BIOCHEMICAL CHANGES OF GREEN PEAS DURING PROCESSING AND STORAGE

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Received September 10, 1985

Summary

Changes of protein and lipid constituents of green peas *(P. sativum L)* at various stage of commercial processing and storage at different temperatures over 6 months period were studied. Blanching had very little effect on protein and protein amino acids of pea, while free amino acids were greatly affected. A slight decrease of protein content and quantity of free amino acids was observed.

Total lipid content did not change during processing and storage, while there was an increase in lysophosphatides and monogalactosyldiglycerides.

Introduction

The great part of the green peas is canned using blanching, thermal processing and storage. The heating of green peas causes from one side an improvement in protein utilization due to the inactivation of trypsin inhibitor present in peas (Deatherage 1975). On the other hand, other processes e.g. Maillard-reactions also take place which may decrease the nutritive value of protein during heating and storage. Furthermore, blanching, heat sterilization and storage may affect also other constituents such as vitamins, lipids etc. Very little is known concerning the fate of small amounts of lipids in a vegetable such as the peas. The aim of the research work whose results are presented in this paper, was the study of some biochemical changes relating to proteins and lipids during processing and storage.

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Materials and methods

Green peas samples and preparation of peas

The sample originate from the Kafr El-Sheikh, Egypt, location. The samples were prepared and blanched for 2 min. in boiling water then divided into two equal groups. One of these groups was bottled in (500 g) glass jam jars with twist-off caps with a filling weight of 200 grams peas. The pH of the final product was brought to ~3.25 with distilled winegar; the overall acid content of the final pack was 1.5%. The vinegar solution was added at temperature of 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 samples of peas were canned, placed in tin-plate, lacquered with a filling weight of 200 grams and added 0.05% citric acid. The pH of the final product was ~4.5 and the closing temperature 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 and Valadon (1981).

The storage conditions

The bottled peas were stored for 6 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

Proteins and amino acids

For protein extraction and determination a procedure of Fraser and Loening (1974) and Lowry et al. (1957) was used. Free amino acids and total amino acid were extracted with the modified methods used by Russel (1944) and Naguib (1964) as follows:

Free amino acids: The peas were dried at 70–80 °C for 12 hrs, and ground to a fine powder, some of which was also used for determining tryptophan. To 50 mg of this powder was added 5 cm^3 of 2% phenol and 10 cm^3 of 30% trichloroacetic acid (TCA), the mixture was left overnight and then filtered through filter paper which then contained protein. The pH of the solution containing free amino acids was adjusted to 2 ± 0.1 with NaOH.

Protein amino acids: The filter paper containing the precipitate was kept at 50 °C overnight. Five mg of the dried residue were collected to which 5 cm^3 of 10 mol/dm^3 HCl were added. This was hydrolyzed in a boiling water bath for

5 hrs, filtered and the pH of the solution adjusted to 2 ± 0.1 with 5 mol/dm^3 NaOH. This sample contained acid-stable protein amino acids. Tryptophan labile under acid hydrolysis, requires a separate method of analysis. Tryptophan was determined using the method of Osborne and Voogt (1978).

The separation and identification of the amino acids were carried out on a Toel Model JLC 6AH fully automatic amino acid analyser. 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 and Winter (1970). The results are expressed as g/100 g protein resp. mg/100 g product (free amino acids).

Lipids

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

Separation of lipid classes: Lipid classes were separated with the help of thin-layer chromatography (TLC) on 0.25 mm polygram Silica gel G/UV 254 (Macherey–Nagel and Company, Düren, Germany).

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

Complex lipids were separated by the use of (1) two-step singledimensional TLC using petroleum ether:acetone (3:1 v/v) as the first solvent which removed the faster-moving simple lipids, and chloroform:methanol:acetic acid:water (170:25:25:6 v/v) which actually separated the complex lipids, and (2) two-dimensional TLC using chloroform:methanol:7 mol/dm^3 ammonium hydroxide (65:30:6 v/v) in the first run and chloroform:methanol:acetic acid:water (170:25:25:6 v/v) in the second run.

Lipid classes were identified by their migration characteristics relative to authentic standards that were chromatographed simultaneously alongside the samples under investigation or cochromatographed with them. Lipid spots were detected by specific spray reagents (Christie, 1973)—ninhydrin for amino phosphatides, molybdenum-blue sulphuric acid for phosphatides (Dittmer and Lester, 1964), acid ferric chloride for sterols and their esters, α-naphthol for glycosides and iodine vapour for neutral lipids. For quantitive 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 color estimated by using a Yoyce-Loebl Chromoscan densitometer (Gasbarro, 1972). Results with the densitometer scans were generally reliable as they compared favourably with those of the weighing method.

Total fatty acids were determined according to the method of Asselineau and Montrozier (1976). Free fatty acids were extracted by the method of

Draper (1969). Fatty acid methyl esters were prepared by using BF_3-methanol reagent according to Metcalfe and Schmitz (1961).

Sterol analysis: The total lipid fraction was saponified at room temperature in 12% KOH in absolute EtOH under N_2 for 20 hr. 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 sterols was then dissolved in a small volume of $CHCl_3$:MeOH (2:1 v/v) and analyzed.

The fatty acid methyl esters and sterols were analyzed using a PYE gas chromatograph. The 1.5 m (i.d. 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^{-1}). N_2 flow was 30 cm^3 min^{-1}. Methyl esters and sterols were identified by comparing their retention times with those of authentic standards and by GC-MS and 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 on a Kratos DS-50S 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 (i.d. 4 mm) glass column used was filled with 10% PEGA on Chromosorb WAW DCMS 60–80 mesh (Pye-Unicam), with the carrier gas helium at 30 cm^3 min^{-1}.

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

Results and discussion

Blanching, sterilization and storage of peas caused only small changes in protein content compared with fresh ones (Table 1). The results showed a loss in blanching, which could be due to extraction of soluble proteins and also to hydrolysis of protein into free amino acids that may couple with carbohydrates especially reducing sugar, to form brown pigments. In the same table it can be observed no losses in total proteins of bottled or canned peas stored at different temperature conditions (10 °C and room temperature) over the 6 months period. The total free amino acids of all samples stored for 6 months at 10 °C and room temperature decreased (see Table 2). The free amino acid most retained in all stored samples were aspartic acid and glutamic acid.

Table 1

Changes of the total protein content during processing and storage of pea

Sample	Protein content	
	g/100 g (75% water content)	Retention (%)
Fresh	7.0	100
After blanching	6.6	94.29
After bottling	6.5	92.86
After canning	6.5	92.86
After storage at 10 °C (6 months)		
bottled	6.4	81.43
canned	6.5	92.86
After storage at room temperature (6 months)		
bottled	6.4	91.43
canned	6.4	91.43

Table 2

Free amino acids contained in peas at different stages of processing and during storage at different temperatures over a 6-month period (as mg/100 g product)

Amino acid	Free amino acids						
	Fresh	After			Stored for 6 months		
		Blanching	Bottling	Canning	Bottled	Canned	
					10 °C	Room temp.	Room temp.
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
Tryptophane	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	224.1	144.3	119.3	92.9	68.9	46.4	44.9

The amino acid composition of protein (see Table 3) changed slightly during storage. A decrease of lysine, cysteine, methionine and tryptophane was observed during storage and relative increase of glutamic acid. Other amino acids showed no significant changes (Table 3).

These results suggest that sugar-amino acid reactions (Maillard-reaction) or other reaction including hydroperoxides formed from unsaturated fat (Carpenter and Booth, 1973).

The total lipid content of fresh sample was 419.0 mg/100 g fresh weight and was not affected by blanching, bottling and canning (Table 4). These results are in agreement with those of Chanem and Hassan (1970). After storage for 6 months, in all samples very little effect on the total lipids was observed. The simple lipids identified in fresh pea were mono-, di-, and triglycerides, free fatty acid esters and the steroles, stigmasterol and β-sitosterol and their esters (Table 4). The percentage of free fatty acids, diglycerides and monoglycerides of total simple lipids were 18%, 8% and 8−4% respectively. These amounts were increased under all conditions tested (Table 4). In the same table it can be seen that phospholipids form 83.9% of total complex lipids in fresh pea. Glycolipids together with phospholipids made up total complex lipids. Phosphatides decreased during storage and an increase of lysophosphatids was observed.

Table 3

Amino acid composition of protein in peas at different stages of processing and after storage at different temperatures over a 6-month period (as g/100 g protein)

Amino acid	Fresh	After			Bottling 10 °C	Bottling R.T.	Canning R.T.
		Blanching	Bottling	Canning			
Glutamic acid	16.96	17.01	17.30	17.48	17.30	17.26	17.80
Arginine	7.09	7.01	6.90	7.10	7.00	6.90	7.05
Aspartic acid	10.87	10.91	10.85	10.30	11.20	11.05	10.70
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
Phenylanine	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
Isoleucina	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	0.48	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	100.2	98.12	98.07	97.86	99.16	97.94	99.08

Table 4

Simple and complex lipids of fresh peas and during storage at different temperatures over a 6-month period (Results are expressed as % total unless otherwise stated)

Components	Fresh	After storage		
		Bottled 10 °C	Bottled Room temperature	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-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
Phosphatic acid	14.4	7.5	0.0	0.0
Monogalactosyl diglyceride	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	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 ethanolamin		1.5	3.5	3.6
Lysophosphatidyl inositol Ceramide		2.5	3.5	5.0
Lysophosph. choline	7.2	18.0	24.0	26.5
total (mg/100 g)	380.0	362.5	316.5	343.0
Total lipids (mg/100 g)	419.0	402.0	368.0	384.0

The monogalactosyldiglyceride (MGDG) and digalactosyl-diglyceride (DGDG) were affected differently by storage. The monogalactosyl-diglyceride content was increased greatly, reaching 26,5% in jars stored at room temperature and DGDG decreased under these conditions of storage.

Glycolipid content increased mostly due to the increase in MGDG, the higher temperature of storage the greater was the increase in MGDG. Fricker et al.'s (1975) studies on spinach at temperatures up to 100 °C suggested that lipid fractions were affected by heat treatment; with increasing heat MGDG content increased and DGDG decreased. This relationship was not stoichiometric and it was possible that further reactions were leading to other

unidentified products. Similar results were obtained in this work and can be suggested recently that MGDG and DGDG may be on 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) recommended 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 high increase in MGDG without the corresponding high decrease in DGDG.

The major fatty acid components of fresh pea were 16:0, 18:0, 18:2 and 18:3 free fatty acids content of stored samples increased at all conditions of storage compared to fresh pea. Total free fatty acids in jars, stored at R.T. (399.1 μg/g) were higher than in cans (350.0 μg/g) when the stored sample in jars at 10 °C was the smaller amount (339.2 μg/g). These results indicated that the total free fatty acids were affected by the storage temperature and the containers.

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