

Size as source of variance in lipid composition of pea

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Changes in crude oil content and composition of *pea seeds (Pisum sativum)* as a function of size during seed growth and commercial canning were studied. Peas were divided into four sizes, chosen according to Spanish commercial classification, from 4.7 to 10.2 mm (Superfine (SF), very fine (VF), fine (FN) and middle (MD)). Fatty acid methyl esters, neutral lipids, glycolipids and phospholipids were determined. The period of crude oil accumulation during seed growth finished at VF size and thereafter began an increase in the degree of saturation and a decrease in the degree of polyunsaturation of fatty acids, mainly due to increases in stearic acid and decreases in linolenic acid. These modifications are discussed in relation to environmental temperature and cotyledon/testa ratio variation during seed growth. Canned MD size should best be used in the manufacture of pea flours, since it presents low levels of linolenic acid and triglycerides, a high neutral/polar lipid coefficient (due to the losses of phospholipids during canning), and low levels of cardiolipin and phosphatidylglycerol. In conclusion, pea size must be taken into consideration in studying the oil composition of raw and canned peas.

INTRODUCTION

Peas contribute substantially to both human and animal nutrition. They are primarily considered as a protein source, but the lipid present is of interest because of its effect on the functional properties of pea protein concentrates or isolates when used as food ingredients (Wright & Bumstead, 1984), the air classification of peas (Reichert, 1982) and the flavour deterioration of pea flour fractions, which might be caused by lipid autoxidation or through the action of lipoxygenase (Coxon & Wright, 1985). Approximately 50-60% of the total lipid content of peas is present in the neutral lipid fraction, but phospholipids and glycolipids are also present in appreciable amounts (Reichert & Mackenzie, 1982).

Crude protein can be used as a quantitative index to distinguish growth period (from 4.7 to 8-2 mm of diameter) and maturation period (from 8.3 to 10.2 mm of diameter) (Ros & Rincón, 1990). Minerals, such as Fe^{$3+$}, Fe^{$2+$}, Cu^{$2+$}, Mn^{$2+$}, Ni^{$2+$}, etc., are known to catalyze the autoxidation of lipids (Pattee *et ai.,* 1980) and the

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mineral content of pea is modified during seed growth and the canning process as a function of pea size (Rincón *et al.,* 1990). For this reason, susceptibility to autoxidative reactions can be different for different pea size. In addition, the content of polyunsaturated fatty acid (particularly linolenic acid) would influence the susceptibility to autoxidative deterioration (Coxon & Wright, 1985). Gervani and Devi (1988) have demonstrated that the lipids of pea are quantitatively modified by maturation, but they did not study qualitative modifications. Qualitative changes in lipid composition during the industrial process have been reported (EI-Refai *et al.,* 1987), but whether these changes are a function of pea size has not been studied. The aim of this paper is to monitor the variation in lipid composition of different sizes of seed pea intended for canning.

MATERIALS AND METHODS

Samples

Manuela variety *pea (Pisum sativum,* L.) seeds (wrinkled, dark green genotype) were obtained from Vanwaveren Inc. (D-3405 Rosdorf, Uber-Gottinge,

Germany) and cultivars and canned produce from Hero España S.A. (Alcantarilla, Spain) in March 1989. Shelled peas were received in truckloads at the processing plant 2 h after harvesting, conveyed through shakers to remove any debris, and classified by diameter into superfine (SF) (4.7-7.5 mm), very fine (VF) (7.6-8.2 mm), fine (FN) (8.3-8.8 mm) and middle (MD) (8.9-10.2 mm). A Bertuzzi field tenderometer (Brugueiro-Milano) was used to obtain the tenderometer value (TV) of each pea size in order to measure maturity, according to the method of Ottoson (1958). This was carried out just as pea seeds were picked using whole seed peas for each determination.

Industrial process

Peas were washed and each diameter separately blanched for 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 was placed in each enamel can, followed by approximately 90 ml of hot filling medium (2% NaCl and 3.65% sucrose in tap water), and the cans sealed. These were 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 fresh samples of 1 kg each and taking l0 processed cans for each size. Sampling was conducted over two consecutive days.

Sample preparation

Peas were lyophilized in a Virtis Quickseal Valves freezedryer (The Virtis Company, New York, USA) and moisture content was then determined (Hemavathy *et al.,* 1987). Fresh samples were lyophilized immediately after receipt and canned samples were lyophilized the day after canning. Subsequently freeze-dried peas were pulverized to a fine powder with mortar and pestle.

Gravimetric determination of total lipids

Gravimetric determination of total lipids was carried out according to the method of Morrison *et al.* (1980). Triplicate pea flour samples (250 mg) were put into 10-ml screw-capped tubes. Each sample was extracted three times with 5 ml of water-saturated *n*-butanol (WSB) for 30 min each in a mechanical inversion mixer. After each extraction period the tubes were centrifuged (10 min at 3000 rpm), the supernatant decanted, and fresh solvent added. The pellet was then dried under a nitrogen stream and weighed. The difference in this weight and that of the original sample is expressed as the total lipid value.

Determination of total lipid as fatty acid methyl esters (FAME)

The procedure for determining total lipids as FAME and the quantitative analysis of lipids was realized according to Coxon and Wright (1985). Triplicate subsamples (250 mg) of pea flour were weighed into separate 10-ml screw-capped tubes. Lipids were extracted by adding 5 ml of WSB mixture to each tube, before flushing with nitrogen, and sealing the tubes with Teflonfaced rubber-lined caps; the sample was dispersed in extraction solvent by using a Vortex mixer (Selectra, Barcelona, Spain) for a few seconds, and then subjecting the sample to continuous gentle mechanical inversion, mixing for 30 min at room temperature. The tubes were then centrifuged (10 min at 3000 rpm) and 200 μ l of solution drawn from each sample and placed in a clean tube. Heptadecanoic acid was added to a final concentration of 0.5 mg ml -1 as internal standard.

The solvent was evaporated under nitrogen. To each residue was added 2 ml of sulphuric acid-methanol $(5\% \text{ v/v})$ and the tubes were flushed under nitrogen and heated for l h at 100°C.

The tubes were then cooled and 3 ml of hexane and 5 ml of water were added to each, followed by mixing and centrifuging. The hexane layer (3 ml) was transferred by pipetting to a clean vial and the solvent evaporated under nitrogen. The residues were dissolved in 100 μ l of hexane for methyl ester analysis by gas chromatography. A stainless steel column $(2 \text{ m} \times \frac{1}{8} \text{ in } i.d.)$ packed with 20% DEGS on Chromasorb W 80-100 mesh was used in a Perkin-Elmer 8300 gas chromatograph equipped with a flame ionization detector. The column was operated at 140-195°C with a nitrogen carrier gas flow rate of 30 ml min⁻¹. FAME were expressed as a percentage of the dry weight of the pea flour.

Identification of FAME

A Hewlett-Packard 5995 gas chromatograph-mass spectrometer, operated with an ionizing current of 100 μ A at 70 eV electron energy in the electron impact mode, a source temperature of 200°C and GC interface at 250- 270°C, was used to identify individual FAME. The column HP-1 (cross-linked methyl silicone gum) of 25 m \times 0.2 mm \times 0.11 μ m film thickness was used with carrier gas (helium).

Separation of lipid classes

Lipids were extracted as described in the preceding sections and separated by thin-layer chromatography (TLC) on plates coated with 0.2 mm layers of aluminium silica gel 60 without fluorescent indicator and activated at 120°C for 1.5 h.

For the separation of non-polar lipids, 70 μ l of lipid extract was applied to each lane (1-5-2.0 cm wide) and the plates were developed to 12 cm in a two-step singledimensional TLC with diethyl ether-toluene-ethanolacetic acid $(40:50:2:0.2)$. Afterwards, they were air-dried with slight warming if necessary and redeveloped in the same direction to 18 cm with diethyl ether-hexane (6:94). For the separation of phospholipids, plates were developed in the solvent system of chloroform-methanolammonia (33% w/v)-water (65:35:5:2.5) (Morrison *et al.,* 1980).

Lipid classes were identified by their migration characteristics relative to standards that were chromatographed simultaneously. Lipid spots were detected by specific reagents; ninhydrin, for phosphatides or lipids having a free amino group, and as little as $1 \mu l$ of aminophosphatide, such as phosphatidyl ethanolamine or phosphatidyl serine (Marinetti, 1964); the Dragendorff stain, for choline-containing lipids which appear as orange spots within a few minutes (Beiss, 1964); ferric chloride solution, for sterols and their esters which appear as violet colour in the oven at 100°C; and iodine vapour for neutral lipid (Kates, 1972). Lipid bands were detected by spraying with 3.5% molybdatophosphoric acid, dried under a stream of hot air and then developed in the oven at 140°C. Heavy bands of lipid could then be seen.

For quantitative determination of lipid classes, the resulting dark colour was estimated by using photocopies on to transparent sheets (Nagata *et al.,* 1988). The density of each spot on the photocopy was measured with a Kontron Analytic gel-scanner 91-00275 (Kontron Instruments, Zurich, Switzerland) for UVIKON 810/ 820 DB and the integrated surfaces were quantified using a IBAS-I interactive image analysis system with a Kontron standard software Y.

RESULTS AND DISCUSSION

The tenderometer values, moisture content, crude oil content and composition of different pea seed sizes are presented in Tables 1 and 2.

Table 1. Characteristics of pea samples

Pea	Size	Tenderometer	Moisture $(\%)$		
	(mm)	value	Raw	Canned	
SF	$4.7 - 7.5$	96.4 ± 2.9		77.29 ± 1.20 83.62 \pm 0.30	
VF	$7.6 - 8.2$	101.6 ± 3.8	76.10 ± 1.96 80.87 ± 0.91		
FN MD	$8.3 - 8.8$ $8.9 - 10.2$	113.2 ± 4.9 126.0 ± 6.0	73.60 ± 1.14 78.09 ± 0.41	72.42 ± 1.03 77.29 ± 0.48	

SF, Superfine; VF, Very fine; FN, Fine; MD, Middle.

Effect of size

Oil content

Similar lipid contents were obtained in fresh samples for SF and VF sizes, but during the growth period from VF to MD size there is an apparently active lipid degradation. However, this does not necessarily mean that there is any destruction of lipids since a significant increase in carbohydrate or protein level would lead to a decrease in % lipid, even if the lipid component actually increased slightly. A previous paper (Ros $&$ Rincón, 1990) showed an increase in protein content during seed growth just to FN size, but decreases in MD size. In the Govorov variety, for the 7-8 mm to 8-9-5 mm growth period, there is an increase of 25% in oil content, but from the $8.9-9.5$ mm to < 9.5 mm growth period a decrease of 4.4% was reported (Bengtsson & Bosund, 1966). Geervani and Devi (1988) obtained lower oil content in immature green pea (14-18 days after flowering) than in mature stages (10 days on the plant for the green colour of the pods to disappear), with values of 0.49 and 0.75%, respectively. During germination, oil content decreases (Chen *et al.,* 1975). Results in Manuela variety obtained here showed that lipid accumulation finished just at VF size (7-6-8.2 mm), before seed germination. These differences are due to the fact that the oil content is determined by genotype (Welch & Griffiths, 1984) and that the activities of the enzymes

Table 2. Crude oil content and composition for different pea sizes and processing phases

Size		Crude oil b			Relative peak area (% methyl esters) ^a		
		$(\%)$	16:0 18:0 18:1 18:2		18:3		
SF	(R)	3.5 ± 0.2	23.4 ± 3.3	19.7 ± 5.2	16.7 ± 5.7	28.2 ± 2.1	12.0 ± 3.9
	(C)	1.5 ± 0.3	17.5 ± 1.4	9.4 ± 2.0	14.6 ± 1.4	42.4 ± 2.9	16.1 ± 2.4
VF	(R)	3.4 ± 0.3	16.4 ± 2.7	15.2 ± 5.3	23.5 ± 4.1	32.9 ± 2.8	12.0 ± 1.9
	$\rm (C)$	2.3 ± 0.6	16.9 ± 0.3	6.1 ± 0.3	18.9 ± 0.5	46.7 ± 1.4	11.4 ± 0.4
FN	(R)	2.6 ± 0.2	18.1 ± 2.8	12.8 ± 0.7	22.8 ± 4.8	32.4 ± 1.8	13.9 ± 4.2
	(C)	2.2 ± 0.3	17.3 ± 0.8	6.8 ± 1.9	18.7 ± 0.9	45.6 ± 2.3	12.6 ± 1.8
MD	(R)	1.6 ± 0.1	28.5 ± 1.8	26.4 ± 1.4	18.1 ± 0.8	20.9 ± 3.8	6.1 ± 2.5
	(C)	1.2 ± 0.1	23.3 ± 2.1	20.2 ± 0.6	18.1 ± 1.2	32.7 ± 2.5	67 ± 1.7

^a Means of three experiments. 16:0, Palmitic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

 b Expressed on a fresh weight basis.</sup>

SF, Superfine; VF, Very fine; FN, Fine; MD, Middle.

R, Raw; C, Canned.

necessary for fatty acid synthesis in seed are influenced by environmental factors (Harwood & Stumpf, 1970).

Fatty acids composition

Results indicate that the major fatty acids in fresh pea are linoleic acid in small and medium types (SF, VF, FN) and palmitic acid in larger (MD), and that the rarest fatty acid is linolenic acid in all sizes (Table 2). In general, this result is in accordance with those obtained by other authors (Welch & Griffith, 1984; Coxon & Wright, 1985; Savage & Deo, 1989). However, the rarest fatty acid in pea seeds may also be stearic acid (Bengtsson & Bosund, 1966; Coxon & Wright, 1985), or oleic acid (EI-Refai *et al.,* 1987). In the majority of 166 pea lines examined by Coxon and Wright (1985) the fatty acid composition was 18:2 > $18:1 > 16:0 = 18:3 > 18:0$. This composition is the same as that of FN size and similar to SF and VF, although data for MD size (Table 2) do not match the typical fatty acid composition of pea seed reported by Coxon and Wright (1985). Bengtsson and Bosund (1966) examined the fatty acid composition of fresh peas in relation to their average diameter at harvest and found that linoleic acid increased, while palmitic and linolenic acids decreased, as the diameter of the pea increased during the growth period from 7.5 to 10 mm. The decrease in linolenic and linoleic acids and the increase in palmitic and stearic acids reported here for MD size can be explained by two hypotheses: during the FN and MD growth period there is (a) an increase in palmitic and stearic acid synthesis or (b) an increase in cotyledon/testa ratio.

Effect of temperature

In the first hypothesis, it must be taken into account that four weeks before harvesting a significant decrease in temperature (reaching almost 0° C) was noted (Fig. 1). In comparison with those results obtained by Bengtsson and Bosund (1966), the differences in results as regards changes in linoleic and linolenic acid contents, in addition to varietal differences in oil content (McCurdy *et aL,* 1983) and composition (Welch & Griffiths, 1984; Coxon & Wright, 1985; Savage & Deo, 1989), may be due to agroclimatic factors, as it is known that the

Fig. 1. Maximum and minimum temperature values occurring during pea culture.

Table 3. Cotyledon/testa ratio for different pea sizes considered

Size	Per $100 g$	Ratio	
	No. of peas	Testa weight	Cotyledon/testa
SF	495	34.2	1.92
VF	399	26.3	2.80
FN	301	25.4	2.94
MD	238	21.4	3.67

SF, Superfine; VF, Very fine; FN, Fine; MD, Middle.

enzymes involved in fatty acid biosynthesis depend as these factors (Harwood & Stumpf, 1970).

Effect of cotyledon/testa ratio

In the second hypothesis, a comparison of the fatty acid composition of lipids from cotyledons and testa revealed great differences (Bengtsson & Bosund, 1966; Welch & Griffiths, 1984; Savage & Deo, 1989). Table 3 shows the cotyledon/testa ratio for the different sizes considered. An increase $(p < 0.01)$ in the cotyledon/ testa ratio was obtained during the growth period from FN to MD size. In the cotyledons, oleic and linoleic acids occurred in about equal amount (8.5 and 9.7%, respectively), while in the testa the amounts of linolenic acid were about 9 times greater $(2.5 \text{ and } 22.5\%$, respectively) (Bengtsson & Bosund, 1966) and the percentage of testa decreased as the diameter of pea increased (Table 3). In addition, palmitic acid in whole seeds ranged from 8.6 to 19.5% and the average content reported in cotyledons was 28.0% (Savage & Deo, 1989), and in some varieties, such as Scout or Vineta, cotyledons showed higher concentrations of palmitic $(\times 2.5)$ and stearic $(\times 6)$ acids than testa (Welch & Griffiths, 1984).

In conclusion, oil content decreases to the end of the growth period considered (MD size) and, comparing the present authors' results in the Manuela variety to those obtained in other varieties reported by different authors, the fatty acid composition depends on the environmental temperature during plant growth and the cotyledon/testa ratio during seed growth.

Nutritional and technological implications

Changes in those ratios of nutritional and technological interest for fatty acids are shown in Fig. 2. The unsaturated/saturated ratio (U/S) increases during the growth period from VF to FN size and decreases sharply in MD size, principally due to a reduction in linolenic acid (Table 2), which is responsible for an increase in the non-essential/essential ratio (N/E) (Fig. 2). The linoleic/oleic ratio (L/O) decreases slowly during the whole growth period studied (from VF to MD size). Significantly lower linolenic acid content was obtained in MD size, and this fact is very important because lipoxygenase can function at low water activity, such as

Fig. 2. Changes in unsaturated/saturated $(\bigcirc$, raw; \bullet , canned), non-essential/essential (\Box , raw; \blacksquare , canned) and linoleic/oleic $(\Delta, \text{raw}; \triangle, \text{canned})$ ratios during growth and later canning, for four sizes (SF, superfine; VF, very fine; FN, fine; MD, middle).

in a finely divided pea flour. Autoxidation would also be expected to occur in such a material and the content of polyunsaturated fatty acids (particularly linolenic acid) would influence the susceptibility to autoxidative deterioration (Coxon & Wright, 1985). For these reasons and according to our results, MD size is principally recommended for the elaboration of flour.

Neutral lipids in raw pea ranged from 70.9% of total lipids in MD size to 77-3% in VF size (Table 4). These results are higher than those obtained by Reichert and Mackenzie (1982), who reported that approximately 50-60% of the total lipid content of peas is present in the neutral lipid fraction, and those obtained by Coxon and Wright (1985) in round peas (from 36.3 to 57.6%) and wrinkled peas (from 41.0 to 55.9%). Higher levels of triglycerides, fatty acid esters, diglycerides and acylsterylglycoside were obtained in SF size and higher levels of steryl esters, free fatty acids and sterols were obtained in VF size (Table 4). Free fatty acid content obtained from immature soybean seeds is higher than that obtained from mature seed, suggesting that triglycerides are being synthesized constantly from the fatty acid pool during the growth process (Yao *et al.,* 1983). As lipoxygenase is active primarily with free polyunsaturated fatty acids (Chompreeda & Fields, 1984) or on ester bound forms such as methyl ester or triglycerides (Sessa, 1979), a reduction in the oil and linolenic acid contents of peas could improve the quality of this crop for processing (Welch & Griffiths, 1984). In the Manuela variety, such a reduction can be obtained by harvesting the pea crop when the MD

a Expressed as ng/100 mg, on a dry weight basis.

SF, Superfine; VF, Very fine; FN, Fine; MD, Middle.

R, Raw; C, Canned.

size is predominant. Because of the above considerations and the lipid composition, MD size is recommended for pea flour elaboration.

Haydar and Hadziyev (1973) noted that the neutral lipid/polar lipid ratio varied considerably between varieties. The present authors' results showed that this ratio varied widely during seed growth (Table 5). A significant increase in glycolipid content was observed in the MD size (Table 4).

Phospholipids ranged from 19.5% in SF size to 14-6% in VF size (Table 4). Higher phospholipid contents are reported by Coxon and Wright (1985) in round peas (from 31-8 to 54.0%) and wrinkled peas (from 38.4 to 54.1%). Phosphatidylethanolamine is the principal phospholipid of pea (Table 4). The larger size (MD) shows lower phospholipid contents than of SF size and similar to that in FN size. In addition, MD size showed a significant decrease in triglycerides, monoglycerides, free fatty acids and in the L/O and U/S ratios (Table 4, Fig. 2).

Effect of canning

Effect of surface area

Differences ($p < 0.01$) in oil content after commercial canning were only observed in SF and VF sizes (blanched for 3 min at 90°C). However, in FN and MD sizes (blanched for 8 min at 90°C) oil content is not modified significantly. This may be explained by the greater surface area exposed to the blanching water and brine solution by the smallest peas, since there is a greater number of peas per 100 g (Table 3). 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 (EI-Refai *et al.,* 1987), according with the results for FN and MD sizes (Table 2). Fricker *et al.* (1975) reported that, as a consequence of heating plant cells, their 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, surface area is a more important factor than blanching time to determine oil content modifications in canned pea.

Nutritional and technological implications

The principal fatty acid occurring on canned pea is linoleic acid (Table 2) in all sizes and the rarest fatty acid is stearic in small and medium sizes (SF, VF, FN)

Table 5. Neutral lipid and polar lipid ratio for raw and canned peas

	SF	VF	FN	MD
Raw	2.5	3.4	3.3	$2-4$
Canned	3. I	2.8	4-1	3·6

or linolenic acid in the higher size (MD). During canning, the U/S ratio increased in all sizes (Fig. 2), but only significantly in small sizes (SF and VF), principally due to a decrease of stearic acid (Table 2). A decrease in the N/E ratio occurred during canning (Fig. 2) principally due to the decrease in stearic and to the increase in linolenic acid (Table 2). The L/O ratio increased in canned peas (Fig. 2) because of the increased linoleic acid (Table 2). In MD size, the increase in the L/O ratio is not significant because little modification in oleic acid is obtained in canned peas (Table 2). These results are in agreement with those obtained by EI-Refai *et al.* (1987), who reported a decrease in percentages of myristic, palmitic and oleic acid (4-I, 5.1 and 6%, respectively) during canning. They also reported an increase in the U/S ratio (from 1.49 to 1.70) and an increase in linoleic and linolenic acids (4.8 and 5.1%, respectively), which increased the L/O ratio (from 1.86 to 3.85).

Tables 4 and 5 show modifications in the lipid classes during canning. Significant decreases of sterol esters, triacylglycerols and free fatty acids have been observed in the SF, VF and FN sizes. As a consequence of the thermic processing, an increase in the contents of diacylglycerols has been observed similarly, and this is probably due to degradation of polar lipids and triacylglycerols; apart from that, an increase in sterols contents was also observed, possibly due to degradation of esters of sterols (Table 4).

A decrease in the content of phospholipid was observed as a consequence of the thermic processing (Table 4). Results show a high neutral lipid/polar liquid ratio in canned peas of the MD size (Table 5), due to phospholipid losses during canning, and also to lower levels of cardiolipin and phosphatidylglycerol, these two phospholipids being rich in polyunsaturated fatty acids. In addition, canned peas of MD size show a lower level in linolenic acid (Table 2) and triacylglycerols (Table 4). Belitz and Grosch (1988) established that the action of phospholipase A_1 (present in vegetables) is a prerequisite to oxidation of phospholipids by pea lipoxygenase. According to the present authors' results, canned peas of the Manuela variety with a tenderometer value in raw pea >120 (size >8.9 mm) should be used in the manufacture of flour, in order to prevent any deterioration of flavour, because lipoxygenase mediates the conversion of polyunsaturated fatty acids to aldehydes and alcohols and these are major contributors to the off-flavours in legume protein products (Sessa, 1979).

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