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FORMATION OF GLYCIDYL ESTERS DURING THE DEODORIZATION OF VEGETABLE OILS

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Glycidyl esters are foodborne contaminants formed during the production of fats and oils, especially during the deodorization of palm oil. The hydrolyzed free form of glycidol has been categorized as probably carcinogenic to humans by the World Health Organization's International Agency for Research on Cancer. The aim of this research was to study the formation of glycidyl esters during the lab-scale deodorization of the three most widely produced seed oils in the world (sunflower, rapeseed and soybean). The effects of two independent factors – temperature and residence time – were analyzed by a 3^2 full factorial experimental design and evaluated by response surface methodology. In accordance with findings in the literature, the greatest amount of glycidyl esters was formed in the soybean oil matrix. For all three oils, the effects of both residence time and temperature were significant, while the latter was more so. To reduce the formation of glycidyl esters, milder deodorization is required, which is limited because of the purposes sought by the thermal operation and removal of volatile minor components and contaminants.

Keywords: glycidyl esters, deodorization, seed oils

1. Introduction

Glycidyl esters (GEs) are foodborne contaminants formed in fat-containing food and food ingredients during high-temperature thermal treatment. According to previous studies, glycidol is produced during digestion from the enzymatic hydrolysis of GEs [1,2]. The IARC (International Agency for Research on Cancer) has listed glycidol as a Group 2A or genotoxic carcinogen [3]. This year, the European Commission adopted the Commission Regulation (EU) 2018/290 that stipulates the maximum level of glycidyl fatty acid esters permitted in vegetable oils and fats, infant formula, follow-on formula and foods for special medical purposes intended for infants and young children. The maximum concentration of glycidyl fatty acid esters is 1 mg/kg in vegetable oils and fats placed on the market for end consumers or for use as an ingredient in food, and 0.5 mg/kg for vegetable oils and fats destined for the production of baby food and processed cereal-based food for infants and young children [4].

GEs are formed in vegetable oils during the refining process in the deodorization step, which is conducted at high temperatures (200-275 °C) under vacuum (of less than 10 mbar residual pressure) [5, 6]. Deodorization is the last step of refining of conventional edible oils and is intended to remove undesirable substances in order to improve the taste, odor, color and oxidative stability of such oils [7]. According to data from the literature, high levels of GEs are primarily measured in refined palm oil and its fractions. Destaillats et al. [8] showed in their study that GEs are formed from di- and monoacylglycerols (DAGs and MAGs), but not from triacylglycerols (TAGs). Accordingly, high levels of GE can be traced back to high levels of DAGs in crude palm oil [8]. The formation of GE starts at about 200 °C [8].

Analytical methods for the determination of GEs can be divided into two main groups: direct and indirect methods [9]. Individual GEs are determined by direct quantitation methods which are mainly based on liquid chromatography-mass spectrometry (LC-MS), requiring a significant number of reference compounds and internal standards [10, 11]. Indirect determination is based on the conversion of GEs into glycidol which is then isolated, derivatized, chromatographically separated and quantified. The result is expressed as the amount of glycidol that can be released from GEs. These methods require only a small number of internal standards [9].

In our study, the quantity of GEs in seed oil during lab-scale deodorization was determined in order to examine the effects of two independent factors – temperature and residence time – on the formation of GEs.

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2. Experimental

2.1 Samples and Measurements

Bleached sunflower, rapeseed and soybean oils were supplied by Bunge Limited (Bunge Zrt. Hungary and Bunge Ibérica, S.A.U.). Diethyl ether, ethyl acetate, *n*-hexane and high-performance liquid chromatography (HPLC)-grade water were obtained from VWR International Kft. (Debrecen, Hungary). Toluene, isohexane, sodium bromide and phenylboronic acid were obtained from Merck Kft. (Budapest, Hungary). Methanol, sodium hydroxide and anhydrous sodium sulfate were purchased from Reanal Laborvegyszer Kft. (Budapest, Hungary). The internal standards glycidyl palmitate-d_5 and 3-chloro-1,2-propanediol-d_5 (3-MCPD-d_5) were obtained from LabStandards (Budapest, Hungary). All reagents and chemicals were of analytical grade.

Lab-scale deodorization trials were conducted in 150 g batches at temperatures between 220 and 260 °C. The bleached oils (sunflower, rapeseed or soybean) were heated to the target temperature (220, 230, 240, 250 or 260 °C) within 10–15 minutes. The process lasted 3 hours at a pressure of 3–4 mbar using nitrogen as a stripping gas. Without breaking the vacuum, sampling was conducted after 0, 15, 30, 45, 60, 90, 120 and 150 minutes had elapsed.

The quantities of glycidyl esters were determined using the American Oil Chemists' Society (AOCS) Official Method Cd 29b-13, which is based on alkaline-catalyzed ester cleavage and transformation of the released glycidol into monobromopropanediol (MBPD) and derived free diols using phenylboronic acid (PBA). These derivatives are measured by the Gas Chromatography/Mass Spectrometry (GC/MS) coupled system (Agilent 6890 coupled with 5973) in the selected ion monitoring (SIM) mode. Quantitative determination was based on the deuterated internal standard using characteristic ions for derivatised glycidol-d_5 at m/z 150 and 245, and derivatised glycidol at m/z 147 and 240.

2.2 Experimental design and statistical analysis

The temperature and residence time were studied using response surface methodology (RSM). The results of the 3^2 full factorial experimental design (see Table 1) were evaluated by analysis of variance (ANOVA) models using Statistica 13. The center point of the 3^2 full factorial design (mid temperature 240 °C) and mid time 90 minutes) was repeated three times.

Only the significant effects (of main effects and interactions) were taken into account in the response surface methodology. The generalized polynomial model for describing the response of independent variables is given in

$$\begin{aligned} y &= \beta_0 + \beta_1 X_1 + \beta_2 X_1^2 + \beta_3 X_2 + \\ &+ \beta_4 X_2^2 + \beta_5 X_1 X_2 + \beta_6 X_1 X_2^2 + \\ &+ \beta_7 X_1^2 X_2 + \beta_8 X_1^2 X_2^2 \end{aligned} \quad (1)$$

Table 1: 3^2 full factorial experimental design

Independent variables		Levels		
		-1	0	+1
X1	Temperature (°C)	220	240	260
X2	Residence time (min)	0	90	180
Dependent Variables (Yi) Glycidyl esters (mg/kg)				

3. Results and Evaluation

3.1 Experiments

The results of the lab-scale investigation of GE formation are shown in Fig. 1. In our experimental design, the greatest amount of GEs formed in soybean oil, in which the concentration of GEs reached 5.5 mg/kg at 260 °C after 180 minutes (Fig. 1A). In the sunflower and rapeseed oils, the maximum concentrations of GEs reached were 1.6 and 1.5 mg/kg, respectively (Figs. 1B and 1C). The GE content of sunflower and rapeseed oils was kept under 1 mg/kg after 120 minutes of deodorization at a temperature of 250 °C or less, but for soybean oil this level was obtained at or below 230 °C. This demonstrates that the amounts of precursors in the oils strongly influence the formation of GE, and consequently the optimal deodorization temperature. The threshold concentration of

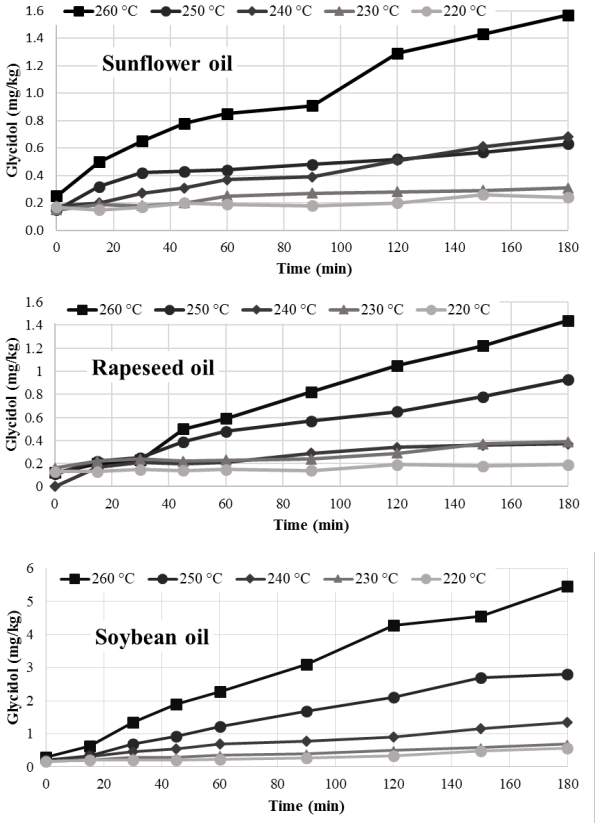

Figure 1: GEs of seed oils during deodorization: A) sunflower oil, B) rapeseed oil, C) soybean oil

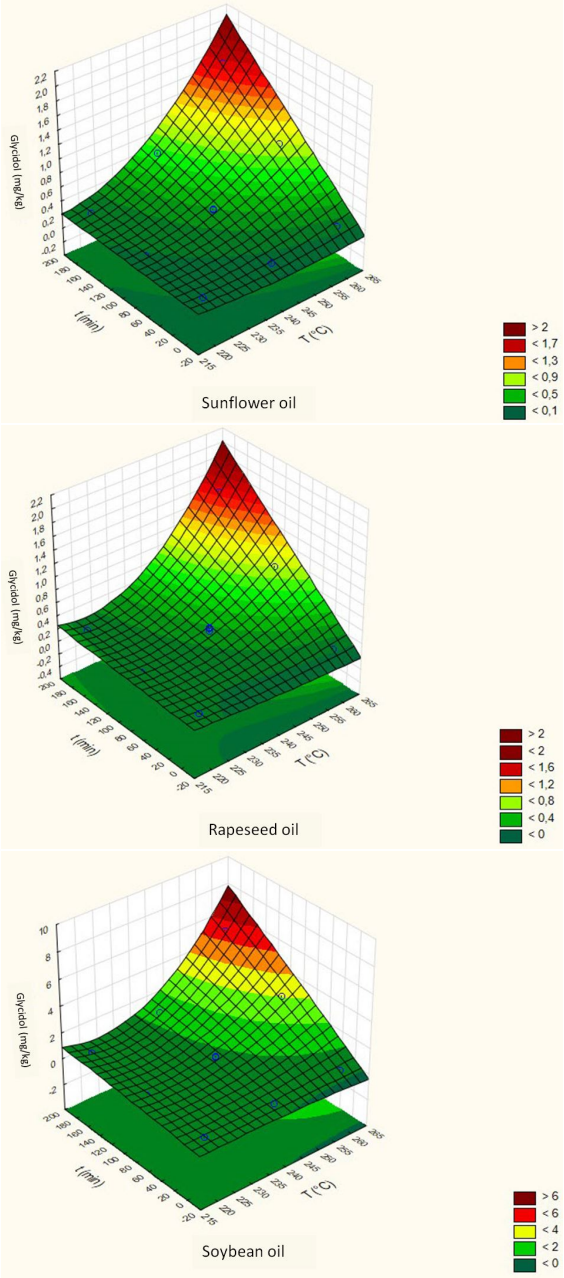

Figure 2: Fitted surfaces for seed oils: A) sunflower oil, B) rapeseed oil, C) soybean oil

0.5 mg/kg permitted for infant food was complied with at 240, 230 and 220 °C for rapeseed, sunflower and soybean oils, respectively (after 120 minutes of deodorization). In the applied experimental setup, up to 0.3 mg/kg of GE formed after 10–15 minutes of heating. At lower deodorization temperatures, the effect of time becomes practically insignificant, especially at 220 and 230 °C.

3.2 Statistical analysis

The application of RSM allowed the main effects and interactions to be determined simultaneously. ANOVA shows the significant effects, which can be used for building the response surface model. The fitted surfaces for sunflower, rapeseed and soybean oils are presented in Figs. 2A-C, respectively. The shapes of the surfaces are very similar, the only difference is in their heights. The interactions between the independent variables can be observed from the fitted surfaces, because at lower temperatures the concentrations of GEs gradually increased over time, while at higher temperatures a more rapid increase occurred.

Table 2: Regression coefficients for intercept (I), linear and quadratic factors, as well as interactions between factors in the fitted models of seed oils

	Sunflower oil	Rapeseed oil	Soybean oil
I	7.95	7.12	10.32
T	-6.72×10^{-2}	-5.9×10^{-2}	-9.02×10^{-2}
T^2	1.45×10^{-4}	1.23×10^{-4}	2×10^{-4}
t	1.17×10^{-1}	2.16×10^{-1}	1.14
t^2	n.s.	n.s.	n.s.
Tt	-1.13×10^{-3}	-1.96×10^{-3}	-1.01×10^{-2}
T^2t	3×10^{-5}	4×10^{-5}	2.2×10^{-5}
Tt^2	n.s.	n.s.	-2.38×10^{-8}

n.s.: effect not significant

For all three seed oils the temperature had the largest effect. The interaction between the independent variables and the effect of time were the second and third most significant, but the quadratic components and their interactions with the other factors were noticeable in most cases, as well. The regression coefficients are shown in Table 2 coefficients in the case of sunflower and rapeseed oils are very similar so the RSM diagrams of these oils fall within the same range of values (Figs. 2A and 2B).

4. Discussion

According to the data from the literature, the oil that has been studied the most in this respect is palm oil along with its fractions [8, 12]. Cheng et al. [13] summarized the data from previous studies and according to this review the highest concentrations of GEs in seed oil were found in soybean oil when compared to rapeseed and sunflower oils. This is in agreement with our observations. The higher concentrations of GEs that formed during deodorization were due to the higher levels of DAGs and MAGs in the raw material.

It was found that the critical temperature range is between 220 and 240 °C, above which more than 0.5 mg/kg of GEs may form, depending on the quality of the raw material. This conclusion is similar to the results of previous investigations. Craft et al. [12] concluded that between 230 and 240 °C, the formation of GE is extensive, consequently this value should be considered as an upper limit for the deodorization process. De Kock et al. [14] suggested conducting deodorization for a longer period

of time at temperatures below 240 °C, which might also minimize the formation of trans fatty acids.

5. Conclusion

The present investigation suggests that the formation of GEs in seed oils during deodorization is not negligible. The rate of formation can be traced back to the level of DAGs and MAGs [15] in the raw material. A simultaneous increase in temperature and time could result in extremely high levels of GEs in oils. On an industrial scale, the formation of GEs can be controlled in the oils examined, meaning that the upper limit of GEs (1 mg/kg) in vegetable oils and fats placed on the market for general consumption can be achieved through preventive measures. The stricter limit imposed on oils destined for the production of food for infants and young children presents greater challenges, and thus requires a combination of high quality raw materials as well as a controlled refining process.

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Symbols

β_{0-8}	Regression coefficients for intercept, linear and quadratic factors and interactions between factors
$X_1,\ X_2$	Independent factors
T	Deodorization temperature
t	Deodorization time

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