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SEED OIL RESPONSE SURFACE METHODOLOGY FOR OPTIMIZATION OF THE EXTRACTION OF FLAX (LINUM USITATISSIMUM) SEED OIL

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ABSTRACT

Flax seed is an important source of ω-3 polyunsaturated fatty acids essential for human physiology. The aim of this paper is to investigate the effects of major parameters of the lipid extraction from flax seed, in relation to the recovery of oil as well as the oil quality properties. The independent variables of extraction were proposed as: organic solvents, temperature, extraction time and solid–liquid ratio. The following quantitative and qualitative parameters were chosen as dependent variables: yield of the lipid fraction, acid value of oil and the absorbance at 490 nm. After calculating the optimal values of the extraction, the validation analysis was carried out and it was found out that the predicted and experimentally verified dependent variables were in agreement with the optimal extraction parameters.

Keywords: food extraction, flax seed, optimization

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)**. The influence of O3PUFA may be categorised into several effects. They have a positive effect to visual, mental and psychomotoric function of human body. They both improve rheology blood properties and decrease systolic pressure. The food additive with O3PUFA, applied in the early postnatal phases is the prevention of the hypertension. Moreover, these components effect as antiarhythmic, antithrombotic, antiinflammative agents and decelerate creation of the atherosclerotic plaques **(Vyhnánková, 2007; Teitelbaum, Walker, 2001; Turnbull, Cullen-Drill, Smaldone, 2008; Jordan, 2010).**

Although the most known sources of the O3PUFA are marine fish **(Gorjão et al., 2009)**, 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, Bang, Aagaard, 1980; Gecgel et al., 2011; Marangoni et al., 2007; Bell et al., 2003)**. From earth plants, the flax seed (*Linum usitatissimum*) has been found out as an important and rich source of the O3PUFA.

The aim of this paper is to determine optimal conditions for the extraction of lipid portion from the flax seed in relation to oil yield and quality. Oil quality parameters were proposed as follows: acid value and presence of the coloured accompanied lipid components (carotenoids, phospholipids etc.). In the literature, there are several scientific papers dedicated to this phase of the edible oil manufacturing **(Rosenthal, Pyle, Niranjan, 1996; Gaur et al., 2007; Tigrine-Kordjani, Meklati, Chemat, 2006; Ayala, Luque de Castro, 2001)** with application of the various physical, chemical techniques and enzyme processing. But the process of the flax seed oil has not been sufficiently published. Extraction solvent choice, temperature, extraction time, solid-liquid ratio (ratio between extracted material and extraction solvent volume) were proposed as independent variables. Yield of the lipid portion, acid value and optical density of the crude oil at 490 nm as a parameter of the quantification of the colour undesired components were dependent variables.

MATERIAL AND METHODOLOGY

Material

Flax seeds (*Linum usitatissimum*) of the food quality were purchased from Ekvia, Ltd. (Czech Republic, harvested in year 2009). Primarily, before experiments was an aliquot amount of the tested material cut at particle size < 0.5 cm.

Extraction procedure

Varied conditions in logical relation to oil yield were as follows: solvent, solid – liquid ratio, temperature, extraction and time. Selection of the suitable solvent (hexane and petroleum ether) and solid – liquid ratio (20, 100 and 500 g of flax seed /L of extraction solvent) were evaluated during 24 hours under room temperature. The temperature influence at extraction process was evaluated at 20, 40 a 60 °C during 300 minutes at solid-liquid ratio 100 g/L (w/v). The obtained lipid portion (crude flax seed oil) was subjected to determination of the acid value.

Extraction experiment design

Three factors five level experiment was carried out with tested independent variables- temperature (17, 22, 30, 38 and 43 °C), extraction time (33, 100, 200, 300 and 367 minutes) solid-liquid ratio (11; 50; 107.5; 165 and 203.7 g/L). Real variables values were transformed into non-dimensional coded values (Table 1).

Table 1 Designed experiments conditions for selected parameters

	Coded expression				
Parameter	-1.682	-1	0	1	1.682
Temperature [°C]	17	22	30	38	43
Extraction time [min.]	33	100	200	300	367
Solid – liquid ratio [g/L]	11	50	107.5	165	203.7

Table 2 Independent variables in original and coded form and experimental results for response variables oil yield [g oil/100 g seeds], acid value [mg KOH/g oil] and optical density at 490 nm (OD 490)

Experiment No.:	Extraction time (X1)	Temperature (X2)	Solid – liquid ratio (X3)	Oil Yield (Y1)	Acid value (Y2)	OD 490 (Y3)
1	300 (1)	38 (1)	50 (-1)	27.45	3.24	0.874
2	100 (-1)	38 (1)	165 (1)	15.03	5.91	0.603
3	300 (1)	22 (-1)	165 (1)	13.75	4.69	0.615
4	100 (-1)	22 (-1)	50 (-1)	18.03	5.62	0.512
5	200 (0)	30 (0)	107.5 (0)	20.83	4.71	0.685
6	300 (1)	22 (-1)	50 (-1)	26.32	3.31	0.713
7	300 (1)	38 (1)	165 (1)	15.42	4.52	0.641
8	200 (0)	30 (0)	107.5 (0)	22.62	3.72	0.718
9	100 (-1)	22 (-1)	165 (1)	14.67	5.02	0.762
10	100 (-1)	38 (1)	50 (-1)	24.51	3.68	0.719
11	200 (0)	30 (0)	204 (1.682)	11.62	4.36	0.693
12	200 (0)	16.6 (-1.682)	107.5 (0)	14.12	4.01	0.679
13	200 (0)	43.4 (1.682)	107.5 (0)	18.31	3.42	0.814
14	200 (0)	30 (0)	107.5 (0)	19.95	3.68	0.705
15	200 (0)	30 (0)	11 (-1.682)	25.61	3.15	0.713
16	33 (-1.682)	30 (0)	107.5 (0)	17.03	4.01	0.531
17	367 (1.682)	30 (0)	107.5 (0)	20.06	4.62	0.673

Measured dependent variables were flax seed oil yield, acid value, expressed as amount of mg KOH per gram of oil and optical density of oil at 490 nm. Experimental data were fit by the polynomial regression of the second order (Equation 1), and regression coefficients bi were calculated:

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

where Xi are independent variables responsible for response Y and bi are regression coefficients, describing relations of the measured properties to coded levels of the selected parameters. For computer and statistical processing, software Statgraphics Plus 5.1 was applied. All experiments were carried out as four parallel attempts.

Acid value determination

Acid value were determined in according N.G.D. C10-1976 method (Equation 2), based upon the titration of the ethanol solution of the oil sample by the 0.1 M solution of sodium hydroxide on the phenolphthalein as indicator (**NGD C10- 1976**).

$$\text{Acid value} = (A * N * 56.1)/W \quad (2)$$

where A is volume (in mL) of 0.1 M KOH consumed for sample, N is normality of KOH and W is weight in grams of the sample.

Oil yield determination

Efficiency of extraction of the flax seed oil by organic solvent was to evaluate in relation to seeds amount and expressed in % in according with Equation 3.

Yield [g oil/100 g flax seed] = (extracted oil [g] *100)/flax seed amount [g] (3)

Oil colour photometric measurement

All prepared crude oil samples were subjected to measurement of the optical density at 490 nm (further OD490nm) aimed to quantify the amount of the accompanied colour components and thus oil sensorial quality.

RESULTS

Extraction procedure

In general, it is well known, that oil extraction efficiency is dependent from four basic parameters: solvent selection, solid –liquid ratio, temperature and time of the extraction. All these parameters were put in positive optimization aimed to maximal oil yield under relevant oil quality.

As a first was processed and tested selection of the extraction solvent (hexane or petroleum ether) and solid – liquid ratio (20, 100 and 500 g of flax seed to L of extraction solvent). Achieved results are presented in Figure 1. As the results show, under applied solid – liquid ratio 20 and 100 g/L, petroleum ether was more effective extraction solvent than hexane. Therefore other experiments were carried out with petroleum ether as extraction solvent. The optimal value of this parameter can be around 20 g/L.

The influence of the temperature and time of the extraction on the flax seeds oil were evaluated by the kinetic measurement of the extraction process under three different temperatures (20, 40 a 60 °C).

Results are presented in Figure 2. From the results, it is evident that the highest yields were achieved in extraction carried out at all temperatures during 300 minutes. During whole extraction, the oil yields at 60 °C were the highest. The following experiments were carried out at adapted parameters: temperature 17 - 43 °C and extraction time 33 to 367 minutes.

Table 3 Regression coefficients of the model polynomial regression of the second order for dependent variables – oil yield [g oil/100g seeds], acid value [mg KOH /g oil] and optical density at 490 nm (OD 490)

Model parameters		Oil yield	Acid value	OD 490
Constant effect		-14,0916	9.1999	0,07385
Linear effect	Extraction time (A)	0,07822	-0.01797	0,00225
	Temperature (B)	1,69605	-0,141477	4,081.10-4
	Solid-liquid ratio (g/L) (C)	0,05003	-0,0206617	0,005842
Quadratic effect	A x A	-5,052.10-5	2,308.10-5	-3,655.10-6
	B x B	-0,0209	2,5809.10-4	2.353.10–4
	C x C	-1,452.10-4	9,3167.10-6	1,436.10-7
Interaction effect	A x B	-6,313.10-4	1,265.10-4	2,1719.10-5
	A x C	-2,556.10-4	2,239.10-5	-1,0109.10-5
	B x C	-1,516.10-3	7,418.10-4	-1,3614.10-4

Reggresion coefficient with statistical significance at p<0,05 are printed as bold

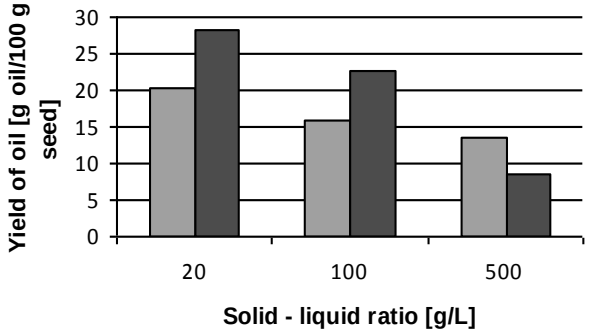

Figure 1 The efficiency of the oil extraction from flax seeds by hexane and petroleum ether, expressed as oil yield [g oil/100 g seeds] during 24 hours at room temperature under following solid-liquid ratio – 20, 100 and 500 g/L; ■ – hexane; ■ – petroleum ether.

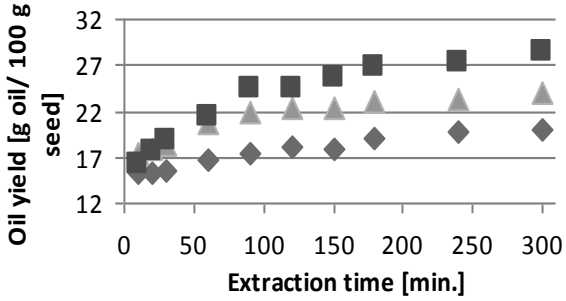

Figure 2 Kinetic of the flax seed oil extraction by petroleum ether, solid –liquid ratio 1:10 during 300 minutes at following temperatures: ♦ - 20, ▲ - 40 a ■ – 60 °C.

Flax seed oil extraction assisted by Response Surface Method (RSM)

Optimal conditions of the extraction were calculated by the software Statgraphics Plus 5.1., processed by RSM approach. In Table 2, flax seed oil yield, acid number and value of OD490nm are presented. Based upon the regression analysis results, we can state, that compared dependent variable expressed self-independent relation.

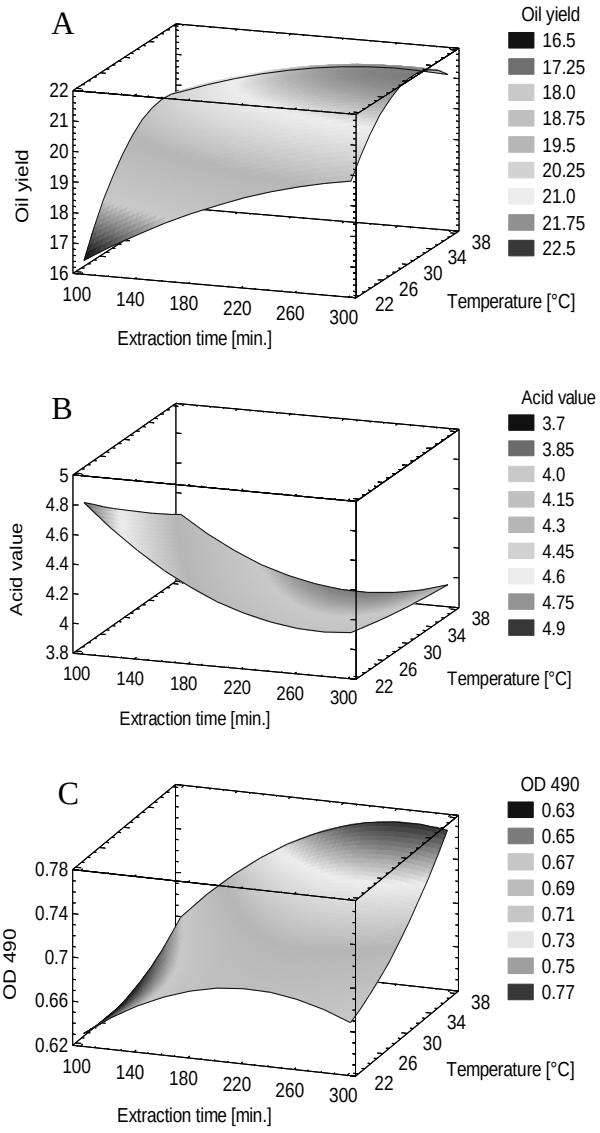

Figure 3 The relation of the dependent variables from the extraction time and temperature ratio at constant solid – liquid ratio 107.5 g/L; A – Yield of the oil [g oil/100 g seed]; B – acid value [mg KOH/g oil]; C – optical density of oil at 490 nm (OD 490).

Table 4 Optimal extraction parameters for maximizing yield of extracted flax seed oil by petroleum ether and comparison of the predicted values of the dependent variables and experimentally measured values at these optimal conditions.

Optimal extraction parameters			
Extraction time [min.]	365		
Temperature [°C]	33,5		
Solid – liquid ratio [g/L]	15.4		
	Oil yield	Acid number	OD 490
Predicted values	32.31	3.01	0.914
Experimental values	33.14 ± 0.02	3.07 ± 0.13	0.897 ± 0.012

Multiple linear regression

For the purpose of the fitting the presented results in Table 2, polynomial regression of the second order (Equation 1) with regression coefficient $R^2 = 0.97$ for yield as parameter Y1, $R^2 = 0.94$ for OD490nm as parameter Y2 and finally $R^2 = 0.85$ for acid number as parameter Y3 was applied.

Regression coefficient analysis

Regression coefficients of the model for yield, acid value and OD490nm obtained by multiple polynomial regression are presented in Table 3. Dependent variable in coded form (Table 1) allow 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 (marked as bold in the Table 3) obtained from statistical analyse.

Determination and experimental validation of the optimal conditions

Optimal values of the parameters for extraction of the flax seed oil by petroleum ether are presented in Table 4. Values of other dependent variables (acid value and OD490) at optimal conditions for flax seed oil extraction were predicted on the base of created models.

Predicted value for extraction yield of oil, acid value and OD 490 as dependent parameters were comparable with experimentally measured value at the level of the statistical significance at $p<0.05$. Achieved results confirm the possibility to predict the course of the extraction flax seeds oil by petroleum ether by the model under particular experimental conditions.

DISCUSION

Commercial production of edible oils is based on mechanical pressing and extraction from oilreach materials **(Pradhan et al., 2010)**. The yield obtained by mechanical pressing is usually lower than those extracted by solvents such as hexane or petroleum ether. The advantage of solvent extraction is the high yield that can be obtained economically with this method (>99 wt.%) **(Bargale et al., 1999; Willems, Kuipers, Haan 2008)**.

Based upon the findings in this work, petroleum ether is better solvent for flax seed oil extraction (Figure 1). The maximum oil recovered was 27.5% with petroleum ether and 20.1% with hexane when the extraction process was carried out for 24 hours. Similar yields were obtained in work **Gutiérrez et al. (2010)**.

The yield oil was increased with increasing temperature up (Figure 2). By increasing the temperature approaching to the boiling point of the solvent, both the diffusion coefficient and the solubility of the oil in the solvent are enhanced, thus improve the extraction rate **(Richardson, Harker, 2002)**.

The solid to liquid ratio of 20 g/L would be sufficient for extraction of the maximum amount of oil (Figure 1). However, when the ratio continued to increase, the yield of oil was decreased. Therefore, 11; 50; 107.5; 165 and 203.7 (g/L) were selected as the variable levels for the solvent-liquid ratio. These results were observed other authors **(Pinelo et al., 2005; Zhang et al., 2009)**.

Flax seed content approximately 31.9 – 37.8 % oil depending on the material **(Carter, 1993)**. The oil yield obtained by 365 minutes extraction at 33.5 °C and with solid – liquid ratio 15.4 g/L, which were computed by response surface methodology, was about 33.14 %. In the work **Pradhan et al. (2010)** the flax seed was extracted using solvent hexane, supercritical carbon dioxide and mechanical pressing. They were found that the hexane extraction gave improved yield in comparing with supercritical CO_2 and screw press (9.02 and 34.3 %, respectively). In the other studies used supercritical fluid extraction of flax seed and they observed oil yield within 26.7 – 30.0 **(Jiao et al. 2008, Özkal, 2009)**.

CONCLUSION

The method of the designed experiment was successfully applied on optimization of the extraction of the oil from flax seeds. Model polynomial regression of the second order gives the successive description of the experiments. Calculated optimal conditions of the extraction process, expressed by the yield of the oil were as follows: temperature 33.5 °C, extraction time 365 minutes and solid-liquid 15.4 g of flax seed to 1 L of petroleum ether. Predicted values of dependent variables were comparable with the experimentally measured values.

REFERENCES

AYALA, R. S., LUQUE DE CASTRO, M. D. 2001. Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. In *Food Chemistry*, vol. 75, 2001, no. 1, p. 109-113.

BARGALE, P. C., FORD, R. J., SOSULSKI, F. W., WULFSOHN, D., IRUDAYARAJ, J. 1999. Mechanical oil expression from extruded soybean samples. In *Journal of the American Oil Chemists Society*, vol. 76, 1999, no. 2, p. 223-229.

BELL, J. G., MCGHEE, F., CAMPBELL, P. J., SARGENT, J. R. 2003. Rapeseed oil as an alternative to

marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil wash out. In *Aquaculture*, vol. 218, 2003, no. 1-4, p. 515-528.

CARTER, J. F. 1993. Potential of flaxseed and flaxseed oil in baked goods and other products in human nutrition. In *American Association of Cereal Chemists*, vol. 38, 1993, no. 10, p. 753-759.

DYERBERG, J., BANG, H. O., AAGAARD, O. 1980. α-Linolenic acid and eicosapentaenoic acid. In *Lancet*, vol. 315, 1980, no. 8161, p. 199.

GAUR, R., SHARMA, A., KHARE, S. K., GUPTA, M. N. 2007. A novel process for extraction of edible oils: Enzyme assisted three phase partitioning (EATPP). In *Bioresource Technology*, vol. 98, 2007, no. 3, p. 696-699.

GECGEL, U., GUMUS, T., TASAN, M., DAGLIOGLU, O., ARICI, M. 2011. Determination of fatty acid composition of γ-irradiated hazelnuts, walnuts, almonds, and pistachios. In *Radiation Physics and Chemistry*, vol. 80, 2011, no. 4, p. 578-581.

GORJÃO, R., AZEVEDO-MARTINS, A. K., RODRIGUES, H. G., ABDULKADER, F., ARCISIO-MIRANDA, M., PROCOPIO, J., CURI, R. 2009. Comparative effects of DHA and EPA on cell function. In *Pharmacology and Therapeutics*, vol. 122, 2009, no. 1, p. 56-64.

GUTIÉRREZ, C., RUBILAR, M., JARA, C., VERDUGO, M., SINEIRO, J., SHENE, C. 2010. Flaxseed and flaxseed cake as a source of compounds for food industry. In *Journal of Plant Nutrition and Soil Science*, vol. 10, 2010, no. 4, p. 454-463.

JIAO, S. S., LI, D., HUANG, Z. G., ZHANG, Z. S., BHANDARI, B., CHEN, X. D., MAO, Z. H. 2008. Optimization of supercritical carbon dioxide extraction of flaxseed oil using response surface methodology. In *International Journal of Food Engineering*, vol. 4, 2008, no. 4, p. 42-56.

JORDAN, R. G. 2010. Prenatal omega-3 fatty acids: review and recommendations original. In *Journal of Midwifery Womens Health*, vol. 55, 2010, no. 6, p. 520-528.

MARANGONI, F., COLOMBO, C., MARTIELLO, A., POLI, A., PAOLETTI, R., GALLI, C. 2007. Levels of the n-3 fatty acid eicosapentaenoic acid in addition to those of alpha linolenic acid are significantly raised in blood lipids by the intake of four walnuts a day in humans. In *Nutrition, Metabolism, and Cardiovascular Diseases*, vol. 17, 2007, no. 6, p. 457-461.

Norme Grassi e Derivati, edited by Stazione Sperimentale per le Industrie degli Oli e Grassi, Milano, Method NGD C10- 1976 (1976).

ÖZKAL, S. G. 2009. Response Surface Analysis and Modeling of Flaxseed Oil Yield in Supercritical Carbon Dioxide. In *Journal of the American Oil Chemists' Society*, vol. 86, 2009, no. 11, p. 1129-1135.

PINELO, M., RUBILAR, M., JEREZ, M., SINEIRO, J., NUNEZ, M. J. 2005. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. In *Journal of Agricultural and Food Chemistry*, vol. 53, 2005, no. 6, p. 2111-2117.

PRADHAN, R. CH., MEDA, V., ROUT, P. K., NAIK, S., DALAI, A. K. 2010. Supercritical CO2 extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. In *Journal of Food Engineering*, vol. 98, 2010, no. 4, p. 393-397.

RICHARDSON, J. F., HARKER J. H. 2002. Coulson and Richardson's Chemical Engineering-Particle. In *Technology and Separation Processes.* Butterworth-Heinemann, ISBN 0750644451, p. 1185.

ROSENTHAL, A., PYLE, D. L., NIRANJAN, K. 1996. Aqueous and enzymatic processes for edible oil extraction. In *Enzyme and Microbial Technology*, vol. 19, 1996, no. 6, p. 402-420.

TEITELBAUM, J. E., WALKER, W. A. 2001. Review: the role of omega 3 fatty acids in intestinal inflammation. In *Journal of Nutritional Biochemistry*, vol. 12, 2001, no. 1, p. 21-32.

TIGRINE-KORDJANI, N., MEKLATI, B. Y., CHEMAT, F. 2006. Microwave 'dry' distillation as an useful tool for extraction of edible essential oils. In *International Journal of Aromatherapy*, vol. 16, 2006, no. 3-4, p. 141-147.

TURNBULL, T., CULLEN-DRILL, M., SMALDONE, A. 2008. Efficacy of omega-3 fatty acid supplementation on improvement of bipolar symptoms: s systematic review. In *Archives of Psychiatric Nursing*, vol. 22, 2008, no. 5, p. 305-311.

VYHNÁNKOVÁ, L. 2007. PUFA omega-3 a jejich působení. In *Pediatrická prax,* vol. 3, 2007, no. 1, p. 141-143, ISSN 1803-5264.

WILLEMS, P., KUIPERS, N. J. M., DE HAAN, A. B. 2008. Hydraulic pressing of oilseeds: experimental determination and modeling of yield and pressing rates. In *Journal of Food Engineering*, vol. 89, 2008, no. 1, p. 8-16.

ZHANG, Q. A , ZHANG, Z. O., YUE, X. F., FAN, X. H., LI, T., CHEN, S. F. 2009. Response surface optimization of ultrasound-assisted oil extraction from autoclaved almond powder. In *Food Chemistry*, vol. 116, 2009, no. 2, p. 513-518.

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