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ORIGINAL PAPER

Camelina sativa Oil Deodorization: Balance Between Free Fatty Acids and Color Reduction and Isomerized Byproducts Formation

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Abstract *Camelina sativa* oil is characterized by its high content (up to 40 wt%) of α-linolenic acid and its unique flavor. It is considered to have beneficial health properties and is suitable for food and cosmetic uses. In the present study, response surface methodology was used to optimize processing parameters for bench-scale deodorization of camelina oil. The mathematical models generated described the effects of process parameters (temperature, steam flow, time) on several deodorization quality indicators: free fatty acids (FFA), *trans* fatty acids (TFA), color, and polymerized triglycerides (PTG). These newly established models can be used as a tool to identify optimum deodorization process conditions within chosen constraints. Based on the optimization of minimum retained FFA with the constraint of a maximum allowable TFA, deodorization parameters can be defined. At a constant steam flow rate of 42 ml/h, a temperature range of 210–220 °C, and deodorization time of 70–120 min were defined. 220 °C appears to be a critical upper temperature limit; above this temperature, isomerization rates significantly increase.

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Introduction

Camelina sativa, an ancient oilseed crop, is a member of the Brassicaceae family with common names like false flax, gold of pleasure, and Leindotter [1]. Because of its past characterization as a weed, its cultivation has been essentially non-existent. Interest in *C. sativa* cultivation in parts of Central and Northern Europe, and North America has been renewed because of the healthful properties of the oil, its suitability for use in biofuel production, its favorable production economics and minimal input requirements [1, 2].

Due to the combination of its unique flavor, its high levels of α-linolenic acid $C_{18:3n-3}$ (30–40%) oleic acid $C_{18:1n-9}$ (15–20%), linoleic acid $C_{18:2n-6}$ (15–20%), and eicosenoic acid $C_{20:1n-9}$ (15–20%) and its low level of erucic acid $C_{22:1n-9}$ (about 3%) camelina oil is considered a value-added product. The presence of high levels of tocopherols (700 mg/kg) and phenolic compounds (128 mg/kg as chlorogenic acid) also makes it more oxidatively stable than other highly unsaturated oils such as flax [1–4]. In order to maximize retention of its valuable minor compounds, particularly tocopherols, cold-pressing may probably be the preferred method for extraction of camelina oil. Because cold-pressed camelina oil exhibits undesirable organoleptic properties, it must be deodorized prior to use in dietary or cosmetic applications.

Deodorization is employed to remove various oxidation and odoriferous compounds. In addition, free fatty acid (FFA) levels are reduced by deodorization. A fully refined oil contains low levels of FFA (usually <0.05%), is low in

peroxide value and *p*-anisidine value, and is bland in flavor and odor. One of the issues to be considered in deodorization of highly unsaturated oils is thermal degradation, which leads to the loss of valuable polyunsaturated fatty acids (PUFA). As the high temperatures used in deodorization favor PUFA degradation reactions such as cyclization, geometrical isomerization and polymerization, the presence of *trans* fatty acids (TFA) and polymerized triacylglycerols (PTG) are indicators that the deodorized oil has undergone thermal degradation [5]. It is a well-known fact that TFA is highly associated with coronary heart disease. There is a strong tendency in Europe to keep the TFA content in edible oil as low as possible [6]. Besides the formation of TFA and PTG, changes in the color of the oil, which can be evaluated through Lovibond color yellow (LCY) and red (LCR), may also be indicative of thermal degradation and accumulation of oxidation products [7]. Therefore, process parameters for deodorization of highly unsaturated oils must be selected to strike a delicate balance between maximizing removal of undesirable compounds (odor, flavor and FFA) and minimizing thermal degradation of PUFA.

To the authors' knowledge, no or limited studies have been done on the deodorization of camelina oil. Thus, an optimization study on deodorization of camelina oil will greatly enhance its market potential. The objective of this work was to optimize the deodorization parameters namely, temperature, steam flow, and time, through response surface methodology (RSM) to obtain a final product with good quality in terms of FFA, TFA, LCY, LCR and PTG. RSM has became a popular tool in lipid studies for product development and process optimization [8, 9]. In short, this method can provide a statistical mathematical model for prediction of a range of variables necessary to achieve desired responses. RSM can be used to predict the optimum deodorization conditions to achieve a finished oil with minimum levels of undesirable compounds while maintaining high levels of the desirable components. The major advantage of this technique is that it enables simultaneous evaluation and optimization of multiple components instead of evaluating a single factor at a time. RSM is an important tool for the design, development and formulation of new products, as well as for the improvement of existing product design [10].

Materials and Methods

Camelina Oil

Camelina seeds, which were purchased from a certified organic seed dealer, were expeller pressed to produce crude camelina oil. The crude oil was centrifuged (4,200 rpm, 30 min). To the crude oil was addedf 3% water and 0.5% citric acid (30% v/v) at 70 °C with 100 rpm agitation for 30 min. The gums were separated by precipitation and decantation. Physical characterization of the crude and degummed oil is presented in Table 1.

Experimental Design

A central composite design with three-factors-five-levels was chosen. The ranges for the variables, namely temperature (180–240 °C), steam flow (3–46 mL/h) and time (30–150 min) were selected to approximate typical deodorization conditions employed in industrial processing of edible vegetable oils. This design generated a total of 20 experimental runs using Design-Expert 7.0.0 (Stat-Ease, Inc.; Minneapolis, USA) software. The effect of deodorization at the central point was repeated 6 times and was combined with the results of 14 other single variations of conditions as presented in Table 2. All experiments were conducted in randomized order as suggested by the software to minimize the effect of unexplained variability in the observed response due to extraneous factors. The data were statistically analyzed and interpreted as described above. First or second order coefficients were generated by regression analysis with hierarchical backward elimination at 95% significance level ($P < 0.05$). Through backward elimination, insignificant ($P > 0.05$) factors and interactions

Table 1 Physical characterization of camelina oil

	Crude oil	Degummed oil
P	28.2	8.8
FFA	0.89	0.90
LCY	60.1	41.0
LCR	4.8	4.3
Fatty acid profile via GC (rel. %)		
C16:0	5.35	5.45
C18:0	2.33	2.58
C18:1n-9	14.33	14.82
C18:2n-6	16.28	16.14
C18:2n-6T	<0.01	<0.01
C18:3n-3	35.53	35.04
C18:3n-3T	<0.01	<0.01
C20:0	1.33	1.40
C20:1n-9	14.60	14.53
C20:2n-6	1.87	1.80
C20:3n-3	1.53	1.46
C22:1n-9	2.55	2.53

P phosphorous content (ppm), *FFA* free fatty acid content (as % of oleic acid), *LCY* Lovibond color yellow (5.25 in Lovibond cell), *LCR* Lovibond color red (5.25 in Lovibond cell), *T trans*

$n = 2$ replicate

Table 2 Actual settings and experimental results by the analysis of the samples of the RSM-generated experimental design for the camelina oil deodorization

Eno	Rno	T	S	t	FFA	C18:2n-6T	C18:3n-3T	TFA	LCY	LCR	PTG
1	11	195	2 (42)	60	0.53	<0.01	0.05	0.05	21.0	1.7	<0.1
2	5	225	2 (42)	60	0.31	0.04	1.15	1.19	14.5	3.3	0.2
3	12	195	6 (18)	60	0.71	<0.01	0.05	0.05	23.4	3.7	<0.1
4	10	225	6 (18)	60	0.42	<0.01	1.23	1.23	13.1	1.6	0.2
5	20	195	2 (42)	120	0.49	<0.01	0.22	0.22	17.0	2.5	<0.1
6	16	225	2 (42)	120	0.16	0.06	2.43	2.49	9.3	2.1	0.2
7	13	195	6 (18)	120	0.62	<0.01	0.22	0.22	19.0	4.0	<0.1
8	6	225	6 (18)	120	0.35	0.06	2.41	2.47	10.3	2.6	0.2
9	4	180	4 (30)	90	0.69	<0.01	0.02	0.02	36.5	4.1	<0.1
10	2	240	4 (30)	90	0.18	0.16	4.19	4.35	11.2	3.1	0.6
11	18	210	0 (46)	90	0.31	<0.01	0.52	0.52	10.0	1.7	<0.1
12	14	210	8 (3)	90	0.74	<0.01	0.56	0.56	11.3	2.1	<0.1
13	3	210	4 (30)	30	0.60	<0.01	0.06	0.06	25.4	3.0	<0.1
14	9	210	4 (30)	150	0.38	<0.01	0.94	0.94	11.3	2.4	<0.1
15	17	210	4 (30)	90	0.37	<0.01	0.51	0.51	13.4	2.2	0.1
16	7	210	4 (30)	90	0.42	<0.01	0.51	0.51	17.3	2.8	<0.1
17	15	210	4 (30)	90	0.37	<0.01	0.49	0.49	13.4	2.4	<0.1
18	8	210	4 (30)	90	0.39	<0.01	0.53	0.53	15.7	3.4	<0.1
19	19	210	4 (30)	90	0.36	<0.01	0.59	0.59	14.0	3.3	<0.1
20	1	210	4 (30)	90	0.45	<0.01	0.46	0.46	15.1	3.4	<0.1

Eno experiment setting number, *Rno* run-order number, *T* temperature (°C), *S* distance of heating body from the glass steam generator (cm), values in brackets show the actual steam flow at that distance (mL/h), *t* time (min), *FFA* free fatty acid content (as % of oleic acid), *T trans* (%), *TFA* a sum of $C_{18:2n\text{-}6T}$ and $C_{18:3n\text{-}3}$ (%), *LCY* Lovibond color yellow (5.25 in Lovibond cell), *LCR* Lovibond color red (5.25 in Lovibond cell), *PTG* polymerized triglycerides (%)

$n = 2$ replicate

between factors were removed. Responses were fitted with the parameters by multiple regression to produce a mathematical model (Eq. 1)

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j. \quad (1)$$

The fit of the model was evaluated by reference to the coefficient of determination (R^2) and analysis of variance (ANOVA). The main effect plot displays the predicted change in the response when parameters varied from their lowest to highest level; the third parameter in the design was set to the average value.

Deodorization Process

Bench-scale deodorization of camelina oil was conducted in 2,000 g batches. The deodorization apparatus has been previously described [11]. It consisted of a 5-L deodorizer flask, a steam generator with the steam tube extending to the bottom of the deodorizer flask, a fully-insulated round bottom condenser flask with a 50-mm cold finger trap containing ethanol, and a mechanical vacuum pump. The velocity of steam was controlled by regulating the distance between the 150 watt heating body and the glass steam generator. The oil was then deaerated by setting the vacuum pump at a constant pressure of 5 mbar. The deaerated oil was heated to 85 °C for 10 min to evaporate any residual water from the degumming process. Finally, the deaerated oil was heated to the desired temperature and deodorized. At the end of the desired deodorization time, the deodorized oil was cooled. Once the deodorized oil reached 60 °C, the vacuum was discontinued by introduction of nitrogen gas to the apparatus.

Determination of FFA

The FFA in camelina oil was determined according to the official AOCS method [12]. Analyses were done in duplicate.

Determination of Color

Lovibond colors were determined in duplicate, according to the official AOCS method [12]. The optical path length of the glass cell was 5.25.

Determination of PTG

PTG were analyzed by gel-permeation chromatography according to the official AOCS method [12]. Samples (approx. 50 mg/mL in tetrahydrofuran) were analyzed with a Hitachi-Merck HPLC Series 7000 (Hitachi-Merck, Japan) with a 10 µL sample loop and a PL-gel column (Polymer Laboratories Ltd., Shropshire, UK) of 7.5 mm internal diameter and 30 cm length. The column packing material was a highly cross-linked styrene-divinylbenzene copolymer with a particle diameter of 5 µm and a pore size of 100 Å. Elution was carried out with THF at 0.5 mL/min. Peaks were detected with a PL-ELS 2100 evaporative light scattering detector (Polymer Laboratories Ltd., Shropshire, UK). The detector conditions were as follows: evaporator temperature of 70 °C, nebulizer temperature of 50 °C and air as the nebulizing gas. Analyses were conducted in duplicate.

Determination of Fatty Acid Composition and TFA

Fatty acid composition and TFA content was determined by preparing fatty acid methyl esters (FAME) from camelina oil according to the method of Hamilton et al. [13]. Briefly, 1 mL of 0.5 M sodium hydroxide in methanol was mixed with 100 µL of oil in a screw-capped test tube and refluxed for 5 min at 80 °C. The sample was cooled and acidified with 1 mL of a 20% solution of boron trifluoride in methanol, and 0.5 mL of 0.1% hydroquinone in methanol was added. The mixture was heated to 80 °C for 2 min. After the mixture was cooled, 2 mL of 0.73% NaCl solution was added and mixed for 10 s. FAME were extracted with two 0.5 ml aliquots of hexane. 1.0 ml of saturated alkaline NaCl solution was added to the two hexane extracts and mixed, and the water phase was removed. The residual water was removed with anhydrous sodium sulfate. An aliquot was transferred to a GC vial for analysis. FAME were separated and analyzed by GC on a 120-m highly polar column (TRACE TR-FAME, 120 m × 0.25 mm ID × 0.25 µm, 70% cyanopropyl polysilphenylene-siloxane phase; Thermo Scientific, USA) following the official AOCS method [12]. FAME were prepared and analyzed in duplicate.

Results and Discussion

Model Evaluation

Table 2 shows the responses (FFA, TFA, LCY, LCR and PTG) for the 20 experimental runs. The best fitting models for all the responses were determined by multiple regression with backward elimination. A model for PTG content could not be established as the values in most of experiments were beyond the limit of quantification. The model coefficients, P and R^2 values for FFA, $\log_{10}$(TFA), LCY and LCR are given in Table 3. Additionally, the relationship between responses and the experimental parameters was tested with the ANOVA lack of fit test, which was insignificant in all situations. This evaluation showed that the models were acceptable and could be used for optimizing the parameters with respect to deodorization quality indicators.

Main Effects of Parameters on Responses

FFA

According to Table 3, all of the deodorization parameters (temperature, steam flow and time) had a significant ($P < 0.05$) effect on FFA. Figure 1 shows the effects of

Table 3 Regression coefficients, P and R^2 of the second order models after hierarchical backward elimination

Variables	FFA		$\log_{10}$(TFA)		LCY		LCR	
	$R^2 = 0.97$		$R^2 = 0.99$		$R^2 = 0.92$		$R^2 = 0.61$	
	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient	P
Intercept	2.52656	<0.0001	−24.33339	<0.0001	483.21250	<0.0001	−11.20147	0.0049
T	-8.78717×10^{-3}	<0.0001	0.15776	<0.0001	−4.08250	<0.0001	0.06042	0.0622
S	−0.01349	<0.0001			2.80625	0.4525	4.76967	0.1669
t	-5.98703×10^{-3}	<0.0001	0.06523	<0.0001	−0.09292	0.0001		
$T \times t$			-1.91539×10^{-4}	0.0003				
$T \times S$							−0.01958	0.0071
$T \times T$			-2.40219×10^{-4}	<0.0001	8.88889×10^{-3}	0.0003		
$S \times S$	7.44012×10^{-3}	0.0006			−0.32500	0.0074	−0.07004	0.0152
$t \times t$	2.40494×10^{-5}	0.0064	8.91760×10^{-5}	<0.0001				

T temperature (°C), S steam flow (mL/min), t time (min)

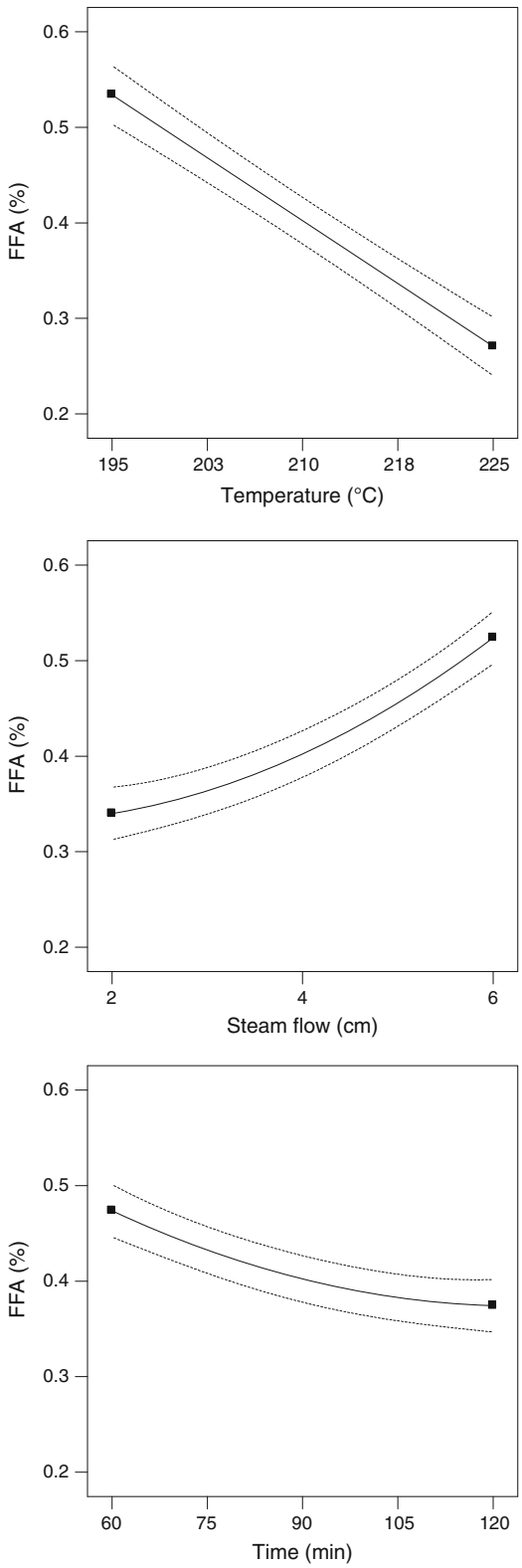

Fig. 1 Main effects of factors on FFA with 95% confidence intervals. The effect of each factor when it is varied from a low to a high level and all other factors that are kept at their averages are displayed. Actual coded values for factor steam flow were the following: 2 cm = 42 mL/h, 4 cm = 30 mL/h, 6 cm = 18 mL/h

these parameters on FFA. Temperature had a linear negative effect on FFA. The removal of FFA increases with increasing deodorization temperature. To reach the required FFA content level of 0.3%, which represents the removal of 66% of the initial FFA, a temperature of 220 °C is needed. Although FFA content can be further lowered by raising the deodorization temperature, this has detrimental effects on oil stability, formation of TFA and loss of valuable minor constituents such as tocopherols [7, 14]. Steam flow had a nonlinear negative effect on FFA, which is indicated by the significant ($P < 0.05$) second order of the steam flow. The optimal steam flow for maximum volatility of FFA is 42 mL/h (3.15% of steam in relation to the oil mass). The shape of the plot indicates that FFA volatility is satisfactory in the flow range between 18 and 42 mL/h of stripping steam. Additionally, the second order of steam flow indicates a lower FFA removal rate with a higher steam flow. This is explained by the fact that, when increasing the steam flow rate, the pressure increases slightly and thus the water or steam concentration is increased. Furthermore, less FFA is volatilized, when the pressure increases. The reaction time also had a nonlinear negative effect on FFA which is indicated by the significant second order of time. This is in correlation with the fact that the concentration of FFA is expected to follow an exponential decrease, i.e. the relative removal is constant. According to Figure 1, FFA decreased greatly from 60 to 100 min. From 100 to 120 min, FFA continued to decrease but to a lesser extent. This is not surprising, as most FFA has been removed by that time.

TFA

GC analysis shows formation of six different TFA, namely four for $C_{18:3n-3}$ and two for $C_{18:2n-6}$. In the present work, all *trans* fatty acids were combined and interpreted as single response. Due to a range of response values, its transformation (base 10 log) was required. Temperature, time, their second order parameters and the interaction between them have a significant effect on the formation of TFA in camelina oil during deodorization, while steam flow does not influence the isomerization of PUFA (Table 3). To evaluate the interaction between temperature and time, a contour plot was constructed (Fig. 2). Temperature is the crucial parameter when setting deodorization limits for oils with high levels of PUFA, due to TFA formation. Although the limits of TFA content in refined vegetable oils have not been clearly defined, oils with TFA below 1.0–1.5% are considered good [15]. A TFA limit of 2.0% of the total fatty acids has been defined for food products in Denmark [16]. From the plot, it is observed that values below 1% TFA can be obtained at deodorization

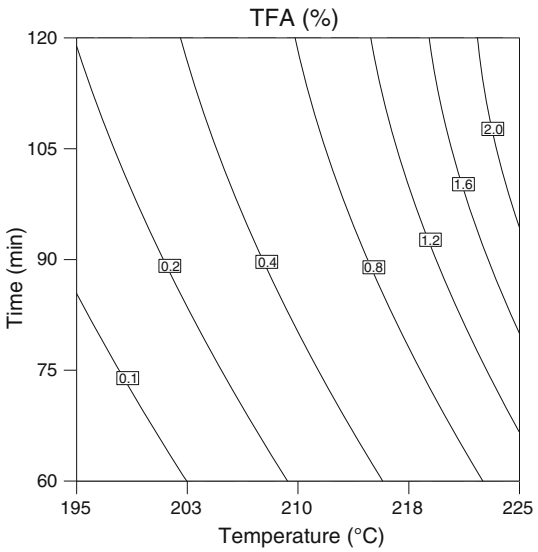

Fig. 2 Contour plot for the evaluation of effects of parameters and the optimization of the TFA. The effect of each factor when it is varied from a low to a high level and third factor that is kept at his average are displayed. Actual coded values for factor steam flow were the following: 2 cm = 42 mL/h, 4 cm = 30 mL/h, 6 cm = 18 mL/h

temperatures at or below 225 °C. TFA levels ranged from 0.02% at 180 °C to 4.35% at 240 °C (Table 2). It appears that a deodorization temperature of about 220 °C appears to be a critical limit beyond which *trans*-isomerization increases very strongly.

TFA formation during deodorization can be expressed on a relative basis by use of the term "Degree of isomerization" (DI) [17]. The DI of a given fatty acid expresses the percentage present in the *trans* configuration compared to the initial content (*cis* + *trans*). The degree of isomerization for camelina oil calculated for $C_{18:3n-3}$ and $C_{18:2n-6}$ at temperatures between 225 and 240 °C is 6.6 to 12% and 0.3 to 1%, respectively (results not shown). This indicates that the probability for a $C_{18:3n-3}$ molecule to be *trans*-isomerized during deodorization at 240 °C is 12 times higher than that for a $C_{18:2n-6}$ molecule. $C_{18:3n-3}$ is more labile than $C_{18:2n-6}$, and degree of isomerization is an important indicator of the intensity of the thermal treatment applied to vegetable oil during deodorization.

LCY and LCR

Table 3 shows that temperature and time significantly ($P < 0.05$) effected LCY (Fig. 3a). The second order of the steam flow also had a significant ($P < 0.05$) effect on LCY. Thus, the first order factor was included in the model due to its hierarchy. The characteristic pale yellow color of camelina oil is due to the presence of carotenoids. Oil used in the present experiment was not adsorptively bleached and therefore, the substantial decrease in color observed in LCY at temperatures up to 225 °C is the result of heat bleaching. Accumulation and formation of PTG at high temperatures may led to a higher LCY above that temperature [7, 18].

First order interaction between temperature and steam flow was an important factor because it significantly affected the response of LCR. Due to the low stability of the model, the prediction for LCR when deodorizing camelina oil was taken into consideration. However, the lowest LCR was obtained from the use of higher temperatures and lower steam flow and vice versa (Fig. 3b). The cause of this low LCR could be the result of hydrolysis reactions (high steam flow, low temperatures) or pigment destruction (low steam flow, high temperatures) in the oil.

PTG

PTG are products of oxidative and thermal degradation. Occurring in the unsaturated acyl moieties of triglycerides, they change the nutritional properties of fats. Determination of polymer levels can be done through size exclusion HPLC analysis, where these compounds (mainly dimers) appear as a small peak that is partly resolved from a huge triglyceride peak. Values below 0.1% could not be quantified. However, the effect of temperature is evident as dimerization mainly occurs only at temperatures at or above 225 °C. Deodorization at 240 °C for 90 min resulted in a PTG level of 0.6%. High initial oxidized triacyglycerols levels, which were mainly formed during pretreatment (degumming, bleaching, etc.) and storage, resulted in high levels of PTG after deodorization [7, 18]. Good quality crude oil (low FFA) and avoidance of the bleaching step resulted low PTG in all experiments.

Optimization of the Deodorization Process

The aim of this optimization process was to maximize removal of FFA and minimize formation of TFA in camelina oil. Figure 4 presents a contour plot, constructed from the selected predictive models for the specific response, for the targeted values of all responses (FFA < 0.3%, TFA < 1%, LCY < 13, and LCR < 2.7) in dependance on temperature and time at constant steam flow. Based on these criteria, the software created an overlay graph highlighting an optimum area of operability. The optimum deodorization conditions were determined to be in a temperature range of 210–220 °C, and deodorization time of 70–120 min at constant steam flow rate of 42 mL/h.

Conclusions

Among the studied process parameters (temperature, steam flow, time) deodorization pressure was set at a constant

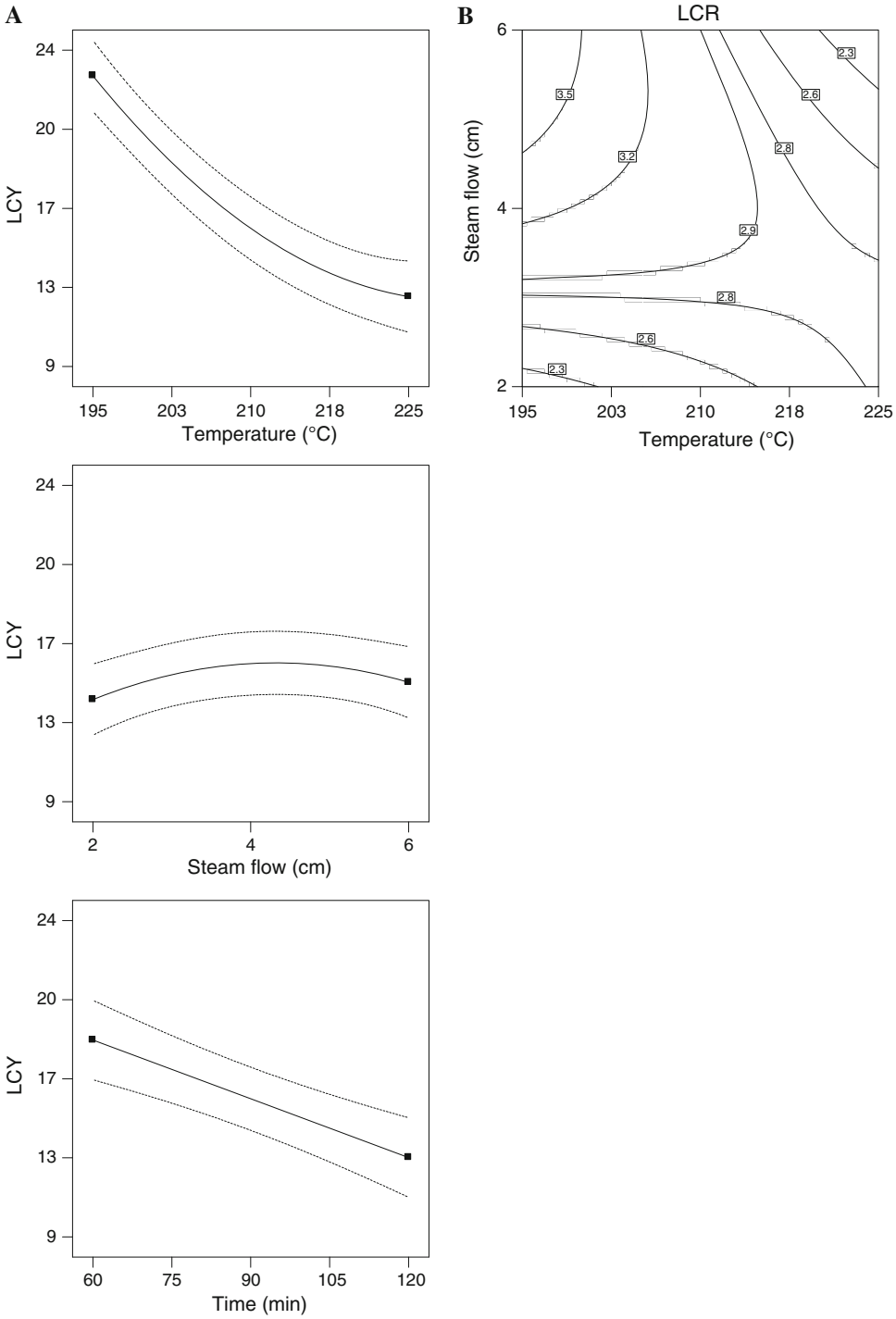

Fig. 3 Main effects of factors on LCY with 95% confidence intervals (**a**) and contour plot for the evaluation of effects of parameters and the optimization of the LCR (**b**). The effect of each factor when it is varied from a low to a high level (and second) and third factor that is kept at his average are displayed. Actual coded values for factor steam flow were the following: 2 cm = 42 mL/h, 4 cm = 30 mL/h, 6 cm = 18 mL/h

value (5 mbar). Typical industrial deodorization is performed at pressures <2 mbar, which is considerably lower than those used in the present study. In any case, a final optimization of deodorization parameters must be determined for each individual deodorizer. However, it can be concluded that single refining step-deodorization of camelina oil can be monitored using RSM. Temperature is the crucial factor for all responses. In combination with parameter time, increasing temperature increases the formation of *trans* PUFA, which is a disputable quality indicator. A compromise must be made in order not to exceed 1% of TFA. The best deodorization conditions are therefore found to be at a temperature range of 210–220 °C, and a time of 70–120 min at constant steam flow of 42 mL/h.

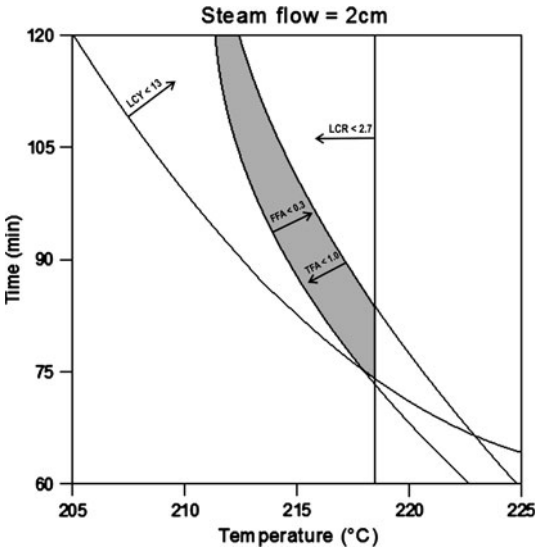

Fig. 4 Graphical optimization of the deodorization process for the desired values of FFA < 0.3%, TFA < 1%, LCY < 13, LCR < 2.7 (*shaded area*). Actual coded values for factor steam flow were the following: 2 cm = 42 mL/h, 4 cm = 30 mL/h, 6 cm = 18 mL/h

Data on the camelina oil deodorization procedure via RSM provide a basis for further investigation of pilot- and industrial-scale deodorization conditions.

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