Chemical and lipidic composition of the Spanish wild rabbit (*Oryctolagus cuniculus*)

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INTRODUCTION

It is well known that wild rabbit is an herbivorous animal eating a great variety of indigenous plants, grains and fruits. Although depending of many factors, the red colour, characteristic flavour, compact texture and dryness are the main organoleptic characteristics of the meat obtained from this species.The same authors (Nath and Rao 1983b) show that texture, flavour, tenderness and juiciness are better in wild than in domestic rabbits although these characteristic are depending of many factors as animal age, nutrition status, slaughtering form, feeding, etc.Chang-Han and Yeon-Hee (1982) studied the chemical composition of various meats (beef, pork, poultry, lamb and rabbit) showing that rabbit meat was the richest in palmitic acid. Similar results were obtained by other workers (Zegarska *et al* 1979; Kostenko *et al* 1980; Matter 1981). Lee and Ahn (1977) have observed that rabbit fat has a higher concentration of linoleic acid than beef, poultry and pork.

This work deals with the chemical composition of Spanish wild rabbit meat at the end of every season of the year. The lipidic fractions and their fatty acids have also been studied.

MATERIAL AND METHODS

Four batches of wild rabbit (*Oryctolagus cuniculus*) caught in the central area of Spain (La Mancha) were used. Animals were caught alive at the end of every season of the year. Each batch was constituted by four animals (males and/or females) with a weight on the carcasses ranging from 400 g to 650 g.

Animals were humanitary slaughtered. The head, viscera and skin of each animal were removed and the meat was obtained from the rabbits removing carefully the flesh from the squeleton. The perirenal and subcutaneous fat were descarted. The meat obtained from the four animals of each batch was finely minced in a blender (Sorvall, Omni-Mixer 17106). The final sample was composed of an homogenate of the meat from the four animals. The samples were kept at -20ºC until analysis.

AOAC (1980) methods were used to quantity moisture (24.002), protein (24.057) and ash (24.009). Lipids were extracted and purified from the former homogenate according to described by Bligh and Duer (1959). The

isolation of apolar lipids, glycolipids and polar lipids was made as described by Vorbeck and Marinetti, 1965. All lipids extracted were kept at -20ºC until analysis. Total lipids were determined by weighing the lipids extracted from the initial homogenate and apolar and polar ones by weighing the fractions eluted with chloroform or methanol from the silicic acid-celite column, respectively.

Separation of apolar and polar fractions and the purification of individual lipid classes were made as previously described (Hoz *et al* 1987, 1989).

Free fatty acids were determined titrimetrically according to Christie (1982) and tri-, di- and monoglycerides after removing them from preparative TLC plates by gravimetric (triglycerides) and by the method of Rapport and Alonzo (1955) the di- and monoglycerides. Cholesterol and cholesteryl esters were quantified by the method of Moore and Baumann (1952). Hydrocarbons were estimated gravimetrically after removal from preparative TLC plates. The estimation of the amount of each individual polar lipid was based on its phosphorus content (Chen *et al* 1956) because all of them were identified as phospholipids.

Free fatty acids were methylated to the procedure of Schlenk and Gellerman (1960). The methyl esters of the glycerides and phospholipids were obtained by the method of Sheata *et al* (1970). Methyl esters were analysed with a Perkin-Elmer 910 chromatograph equipped with a dual flame-ionization detector and glass columns packed with Chromosorb W coated with 10% DEGS.

RESULTS

The chemical composition and the total, apolar and polar lipid contents in the muscle of the four batches of wild rabbit are shown in Table 1.

TLC of the apolar lipid extracts (eluted by chloroform) revealed the presence of ten spots in all batches. According to their Rfs and behaviour against general and specific reagents, seven of them were characterized as monoglycerides, diglycerides, free cholesterol, free fatty acids, triglycerides, hydrocarbons and cholesteryl esters. Three spots were not identified. Percentages of individual apolar lipids are shown in Table 2 Triglycerides were always the major components. The lowest percentage corresponded to the Wintry samples and the highest to the Spring batch although the later showed similar values to the Summer one. The cholesterol content ranged from 1.19 to 8.39%, whilst the amount of cholesteryl esters varied from 2.74 to 9.49%.

TLC of the polar fraction (eluted by methanol) revealed the presence of seven phospholipids in all batches. The tentative identification of spots was made according to their Rfs and behaviour against general and specific

reagents. The phospholipids detected and the percentage of each individual phospholipid is shown in Table 3. Phosphatidylcholine (PC) reached the highest concentration followed by phosphatidylethanolamine (PE), accounting average values of 46% and 36%, respectively.

GLC analysis of fatty acid methyl esters from total, apolar and polar lipids of the four batches of wild rabbits revealed the presence of more than twenty fatty acids; the more abundant ones are shown in Table 4. The fatty acid C-16:0, 18:0, 18:1 and 18:2 were almost always the more abundant fatty acids. However, in a few samples, the C-20:0 reached similar or higher concentrations than those found for the fatty acids formerly mentioned.

The apolar lipids accounted more than 60% of total fat (Table 1). Therefore, as could be expected, they always showed a fatty acid composition similar to the total lipids (Table 4). However, it was observed, in general, a greater concentration of C-18:1 because of the lower C-18:2 and C-18:0 percentages.

DISCUSSION

The geographical area where rabbits were caught is characterized by a continental climate i.e. relatively low temperature in winter (max. 8-10ºC, min. about -3ºC) and very warm in summer (max. 35-40ºC, min. 10-12ºC) being the spring the main rainning period. Then, it must be expected that wild animals have many feed available at the end of the spring and the beginning of the summer which implies the animals become fatness in this period. It is that just happened, reflected by the highest concentration of fat (Table 1) and, in turn, by the highest concentration of triglycerides (Table 3). Despite the increase observed in fat content at the Spring batch, the values were still lower than those reported by other authors (Nath and Rao 1983a) for farmed rabbits which obtained fat contents about two-fold higher. However, the protein content of wild rabbit were similar than those found in farmed animals (Whiting and Jenkins 1981).

The spots tentatively identified by TLC on the apolar lipid fraction are, in general, the same than those described by other authors as in rabbits as in other animals (Chan-Haan and Yeon-Hee 1982) even fish (de la Hoz *et al* 1987). The percentages of triglycerides on wild rabbit meat (Table 3) were lower than those reported by other authors (Otake *et al* 1971; Chang-Haan and Yeon-Hee 1982) for farmed rabbit which is due, without doubt, to a less amount of depot lipids. Interesting enough is the composition of individual apolar lipids of the wintry batch. The triglycerides are the major components but its concentration is meaningly lower than the concentrations achieved in the rabbits caught at the end of the other seasons. On the contrary, the contents reached in winter by the

other components (mono- and diglycerides, cholesterol, hydrocarbons and cholesteryl esters) are greater than those of other batches. It has been attributed to the fact that the winter is the season in which the animals have available the poorest and scarcest diet and, therefore, the majority of the necessary energy for the vital activities has to be derived from the corporal depot fat.

As in other papers deal with lipids of rabbit (Gray and MacFarlane 1961; Kostetskii *et al* 1977) the major phospholipid was phosphatidylcholine followed by phosphatidylethanolamine, representing both together (Table 3) more than the 80% of the polar fraction. Similar phospholipids classes have been identified in ovine (Dawson 1960), porcine (Wood and Lister 1973), bovine (Evarts and Oksanen 1973) and poultry (Hay *et al* 1974).

Many seasonal differences may be observed in the fatty acid composition of total and apolar and polar fractions which are obviously related with the classes of feed available in each season. For example, the C-18:1 accounted for more than 50% in the apolar lipids from rabbits caught in Autumn whereas that fatty acid never reached the 30% in the other batches. On the contrary, the C-18:2 and C-20:0 achieved a minor concentration in the Autumnal batch in comparison to those of the other seasons. Similar considerations may be made in the fatty acid concentration of polar lipids. One of the more relevant differences is that related to the C-16:0. This fatty acid accounted for a 8% in the Spring batch while the concentrations in the same fraction from rabbits caught in the other seasons were close to the 30%. Nevertheless, this value was lower than those found for farmed rabbits which fat is characterized by a high palmitic acid content (about 40%) being always the major fatty acid (Zegarska *et a*, 1979; Matter 1981). This circunstance implies a high saturation degree on farmed rabbit fat. It is a dissimilar situation in wild rabbits in which the percentage of C-16:0 was lower and those of C-18:1 and/or C-18:2 were higher. Thus, the insaturation level is greater in fat from wild rabbits than farmed ones. A similar phenomenon has been observed in other animals, like deer (*Cervus elaphus*); i.e., a decrease of poliunsaturated fatty acids when the animal changes from wild to farmed life (Manley and Forss 1979).

The polyunsaturated fatty acids from fat of the main domestic animals are essentially represented by C-18:2 and C-18:3 (Otake *et al* 1971; Lin *et al* 1988; Rhee *et al* 1988). However, in the rabbit it seem to be that the C-20:4 is also characteristic of their fat at least in farmed animals (Otake *et al* 1971; Chang-Han and Yeon-Hee 1982). In this investigation the fatty acid C-20:4 has been only observed in significant concentration (>3%) in the polar fraction (PC and PE) but it was detected in minor percentages in the apolar lipids. It coincides with the findings of Otake *et al*. (1971).

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Table 1. Chemical composition, apolar and polar lipids of the meat of Spanish wild rabbit (Oryctolagus cuniculus)

Batch	Moisture	Lipids					Protein*	Ash*
		Total*	Apolar		Polar			
			WM*	TL**	WM*	TL**		
Wintry (1)	77.0	1.4	0.84	60.25	0.48	34.34	20.8	1.1
Spring (2)	75.1	3.7	3.01	81.54	0.59	16.19	20.9	1.1
Summer (3)	75.0	3.2	2.04	64.59	0.90	28.61	20.9	1.4
Autumnal (4)	74.2	2.7	1.66	61.29	0.83	30.64	22.2	1.6

*: % of wet matter ** % of total lipids

(1), (2), (3) and (4): Wild rabbits were caught at the end of winter, spring, summer or autum, respectively

Table 2. Concentration (% apolar lipids) of major components isolated from apolar lipids of Spanish wild rabbit (Oryctolagus cuniculus) meat.

Component	Batch			
	Wintry (1)	Spring (2)	Summer (3)	Autumnal (4)
Monoglycerides (a)	5.20	2.96	2.09	3.92
Diglycerides (b)	5.40	1.79	1.33	3.30
Cholesterol	8.39	1.71	1.19	2.03
Free fatty acids (c)	2.34	2.78	1.93	3.63
Triglycerides (d)	59.43	83.91	83.12	78.60
Hydrocarbons	5.55	4.08	2.49	3.26
Cholesteryl esters (f)	9.49	2.74	2.77	2.96

(1), (2), (3) and (4): Legend as in Table 1

(a), (b), (c), (d) and (f): expresed as monostearin, dipalmitin, palmitic acid, tripalmitin and cholesterol esters, respectively.

Table 3. Phospholipid composition (% of total phospholipid) of Spanish wild rabbit (Oryctolagus cuniculus) meat.

Component*	Batch			
	Wintry (1)	Spring (2)	Summer (3)	Autumnal (4)
PS + LPC	4.92	2.37	2.66	5.78
S+ LPE	4.98	7.37	4.44	6.19
PC	44.04	45.46	51.71	45.54
PE	38.11	37.00	35.34	35.51
C	7.95	7.81	5.94	7.00

(1), (2), (3) and (4): Legend as in Table 1

* PS: Phosphatidylserine; LPC: Lisophosphatidylcholine; S: Sphingomyelin; LPE: Lisophosphatidylethanolamine; PC Phosphatidylcholine; PE; Phosphatidylethanolamine; CL: Cardiolopin

Table 4. Fatty acid composition (weight %) of the total, apolar and polar lipid fraction of Spanish wild rabbit (Oryctolagus cuniculus) meat

	Batch											
	Wintry (1)			Spring (2)			Summer (3)			Autumnal (4)		
Fatty acid	Total	Apolar	Polar	Total	Apolar	Polar	Total	Apolar	Polar	Total	Apolar	Polar
14:0	1.60	2.64	0.16	3.18	4.00	0.15	1.78	2.69	1.97	1.03	1.70	0.17
16:0	25.05	27.81	26.81	25.55	29.20	8.30	23.73	26.73	21.15	21.79	21.16	28.65
16:1	1.19	4.65	0.68	1.03	1.09	0.42	1.99	2.05	2.18	2.65	4.17	1.06
18:0	13.29	8.82	20.26	10.48	11.52	19.39	8.87	7.98	11.13	8.06	6.94	25.01
18:1	22.52	28.49	15.86	14.59	14.52	19.45	24.91	27.28	12.68	42.68	51.90	23.29
18:2	21.82	19.83	18.88	18.23	16.06	18.52	23.20	23.11	18.92	17.03	9.20	15.08
18:3	0.35	0.12	0.15	0.23	Tr	1.04	0.23	0.50	0.16	1.05	0.43	0.44
20:0	4.02	2.87	2.95	15.52	17.30	3.42	4.60	5.70	7.83	1.22	0.97	0.15
20:1	0.27	0.35	0.26	0.34	0.59	0.19	1.11	1.12	0.37	0.22	1.13	0.30
20:2	0.32	0.12	0.17	1.97	---	9.04	0.21	0.31	0.52	Tr	Tr	Tr
20:4	4.50	0.32	6.91	2.22	Tr	12.05	3.64	0.43	8.91	1.35	Tr	3.52
Others	5.07	3.98	4.91	6.66	5.52	7.97	5.73	2.10	14.18	2.92	2.40	2.33

(1), (2), (3) and (4): Legend as in Table 1

Tr: Trace amounts, less than 0.1%