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Characteristics of denatured rapeseed oil during storage and refining processes

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RESUMEN

Características del aceite de colza desnaturalizado durante los procesos de almacenamiento y refinación.

En 1981 tuvo lugar en España el llamado «Síndrome del Aceite Tóxico», una enfermedad progresiva multi-sistemática causada por el consumo de aceite de colza desnaturalizado con anilina. Hasta la fecha, el agente o agentes tóxicos causantes permanecen desconocidos. Se han realizado medidas de acidez, humedad, impurezas, fósforo, jabones y determinaciones espectrofotométricas de color a 409 nm. Puesto que las concentraciones de anilidas de los ácidos grasos en estos aceites se asocian al riesgo de padecer la enfermedad, se estudiaron la formación de compuestos derivados de anilina durante el tiempo de desnaturalización y con las temperaturas y tiempos de desodorización (temperaturas: 200°C, 215°C, 230°C, 245°C, 260°C, y 270°C y tiempos: 3, 4, 4.5, 5, 5.5 y 6 horas). La formación de anilidas de ácidos grasos aumenta con el tiempo de almacenamiento. La desodorización conlleva una reducción de la cantidad total de anilidas en todas las muestras, particularmente cuando la temperatura es superior a 245°C. No fueron detectados ésteres de 3-(N-fenilamino)-1,2-propanodiol.

PALABRAS-CLAVE: Aceite de colza - Anilidas de ácidos grasos - Síndrome del aceite tóxico - 3-(N-fenilamino)-1,2-propanodiol.

SUMMARY

Characteristics of denatured rapeseed oil during storage and refining processes.

In 1981, toxic oil syndrome, a progressive multi-system disease caused by consumption of rapeseed oil denatured with aniline occurred in Spain. To date, the causal toxic agent or agents remain unknown. Measures of acidity, moisture, impurities, phosphorous, soaps, and spectrophotometric determinations of color at 409 nm were performed. Since fatty acid anilide concentrations in these oils are associated with risk of disease, we studied the formation of aniline-derived compounds over time after oil denaturation and by oil deodorization temperatures (200°C, 215°C, 230°C, 245°C, 260°C, y 270°C) and times (3, 4, 4.5, 5, 5.5 and 6 hours). Formation of fatty acid anilide compounds increased with storage time. Deodorization led to a reduction of total anilides in all the samples, particularly at temperatures above 245°C. Esters of 3-(N-phenylamino)-1,2-propanediol were not detected.

KEY-WORDS: Fatty acid anilides - Rapeseed oil - 3-phenylamino -1,2-propanediol - Toxic oil syndrome.

1. INTRODUCTION

In May 1981, an epidemic occurred in Spain as a result of consumption of rapeseed oil that was denatured with aniline and sold as cooking oil (Tabuenca, 1981). The disease became known as toxic oil syndrome (TOS) (Grandjean *et al.*, 1984). The vast majority (99%) of the TOS cases occurred in the central and northwestern provinces of Spain. Three cases, however, were identified in the southern and were the result of ingestion of oil from the *Industria Trianera de Hidrogenación* (ITH) oil refinery in Seville (Posada *et al.*, 1987). Previous work has shown that the oil refined by ITH and distributed by the RAELCA company of Madrid was the principal, and probably the only, oil responsible for the TOS epidemic (Posada *et al.*, 1996).

Although several contaminants, mainly those formed by reaction of aniline and oil components in suspect oils have been identified (Hill *et al.*, 1995; Bernert *et al.*, 1987), the precise etiologic agent or agents remains unknown; however, researchers have established that increasing concentrations of fatty acid anilides in the oils are associated with an increased risk of disease (Kilbourne *et al.*, 1988; Posada *et al.*, 1994).

Compounds other than fatty acid anilides, in particular, 3-(N-phenylamino)-1,2-propanediol (PAP), the 3-oleyl-ester of PAP (OPAP), and the 1,2-dioleyl ester of PAP (OOPAP), were found in aniline denatured rapeseed oil refined at ITH, but not in other stocks of denatured rapeseed oil refined elsewhere (Posada *et al.*, 1991; Posada *et al.*, 1996). Moreover, these PAP esters were not detected in unrefined aniline denatured samples of rapeseed oil delivered to ITH (Hill *et al.*, 1995). Hence, it is likely that the causal toxic agent(s) were formed in the denatured rapeseed oil during the refining process. Studies of denatured rapeseed oil during oil transport and refining were conducted by Vázquez-Roncero *et al.* in 1983, but the formation of the PAP compounds mentioned above has not previously been studied.

Our objective was to reproduce the refining process that took place at the ITH refinery in 1981 under laboratory conditions, and to produce denatured, refined rapeseed oils that have specific characteristics similar to those of known toxic oils. In this work we studied the effects of deodorization temperatures and deodorization operation times on the oil in order to identify the refining conditions necessary for toxin formation.

2. MATERIALS AND METHODS

The processing conditions used by ITH and described in the third ITH report to the World Health Organization - Fondo de Investigaciones Sanitarias (WHO-FIS) committee were used as the starting point for determining experimental conditions (Posada *et al.*, 1995).

2.1. Samples

A 50 kg lot of low erucic acid (< 1%) rapeseed oil (Koipesol, SA) from the Andalusian region of Spain was divided into 5 kg batches and each batch was denatured with 2% w/w aniline (99.5% purity). After denaturing but prior to refining, each batch was stored at room temperature for one of five different time periods: 1, 2, 3, 4, and 8 weeks. After storage, the following samples were analyzed: (a) denatured crude oil; deodorized oils at (b) 270°C/6h; (c) 260°C/6h; (d) 260°C/5.5h; (e) 245°C/6h; (f) 245°C/4.5h; (g) 230°C/5h; (h) 215°C/4.5h; and (j) 200°C/4h.

2.2. Treatment

Each sample of oil was processed as follows:

(a) Neutralization. Approximately 4.5 kilogram (kg) of crude oil was charged into a 5-liter, three-necked, round-bottomed stirred tank reactor. The tank reactor was fitted with an outlet in the lower part, a thermometer and a stirring shaft with two stainless steel blades driven by a 70 rpm stirring motor. The stirring motor was started, the oil temperature was brought to 40°C and the required amount of 20° Bé (4.2 M) sodium hydroxide was added (volume of sodium hydroxide in liters to be added = kg of oil x acidity x 0.85), The mixture were then heated to 80°C over a maximum time of 35 min. When the mixture reached 70°C a brine solution [10% ClNa (w/w)] was added in an amount equal to 75% of the volume of sodium hydroxide previously added. After stirring for 20 min at 70°C, the soap stocks were separated from the refined oil by centrifugation (10 min at 3000 rpm).

(b) Washing. The oil was washed twice at 70°C with 10% brine in an amount equal to 3% by weight of oil. The contents were then heated to 90°C, stirred at 95 rpm for 10 min, and the oil separated by centrifugation for 10 min at 3000 rpm.

(c) Bleaching. The oil was dried by increasing the temperature of the 2.4 kg of washed oil from 60°C to 90°C under 60 Torr vacuum. After stirring for 15 min at 90°C, 1% by weight bleaching earth (Type C, Minas de Gador, Almería, Spain) was added. The oil was then heated to 110°C, held at 110°C for 20 minutes with continuous stirring, filtered through filter paper (Esteryfil 123, Barcelona, Spain) in a laboratory filter press and allowed to cool. The bleached oil was divided into 6 lots of 260 g each which were stored at -24° C until deodorization.

(d) For each deodorization, 200 grams of the prepared rapeseed oil sample were placed in a round-bottomed, three-necked flask, which was then heated using a hemispherical heating mantle with thermo regulator connected by a PID-controller with a thermocouple. The flask was connected with the steam source regulated by a stopcock; with the vacuum pump by cold traps and with a sample collector. Deodorization was performed for 60 hours under a vacuum of less than 3 mm Hg. The oil temperatures during deodorization were 200, 215, 230, 245, 260 and 270°C and the total amount of steam was 3% in all cases. After cooling the oil to 40°C, the vacuum in the flask was broken with nitrogen. Samples were collected during deodorization at 3.5, 4, 4.5, 5, 5.5, and 6 h.

2.3. Analytical determinations

The classical measures for evaluating oils, that is, measures of free fatty acids (FFA), moisture, impurities, phosphorous, soaps, and spectrophotometric determinations of color at 409 nm, were performed according to American Oil Chemists' Society analytical methods (AOCS, 1987).

Additionally, liquid chromatography combined with atmospheric pressure ionization tandem mass spectrometry was used to analyze the samples for the presence of PAP, 1-oleyl-ester of PAP (OPAP), and OOPAP and the following fatty acid anilides: linolenanilide (LNA); linoleanilide (LA); oleanilide (OA); and eicosenanilide (ECA). Detection limits for anilides were 2 ppm (S/N=3). Their Relative Standard Deviations (% SD) for anilides depended on concentrations and ranged from 5% to 3% for concentrations between 16 ppm and 80 ppm (Hill *et al.*, 1995).

3. RESULTS AND DISCUSSION

Table I shows the acidity, phosphorous concentration, and soap concentration in crude, neutralized, and bleached oils by time before refining.

Table I

Characteristics of aniline-denatured rapeseed oil stored for varying amounts of time before refining process

Storage Time (weeks)	Crude rapessed oil with 2% aniline Acidity (% oleic acid)	Neutralized and washed oil		Bleached oil	
		Acidity (% oleic acid)	Soaps (ppm)	Acidity (% oleic acid)	Phosphorous (ppm)
1 week	1.52	0.35	45	0.41	95
2 weeks	1.41	0.36	42	0.43	95
3 weeks	1.42	0.41	64	0.44	84
4 weeks	1.45	0.42	52	0.47	99
8 weeks	1.48	0.42	56	0.53	99

Note: Mean of two replicates ± 0.02
Crude rapeseed oil with 2% aniline (before storage time): Acidity=1,75% oleic acid; Moisture = 1.68% and Phosphorous = 300 ppm. Percentage of fatty acids: C16:0 = 4.5%; C18:0 = 2%; C20:0 = 0,5%; C16:1 = 0.25%, C18:1 = 58%, C18:2 = 20%, C18:3 = 10%, C20:1 = 1.5%, C22:1 = 0,5%.

The color and moisture of the oils we produced were almost identical to those of normal crude rapeseed oil, although the acidity of our oils diminished slightly during the first two weeks of storage period. The crude oil sampled from ITH was reported to have been characterized by a unpleasant color and smell (Kochhar and Rossell, 1984); however, the denatured crude oils in this study had neither of these characteristics.

The aim of the refining process is to improve the edible oil quality by removing undesirable compounds. Separation of phospholipids, FFA, pigments and volatile compounds responsible for off-flavours from the oil are foreseen in the different steps of the process: degumming, neutralization, washing, bleaching, and deodorization.

The operation conditions for neutralization described in the WHO-FIS committee report as those used for the neutralization step at ITH are similar to conditions used in the refining of olive oil, that is, no degumming step was used. However, the recommended conditions during caustic refining of rapeseed oil should be similar to those used for soybean oil, including the use of a degumming step prior to neutralization. Although it is possible to do caustic refining without acid pretreatment, degumming is used for a better reduction of phosphatides, color, and FFA (Carr, 1990).

Typically rapeseed oil is refined using 17°Bé sodium hydroxide mixed with the oil in an amount 0.07% in excess of the theoretical amount necessary to neutralize the FFA, however, because equipment may vary, it is prudent to carefully optimize the amount of sodium hydroxide used to refine the rapeseed oil (Carr, 1990). Nevertheless, excess lye was not used at ITH; the process ITH followed was similar to that used for olive oil, which is partial neutralization, followed by removal of residual FFA in the deodorization step.

After caustic refining samples were washed with water to reduce the amount of residual soaps to between 10 ppm and 15 ppm and to remove any phosphatides remaining in the oil since residual soaps would increase the amount of bleaching earth needed in the next step.

In these assays, stable emulsions were formed with water in the washing step, which forced us to use brine as the wash solution. The final acidity in the neutralized and washed oils we produced was above 0.30% in most of the samples, and the phosphorous level was approximately 100 ppm in the bleached oils, which is indicative of an ineffective neutralization step. Despite having used a brine solution to wash the oils, approximately 50 ppm of soaps was detected in washed oils.

Table II shows changes in the color index of the samples. The samples showed green color indicated that the bleaching step had not been carried out properly, partly because of the presence of phosphorous (approximately 100 ppm in the bleached oils) and soaps and partly because of the low acidity of the bleaching agent used. The conditions used led to only a 20% reduction of color.

Compared to soybean oil, rapeseed oil is bleached at a higher temperature using higher levels of bleaching agents due to its larger quantity of chlorophyll, or chlorophyll derivatives, which impart a green color to the oil. Yellow and red carotenoid pigments normally present in the oil usually mask the green color. Once the yellow and red pigments are removed in the refining process, the green color becomes apparent, often as late in the process as

Table II

Color Index * in denatured rapeseed oil samples stored for different time periods before refining and deodorizing at different temperatures and operation times

		Storage time (weeks)				
		1	2	3	4	8
Crude rapeseed oil with 2% aniline		1.63	1.64	2.04	1.70	1.62
Neutralized and Washed oil		1.53	1.53	1.47	1.53	1.52
Bleached oil		1.18	1.18	1.13	1.02	136
Deodorizing						
Temperature (°C)	Time (h)					
200	4	0.73	0.69	0.62	0.69	0.81
215	4.5	0.62	0.61	0.54	0.56	0.56
230	5	0.43	0.46	0.43	0.47	0.50
245	4.5	0.38	0.45	0.38	0.36	0.42
245	6	0.38	0.44	0.39	0.40	0.40
260	4.5	0.31	0.39	0.33	0.39	0.32
260	5.5	0.30	0.37	0.31	0.38	0.34
270	6	0.27	0.26	0.25	0.33	0.28

* Color Index:, 409 nm absorption per g of oil; Mean of two replicates, S.E. ± 0.01

deodorization (Ericksson, 1995). This is what could have occurred in our case; throughout the refining process, the red and yellow pigments diminished and the final color of the oil was green. As the denatured oil was to be sold as olive oil, that point should even be positive for a fraudulent distribution.

Deodorization is the last step in oil processing, and its goal is to produce an oil with little or no flavor, little color, and a low FFA level (0.02% - 0.10%). Table III lists the acidity of the 6 h deodorized oils at different temperatures. Although the oil's odor disappeared during the deodorization process, the minimum level of FFA was 0.12%. This elevated FFA level might be attributed to triglyceride hydrolysis during deodorization which is catalytically accelerated by the FFA originally present and modifies the vapor-liquid phase equilibrium during distillative deacidification by means of steam stripping (Szabo, 1957).

Figure 1 shows the anilide formation in denatured rapeseed oil with storage time. The level of anilides obtained in samples stored for 8 weeks was higher than in those stored for 1 week in all of the samples evaluated. The levels of each fatty acid anilide were related to the fatty acid composition of the oil, with the level of oleyl anilide being the highest; thus, we found it curious that the linoleyl and linolenyl anilides seemed to be formed in similar amounts, since the percentage of linolenic acid is lower than that of linoleic acid.

Table III

Acidity (% of oleic acid) in denatured rapeseed oil samples stored for different periods of time before the refining and deodorizing at different temperatures for 6 hours

Acidity (% Oleic Acid)

	Storage time (weeks)				
T (°C)	1	2	3	4	8
200	0.31	0.32	0.45	0.42	0.44
215	0.28	0.32	0.35	0.31	0.32
230	0.32	0.24	0.43	0.36	0.28
245	0.25	0.24	0.25	0.32	0.28
260	0.31	0.20	0.20	0.21	0.22
270	0.12	0.16	0.22	0.21	0.27

Mean of two replicates, S.E. ± 0.02.

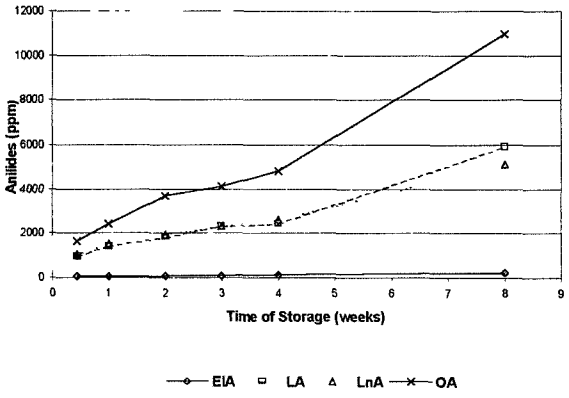

Figure 1
Anilide formation in aniline-denatured rapeseed oil following storage at 35 ± 2°C.
Abbreviations: LNA, Linolenanilide; LA, linoleanilide; OA, oleanilide and ECA, eicosenanilide; n.d., not detected (< 2ppm); ppm, parts per million, %Standard Deviation: 5% to 3% for concentrations between 16 ppm and 80 ppm.

Table IV lists by deodorization time and temperature the total anilide levels in the samples stored for different amounts of time prior to refining and deodorization. The deodorization step appears to lead to a reduction of total anilides in all of the samples. This reduction is most pronounced when the deodorization temperature is above 260°C. Relative proportions of individual anilides were not significantly altered during this step (Figure 2). Note that the total anilide content decreased substantially (92%) after deodorization. With the exception of eicosanoyl anilide, which showed only a marginal decrease following deodorization, all individual anilides decreased substantially in concentration after this step. These results are consistent with studies carried out by Vázquez-Roncero *et al.* (1982).

Table IV
Total content of anilides in denatured rapeseed oil samples stored for different periods of time before the refining and deodorizing at different temperatures and operation times

Anilide contents (mg/kg)						
		Storage Time (weeks)				
		1	2	3	4	8
Initial[a]		5355	7475	9174	9896	22210
T (°C)[b]	t (h)[c]					
200	4	3176	5458	6170	8583	23300
215	4.5	3102	4859	5769	8483	19800
230	5	2291	5652	6470	8590	19590
245	4.5	2233	4448	5866	7582	17680
245	6	2220	4395	5767	7383	18090
260	4.5	1787	1085	3616	6373	13180
260	5.5	1553	1735	2383	6283	11860
270	6	249	1069	1042	2560	1798

% Standard Deviation: 5% to 3% for concentrations between 16 ppm and 80 ppm.
[a] Crude rapeseed oil with 2% of aniline added; [b] Temperature of deodorization; [c] Length of deodorization

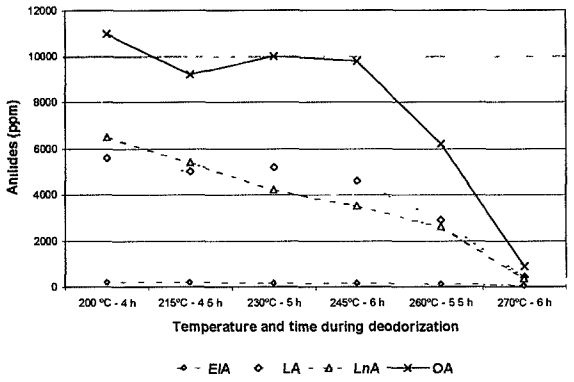

Figure 2
Anilides in deodorized samples from initial oil denatured and stored 8 weeks before the refining process.
Abbreviations: LNA, Linolenanilide; LA, linoleanilide; OA, oleanilide and ECA, eicosenanilide; n.d., not detected (< 2ppm); ppm, parts per million, %Standard Deviation: 5% to 3% for concentrations between 16 ppm and 80 ppm.

Neither OOPAP nor other diesters of PAP were detected in the samples we produced; however, these compounds are present in the oils obtained from ITH and are stable over long periods of time (Hill *et al.*, 1995). One hypothesis is that these types of compounds are formed by a reaction between aniline and diglycerides during the deodorization step (Kochhar and Rosell, 1984). Another possibility is that PAP-esters form initially upon heating but later decompose during the deodorization process. We investigated this possibility by measuring PAP-esters levels at different times during the deodorization process but found no evidence of PAP-esters formation (data not shown).

In conclusion, despite having used the refining conditions reported by ITH and recommended by the WHO-FIS committee, the oils we obtained did not have the same characteristics as those obtained at ITH, and PAP-esters was not detected in the oil samples we produced. We believe that the refining process followed at ITH in 1981 was not the appropriate process for a rapeseed oil, and that the process used led to the production of poor quality oil. Assuming that ITH followed the typical olive oil refining process, PAP-esters should not have been produced, as we have shown here. Thus, other conditions likely existed at the time of the refining of the toxic oil, which, owing to the presence of aniline, would have led to the formation of PAP-esters and other PAP related compounds. Additional studies to investigate these conditions are in progress.

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