Fatty acid composition of pea (Pisum sativum) L., var. Citrina) during growth

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RESUMEN

Composición en ácidos grasos del guisante (Pisum sativum L, var. Citrina) durante el desarrollo de la semilla.

Se ha determinado el contenido lipídico y la composición en ácidos grasos de cuatro tamaños de guisantes crudos y enlatados. Durante el período de crecimiento considerado (4.7-10.2 mm de diámetro), el contenido lipídico no es modificado, y el contenido medio fue de 2.52% expresado en peso seco. En general, el predominio de los ácidos grasos en el guisante crudo se ajusta a la fórmula C18:2 > C18:1 > C16:0 > C18:3 > C18:0; sin embargo, durante el crecimiento de la semilla incrementa C18:2 y decrecen C18:0 y C18:3. Estas variaciones son discutidas en relación a la temperatura ambiental y a la modificación del cociente cotiledón/testa durante el crecimiento de la semilla. Un bajo contenido lipídico, que permanece constante durante el crecimiento de la semilla, y el menor contenido de C18:3 en el tamaño FN (8.3-8.8 mm), ponen de manifiesto que cuando la variedad Citrina es utilizada para la elaboración de productos procesados, la cosecha debe ser realizada cuando existe un predominio del tamaño FN. El contenido lipídico durante el enlatado no es modificado en ningún tamaño, aunque se producen algunas modificaciones en la composición de ácidos grasos debidas, en general, a que durante el enlatado se produce una disminución en el grado de saturación.

PALABRAS-CLAVE: Acido graso (composición en) – Guisante (Pisum sativum L.) – Lípido (contenido).

SUMMARY

Fatty acid composition of pea (Pisum sativum L., var. Citrina) during seed growth.

Crude oil content and fatty acid composition of four sizes of raw and canned pea were determined. In the seed growth period, considered (from 4.7 mm to 10.2 mm of diameter), crude oil content was not modified and its level showed a mean value of 2.52% on a dry weight basis. Generally, in raw pea the following fatty acid composition was obtained C18:2 > C18:1 > C16:0 > C18:3 > C18:0; however, during seed growth C18:2 increased and C18:3 decreased. These variations are discussed in relation to environmental temperature and cotyledon/testa ratio modifications during seed growth. A low oil content, which remains constant during seed growth, and a lower linolenic acid content in fine (FN) size showed that when Citrina variety is used for processing, peas should be harvested when a FN size are predominant in crop. During canning, crude oil content is not modified in any size, but some differences in fatty acid composition occur between raw and canned peas because, in general, the degree of saturation decreases during canning.

KEY-WORDS: Fatty acid (composition in) – Lipid (content) – Pea (Pisum sativum L.).

1. INTRODUCTION

The lipid content of peas is low and ranges from 0.8 to 6.1% for whole seeds (Savage and Deo, 1989). The principal varieties used for canning, such as Citrina or Manuela, are wrinkled and Coxon and Davies (1982) have shown that wrinkled peas contain between 4.5 and 5.2% as total lipid content, while round-seeded varieties contain only 2.8 to 3.1%. Although low, crude oil content may be of importance in the flavour of peas (McCurdy et al., 1983). In canned pea, the formation of volatile flavour compounds can be prevented by blanching and this process also inactivates lipoxygenase and other enzymes that degrade lipids. Autooxidation of lipid-bound polyunsaturated fatty acids is usually initiated by the catalytic action of trace metals with abstraction of a proton and formation of fatty acid free-radical (Uri, 1961), and mineral composition is modified during seed growth and during canning (Rincón et al., 1990). Chowdhury et al. (1984) reported that in leguminous seeds the major saturated fatty acid was palmitic, while the major unsaturated fatty acid was linoleic. In the majority of 166 pea lines examined by Coxón and Wright (1985), the fatty acid composition was such that 18:2 > 18:1 > 16:0 = 18:3 > 18:0. An earlier study of pea seeds of Manuela variety (Murcia and Rincón, 1991) showed that this general composition is modified during seed growth in Manuela variety. In addition, the tenderometer value has been shown relevant for measuring the growth and maturity of peas destined for canning (Ottosson, 1958), and the variations in tenderometric value indicate substantial variations in pea quality (Martens, 1986). The purpose of the present investigation was to study the fatty acid composition of

different sizes of pea seed (var. Citrina) for canning.

2. MATERIALS AND METHODS

2.1. Samples

Citrina variety pea (Pisum sativum, L.) seeds (wrinkled, dark green genotype) were obtained from Van-waveren Inc. (D-3405-Rosdorf, Uber-Gottinge, Germany) and cultivars and canned samples from Hero España, S. A. (Alcantarilla, Spain) in March 1989. Shelled peas were received in truckloads at the processing plant two hours after being harvested, conveyed through shakers to remove any debris and classified by diameter into superfine (SF) (4.7 to 7.5 mm), very fine (VF) (7.6 to 8.2 mm), fine (FN) (8.3 to 8.8 mm) and middle (MD) (8.9 to 10.2 mm). Tenderometer value (TV) was determined using a Bertuzzi field tenderometer (Brugueiro-Milano) and was carried out just as pea seeds were picked using whole seed peas for each determination.

2.2. Industrial process

The peas are washed and each diameter separately blanched: 3 min at 90°C for SF and VF samples and 8 min at 90°C for FN and MD samples. Approximately 120 g of peas are introduced into each enameled can, followed by approximately 90 ml of hot filling medium (solution of 2% NaCl and 3.65% sucrose in tap water). The cans are then placed in retorts, processed for 25 min at 121°C and cooled in water. Each diameter was inspected fresh and after canning, using three samples of approximately 1 Kg and by taking ten cans of each of the four sizes. Sampling was realized over two consecutive days.

2.3. Sample preparation

Peas were lyophilized in a Virtis Quickseal Valves freeze-dryer and moisture determined (Hemavathy et col., 1987). Fresh samples were lyophilized immediately after receipt and canned samples were lyophilized one day after canning. Subsequently the freeze-dried peas were pulverized to a fine powder with mortar and pestle.

2.4. Gravimetric determination of total lipids.

Replicate pea flour samples (250 mg correct to 1 mg) were weighed into 10 ml screw-capped tubes in triplicate. Each sample was extracted three times with 5 ml of a water-saturated n-butanol (WSB) (Coxon and Wright, 1985) for 30 min in a mechanical inversion mixer. After each extraction period the tubes were centrifuged (10 min at 3000 rpm) and the extracts decanted before fresh solvent was added. The pellet was dessicated under a nitrogen stream and then weighed by a balance. The difference in weight from the original sample expressed the total lipid value.

2.5. Fatty acid methyl esters (FAME) of the lipid

The determination of fatty acid methyl esters (FAME) of lipids was done according to Coxon and Wright (1985). Triplicate samples of pea flour (250 mg correct to 1 mg) were weighed into 10 ml screw-capped tubes. The extraction was realized with 5 ml of WSB mixture added to each tube. The tubes were flushed with nitrogen and sealed with teflon-faced rubber-lined caps. The pea flour was dispersed in the extraction solvent by placing the tubes in a Vortex mixer for a few seconds and then subjecting them to continuous gentle mechanical inversion mixing for 30 min at room temperature. The tubes were then centrifuged (10 min at 3000 rpm) and 200 µl of solution was drawn off from each sample into a clean tube. In this moment 0.5 mg ml^{-1} of heptadecanoic acid (C_{17}) was added as internal standard.

The solvent was evaporated under nitrogen. To each residue was added 2 ml of sulphuric acid-methanol (5% v/v) and the tubes flushed under nitrogen and heated for 1 h at 100°C. The tubes were then cooled and 3 ml of hexane and 5 ml of water were added to each, before mixing and centrifuging. The hexane layers (3 ml) were transferred by pipette into vials and the solvent evaporated under nitrogen. The residues were dissolved in 100 µl of hexane for the analysis of methyl esters by gas chromatography. A steel column (2 m x 1/8 inch i. d.) packed with 20% DEGS on cromosorb W 80-100 mesh was used in a Perkin-Elmer 8300 gas chromatograph equipped with a flame ionisation detector. The column was operated at (140°C - 195°C) with nitrogen carrier gas flow rate of 30 ml min^{-1}. FAME were expressed as a percentage of total in each type of sample.

2. 6. Identification of fatty acids

A Hewlett-Packard 5995 gas chromatograph-mass spectrometer was used, operated with an ionizing current of 100 μA at 70 eV electron energy in electron impact mode, with a source temperature of 200°C and GC interface at 250-270°C. The HP-1 column (crosslinked methyl silicone gum) of 25 m x 0.2 mm x 0.11 μm filum thickness was used with carrier gas (helium). The identification of fatty acids was realized according to the mass spectral data (Table I).

Table I
Mass spectral data used for fatty acid identification.

	Peak nº	RRT[a]	Fatty acids	Molecular weight	Mass spectral data[b]
$C_{14}H_{28}O_2$	1	0.796	tetradecanoic	238.36	74,87,55,143,57,67
$C_{16}H_{32}O_2$	2	1.158	hexadecanoic	256.42	74,87,55,57,59,143
$C_{18}H_{36}O_2$	3	1.549	octadecanoic	284.47	74,87,55,57,75,143
$C_{18}H_{34}O_2$	4	1.629	9-octadecanoic	282.45	55,74,69,83,84,96
$C_{18}H_{32}O_2$	5	1.810	9,12-octadecadienoic	280.44	55,67,81,95,74,79
$C_{18}H_{30}O_2$	6	2.093	9,12,15-octadecatrienoic	278.42	55,57,67,79,33,95
$C_{20}H_{40}O_2$	7	2.322	eicosanoic	312.52	74,87,55,57,69,75

[a] Relative retention time in relation to the injection.

[b] Major fragmentation ions, base peak and five other ions in decreasing order of relative abundance.

3. RESULTS AND DISCUSSION

Crude oil content and fatty acid composition for raw and canned pea are shown in Table II. Crude oil content is not modified during the seed growth period considered. An earlier paper (Murcia and Rincón, 1991) showed that for the growth period VF-MD there exists an active lipid destruction in Manuela variety; in Govorov variety, during the 7-8 mm to 8-9.5 mm growth period there is an increase of 25% in oil content (Bengtson and Bosund, 1966). In several different field pea cultivars, a negative correlation is reported between mean seed weight and oil content (Welch and Griffiths, 1984), but Garfiel peas were larger and had a higher lipid content than Alaska pea (McCurdy et al., 1983). Coxon and Wright (1985) reported lower oil content in round peas (from 2.24 to 3.14%) than in wrinkled peas (from 3.7 to 4.97%). In conclusion, oil content and its variation during seed growth is a function of variety. Citrina variety shows lower oil content (from 2.3 to 2.6%) that other varieties (Coxon and Wright, 1985; Welch and Griffiths, 1984); in addition, oil content remains constant during seed growth, and a reduction of the oil or linolenic acid content of peas could improve the quality of this crop for processing (Welch and Griffiths, 1984).

Table II
Crude oil content and fatty acids composition for differents sizes (SF = Super fine, VF = Very Fine, FN = Fine, MD = Medium) and processing phase (R = Raw, C = Canned).

Size		Oil[b] (%)	Component fatty acids (% of total)[a] C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
SF	(R)	2.46	0.4	22.4	17.6	19.2	28.2	12.2	Tr.
	(C)	2.20	1.7	27.3	21.2	14.9	25.2	9.7	Tr.
VF	(R)	2.31	Tr.	21.1	16.6	17.3	29.4	13.6	Tr.
	(C)	2.16	Tr.	17.6	12.6	16.0	39.3	14.5	Tr.
FN	(R)	2.55	Tr.	22.3	11.5	21.4	35.9	7.9	Tr.
	(C)	2.25	Tr.	18.8	13.0	21.7	38.9	7.8	Tr.
MD	(R)	2.64	Tr.	24.9	10.9	21.7	35.2	7.3	Tr.
	(C)	2.24	Tr.	16.7	12.6	17.4	40.3	13.0	Tr.

14:0, myristic; 16:0, palmitic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic; 20:0, eicosanoic.

(a) Means of three determinations.

(b) Expresed on dry weight.

A comparison with fatty acid compositions obtained by other authors is shown in Table III and shows the wide variability as a function of pea cultivar. In raw pea a similar composition (18:2 > 18:1 > 16:0 = 18:3 > 18:0) was found for all sizes, but 14:0 was only detected in any significant quantity in SF size, although trace amounts of myristic and arachidic acids were present (Table II) in all others sizes. This general composition in raw pea coincides with thoses results obtained in the majority of 166 pea lines examined by Coxon and Wright (1985). Similar linolenic (from 6.4 to 13.4%), but higher linoleic (from 43.7 to 60.9%) and oleic (from

Table III

Fatty acid composition of raw pea obtained by other authors.

Lower fatty[a] acids	Fatty acids composition[b] C16:0	C18:0	C18:1	C18:2	C18:3	Variety	Author
2	21	2	16	51	8	Govorov	Bengtson & Bosund 1966
3	20	17.1	14	26.1	18.9	-----	El-Refai et al. 1987
--	--	5.6	45.4	32.1	--	Citation	Coxon & Wright 1985
--	--	5.6	47	32.9	--	Carpo	Coxon & Wright 1985
--	9-20	2-5	14-33	38-61	6-13	-----	Savage & Deo 1989
--	12.0	4.2	22.8	51.1	9.9	Minerva	Welch & Griffiths 1984
--	13.5	2.5	25.3	48.7	10.1	Filby	Welch & Griffiths 1984
--	12.8	3.5	14.2	60.9	8.8	Marathon	Welch & Griffiths 1984
--	14.1	2.7	19.4	54.2	9.8	Brite	Welch & Griffiths 1984
--	16-28	12-26	16-23	21-33	6-14	Manuela	Murcia & Rincón 1991

[a] Mainly myristic acid.
[b] Relative peak area (% methyl esters).

Table IV

F-Values and their significance obtained for each fatty acid.[a]

Source of variance	Fatty acids C16:0	C18:0	C18:1	C18:2	C18:3
Size	1.3 (ns)	5.9 (*)	1.6 (ns)	5.2 (*)	14.4 (***)
Canning	18.2 (***)	7.4 (*)	4.4 (*)	17.4 (***)	3.2 (ns)
Interaction	0.6 (ns)	5.6 (**)	1.5 (ns)	2.6 (ns)	5.1 (**)

[a] Statistical differences as analyzed by ANOVA: ns= no significant difference at 5%
* Significant difference at 5% level.
** Significant difference at 5% level.
*** Significant difference at 5% level.

14.2 to 33.3%) and lower stearic (from 2.7 to 4.2%) and palmitic (from 12.0 to 16.6%) acid contents were reported by Welch and Griffiths (1984) in different field pea cultivars. Two-way ANOVA (Table IV) showed that during seed growth 18:2 increases and 18:0 and 18:3 decrease (Table II). These variations are similar to those obtained from the 7.0 mm to more than 9.5 mm growth period in Govorov variety, where oleic acid does not vary significantly with maturation and linoleic acid apparently increases, while palmitic and linolenic acids decrease (Bengtsson and Bosund, 1966).

In the discussion of the results obtained here we must consider two factors: (1) temperature

values during pea growth and (2) value of cotyledon/testa ratio during seed growth. It is known that the enzymes involved in fatty acid biosynthesis depend on agroclimatic factors (Harwood and Stumpf, 1970), and lipid content varies with climatic and environmental conditions (Worthington et al., 1972). For this reason, a significant decrease in temperature values during the four weeks before harvest must be taken into account (Figure 1). In adition, it was shown that the total fatty acid content of Manuela pea is partly determined by modifications in the cotyledon/testa ratio during seed growth (Murcia and Rincón, 1991), because the fatty acid composition of lipids from cotyledons and testas revealed great differences (Bengtsson and Bosund, 1966; Welch and Griffiths, 1984; Savage and Deo, 1989). Both temperature and cotyledon/testa ratio factors can explain the differences in fatty acid composition during pea seed growth obtained here and those obtained by Bengtsoon and Bosund (1966), mainly in relation to oleic and linoleic acids. Table V shows the cotyledon/testa ratio for the different sizes considered. Significant increases ($p<0.01$) in the cotyledon/testa ratio are obtained during the SF to VF and VF to FN growth periods. A low oil content, which is not significatively modified during seed growth, and a low linolenic acid content in FN size showed that when Citrina variety is used to obtain peas for processing, peas should be harvested when FN size is predominant, because a deterioration in flavour might be caused by lipid autoxidation or through the action of lipoxygenase (EC 1.13.1.13) (Coxon and Wright, 1985), which mediates the conversion of polyunsaturated fatty acids to aldehydes and alcohols, both major contributors to the off-flavors in legume protein products (Sessa, 1979).

During canning the crude oil content is not modified significantly in any size (Table II). In Manuela variety (VF size), processed in the same

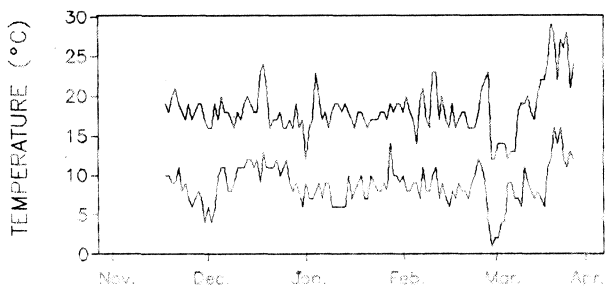

Figure 1
Maximum and minimum temperature values during pea crop.

Table V
Cotyledon/Testa ratio for different sizes considered. (SF = Super Fine, VF = Very Fine, FN = Fine, MD = Medium).

Size	Per 100 gr. Number of peas	Per 100 gr. Testa weight	Ratio Cotyledon/testa
SF	494	35.2	1.67
VF	346	27.8	2.36
FN	282	22.8	3.28
MD	200	22.4	3.46

conditions, losses in crude oil content occurred (Murcia and Rincón, 1991) so it can be concluded that losses in oil content during commercial canning are a function of pea variety. In peas blanched for 2 min in boiling water and later pasteurized in water at 85°C for 13 min, the total oil content was not affected (El-Refai et al, 1987). Fricker et al. (1975) reported that as a consequence of heating, plant cell membranes may be changed in such a way that lipids not accessible to the solvent in the fresh product may become more readily extractable. In conclusion, these changes are a function of pea variety because testa is the most resistant tissue in pea due to the single layer of macroscleroids (Schoonerns, 1977), and the cotyledon/testa ratio varies between varieties such as Citrina (Table III) and Manuela (Murcia and Rincón, 1991).

Some differences exist in the fatty acid composition between raw and canned peas (Table II) and, in general, the palmitic acid contents decreased after canning in the sizes (VF, FN y MD). However the stearic acid contents did not change significantly in these same sizes (Table II). This can be appreciated in Table VI where it is shown a decrease in the contents of saturated fatty acids during canning (for VF, FN and MD size, $p<0.05$). This can be due to the loss of complex lipids which may contain saturated fatty acids or simply to the loss of saturated free fatty acids. The major fatty acid occurring in canned pea is linoleic acid in all sizes except in the SF size. Furthermore, the SF size showed an important decrease during canning in linolenic acid and for these reasons, autoxidation would also be expected to occur in this size of canned pea. The FN size shows a low linolenic acid content which does not change during processing, for which reason it is principally recommended for the elaboration of flour.

Table VI

Percent of lipids in pea (Raw/Canned) for different sizes (SF = Super Fine, VF = Very Fine, FN = Fine, MD = Medium).

Lipids	Sizes			
	SF	VF	FN	MD
Saturated	40.4 / 50.2	37.7 / 30.2	33.8 / 31.8	35.8 / 29.3
Monoinsat.	19.2 / 14.9	17.3 / 16.0	21.4 / 21.7	21.7 / 17.4
Poliinsat.	40.4 / 34.9	45.06 / 53.8	44.8 / 46.5	42.8 / 53.3

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