Food Chemistry **23** (1987) 117–127

Effect of Processing and Storage on Protein and Lipid Composition of Peas

Ahmed A. El-Refai, Mahmoud S. Gouda & Kamal A. Ammar

Food Science and Technology Department, Faculty of Agriculture,
Tanta University, Kafr El-Sheikh, Egypt

(Received 14 March 1986; accepted 10 July 1986)

ABSTRACT

*Changes in protein and lipid contents of peas (*P. sativum *L.) at various stages of processing and storage at different temperatures over a six-month period were studied. Blanching had very little effect on protein or protein amino acids of peas while free amino acids were greatly affected. Immediately after bottling and canning peas, total protein differed little from that of blanched, unpackaged peas. However, free amino acids decreased much more than did protein amino acids. Storage of canned peas for six months at room temperature caused changes in some protein amino acids.*

Total lipids of fresh peas did not change after blanching, bottling or canning. After storage for six months, no changes were observed in the total lipids while there were increases in glycolipids and decreases in phosphatides.

Increases in monogalactosyl diglycerides and decreases in digalactosyl diglycerides were also observed.

INTRODUCTION

Various factors affecting protein and amino acid losses during blanching, thermal processing and storage have been well reviewed recently by Bender (1978) but his review was restricted mainly to high protein foods. Peas are known to contain trypsin inhibitors which can be inactivated by heating and this causes an improvement in protein utilization (Deatherage, 1975). On the other hand, other activities (for example, the Maillard reactions) also take place, which may decrease the amount of protein during

Food Chemistry 0308-8146/87/$03·50

processing and storage. Furthermore, blanching, processing and storage conditions of vegetables are known to influence other nutritive compounds, as well as lipids. Very little is known about the fate of small amounts of lipids in vegetables such as peas (Deatherage, 1975). On heat treatment lipoxygenase is inactivated. Lipoxygenase is active primarily with free unsaturated fatty acids resulting in off-flavours (Fujimake *et al.*, 1968; Mustakas *et al.*, 1970; Eriksson, 1972; Svensson & Eriksson, 1972; Chompreeda & Fields, 1984).

The purpose of this investigation was to study the influence of processing and storage conditions of green peas on their protein and amino acid composition. Also, the behaviour of small amounts of lipids and fatty acids present in peas during processing and storage was studied.

MATERIALS AND METHODS

Green pea samples and preparation of peas

The green pea samples were obtained from Kafr El-Sheikh location, Egypt. Samples were prepared and blanched for 2 min in boiling water. Peas were divided into two equal groups. One of these groups was bottled in glass jars (500 g) with twist-off caps and a filled weight of 200 g of peas. The pH of the final product was brought to 3·25 with distilled vinegar; the overall acid content of the final pack was 1·5%. The vinegar solution was added at 70°C and the capped glass jars had an equilibrium temperature of ~54°C. The jars were pasteurized in water at 85°C for 13 min and then air cooled. The other group of peas was canned (placed in a tin-plate, lacquered can to a filled weight of 200 g and filled with 0·05% citric acid). The pH of the final product was ~4·5 and the closing temperature was 85°C. The peas were sterilized at 120°C for 20 min, cooled and stored. Blanching, bottling and canning were exactly as described by Farhangi & Valadon (1981).

The storage conditions

The bottled peas were stored for six months at 10°C and room temperature. The canned peas were stored for the same period of time at room temperature (about 30°C).

Analytical methods

Protein extraction and determination were carried out according to the methods of Fraser & Loening (1974) and Lowry *et al.* (1957).

Free amino acids and total amino acid contents were estimated as mentioned by Russel (1944) and Naguib (1964) with modifications as follows.

Free amino acids

The peas were dried at 70–80°C for 12 h and ground to a fine powder, some of which was also used for the determination of tryptophan. To 50 mg of this powder 5 ml of 2% phenol and 10 ml of 30% trichloroacetic acid (TCA) were added; the mixture was left overnight and then filtered. The filtrate, containing free amino acids, was adjusted to pH $2 \pm 0{\cdot}1$ with 5N NaOH.

Protein amino acids

The filter paper containing the precipitate was kept at 50°C overnight. The dried residue (5 mg) was hydrolyzed with 5 ml of HCl (10N) in a sealed tube at 110°C for 5 h. The hydrolyzate was filtered and adjusted to pH $2 \pm 0{\cdot}1$ with NaOH (5N). This solution contains acid stable protein amino acids. Tryptophan, which is destroyed under acid hydrolysis, requires a separate method of analysis. Tryptophan was determined using the method of Osborne & Voogt (1978).

The separation and identification of the amino acids were carried out on a Toel Model JLC 6AH fully automatic amino acid analyzer. The amount of amino acid in each sample was calculated by comparison of peak areas with those obtained using a calibration mixture as described by Everleigh & Winter (1970).

For lipid extraction and determination of total lipids, the methods of Folch *et al.* (1957) and Deven & Manocha (1975), respectively, were used.

Separation of lipid classes

Lipid classes were separated by thin-layer chromatography (TLC) on 0·25 mm polygram silica gel G/uv 254 (Macherey-Nageland Company, Düren, FRG).

For the separation of simple lipids, plates were developed in the solvent system of hexane:diethylether:formic acid (80:20:2), where the complex lipids stayed at the origin.

Complex lipids were separated by the use of (i) two-step single dimensional TLC using petroleum ether:acetone (3:1) as the first solvent with which the faster moving simple lipids were removed and chloroform: methanol:acetic acid:water (170:25:25:6) which actually separated the complex lipids and (ii) two-dimensional TLC using chloroform:methanol: 7N ammonium hydroxide (65:30:6) in the first run and chloroform: methanol:acetic acid:water (170:25:25:6) in the second run.

Lipid classes were identified by their migration characteristics relative to authentic standards that were chromatographed either simultaneously alongside the samples under investigation or with them. Lipid spots were detected by specific spray reagents (Christie, 1973), ninhydrin for amino phosphatides, molybdenum-blue sulphuric acid for phosphatides (Dittmer & Lester, 1964), acid ferric chloride for sterols and their esters, α-naphthol for glycosides and iodine vapour for neutral lipids. For quantitative determination of lipid classes, the developed TLC plates were sprayed with 3% cupric acetate in 8% phosphoric acid heated at 180°C for 25 min (Fewster *et al.*, 1949) and the resulting dark colour estimated by using a Toycel-Loebl Chromoscan densitometer (Gasbarro, 1972). Results with the densitometer scans were generally realiable as they compared favourably with those of the weighing method.

Total fatty acids were determined according to the method of Asselineau & Montrozier (1976). Free fatty acids were extracted by the method of Draper (1969). Fatty acid methyl esters were prepared using BF_3-methanol reagent according to Metcalfe & Schmitz (1961).

Sterol analysis

The total lipids fraction was saponified at room temperature in 12% KOH in absolute EtOH under N_2 for 20 h. Steroids were extracted with ether after dilution with water. The ether layer was washed with water to remove the alkali, dried with Na_2SO_4, evaporated to dryness and weighed.

This extract (containing steroids) was then dissolved in a small volume of $CHCl_3$:MeOH (2:1) and analyzed.

The fatty acid methyl esters and sterols were analyzed using a Pye-Unicam gas chromatograph. The 1·5-m (inside diameter, 4 mm) glass column was packed with 10% PEGA on Chromosorb WAW DCMS 60–80 mesh (Pye-Unicam). The column temperature was programmed for 75–180°C (8°C/min). N_2 flow was 30 ml/min. Methyl esters and sterols were identified by comparing their retention times with those of authentic standards and by GC–MS quantified by the peak area method.

GC–MS of fatty acid methyl esters

The apparatus used was a Kratos MS 25 mass spectrometer interfaced to a Perkin-Elmer Sigma 3 gas chromatograph. Mass spectral data were obtained by a Kratox DS-50 S computer data system.

The instrument was operated with an ionizing current of 100 μA at 70 eV electron energy in electron impact mode, with a source temperature of 250°C and the GC interface (all-glass jet separator) at 250–270°C. The 1·5-m (inside diameter, 4 mm) WAW DCMS 60–80 mesh (Pye-Unicam) column was fed with carrier gas (helium) at 30 ml/min.

All the experiments were repeated several times and the results are the average ±SD of at least three determinations.

RESULTS AND DISCUSSION

Effect of processing and storage conditions on protein and amino acid composition of green peas

The protein contents of green peas at different stages of processing and protein percentage retentions of processed green peas (compared with fresh samples) over a six-month storage period are given in Table 1. The results indicate that blanching, sterilization and storage of peas showed no noticeable effect on the protein content compared with the fresh ones. These results are in accordance with those of Adam *et al.* (1942), who reported that a slight loss of protein was found (about 4%) after water-blanching in a wide range of vegetables which could be attributed to extraction of soluble proteins and also to hydrolysis of protein into free amino acids that may couple with carbohydrates, especially reducing sugars, to form pigments. From Table 1, also, it can be observed that no losses occurred in total protein content of bottled or canned peas stored under different temperature conditions (10°C and room temperature) over the six-month period.

TABLE 1
Changes of the Total Protein Content during Processing and Storage of Green Peas

Sample	*Protein content*	
	g/100 g (75% water content)	*Retention (%)*
Fresh	7·0	100
After blanching	6·6	94·3
After bottling	6·5	92·9
After canning	6·5	92·9
After storage at 10°C (6 months)		
Bottled	6·4	91·4
Canned	6·5	92·9
After storage at room temperature (6 months)		
Bottled	6·4	91·4
Canned	6·4	91·4

TABLE 2
Free Amino Acid Composition of Green Peas at Different Stages of Processing and during Storage at Different Temperatures over a Six-month Period (as mg/100 g product)

Amino acid	*Fresh peas*	*After*			*Stored peas*		
		Blanching	*Bottling*	*Canning*	*Bottled*		*Canned (room temperature)*
					10°C	*Room temperature*	
Glutamic acid	34·2	28·8	24·5	18·7	16·8	9·6	9·8
Arginine	29·5	18·0	11·4	9·3	9·0	4·2	4·2
Aspartic acid	22·2	15·0	11·9	12·0	12·0	12·0	12·0
Lysine	18·0	12·0	11·7	10·5	3·0	2·4	2·4
Leucine	19·2	9·6	7·2	4·3	5·3	3·8	3·9
Alanine	15·0	5·0	4·8	4·3	3·5	3·4	3·4
Threonine	13·1	11·2	10·3	6·7	1·0	0·3	0·3
Valine	11·5	6·4	4·0	2·0	1·8	1·1	1·1
Phenylalanine	9·6	6·0	5·8	2·7	2·1	1·0	0·8
Serine	9·6	5·6	4·8	4·1	1·7	1·4	1·4
Proline	8·2	5·6	5·3	4·2	1·2	0·6	0·3
Glycine	9·5	5·0	3·1	1·5	1·3	0·0	0·0
Isoleucine	9·4	5·8	5·0	4·5	4·8	4·0	3·0
Tyrosine	6·8	3·2	2·9	1·8	1·9	0·6	0·6
Histidine	4·8	4·5	4·3	4·3	1·8	1·0	0·7
Tryptophan	2·5	2·0	2·0	1·7	1·5	1·0	1·0
Cystine	0·6	0·4	0·2	0·2	0·2	Traces	Traces
Methionine	0·4	0·2	0·1	0·1	Traces	Traces	Traces
Total	224	144	119	92·9	68·9	46·4	44·9

The total free amino acids of all samples stored for six months at 10°C and room temperature decreased (Table 2). The free amino acids most retained in all stored samples were aspartic acid and glutamic acid.

The amino acid composition of protein changed slightly during storage (Table 3). A decrease of lysine, cysteine, methionine and tryptophan was observed during storage, and a relative increase of glutamic acid. Other protein amino acids showed no significant changes. These results suggest that sugar–amino acid reactions (Maillard reactions) or other reactions, including hydroperoxides formed from unsaturated fats, had occurred (Carpenter & Booth, 1973).

Effect of processing and storage on lipid composition of green peas

The total lipid content of fresh peas was 419·0 mg/100 g fresh weight and was not affected by blanching, bottling or canning (Table 4). These results are in agreement with those of Ghanem & Hassan (1970). After storage for

TABLE 3
Amino Acid Composition of Protein in Green Peas at Different Stages of Processing and after Storage at Different Temperatures over a Six-month Period (as g/100 g protein)

Amino acid	*Fresh peas*	*After*			*Stored peas*		
		Blanching	*Bottling*	*Canning*	*Bottled*		*Canned (room temperature)*
					10°C	*Room temperature*	
Glutamic acid	17·0	17·0	17·3	17·5	17·3	17·3	17·8
Arginine	7·09	7·01	6·90	7·10	7·00	6·90	7·05
Aspartic acid	10·9	10·9	10·9	10·3	11·2	11·1	10·7
Lysine	6·96	6·72	6·20	6·41	6·23	5·82	6·02
Leucine	7·83	7·81	7·52	7·96	8·02	7·75	7·68
Alanine	2·95	3·10	3·21	2·91	3·05	2·75	2·84
Threonine	4·13	3·80	4·25	4·29	3·95	4·23	4·00
Valine	6·09	6·10	6·21	5·95	6·05	6·03	6·11
Phenylalanine	3·43	3·65	3·51	3·70	3·41	3·72	3·54
Serine	6·09	5·80	5·75	6·05	6·10	5·70	5·95
Proline	5·22	5·30	5·41	6·43	5·30	5·45	5·61
Glycine	6·96	6·91	7·05	7·40	7·10	6·90	7·21
Isoleucine	6·52	5·50	5·80	6·70	6·85	6·49	7·01
Tyrosine	3·98	4·10	3·70	4·48	3·75	3·68	3·61
Histidine	1·96	1·70	1·58	1·49	1·50	1·80	1·62
Tryptophan	1·01	0·90	0·98	0·90	0·85	0·91	0·83
Cystine	1·17	1·00	0·90	0·90	0·85	0·90	0·75
Methionine	0·80	0·80	0·95	0·81	0·65	0·60	0·75
Total	100	98·1	98·1	97·9	99·2	97·9	99·1

six months, very little effect on the total lipids was noticeable in any of the samples.

The simple lipids identified in fresh peas were mono-, di- and triglycerides, free fatty acid esters and the sterols, stigmasterol and β-sitosterol, and their esters (Table 4). The percentage of free fatty acids, monoglycerides and diglycerides of total simple lipids were 18·0%, 8·4% and 8·0%, respectively. These amounts were increased under all conditions tested (Table 4). As shown in Table 4, the phospholipids represent 89·9% of the total complex lipids in fresh peas. Glycolipids, together with phospholipids, made up the total complex lipids. Phosphatides decreased during storage and an increase of lysophosphatides was observed. The monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) were affected differently during storage. The monogalactosyl diglyceride content increased markedly to reach a high of 26·5% in peas stored in jars at room temperature and DGDG decreased under the three conditions of storage.

TABLE 4
Simple and Complex Lipids of Fresh and Stored Peas at Different Temperatures over a Six-month Period. (Results are expressed as % total unless otherwise stated.)

Components	*Fresh peas*	*After storage*		
		Bottled (10°C)	*Bottled*	*Canned*
			Room temperature	
Simple lipids				
Sterol esters	18·0	10·8	10·0	10·4
Fatty acid esters	1·2	1·2	1·2	1·0
Triglycerides	10·8	10·8	10·4	10·8
Free fatty acids	18·0	33·5	40·0	35·0
Stigmasterol	12·0	7·2	8·5	7·0
β-Sitosterol	43·2	17·0	15·5	16·7
Diglycerides	8·0	14·5	13·0	10·8
Monoglycerides	8·4	25·0	18·0	27·0
Total (mg/100 g)	39·0	39·5	51·5	41·0
Complex lipids				
Sterol glycoside	9·6	9·0	9·5	9·6
Cardiolipin	9·6	Traces	0·0	0·0
Phosphatidic acid	14·4	7·5	0·0	0·0
Monogalactosyl diglyceride (MGDG)	6·0	19·0	26·5	20·5
Ceramide monohexoside	7·2	4·8	3·5	3·5
Phosphatidyl glycerol	6·0	14·5	18·0	18·0
Phosphatidyl ethanolamine	14·3	14·7	11·0	12·0
Digalactosyl diglyceride (DGDG)	3·6	2·5	2·5	1·5
Sulpholipid	1·2	1·2	2·5	2·5
Phosphatidyl inositol	2·4	1·2	1·2	2·5
Phosphatidyl choline	38·4	24·0	14·5	15·5
Lysophosphatidyl ethanolamine		1·5	3·5	3·6
Lysophosphatidyl inositol ceramide		2·5	3·5	5·0
Lysophosphatidyl choline	7·2	18·0	24·0	26·5
Total (mg/100 g)	380	363	317	343
Total lipids (mg/100 g)	419	402	368	384

Glycolipid content increased mostly due to increase in MGDG. The higher the temperature of storage, the greater was the increase in MGDG. Fricker *et al.*'s (1975) studies on spinach at temperatures of up to 100°C suggested that lipid fractions were affected by heat treatment; with increasing heat the MGDG content increased and DGDG contents decreased. This relationship was not stoichiometric and it was possible that further reactions led to the formation of other unidentified products. Similar results were obtained in this work and it can be suggested that

TABLE 5
Free Fatty Acids of Fresh and Stored Peas at Different Temperatures over a Six-month Period. (Results are expressed as μg/g wet weight.)

Fatty acids	*Fresh peas*	*Stored peas*		
		Bottled (10°C)	*Bottled*	*Canned*
			Room temperature	
14:0	5·3	23·8	32·0	29·0
16:0	35·5	60·5	75·7	56·0
18:0	30·2	41·0	44·0	42·0
18:1	24·8	30·5	31·8	28·0
18:2	46·2	95·0	112	108
18:3	35·0	88·4	104	87·0
Total FAA (μg/g)	177	339	399	350

MGDG and DGDG may be formed through separate pathways but may be formed from the same precursor (1,2-diglyceride).

Under the conditions of the present study, it is possible that 1,2-diglyceride could combine with free galactose (Farhangi, 1980) to give rise to MGDG. Fricker *et al.* (1975) reported that the influence of heat may change plant cells and their membranes in such a way that lipids not accessible to the solvent in the fresh product become more readily extractable. This would account for the higher increase in MGDG without a corresponding decrease in DGDG.

Table 5 shows the fatty acid composition of pea samples under investigation. These results indicate that the major fatty acids occurring in fresh pea were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3). Myristic acid (14:0) occurred as a minor component. As shown in Table 5, the total free fatty acid content increased under all conditions of storage compared with fresh peas. The total free fatty acid content of bottled peas stored at room temperature (399 μg/g) was higher than that of canned peas (350 μg/g), while the stored peas in jars at 10°C had the smallest amount (339 μg/g). These results indicate that the total free fatty acids were affected by the storage temperature and containers.

REFERENCES

Adam, W. B., Horner, G. & Stanworth, I. (1942). Changes occurring during the blanching of vegetables. *T. Soc. Chem. Ind.*, **61**, 96.

Asselineau, C. P. & Montrozier, H. L. (1976). Etude du processus de biosynthèse des acides phléiques, acides polyunsaturés synthétisés par *Mycobacterium phlei. Eur. J. Biochem.*, **63**, 509.

Bender, A. E. (1978). *Food processing and nutrition*, Academic Press, London and New York.

Carpenter, K. J. & Booth, V. H. (1973). Damage to lysine in food processing: Its measurement and its significance. *Nut. Abstr. Rev.*, **43**, 423.

Chompreeda, P. T. & Fields, M. L. (1984). Effect of heat and natural fermentation on amino acids, flatus producing compounds, lipid oxidation and trypsin inhibitor in blends of soybean and cornmeal. *J. Food Sci.*, **49**, 563.

Christie, W. W. (1973), *Lipid analysis*, Pergamon Press, Oxford and New York.

Deatherage, F. E. (1975). *Food for life*, Plenum Press, New York and London.

Deven, J. M. & Manocha, M. S. (1975). Effect of glutamic acid on the fatty acid and lipid composition of *Choanephora cucurbitarum. Can. J. Microbiol.*, **21**, 1827.

Dittmer, J. C. & Lester, R. L. (1964). A simple specific spray for the detection of phospholipids on TLC. *J. Lipid Res.*, **5**, 126.

Draper, P. (1969). Lipid changes in senescing cucumber cotyledons. *Phytochemistry*, **8**, 1641.

Eriksson, C. E. (1972). Aroma compounds derived from oxidized lipids. Some biochemical and analytical aspects. *J. Agr. Food Chem.*, **23**, 126.

Everleigh, W. T. & Winter, G. D. (1970). Amino acid composition determination. In *Protein sequence determination* (Needleman, S. B. (Ed.)), Chapman and Hall, London, 91.

Farhangi, M. (1980). *Light and processing effect on protein, lipids, carbohydrates, carotenoids, vitamin C and on plastid ultrastructure of* Phaseolus aureus Roxb. *(mung bean) seedlings.* PhD Thesis, University of London, Great Britain.

Farhangi, M. & Valadon, L. R. G. (1981). The effect of acidified processing and storage on carotenoids (provitamin A) and vitamin C in mung bean sprouts. *J. Food Sci.*, **46**, 1464.

Fewster, J. E., Mudra, A. E., Ives, M. & Tompkins, M. D. (1949). Effect of blanching time on vitamin retention in canned peas. *Canner*, **108**, 27.

Folch, J., Lees, M. & Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497.

Fraser, R. S. S. & Loening, U. E. (1974). RNA synthesis during synchronous cell division in cultured explants of Jerusalem artichoke tuber. *J. Exp. Bot.*, **25**, 847.

Fricker, A., Duben, R., Heintze, K., Panlas, K. & Zohm, H. (1975). Influence of heat treatment of spinach at temperatures up to 100°C on important constituents. Total lipids and glycolipids. *Lebensm. Wiss. u. Technol.*, **8**, 172.

Fujimake, M., Kato, H., Aria, S. & Tamaki, E. (1968). Applying proteolytic enzymes on soybeans. *Food Technol.*, **22**, 889.

Gasbarro, L. (1972). Simultaneous evaluation of phospholipids, free fatty acids, free cholesterol, triglycerides and esterified cholesterol by thin-layer chromatography and densitometry. *Clin. Chim. Acta*, **37**, 271.

Ghanem, S. S. & Hassan, F. M. (1970). Effect of pickling on the nutritive value of different vegetables used in Egyptian pickles. *U.A.R.J. Bot.*, **13**, 191.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1957). Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.*, **193**, 265.

Metcalfe, I. D. & Schmitz, A. A. (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Anal. Chem.*, **33**, 363.

Mustakas, G. C., Albrecht, W. J., McGhee, J. E., Kwoleh, W. F. & Griffin, E. L. Jr (1970). Extruder-processing to improve nutritional quality, flavor, and keeping quality of full-fat soy flours. *J. Amer. Oil Chemists' Soc.*, **46**, 623.

Naguib, M. I. (1964). Effect of benzoic acid and its derivatives on plant metabolism. *Can. J. Bot.*, **42**, 197.

Osborne, D. R. & Voogt, P. (1978). *The analysis of nutrients in foods*, Academic Press, London.

Russel, J. A. (1944). Note on the colorimetric determination of amino nitrogen. *J. Biol. Chem.*, **156**, 467.

Svensson, S. G. & Eriksson, C. E. (1972). Thermal inactivation of lipoxygenase from peas (*Pisium sativum* L.). *Lebensm. Wiss. u. Technol.*, **5**, 118.