order gives the successive description of the experiments. Calculated optimal conditions for the bleaching expressed by the optical density of the oil were as follows: temperature 50 °C, bleaching time 77 minutes and solid-liquid ratio 56 g of bleaching agent to 1 Liter of oil. Predicted value as parameters was comparable with the experimentally measured value.

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