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Contribution of denaturing and deodorization processes of oils to toxic oil syndrome

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ABSTRACT

In this article, denaturing with aniline and refining process of the oil responsible for toxic oil syndrome is briefly reviewed. The aspects considered include description of conditions of the main refining steps in relation to aniline derivatives formation, with special focus on the deodorization stage. In this last step of the refining process, an increase in the temperature, applied to such an unusual oil, could have given rise to the formation of anilides and 3-N-phenylamino-1,2-propanediol esters.

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1. Introduction

Olive oil is an expensive oil and hence its adulteration with cheaper vegetable oils has been a traditional fraud problem in the food sector. The most dramatic health consequence of this practice occurred in 1981, when denatured rapeseed oil intended for industrial use was fraudulently included in the food chain mixed with olive oil. The toxic oil syndrome (TOS) continues to have a special relevance in the edible oil field, mainly for two reasons: firstly, because of the high number of people who suffered and still suffer the consequences of this poisoning and, secondly, because determination of the toxic agent(s) that caused it remains unclear yet [1].

The denaturing agent used was aniline and, at first, the content of anilides, resulting from the reaction of aniline with oil, was the marker that allowed to distinguish the adulterated oil. In the 1990s, especially by virtue of great advances in analytical techniques, it was demonstrated that some derivatives that had been described years before (PAPs, 3-N-phenylamino-1,2-propanediol esters) [2] showed a higher risk than did the fatty acid anilides [3]. On the other hand, from epidemiological studies, it was established that no cases were registered in the Catalanian area of delivery. The vast majority (99%) of the TOS cases occurred in the central and north-western provinces of Spain. Three cases, however, were identified in the south and were the result of ingestion of oil from the *Industria Trianera Hidrogenación* (ITH) refinery in Seville [4].

The oil refined by the ITH company and distributed by commercial companies in Madrid was identified as the main, and probably

the only, oil responsible for the TOS epidemic [2]. Moreover, PAP esters were not detected in unrefined aniline-denatured samples of rapeseed oil delivered to ITH [5]. Hence it is likely that the causal toxic agent(s) were formed in the denatured rapeseed oil during the refining process, and that the process varied from one factory to another.

At that time, a systematic work plan was established between the *Instituto de la Grasa* in Seville (IGS) and *Centro de Investigación sobre el Síndrome del Aceite Tóxico* (CISAT) for determining, at both laboratory and industrial scale, those variables that led to the production of the toxic oil. The contents of oleoyl anilide (OA), mono-oleyl 3-N-phenylamino-1,2-propanediol (OPAP) and dioleoyl 3-N-phenylamino-1,2-propanediol (OOPAP) in the ITH oil were used as the gold standard for these experiments.

2. Refining process

Oil consists of a very complex mixture of triacylglycerols, partial glycerides, hydrocarbons, tocopherols, pigments, sterols, alcohols, triterpene acids, volatile compounds, phenolic compounds, phospholipids, and proteins. Refining of crude oil is done to remove unwanted minor components that make oils unappealing to consumers, trying to cause the least possible damage to the neutral oil as well as minimum refining loss. The components to be removed are all those glyceridic and non-glyceridic compounds detrimental to flavour, colour or stability of the refined oils and safety. They are primarily phosphoracylglycerols, free fatty acids, pigments, volatiles and contaminants [6].

Contaminants found in most crude oils are minor components commonly called persistent organic pollutants or POP. These include primarily chlorinated pesticides (DDT, toxaphene, aldrin, etc.), polycyclic aromatic hydrocarbons, dioxins, furans, polychlorinated biphenyls and polybrominated flame retardants

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Table 1
Basic steps of the refining process.

Alkali or chemical refining	Main groups of compounds removed	Physical refining
Degumming	Phospholipids	Degumming
Neutralisation	Free fatty acids	–
Bleaching	Pigments/metals/soaps/	Bleaching
Winterisation	Waxes/saturated triacylglycerols	Winterisation
Deodorisation	Volatiles/free fatty acids	Deacidification/distillation

(polybrominated diphenyl ethers). The persistent nature of POPs related to their great chemical stability leads to their significant accumulation in the food chain. The high liposolubility of these molecules also favours their high concentration in oils and fatty foods. The toxicity of POPs is clear and characterized by low daily dose tolerance hence the maximum residue amounts permitted in oils is very low [7].

The major steps involved in the refining process, and the main components removed are shown in Table 1.

- The purpose of degumming is to remove phospholipids or gums from the crude oil.
- The main objective of neutralization is the removal of free fatty acids, which can be made either by means of an alkali reaction (chemical refining) or by distillation (physical refining). Both are standard processes.
- A bleaching step is common to both physical and alkali refinings; the hot oil (around 100 °C) is slurried, with acid activated bleaching earth (1–2%), normally montmorillonite or natural hydrated aluminum silicate (bentonite). Under these conditions, adsorption of colour bodies, trace metals and oxidation products, as well as residual soaps and phospholipids remaining after washing neutralized oils, takes place. Treatment with an apolar adsorbent, e.g., activated carbon seems to be the most efficient process to eliminate dioxins and dioxin-like polychlorinated biphenyls, as well as heavy poly aromatic hydrocarbons [8].
- Deodorization/distillation of fats and oils normally consists of steam distillation at elevated temperature under vacuum, although nitrogen has also been used. The purpose of this step is to remove volatile compounds (mainly ketones and aldehydes) thus improving the oil taste and odour, and to eliminate the free fatty acids in physical refining and the residual free fatty acids from neutralized bleached oils. The deodorization conditions also contribute to remove contaminants, prolong shelf life, improve taste stability, and reduce the oil colour due to the breakdown of the remaining carotenes at high temperature. The efficiency of deodorization is a function of pressure (1–5 Torr), temperature (200–260 °C), residence time (0.5–3 h) and volume of stripping gas (1–3%) [8].

The main difference between alkali and physical refining processes is that alkali refining procedure includes caustic treatment to neutralise the oil while, following physical refining, free fatty acid are eliminated by distillation during deodorization.

The physical refining reduces the loss of neutral oil, minimises pollution and enables recovery of high quality free fatty acids. Nevertheless, not all the oils can be physically refined and, as a general rule, the lesser the phosphorous content and double bond fatty acids in the oil, the easier for the oil to be physically refined.

From the point of view of the refiner, the main characteristic distinguishing olive oil and seed oils is phosphorous compounds content, which requires a degumming stage. Phosphatides are not present in virgin olive oils since they are obtained by mechanical means of a mass of crushed olives whipped with water. Water treat-

ment taking place during the mixing of the pulp of olives is enough to flocculate and separate these compounds in the aqueous phase. Prior to olive oil refining, a water wash is commonly applied to remove mechanical impurities [6]. Therefore olive oil is normally physically refined.

2.1. TOS oil refining process

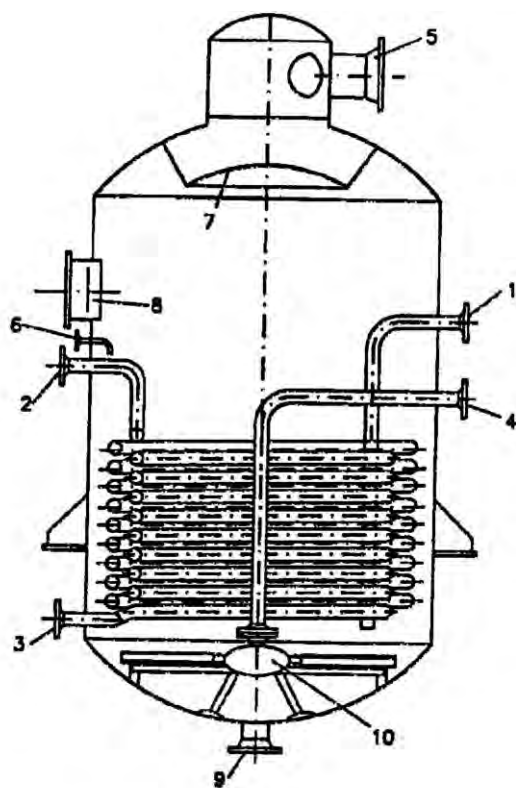
A study was undertaken to establish the importance of the different refining steps on OOPAP formation. The effects of degumming conditions, varying acidity and the percentage of bleaching earth, the addition procedure and operation time were tested. Also, the possible influence of the oxidation grade of the aniline used to denature the oil was evaluated. Apart from the generally poor oil quality resulting from inappropriate refining procedures, the samples had no other notable characteristics. Complete chemical characterization was performed at the *Center for Disease Control and Prevention* (CDC) in the United States and at the *Instituto de Investigaciones Biomédicas de Barcelona* (IIBB). Intermediate and final samples checked [9] had no trace of OOPAP.

The ITH workers described the industrial process they used with the TOS oil [10]. The processing conditions used consisted of three steps: neutralization without excess of lye; bleaching without specified conditions (although it was stated that owing to the colour of the oil this step had been repeated); and finally deodorization in a discontinuous device of 10-ton capacity. Based on these conditions, partial neutralization followed by removal of residual free fatty acids at the deodorization stage did not result in oils with the same characteristics as those obtained at ITH. In addition, these assays confirmed that bleaching negatively affected the presence of OOPAPs. But deodorization seemed to be positive, demonstrating that once formed, OOPAPs were stable under standard deodorization conditions.

At this point, the deodorization process carried out at ITH was taken into account. In initial studies, Hill et al. [5] performed experiments using two different samples of oil: a locally purchased canola oil (a variety of rapeseed oil produced in Canada) with 1% aniline added and a Catalonian rapeseed oil manufactured during the time of the epidemic, which contained anilides but not PAP esters or additional aniline. Both samples were heated at 300 °C for 4 h, and PAP esters were formed in both cases. This suggests that high temperature might be necessary to form the PAP compounds. The main difference between the distillation assays performed by Hill and normal conditions of the deodorization step in a factory were the extremely high temperatures used and the fact that stripping steam was not introduced.

If we consider the design of devices used for industrial deodorization at that time at ITH, it is important to remark on several issues. Discontinuous deodorizers were vessels capable of holding 10 ton of oil while withstanding a vacuum of 10 Torr, similar to the one shown in Fig. 1 [11]. The deodorization process began at around 160 °C as steam was blown in through perforated pipes fixed to the bottom of the vessel. The oil was heated to between 180 and 260 °C by circulating hot mineral oil through coils inside the deodorization vessel. Mineral oils and mineral oil derivatives with a working range of between –10 °C and 330 °C were the most commonly used heating fluids in the edible oil industry at the time of the TOS epidemic [10]. An analogous situation to distillation without gas stripping could occur in an industrial-scale apparatus if the agitation produced by the stripping steam was insufficient to frequently renew the layer of oil near the heating coil. In this way, the stagnant layer of oil could reach temperatures of over 300 °C.

Further systematic assays were carried out to evaluate this hypothesis and checked the influence of elevated refining temperature, such as it might occur in closer proximity to a deodorizer coil, and to account for storage time prior to refining [12]. Two



- 1.-Inlet for vegetable oil
2. Inlet for heating fluid
3. Outlet for heating fluid
- 4 Inlet for stripping steam
5. Outlet for volatiles by vacuum

Fig. 1. Discontinuous deodorizer apparatus [11].

samples of rapeseed oil were initially prepared. The first sample was denatured with 2% (w/w) aniline (99.5% purity) and stored for one week prior to neutralizing and bleaching. The second sample was denatured with 2% (w/w) aniline (99.5% purity) and stored for three weeks before neutralizing and bleaching. The results related to PAP derivatives obtained in these assays were very promising. Fig. 2 shows the results obtained for the quantification of the PAP esters. The amount of PAP esters in aniline-denatured oil increased dramatically when the temperature of the oil was raised from

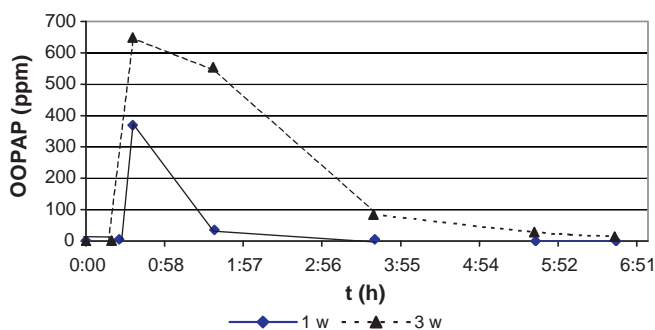


Fig. 2. Amount of OOPAP before, during and after distillation at 300 °C in rapeseed oil denatured and stored for 1 week and 3 weeks before neutralizing and bleaching [12].

250 °C to 300 °C. Those formed at 300 °C, however, were lost during processing at that temperature. The level maintained during operation at 300 °C was higher in the samples stored for three weeks before refining than in those stored for only one week. This suggests that the length of storage time before oil refining also affected the quantity of contaminants formed.

These studies have provided a number of conflicting conclusions as to the route for the formation of PAP esters. It is interesting to note that the amount of OPAP seemed to increase more than that of the other related compounds, as OA or OOPAP, during the heating period [12]. This behaviour was further studied by Escabrós et al. [13] who found that while anilide OA and PAP were thermostable until 300 °C, compounds like monoesters, OPAP, as well as diesters, OOPAP, suffered thermal decomposition in two phases. The first phase coincides with the split of the molecule to give the corresponding anilide and the second phase with the evaporation of the generated by-product.

In conclusion, the resulting hypothesis is that the ITH refinery most likely followed the normal procedure for deodorizing poor quality oil. The process ITH followed conceivably involved enormous difficulties in the bleaching step, and the strong undesirable flavour could subsequently have required an increase in the deodorization temperature thus involving an increase in the temperature of the thermal heating fluid. These circumstances, applied to such an unusual oil, could have given rise to the formation of anilides and PAP esters in the area near the heating coil, followed by the dilution of these compounds and other derivatives in the oil through the action of the stripping gas.

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