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Lipid Composition of Lees from Sherry Wine

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In this paper, we describe the study and characterization of the lipids from lees of Sherry wine, one of the main byproducts from the wine-making industry in the Jerez/Xeres/Sherry denomination of the origin zone in Jerez de la Frontera, Spain. The lipid content, extractability, classification, fatty acid composition, and its main chemical characteristics have been determined in order to evaluate their potential use as a food or food additive.

KEYWORDS: Lipids; Sherry wine; lees; lipid extractability; fatty acid composition; lipid classification; physicochemical parameters of lipids

INTRODUCTION

The most important agroalimentary activity in the Cádiz province of Spain is the wine-making industry, located at the Jerez/Xeres/Sherry denomination of the origin zone. This process generates two main residuals or byproducts: the bagasse or solid residue generated when the grapes (mainly *Vitis vinifera* cv. Palomino) are pressed to obtain the must and wine lees that are generated during the fermentation of must and its transformation into wine.

Lees from Sherry wine are a byproduct produced during the fermentation that converts the must, mainly from the Palomino grape, into young wine, which is later submitted to aging in the Soleras system. Wine lees are then constituted by yeast cells (alive and deactivated) produced during alcoholic fermentation and deposited at the bottom of fermentation tanks together with the tartrates, proteins, polysaccharides, and the rest of materials that precipitate in the tank as long as the alcohol concentration is increased.

During the pressing of grapes, part of its lipids (mainly from the skin and the seeds) passes to the must (*1*), and as long as fermentation is produced, there is a lipid exchange between wine and yeasts. Ferrari (*2*) has studied the variation on the fatty acids content of a wine that is kept in contact with the lees and observed that at the end of the third month the wine had suffered an increase in the amount of fatty acids of 1.7 μmol/L when the fermentation was conducted in stainless steel tanks and of 6.5 mmol/L when an oak tree cask was used. Lipids from different varieties of grape seeds and musts have shown a high content of C16:0 and of polyunsaturated fatty acids, mainly, C18:2 and C18:3 (*3*).

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Table 1. Composition of Lees from Sherry Wine

fraction	relative abundance (%)	fraction	relative abundance (%)
proteins	15.11 ± 0.57	tartaric acid	24.60 ± 0.12
lipids	5.40 ± 0.46	ash	10.55 ± 0.05
sugars	4.13 ± 0.63	others[a]	18.71
dietary fiber	21.50 ± 0.35		

[a] Others are given by difference.

Lees of Sherry wine constitute a byproduct of the wine-making industry that is actually undervaluated. Its main actual use is the recovery of tartaric acid, which is reused in wine manufacturing, to correct the initial pH of the musts before its fermentation and their distillation to obtain alcohol, which could be obtained more easily by distillation of the excedents from the wine production. This barely used byproduct contains about 5% lipids, according to studies carried out by our group (**Table 1**). In this paper, we board on the study and characterization of the lipid fraction of lees from Sherry wine, to evaluate its possible utilization.

EXPERIMENTAL PROCEDURES

Wine lees were obtained from Bodegas Domecq S. A., and we studied lees from two different harvests (1997 and 1999), not finding any significant differences between both of them. Lees were decanted from fermentation tanks at the bodega and then filtered through filter cloth to remove the excess of wine, and then, the solid residue was freeze-dried.

Extractability of Lipids from Sherry Wine Lees. The extractability of lipids from Sherry wine has been studied by using the modified Folch method (*4*) and five different solvent systems: petroleum ether, chloroform, chloroform:methanol (1:1 V/V), chloroform:methanol (2:1 V/V), and *n*-butanol saturated with water (*5*−*7*). A quantity of 2 g of dried lees was stirred with 20 mL of solvent for 30 min at room

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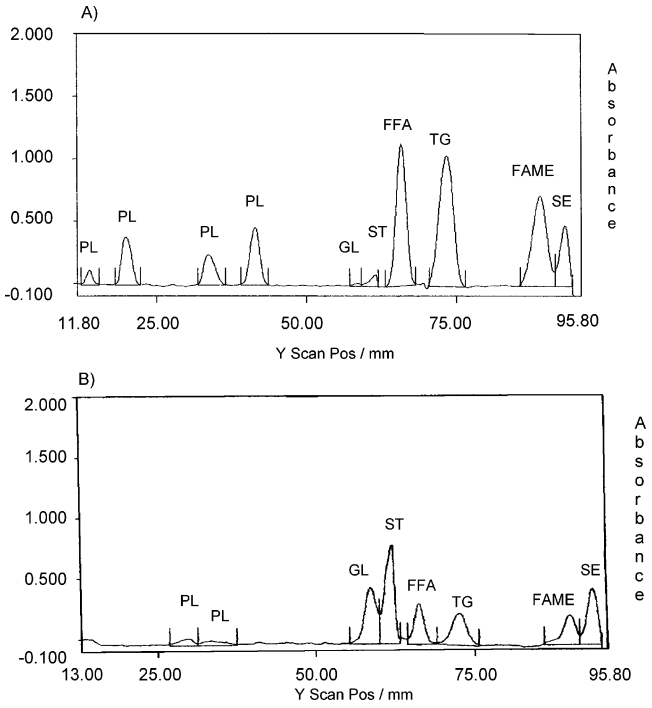

Figure 1. Lipid classes analysis of lipids of lees from Sherry wine. (**A**) Standard lipids; (**B**) lipids from lees of Sherry wine.

temperature and then vacuum filtered through two layers of Wathman 1 filter paper. The solid residue was then washed with 2 portions of 10 mL of the solvent. The combined filtrates were taken to dryness in a rotatory evaporator at 40 °C, until constant weight, and then overnight on a vacuum desecator. All of the extractions were made in triplicate.

Separation and Quantification of Lipid Classes (*8*). A quantity of 2 g of dried lees was extracted as described before with *n*-ButOH saturated with water. The obtained lipids (102.8 mg) were dissolved in 10 mL of chloroform:methanol (2:1), and 2 μL of this solution was applied to a high-performance thin-layer chromatography silica gel 60 plate of 10 cm × 10 cm (Merck) without a fluorescent indicator. The plates were prewashed with hexane:diethyl ether (1:1), dried in air, and then activated by heating at 110 °C for 30 min in an oven.

Neutral and polar lipids were separated by using a double-development system in one dimension. The plates were first eluted up to 6 cm with a solvent system for polar lipids, formed by methyl acetate:2-propanol:chloroform:methanol:aqueous 0.25% KCl (25:25:25:10:9), then dried under vacuum in a desiccator for 30 min, and then developed up to 8.5 cm with a solvent system for neutral lipids constituted by hexane:diethyl ether:acetic acid (80:20:2).

After development, the plates were dried overnight under vacuum on a desiccator and detection was made by spraying the plate with a solution of 3% (w/V) cupric acetate in 8% (V/V) aqueous orthophosphoric acid followed by charring at 160 °C for 20 min in an oven.

Quantification was made by scanning the photodensitometry of the charred lipids on the plate with a Shimadzu Dual Wavelength Flying-Spot Scanner CS 9000 equipped with an integrator recorder and computer Shimadzu DI13 (**Figure 1**).

Calibration and identification of the different lipid classes were made by running standards in parallel with the samples. The standards used were obtained from Sigma (products 178-I for neutral lipids, G-9523 for glycolipids, and P-3817 for phospholipids).

Fatty Acids Composition. A quantity of 2 g of dried lees was extracted as described before with *n*-ButOH saturated with water. The obtained lipids (103.5 mg) were transferred to a hydrolysis tube (a 16 cm × 2 cm borosilicate glass tube provided with a screw cap with a Teflon fitting) and transformed into its corresponding methyl ester by reaction with 10 mL of 1% H_2SO_4 in dry ethanol for 10 h, at 50 °C under dry nitrogen (*9*). Once finished, the reaction mixture was cooled to room temperature; the methanol was evaporated by blowing nitrogen, and the methyl esters were then extracted twice with 3 mL of hexane and 2 mL of water. The combined organic layers were taken to dryness by blowing N_2 and were dissolved in 100 μL of hexane for gas chromatography (GC) analysis.

GC analysis was performed on a Hewlett-Packard 5890A gas chromatograph equipped with a Supelcowax 10 M WCOT column (30 m × 0.53 mm i.d.) using a temperature program from 100 to 240 °C at 4 °C/min. The temperatures of the injection port and flame ionization detector were 250 and 350 °C, respectively. Hydrogen was used as the carrier gas at a flow rate of 4.6 mL/min.

Chemical Characteristics of the Lipids. The acid value, iodine value, saponification value, peroxide index, and insaponificable fraction of lipids extracted from Sherry wine lees with *n*-ButOH saturated with water were determined according to the Official Standard Methods issued by the IUPAC for the analysis of oils, fats, and derivatives (*10*).

Table 2. Extractability of Lipids from Lees of Sherry Wine

solvent	extracted lipids (%)
petroleum ether	1.4
chloroform	1.6
chloroform:methanol (2:1)	3.0
folch	3.4
chloroform:methanol (1:1)	5.0
n-butanol saturated of water	5.4

Table 3. Classification and Quantification of Lipids from Lees of Sherry Wine

lipid classes	relative amount (%)	lipid classes	relative amount (%)
SE	15.9	ST	25.3
FAME	10.8	GL	18.2
TG	13.0	PL	5.6
FFA	11.2		

RESULTS AND DISCUSSION

The composition of lees from Sherry wine is shown in **Table 1**. In previous works, we have described the study and characterization of the protein fraction of this material (*11*). Lipids represent about a 5 wt % of the dry lees. As lees from Sherry wine are formed by all of those materials that are deposited in the bottom of fermentation tanks, its main constituents are the yeast cells and their membranes and all of those materials that have precipitated as long alcohol concentration was increasing. Lipids have tensioactive properties that play an essential role during the alcoholic fermentation, mainly in those wines that keep contact with its lees for a long time such as the sparkling wines and Sherries. A persistent foaming in fermentation tanks could be a source of problems due to the limited capacity of the tanks but is quite important in the elaboration of high quality sparkling wines (*12*). Lipids, mainly oleic acid and its esters, are also associated with the aggregation of the yeast cells from the yeast layer that characterize the aging of Sherry wines through the Soleras system (*13*).

The extractability of lipids from Sherry wine in different solvent systems is shown in **Table 2**. We have employed six different solvent systems in order to evaluate the best way of recovery of the lipid fraction present in this stuff. The standard Folch method was used as a reference method. As we can observe, the higher the polarity of the extracting solvent, the greater is the amount of extracted lipids. Low polarity solvents extract mainly neutral lipids, and as long the polarity of the solvent increased, the extraction of the polar lipids started.

Table 3 shows the distribution of the extracted lipids in its different classes: sterol esters (SE), fatty acids methyl esters

Table 4. Fatty Acids Composition of Lipids from Lees of Sherry Wine

fatty acid		relative amount (%)
capric acid	C10:0	2.32
lauric acid	C12:0	4.42
miristic acid	C14:0	1.98
palmitic acid	C16:0	33.29
palmitoleic acid	C16:1	1.80
margaric acid	C17:0	0.30
stearic acid	C18:0	10.40
oleic acid	C18:1	7.82
linoleic acid	C18:2	21.26
linolenic acid	C18:3	5.88
araquidonic acid	C20:0	2.10
erucic acid	C22:0	6.10
lignoceric acid	C24:0	2.32

Table 5. Main Chemical Characteristics of the Lipids from the Lees of Sherry Wine

acid value	39.85% as oleic acid
saponification value	64.25 g KOH/g fat
iodine value	80.55 g iodine/100 g fat
insaponificable	6.87%
peroxide index	26.97 mequiv oxygen/kg fat

(FAME), triglycerides (TG), free fatty acids (FFA), sterols (ST), glycolipids (GL), and phospholipids (PL). The identification of the different classes was made by comparison with known standards, and quantification was made by densitometry (**Figure 1**). From this table, we can appreciate that the major fraction of lipids from lees of Sherry wine was that formed by ST (about 25%) followed by the GL and SE (18 and 16%, respectively), and the minor fraction corresponds to the PL.

These results are in line with what we could anticipate on the basis of the origin of this lipid fraction, which must come from components of the must, the wine, and the yeasts that produce this transformation. A big part of the materials forming parts of lees are remains from the cell walls of these yeasts, and ST form part of these membranes together with TG and esters of ST and fatty acids (*14*−*16*). GL could be coming from grapes and have been found both in the pulp and in the skin and seeds (*3*, *13*, *17*). In wine, the most important lipid fraction corresponds to FFA and could pass to form a pair of the lipids of the lees forming associations with the components of the cell walls of the yeasts. This could be supported by the observations made by Bertrand and Geneix (*13*, *17*) (who have shown how the addition of yeast cell walls to a wine produces a reduction on its content on long chain fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3). FAME, TG, and PL have been observed as common components of musts and yeasts and could be incorporated into the lees (*18*, *19*).

Fatty acids composition of lipids from lees of Sherry wine is shown in **Table 4**. The major components of these fatty acids are those having 16 and 18 carbon atoms. Thus, the most abundant was palmitic acid (about a 33%) followed by linolenic acid (about 21%). These results are in good agreement with its suggested origin and with those obtained for several strains of yeasts and for musts, which showed that the major fatty acid components of these materials were those having 16 and 18 carbon atoms.

Table 5 shows the main chemical characteristics of the lipids extracted from lees of Sherry wine. We have not found antecedents about the determination of these parameters in lees from other wine varieties or in yeasts. If we compare these values with those obtained by our group for the lipids from the seeds of the Palomino grape (*20*), we can observe that the acid value is higher in the lipids from lees than in those coming from the seeds, indicating a higher content in FFA. The saponification value is lower than in lipids from grape seeds meaning a higher average chain length in the fatty acids than from part of the lipids from lees. The iodine value was similar in both materials indicating that the unsaturation degree is close on them. The percentage of insaponificable matter is higher in the lipids from lees, showing their different origins; while the lipids from the grape seeds have their main function as energy storage for the early stages of the development of plant embryos, lipids from lees are coming from the yeasts cell walls, where they play a structural function. The first ones are mainly formed by TG, and the latest have a higher percentage of ST. The peroxide index was lower than the one obtained for the lipids of grape seeds and can be an indication of the presence of antioxidant substances between the components of this lipids fraction.

All of these parameters, mainly the high content of polyunsaturated fatty acids, like linolenic acid, could allow the use of these lipids as a complement in feeding for both human and animals.

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