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Influence of variety and geographical origin on the lipid fraction of hazelnuts (*Coryllus avellana* L.) from Spain: (II). Triglyceride composition

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In a former paper (Parcerisa *et al.* (1993). *Food Chem.*, **48**, 411–14), the fat content and fatty acid composition of hazelnut samples corresponding to four different varieties, all cultivated in two different areas of Catalonia (Spain) during three consecutive years, were analysed. Those results showed that the fatty acid composition was influenced by the geographical origin and harvesting year of samples. In the present paper, results of triglyceride composition corresponding to the same varieties and harvesting years are presented using HPLC. Twelve different triglycerides have been identified. Main proportions correspond to triolein ($\bar{X}$ = 46·9%) and linoleodiolein ($\bar{X}$ = 18·4%). Triglyceride composition of samples changed significantly as a function of harvest and geographical origin but not in relation to the hazelnut variety, in agreement with previous results. It is thus established that these factors have a great influence on the characteristics and quality of nuts.

In addition, a correlation study with previous fatty acid composition data was carried out, in order to check some aspects related to the biosynthesis of glycerides in these nuts. OOO, POO and LOO are always correlated with all glyceride and fatty acid contents, except with PLP and palmitic acid.

INTRODUCTION

Several authors have studied the influence of environmental conditions on the fatty acid composition in different nuts and seeds (Slack & Roughan, 1978; Slack *et al.*, 1978; Chaiserie & Dimick, 1989; Branch *et al.*, 1990; Lajara *et al.*, 1990). Furthermore, many authors have published results in relation to hazelnut oil and its glyceride composition (Shewry *et al.*, 1972; Bazan *et al.*, 1975; Van Dijk *et al.*, 1975, Fincke, 1980; Geeraert & Slopper, 1983; Bhati *et al.*, 1986; Geeraert & Sandra, 1987), although there is no information about the variability of glyceride composition in relation to the hazelnut varieties and their geographical origin. In this paper we show the experimental results corresponding to three consecutive harvesting years of four varieties, which were collected in two different cultivar areas which are principal hazelnut-producing regions of Catalonia (Spain).

MATERIALS AND METHODS

Samples

Four varieties of hazelnut (*Coryllus avellana* L.), Gironell, Negret, Pauetet and Tonda Romana were harvested in two different geographical areas (Reus, near the sea and Falset, in the mountains), during three consecutive years (1990, 1991 and 1992). These samples (n = 24) were collected by trained workers of the Institut de Recerca i Tecnologia Agroalimentàries (I.R.T.A.) during the second half of September, and they were stored unshelled in a refrigerator at 0°C until analysis.

Sample preparation

Approximately 0·2 g of hazelnut oil, which was pressed from crushed nuts (300 kg/cm^2), was weighed and then

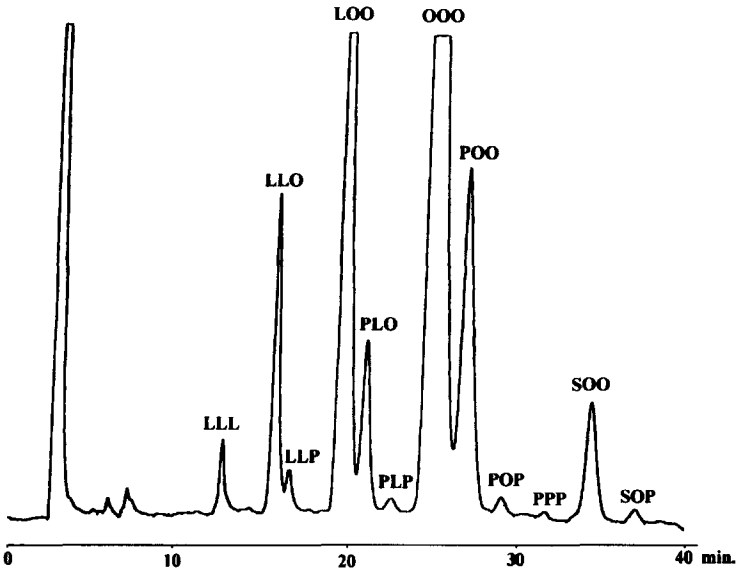

Fig. 1. Separation of triglycerides in a hazelnut oil (Tonda di Giffoni from Reus, 1991), according to the HPLC conditions described in the text (LLL = trilinolein, LLO = oleo-diolein, LLP = dilinolein-palmitin, LOO = dioleo-linolein, PLO = palmito-oleo-linolein, PLP = dipalmito-linolein, OOO = triolein, POO = palmito-diolein, POP = dipalmito-olein, PPP = tripalmitin, SOO = dioleo-stearin, SOP = palmito-oleo-stearin).

dissolved in 2 ml of acetone (HPLC grade), and the solution passed through a Nylon filter, 13 mm diameter and 0·45 μm pore (Lida Manufacturing Corp.). In this way the sample was prepared for chromatographic analysis.

Determination of triglycerides

The composition of hazelnut samples was determined by HPLC using a Perkin Elmer chromatograph (Series 10) fitted with a Rheodyne Loop (150 μl) injector, a Perkin Elmer LC-25 refraction-index detector and an HP3396A Hewlett Packard integrator.

The mobile phase used was an acetone–acetonitrile mixture (64 : 36 (v/v)) with a flow rate of 1 ml/min at 25°C. The volume of sample injected was 10 μl. A Spherisorb ODS-2 (5 μm) column, 25 cm length and 4 mm internal diameter was used (Tracer Analítica).

Peaks were identified by the comparison of logarithms of selectivities (log α) relative to the triolein in relation to the corresponding values of standard homogeneous triglycerides (Sigma Chemicals, Goiffon *et al.*,

Table 1. Triglyceride composition of hazelnut samples, according to their variety, location and harvesting year

Variety	Location	Harvest	LLL	LLO	LLP	LOO	PLO	PLP	OOO	POO	POP	PPP	SOO	SOP[a]
Gironell	Reus	1990	0·99	4·19	0·73	16·7	3·9	0·63	49·1	14·6	1·59	0·29	5·1	1·01
		1991	2·04	7·92	1·39	22·9	5·56	0·57	40·1	12·2	0·97	0·5	4·9	0·97
		1992	3·58	10·8	2·24	24·2	6·19	0·92	35·6	9·99	2·0	0·65	3·28	0·5
	Falset	1990	0·89	2·6	0·72	12·5	3·01	0·57	54·7	14·7	1·22	0·29	5·54	1·32
		1991	1·08	3·02	0·84	14·2	3·34	0·67	55·1	14·7	1·12	0·55	4·72	1·05
		1992	2·47	8·44	1·41	23·3	4·68	0·19	43·7	10·6	1·08	0·42	3·3	0·4
Negret	Reus	1990	1·33	5·66	1·08	20·1	5·53	0·39	43·8	15·3	1·64	0	3·64	0·99
		1991	1·9	7·94	1·52	23·4	6·07	0·0	41·4	12·7	0·71	0·15	3·56	0·74
		1992	2·52	9·9	2·13	24·2	6·17	0·83	38·5	11·4	1·11	0·42	2·64	0·21
	Falset	1990	0·82	2·98	0·56	14·3	3·17	0·63	54·7	15·6	0·66	0·12	4·6	0·79
		1991	1·07	3·66	0·91	16·3	5·37	0·0	50·3	16·7	0·0	0·28	4·91	0·56
		1992	1·78	6·57	1·14	21·0	4·55	0·3	47·1	11·4	0·76	0·61	4·2	0·64
Pauetet	Reus	1990	1·72	7·26	1·55	21·5	6·42	0·39	38·8	14·5	1·51	0·29	3·96	0·68
		1991	2·11	7·85	1·4	23·8	5·65	0·27	41·6	13·0	0·77	0·23	2·95	0·49
		1992	2·12	8·53	1·45	23·6	5·65	0·32	41·7	11·6	0·69	0·56	3·46	0·43
	Falset	1990	0·69	2·48	0·84	12·0	3·21	0·49	56·6	16·5	1·4	0·09	4·27	1·04
		1991	0·89	3·65	0·94	14·6	4·14	0·3	50·1	14·5	2·4	0·88	5·91	1·6
		1992	1·59	6·19	1·12	19·5	4·42	0·16	45·4	11·9	1·14	0·35	6·32	1·92
T. Romana	Reus	1990	1·73	7·35	1·41	21·1	4·98	0·7	44·5	12·4	0·87	0·09	3·62	0·37
		1991	0·81	3·51	0·83	16·6	4·8	0·0	49·9	16·7	2·0	0·74	3·89	0·31
		1992	2·21	7·78	1·35	22·9	5·3	0·39	40·5	12·3	1·87	1·08	3·41	0·94
	Falset	1990	0·51	1·83	0·56	10·2	2·67	0·46	56·6	18·3	1·76	0·28	5·05	0·81
		1991	0·72	2·07	0·0	11·7	3·14	0·16	57·0	17·4	1·11	0·48	5·58	1·19
		1992	0·5	2·36	0·52	11·2	3·26	0·6	49·4	15·8	2·44	2·66	7·39	3·98

[a] See Fig. 1. caption for abbreviations.

Table 2. Statistical data (mean, standard error of mean and significance level) for triglyceride composition, in respect to the location and harvesting year

	1990 $\bar{X}$	1991 $\bar{X}$	1992 $\bar{X}$	SE	*p*	REUS $\bar{X}$	FALSET $\bar{X}$	SE	*p*
LLL	1·09	1·33	2·10	0·179	0·0019	1·92	1·08	0·146	0·0006
LLO	4·29	4·95	7·57	0·552	0·001	7·39	3·82	0·451	<0·0001
LLP	0·93	1·079	1·42	0·112	0·0186	1·42	0·863	0·0918	0·0005
LOO	16·1	17·9	21·2	1·009	0·0057	21·74	15·1	0·824	<0·0001
PLO	4·11	4·76	5·03	0·248	N.S.	5·52	3·75	0·203	<0·0001
PLP	0·533	0·406	0·464	0·0966	N.S.	0·529	0·406	0·0695	N.S.
OOO	49·8	48·2	42·7	1·15	0·0008	42·1	51·7	0·938	<0·0001
POO	15·2	14·7	11·9	0·561	0·0008	13·0	14·8	0·458	0·0115
POP	1·33	1·30	1·39	0·220	N.S.	1·31	1·37	0·175	N.S.
PPP	0·181	0·476	0·843	0·168	0·0368	0·417	0·584	0·137	N.S.
SOO	4·47	4·55	4·25	0·336	N.S.	3·70	5·15	0·275	0·0013
SOP	0·876	0·864	1·13	0·258	N.S.	0·637	1·28	0·211	0·0447

N.S. = not significant.

1981; Hernàndez *et al.*, 1991; Parreño *et al.*, 1993). Quantification was accomplished by internal normalization, assuming the same detector response for all triglycerides.

RESULTS

We have identified and quantified twelve triglycerides: LLL, LLO, LLP, LOO, PLO, PLP, OOO, POO, POP, PPP, SOO and SOP, (P = C16 : 0, S = C18 : 0, O = C18 : 1, L = C18 : 2) (Fig. 1). The average values of triglyceride content expressed as a percent, for every variety, geographical origin and harvesting year are shown in Table 1. The main triglycerides are: triolein ($\bar{X}$ = 46·9%, SD = 6·5) and LOO ($\bar{X}$ = 18·4%, SD = 4·8), and the triglycerides that have two or three saturated fatty acids show the lowest proportions. In this last case, the only representative is tripalmitin, PPP ($\bar{X}$ = 0·5%, SD = 0·5).

The two-way ANOVA of results (Table 2), shows that there are significant differences between years only for the triglycerides LLL, LLO, LLP, LOO, OOO, POO and PPP. There are significant differences between the two cultivar areas, Reus and Falset, for the triglycerides LLL, LLO, LLP, LOO, PLO, OOO, POO, SOO and SOP (Table 2). In contrast, no significant differences were found for PLP, POP or PPP.

Nevertheless, no significant differences were found for any triglyceride between the four varieties studied.

DISCUSSION

The HPLC method shows a good resolution for hazelnut oil samples, although the position of the fatty acid chain in the triglyceride cannot be discriminated. However, our results are in agreement with those published by Bazan *et al.* (1975), who used lipases and further analysis of fatty acids to establish the composition and structure of hazelnut oil triglycerides.

It should be emphasized that the proportion of triglycerides having at least one linoleic acid chain in their structure (LLL, LLO, LLP, LOO and PLO) is significantly higher in the samples from Reus than in the samples from Falset. Significant differences were also found in relation to the harvesting year for the same triglycerides, except PLO. This suggests that there is a strong influence of environmental and harvest factors on the synthesis of these triglycerides in hazelnuts. This was pointed out by different authors in other fruits (Slack & Roughan, 1978; Tremolieres, *et al.*, 1978, Tremolieres *et al.*, 1982; Lajara *et al.*, 1990).

These conclusions are in accordance with our results on the study of hazelnut fatty acid composition (Parcerisa *et al.*, 1993), where we also observed significant differences between oleic and linoleic acids in relation to the same factors.

A correlation study between proportions of glycerides (Table 3), and also of some fatty acids, shows that those triglycerides that have a linoleic chain in their molecule are inversely correlated with those that do not. Moreover, the triglycerides which have a linoleic chain in their molecule are positively correlated with linoleic acid content, except the triglyceride PLP.

On the other hand, OOO is positively correlated with POO, whereas there is a negative correlation between triolein and PLO, LOO, LLO, LLP and LLL, but there is no correlation with the rest of the glycerides. Oleic acid is positively correlated with OOO and POO with high significance and negatively correlated with the triglycerides that have at least one linoleic acid chain, except the triglyceride PLP.

These correlations, and those obtained between fatty acids in the same hazelnut samples (Parcerisa *et al.*, 1993), are explained with regard to linoleic acid synthesis from oleic acid, which determines their relative proportions, since their sum (oleic + linoleic) is always constant.

It should be pointed out that there is no correlation between the triglycerides POP, PPP or PLP with the rest of the triglycerides, or palmitic acid. Palmitic acid is only correlated with POO, therefore this triglyceride is the most sensitive when the palmitic acid concentration changes.

Table 3. Correlations between triglyceride and fatty acid proportions and between triglyceride proportions (r, correlation coefficient and p, significance level)

		LLL	LLO	LLP	LOO	PLO	PLP	OOO	POO	POP	PPP	SOO	SOP[a]
LINOLEIC	r	0.8959	0.9517	0.8722	0.9537	0·9072	0.0593	−0.9255	−0.8184	−0.2414	−0.198	−0.6779	−0.5351
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N.S.	<0.0001	<0.0001	N.S.	N.S.	0.001	0.0151
PALMITIC	r	−0.4467	−0.3701	−0.2777	−0.3782	−0.1157	−0.1884	0.1844	0.5706	0.3042	0.2399	0.3499	0.4362
	p	0.0483	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.0086	N.S.	N.S.	N.S.	N.S.
OLEIC	r	−0.8875	−0.9524	−0.8771	−0.9537	−0.9279	−0.0493	0.9429	0.7995	0.2261	0.1838	0.6623	0.5099
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N.S.	<0.0001	<0.0001	N.S	N.S.	0.0015	0.0216
LLL	r		0.9641	0.9361	0.908	0.8266	0.1229	−0.8692	−0.8915	−0.2086	−0.1147	−0.692	−0.534
	p		<0.0001	<0.0001	<0.0001	<0.0001	N.S.	<0.0001	<0.0001	N.S.	N.S.	0.0007	0.0153
LLO	r			0.9459	0.9706	0.9147	0.0462	−0.9384	−0.8841	−0.2632	−0.132	−0.702	−0.5335
	p			<0.0001	<0.0001	<0.0001	N.S.	<0.0001	<0.0001	N.S.	N.S.	0.0006	0.0154
LLP	r				0.8673	0.8904	0.2149	−0.8802	−0.7921	−0.1476	−0.1582	−0.7059	−0.542
	p				<0.0001	<0.0001	N.S.	<0.0001	<0.0001	N.S.	N.S.	0.0005	0.0136
LOO	r					0.9204	−0.0923	−0.9204	−0.862	−0.3612	−0.2021	−0.717	−0.561
	p					<0.0001	N.S.	<0.0001	<0.0001	N.S.	N.S.	0.0004	0.01
PLO	r						0.0016	−0.9604	−0.6905	−0.1408	−0.1415	−0.6566	−0.459
	p						N.S.	<0.0001	0.0008	N.S.	N.S.	0.0017	0.0418
PLP	r							−0.0251	0.0218	0.1244	0.0656	−0.0981	−0.0616
	p							N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OOO	r								0.7646	0.0497	−0.0375	0.5443	0.3171
	p								0.0001	N.S.	N.S.	N.S.	N.S.
POO	r									0.3226	0.0104	0.4778	0.3769
	p									N.S.	N.S.	0.0331	N.S.
POP	r										0.5823	0.4348	0.5769
	p										0.0071	N.S.	0.0077
PPP	r											0.5114	0.7834
	p											0.0212	<0.0001
SOO	r												0.8637
	P												<0.0001

[a] See Fig. 1 caption for abbreviations.
N.S. = not significant.

To sum up, the most important glycerides are OOO, POO and LOO, since they always show the highest proportions, and they are also correlated with all glyceride and fatty acid contents, except the triglyceride PLP and palmitic acid.

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