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Effect of ripening stage and technological treatments on the lipid composition, lipase and lipoxygenase activities of chickpea (*Cicer arietinum* L.)

R. S. Attia, M. E. Aman,* A. M. El-Tabey Shehata & M. A. Hamza

Department of Food Science and Technology, Faculty of Agriculture, University of Alexandria, Alexandria, El-Chatpy 21545, Egypt

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Lipid composition, lipase and lipoxygenase activities of chickpea seeds were determined in the green (Malana) and fully ripe (dry) chickpea seeds for Giza 1 and Giza 2 cultivars before and after some technological treatments (decortication, cooking and parching). Ripening resulted in marked increase in the content of triglycerides, phosphatidyl choline and phosphatidyl ethanolamine as major components of chickpea lipids. Also, there was a marked rise in lipase and lipoxygenase activities with slight changes in fatty acid composition of chickpea lipids. Minor changes in lipid composition owing to the decortication process applied to the two tested chickpea cultivars (Giza 1 and 2) were also noticed. Cooking and parching caused only slight changes in lipid classes and fatty acids composition, but yielded a significant increase in peroxide value of chickpea lipids. There was a marked drop in lipoxygenase activity with complete inhibition of lipase activity due to cooking and parching. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Mature dry legumes are important in the diets of many countries (Aykroud & Doughty, 1964). Among the world's grain food legumes, chickpea (*Cicer arietinum* L.) is second to dry beans in area grown and third in production to dry beans and peas (Singh, 1985).

Little attention has been given to the lipid components of dry legume seeds, especially minor components. Chickpea seeds contain lipids ranging from 5.4 to 7.0% as reported by Attia (1992). Such lipids can undergo both chemical and enzymatic changes that could influence the quality and acceptability of the cooked seeds or other foods made from their flours (Haydar *et al.*, 1975; Mahadevappa & Rain, 1978; El-Tabey Shehata, 1992).

Giza 1 and Giza 2 are considered the two main chickpea cultivars grown in Egypt and are usually consumed at the raw green and tender stage (unripe stage), called Malana, or in the form of mature dry seeds after parching as a popular snack food. Moreover, the mature seeds can be consumed as whole or decorticated seeds after cooking or processing by different methods.

*To whom correspondence should be addressed.

As a matter of fact studies involving the key role of lipids in the quality as well as on the storage stability of legumes, in general and chickpea in particular, are limited (Onaymi *et al.*, 1986; Sosulski *et al.*, 1987; Bassyony, 1988). Moreover, lipase (E.C. 3.1.1.3) and lipoxygenase (E.C. 1.13.11.12) were found to be determinant factors affecting the overall quality of lipids of legumes and cereals (Hinchcliffe *et al.*, 1977; Sessa, 1979; Sosulski *et al.*, 1987).

The neutral lipids are the predominant class of lipids in most of the legume seeds. However, phospholipids and glycolipids are present in appreciable amounts (Salunkhe *et al.*, 1983). The prevailing constituents of chickpea lipids were triglycerides, while phosphatidyl choline (lecithin) was the predominant polar lipid (Krivelevich *et al.*, 1981; Singh, 1985). The lipids of chickpeas are rich in polyunsaturated fatty acids, especially linoleic acid (Krivelevich *et al.*, 1981, 1982). The high level of unsaturated fatty acids may influence the functional properties as well as the storage stability of processed flours obtained from chickpea (Sosulski & Gadan, 1988).

Linoleic acid is an essential fatty acid (Salunkhe *et al.*, 1983). The hypocholesterolaemic effect of chickpea is a result of its high content of essential fatty acids (Singh, 1985).

Chickpea flour contains an active lipase and lipoxygenase responsible for release and oxidation of free fatty acids. The oxidation of lipids leads to the production of several hydroperoxides damaging the quality of seed/flour during storage (Salunkhe *et al.*, 1983; Sharma *et al.*, 1985).

Information on the effects of ripening and processing on lipid composition of legumes is limited. The present work was conducted to study the effect of ripening, decortication, cooking and parching on the lipid composition in relation to lipase and lipoxygenase activities of the two common chickpea cultivars grown in Egypt, namely Giza 1 and Giza 2.

MATERIALS AND METHODS

Materials

Mature samples of two chickpea cultivars, namely Giza 1 and Giza 2, were obtained from two farms. Fully ripe, dry seeds of Giza 2, which are used mainly for parching, were grown in Upper Egypt (hot and dry climate). Seeds of Giza 1 cultivar which is cultivated in Lower Egypt (cool climate), were collected at physiological maturity, i.e. green, tender seeds (Malana), about 4 weeks before completion of ripening.

Methods

Decortication

The clean, dry chickpea seeds were decorticated by abrasion using a PRL mini-dehuller (National Research Council, Canada). A sample (500 g) of raw dry seeds was dehulled for 45 s for Giza 1. Giza 2, having relatively smaller seeds, was dehulled for 30 s only.

Cooking

The clean, dry whole chickpea seeds were cooked by boiling in tap water until they became soft (about 75 min). The cooked seeds were drained and mashed in a blender before drying at 50°C.

Parching

The traditional commercial procedure of processing dry chickpea seeds was applied. A batch of Giza 2 seeds was sprayed heavily with a 40% lime solution and left to drip for about 24 h at room temperature. Seeds were sieved to remove excess of dry lime residue. The seeds were mixed with sand to which a very small amount of fresh edible oil was added. Parching at 240°C for 1 min was carried out on a metal tray placed on an open fire and provided with a mechanical stirrer. The parched chickpea seeds were separated from sand by sieving.

Extraction of total lipids

The whole dry decorticated, cooked and parched chickpea seeds were ground in an electric mill (Braun, type 4248) to pass through a screen with 0.25 mm openings. Malana buds, i.e. the green seeds, were obtained from the pods, crushed in a blender and dried at 55°C for 8 h before grinding. Lipids were extracted with chloroform–methanol (2:1, v/v) using the procedure of Folch *et al.* (1957).

Lipid fractionation

Polar lipids (phospholipids and glycolipids) were separated from the non-polar lipids of the total lipid extract by preparative thin-layer chromatography (TLC) using petroleum ether–diethyl ether (90:10, v/v) as developing solvent. Polar lipids as well as the total lipid extracts were fractionated using a TLC technique using the method of Mangold & Malins (1960) on glass plates (20×20 cm) precoated with 0.25 mm silica gel, G 60. Neutral (non-polar) lipids were developed in petroleum ether–diethyl ether–glacial acetic acid (70:30:2, v/v/v) and polar lipids in chloroform–methanol–water (65:25:5, v/v/v). After running, the plates were air dried and separated spots were visualized by phosphomolybdic acid (10%) in ethanol.

Neutral (non-polar) lipids were identified by their R_f values (Rahma & Abd El-Aal, 1988). Phospholipids and glycolipids were identified using rat liver lipids extract as a natural source of such components as reported by Aman *et al.* (1970). Non-polar and polar lipids were quantified by the Shimadzu TLC-scanner (C.S. 910) according to Blank *et al.* (1964). The area under each peak of non-polar and polar lipid fractions was measured by triangulation and the percentage of each component was calculated with regard to the total area.

Fatty acid composition

Preparation of fatty acid methyl esters from total lipids of chickpea was performed according to the procedure of Radwan (1978), using 1% sulphuric acid in absolute methanol. The fatty acid methyl esters obtained were separated by (Shimadzu) gas chromatography (GC-4CM, PFE) with a flame ionization detector. Standard fatty acid methyl esters were used for identification. The area under each peak was measured by the triangulation method and percentage of each fatty acid was expressed with regard to the total area.

Lipid changes of raw and processed chickpea seeds in relation to lipase and lipoxygenase activities

Free fatty acids (% as oleic acid) and peroxide value (mEq/kg oil) of chickpea seed oil were determined as described by the AOAC (1980). The method of Pokorny *et al.* (1985) was followed to estimate the thiobarbituric acid value (TBA) colorimetric at 530 nm using the Specol Spectrophotometer (Carl Zeiss, Jena 32-G34). The TBA values were expressed as absorbance of 1 g oil sample in 10 ml reaction mixture.

Lipase activity

Crude lipase was extracted from chickpea samples by stirring with an extraction solution (0.25 M sucrose, 0.175 M KCl, 1 mM EDTA). The method described by Bier (1955) for the determination of lipase activity was used. One millilitre of 0.02 N NaOH corresponds to 100 lipase units.

Lipoxygenase activity

Crude lipoxygenase was extracted by shaking chickpea samples with 0.2 M phosphate buffer (pH 6.6) at 4°C for 60 min as described by Sosulski & Gadan (1988). Lipoxygenase activity was determined spectrophotometrically by measuring the increase in absorbance at 234 nm using the SP8-100 Ultraviolet spectrophotometer (PYE UNICAM) as described by Troung *et al.* (1982).

RESULTS AND DISCUSSION

Lipid fractions

Data in Table 1 reveal that the neutral lipids of chickpea seeds consist mainly of six fractions, namely 1,2- and 2,3-diglycerides, free sterols, 1,3-diglycerides, free fatty acids, triglycerides, sterol esters and hydrocarbons. Two unidentified fractions were also separated in addition to the polar lipids that remained at the base line. Triglycerides were the major class of total lipids. Malana (green seeds) had lower percentages of triglycerides but higher values of diglycerides, free fatty acids and unknown fractions than the dry seeds. Ripening increased triglycerides by 26.2% with a parallel decrease in diglycerides and free fatty acids by 20.2 and 38.5%, respectively. The marked differences in composition of neutral lipids might be related to the developmental stage of chickpea seed ripening for both the whole dry (fully mature) and green (immature) stage. This can be followed by determining the triglycerides content, the main storage fat deposit in the fully ripened chickpea seeds. Slight differences were noticed between decorticated and whole dry chickpea seeds of the two cultivars (Giza 1 and Giza 2). The decorticated chickpea seeds had higher values of triglycerides, but lower quantities of polar lipids than the whole dry seeds. Cooked chickpea samples contained relatively higher percentages of triglycerides and lower values of free fatty acids, polar lipids and unknown fractions than the whole dry seeds. A similar trend but with different values was noticed between parched and whole seeds of the Giza 2 cultivar.

As shown in Table 2, phospholipids and glycolipids presented the major component among the polar lipids in chickpea seeds. When fractionated on TLC plates, as shown in Table 2, eight fractions were separated from polar lipids of both Giza 1 and Giza 2 cultivars. These contained sphingomyelins, phosphatidic acid and glycerophosphatids, cardiolipins, and glycolipids. The major fractions of Malana sample were the glycolipids, phosphatidyl inositol and phosphatidyl ethanolamine. The major fractions of whole seeds of the two cultivars were phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelins. Malana had less phosphatidyl choline, sphingomyelins and phosphatidyl ethanolamine, but more of the other phospholipid fractions than the dry seeds. Ripening resulted in a decrease in phosphatidyl inositol by about 95.3% with a parallel increase in the content of phosphatidyl choline, sphingomyelins and phosphatidyl ethanolamine contents by about 386.1, 279.6 and 142.3%, respectively. Small variations between whole dry, cooked and parched chickpea seeds of the two cultivars were noticed. Decortication decreased the content of sphingomyelins by about 25.1 and 20.5% for Giza 1 and 2, respectively. The percentages of phosphatidyl choline decreased by about 4.9 and 3.2% for Giza 1 and Giza 2, respectively. The content of phosphatidyl inositol increased by about 100 and 37.3% for Giza 1 and Giza 2. Consequently, the quantity of phosphatidyl ethanolamine increased by 9.5% for Giza 1 and 3.2% for Giza 2. According to Singh (1985), phosphatidyl choline is the predominant polar lipid of chickpeas.

Fatty acids composition

Table 3 shows the fatty acids composition of raw and processed chickpea seeds of the two cultivars. The total content of unsaturated fatty acids ranged from 77 to 80%, while that of the saturated varied from 20 to 23%. The unsaturated–saturated fatty acids ratio ranged from 3.4 to 4.1. The total amount of unsaturated fatty acids, as well as the unsaturated–saturated fatty acid ratio of the Malana sample, were lower than those for the other chickpea samples.

There were slight differences in fatty acid composition among the samples of the two cultivars (Giza 1 and Giza 2). Ripening reduced the content of palmitic and linolenic acids, by about 13.5 and 11.8%, respectively.

There was an increase by about 20.9, 6.6 and 2.4% for stearic, oleic and linoleic acids, respectively. Cooking decreased linoleic acid by about 3.8 and 2.8% for Giza 1 and Giza 2, respectively. Parching decreased (by about 4.7%) linoleic acid and increased (by about 6.9%) oleic acid. Generally, palmitic acid was the predominant saturated fatty acid. Linoleic acid was the major unsaturated fatty acid. Krivelevich *et al.* (1981) reported that chickpea lipids were high in polyunsaturated fatty acids, especially linoleic acid. The content of the major fatty acids present in chickpeas agrees with those given by Abd El-Rahman (1981) and Sosulski & Gadan (1988) except for palmitic acid, which was higher in the present study.

Lipid in relation to lipase and lipoxygenase activities

Table 4 indicates that Giza 1 cultivar contained appreciable amounts of free fatty acids, these being 2.84, 2.22, 2.28 and 2.39% for Malana, whole, dehulled and cooked seeds, respectively. For Giza 1 cultivar, the percentages of free fatty acids were 2.53, 2.60, 2.67 and 2.35 for whole dry, dehulled dry, cooked and parched seeds, respectively. Sharma *et al.* (1985) reported that chickpea flour contains an active lipase, which is mainly responsible for the release of free fatty acids and impairs the quality of chickpea grain/flour during storage. Heat treatment (cooking and/or parching) inhibited lipase activity for both Giza 1 and 2 cultivars.

Table 1. Neutral lipids of raw and processed chickpea seeds

Cultivars and treatments	Hydrocarbons and sterols esters	Fraction composition (% of total lipids)[1]							
		Triglycerides	Unknown (1)	Free fatty acids	Unknown (2)	1,3 Diglycerides	Sterols	1,2-2,3 Diglycerides	Polar lipids
Giza 1									
Malana	1.84 ± 0.07	59.4 ± 0.92	2.91 ± 0.12	4.36 ± 0.17	13.13 ± 0.23	4.75 ± 0.16	7.44 ± 0.12	3.22 ± 0.10	2.99 ± 0.08
Whole dry	2.81 ± 0.10	74.9 ± 1.20	1.50 ± 0.07	2.68 ± 0.10	1.71 ± 0.04	3.71 ± 0.10	6.69 ± 0.09	2.65 ± 0.03	3.34 ± 0.05
Decorticated	2.55 ± 0.12	78 ± 1.35	1.05 ± 0.08	1.57 ± 0.11	0.99 ± 0.07	4.71 ± 0.08	5.96 ± 0.05	2.21 ± 0.07	2.91 ± 0.02
Cooked	2.31 ± 0.09	79.4 ± 1.10	0.77 ± 0.05	1.45 ± 0.08	0.66 ± 0.06	4.64 ± 0.012	5.74 ± 0.10	2.05 ± 0.06	2.99 ± 0.05
Giza 2									
Whole dry	4.36 ± 0.13	70.6 ± 1.22	2.76 ± 0.10	3.39 ± 0.09	1.23 ± 0.05	4.84 ± 0.11	7.42 ± 0.07	1.94 ± 0.05	3.47 ± 0.04
Decorticated	4.23 ± 0.11	74.6 ± 1.41	1.69 ± 0.06	2.41 ± 0.12	0.91 ± 0.03	5.31 ± 0.09	6.42 ± 0.13	1.69 ± 0.04	2.79 ± 0.05
Cooked	5.87 ± 0.15	74.1 ± 0.85	1.87 ± 0.06	2.00 ± 0.08	0.77 ± 0.07	5.40 ± 0.05	5.14 ± 0.06	2.00 ± 0.08	2.86 ± 0.04
Parched	6.17 ± 0.12	73.6 ± 0.98	1.49 ± 0.03	2.41 ± 0.09	0.84 ± 0.03	4.86 ± 0.14	5.34 ± 0.09	2.15 ± 0.03	3.13 ± 0.06

Table 2. Phospholipids of raw and processed chickpea seeds

Cultivars and treatments	Sphingomyelins	Fraction composition (% of total polar lipids)[1]						
		Phosphatidyl choline	Phosphatidyl inositol	Phosphatidyl ethanolamine	Phosphatidic acid and glycero-phosphatids	Cardiolipins	Unknown	Glycolipids
Giza 1								
Malana	4.31 ± 0.12	8.08 ± 0.15	18.4 ± 0.34	12.6 ± 0.20	3.23 ± 0.04	4.85 ± 0.05	2.16 ± 0.08	46.34 ± 0.73
Whole dry	16.4 ± 0.19	39.3 ± 0.61	0.87 ± 0.06	30.6 ± 0.38	1.44 ± 0.01	4.08 ± 0.02	0.87 ± 0.03	6.55 ± 0.10
Decorticated	12.3 ± 0.10	37.4 ± 0.52	1.74 ± 0.08	33.4 ± 0.51	1.09 ± 0.02	4.70 ± 0.01	1.04 ± 0.05	8.36 ± 0.12
Cooked	16.8 ± 0.23	36.1 ± 0.50	1.11 ± 0.10	32.4 ± 0.30	1.15 ± 0.01	3.18 ± 0.01	1.33 ± 0.01	7.96 ± 0.08
Giza 2								
Whole dry	17.4 ± 0.25	38.7 ± 0.43	1.34 ± 0.05	28.5 ± 0.27	2.99 ± 0.02	4.78 ± 0.03	1.29 ± 0.04	4.98 ± 0.06
Decorticated	15.6 ± 0.17	37.5 ± 0.62	1.84 ± 0.03	29.4 ± 0.23	1.98 ± 0.02	5.82 ± 0.02	1.50 ± 0.03	6.37 ± 0.11
Cooked	16.8 ± 0.20	38.4 ± 0.55	1.77 ± 0.06	28.7 ± 0.35	2.64 ± 0.01	3.94 ± 0.01	1.61 ± 0.03	6.11 ± 0.02
Parched	16.3 ± 0.31	39.4 ± 0.30	1.54 ± 0.03	28.4 ± 0.31	2.55 ± 0.03	3.57 ± 0.03	1.73 ± 0.06	6.12 ± 0.07

Table 3. Fatty acid composition of lipids of raw and processed chickpea seeds[1]

Cultivars and treatments	Fatty acids (%)								
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	Others[2]
Giza 1									
Malana	0.42 ± 0.03	18.4 ± 0.15	2.68 ± 0.06	25.1 ± 0.23	45.4 ± 0.51	6.20 ± 0.09	0.56 ± 0.01	0.65 ± 0.03	0.57 ± 0.02
Whole dry	0.34 ± 0.01	16.0 ± 0.18	3.24 ± 0.08	26.8 ± 0.25	46.5 ± 0.60	5.47 ± 0.11	0.68 ± 0.01	0.62 ± 0.02	0.45 ± 0.04
Decorticated	0.28 ± 0.02	15.5 ± 0.12	2.99 ± 0.06	26.6 ± 0.13	47.4 ± 0.55	5.61 ± 0.14	0.62 ± 0.02	0.53 ± 0.03	0.54 ± 0.03
Cooked	0.30 ± 0.02	15.9 ± 0.22	3.20 ± 0.07	28.4 ± 0.19	44.7 ± 0.81	5.76 ± 0.10	0.50 ± 0.01	0.63 ± 0.03	0.59 ± 0.02
Giza 2									
Whole dry	0.50 ± 0.03	15.9 ± 0.21	2.37 ± 0.06	25.1 ± 0.20	48.8 ± 0.73	5.34 ± 0.18	0.85 ± 0.02	0.64 ± 0.02	0.45 ± 0.03
Decorticated	0.51 ± 0.01	15.9 ± 0.17	2.18 ± 0.06	25.3 ± 0.17	48.7 ± 0.49	5.57 ± 0.12	0.78 ± 0.03	0.58 ± 0.01	0.49 ± 0.02
Cooked	0.46 ± 0.01	15.6 ± 0.13	2.43 ± 0.09	26.4 ± 0.22	47.5 ± 0.62	5.93 ± 0.08	0.69 ± 0.01	0.46 ± 0.02	0.56 ± 0.02
Parched	0.51 ± 0.02	16.5 ± 0.20	2.51 ± 0.07	26.8 ± 0.20	47.5 ± 0.94	5.44 ± 0.20	0.60 ± 0.02	0.53 ± 0.03	0.54 ± 0.01

[1]Values are means ± standard deviations.

Table 4. Changes in lipids of raw and processed chickpea seeds in relation to lipase and lipoxygenase activities[1]

Cultivars and treatments	Free fatty acids (% as oleic acid)	Peroxide value (meq/kg oil)	TBA value (absorbance/g oil)	Lipase activity (U/g dry matter)	Lipoxygenase activity (U/g dry matter)
Giza 1					
Malana	2.84 ± 0.37	20.5 ± 0.09	8.95 ± 0.16	107 ± 0.70	19 343 ± 83.1
Whole dry	2.22 ± 0.05	9.58 ± 0.09	4.44 ± 0.08	155 ± 0.65	24 726 ± 93.4
Decorticated	2.28 ± 0.15	8.94 ± 0.06	4.39 ± 0.11	162 ± 0.31	25 615 ± 63.5
Cooked	2.29 ± 0.08	11.1 ± 0.13	4.52 ± 0.16	0.0[2]	179 ± 5.6
Giza 2					
Whole dry	2.53 ± 0.09	17.3 ± 0.04	6.53 ± 0.04	178 ± 0.49	22 051 ± 83.2
Decorticated	2.60 ± 0.13	17.0 ± 0.18	5.57 ± 0.12	185 ± 0.56	25 796 ± 37.2
Cooked	2.67 ± 0.19	19.2 ± 0.47	6.51 ± 0.13	0.0[2]	134 ± 5.6
Parched	2.35 ± 0.07	21.0 ± 0.18	6.82 ± 0.06	0.0[2]	167 ± 4.7

[1]Values are means ± standard deviations.
[2]Complete inhibition.

Dehulling slightly increased lipase activity. Peroxide and TBA values for Giza 1 cultivar were lower than those for Giza 2.

Peroxide and TBA values of Malana were much higher (20.5 and 8.95, respectively) than those of the whole dry Giza 1 sample (9.6 and 4.4). This might be a result of the drying of the Malana in an open-air oven at 55°C. Generally, slight changes in oil characteristics were due to decortication. In contrast, slight increases in free fatty acids, peroxide and TBA values were a result of either cooking or parching.

Lipase and lipoxygenase activities

Giza 1 was relatively lower in lipase activity than Giza 2 (Table 4). There were slight variations between whole and decorticated chickpea seeds of the two cultivars. In general, the whole dry chickpea seeds were relatively lower in lipase activity than the decorticated, dry seeds. Also, Malana was lower in lipase activity than the dry seeds. Cooking and parching inhibited lipase activity, in agreement with Bassyony (1988), who reported that dry preheating of raw faba bean at 100 and 120°C for 90 or 120 min inhibited lipase activity.

The oxidation of legume lipids can be enzymic and non-enzymic. Both lead to the production of several hydroperoxides, which in turn, undergo decomposition to yield several products (Salunkhe *et al.*, 1983). The Malana sample contained a lower lipoxygenase activity (19.3×10^3) than the whole dry chickpea seeds (24.7×10^3) of the Giza 1 cultivar (Table 4). Decortication of dry chickpea seeds slightly increased lipoxygenase activity. Cooking chickpea seeds for 75 min at 100°C led to a sharp drop to 179.0 ± 5.6 units/g dry matter in lipoxygenase activity.

A similar trend was observed for Giza 2. Chang & McCudry (1985), reported that lipoxygenase activity of chickpea was 1.7×10^6 units/mg extractable protein. Sosulski & Gadan (1988) stated that lipoxygenase activity ranged from 21 570 to 86 960 units/g chickpea seeds.

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