PERIODICA POLYTECHNICA SER. CHEM. ENG VOL. 36, NO. 1, PP. 71-15 (1992)

# BIOCHEMICAL EVALUATION OF CHICKPEA CAKES AS ADMINISTERED TO ALBINO RATS

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Received February 28, 1991.

## Abstract

Chickpea cakes were prepared according to the procedure of the traditional cake after replacing 0, 25, 50, 75 and 100% of wheat flour by chickpea flour. Ingestion of chickpea cakes did not alter the levels of serum proteins, globulins or albumins. Chickpea cakes had a lowering effect on the serum glucose levels of rats. Serum creatinine and uric acid of different rat groups were within normal limits and did not change in response to chickpea cakes. Chickpea cakes did not affect liver function and had a cholesterol lowering effect either in the rat serum or organs: liver, kidney and heart.

*Keywords:* chickpea cakes, albino rats, biochemical evaluation, effect on serum protein and cholesterol level.

## Introduction

Chickpea is a popular entertaining food in many countries which has a good palatability and excellent flavour. Chickpea flour contains around 17–25% protein.

Replacing different proportions of wheat flour by an equivalent weight of chickpea flour when preparing the Egyptian cakes might have many desirable effects; good organoleptic properties and enhanced nutritional value because of the higher protein content and better protein quality of chickpea (ROSSI et al., 1984)

Moreover, vegetable proteins especially legumes were found to be less cholesterolemic than animal protein even when supplemented by their limiting amino acids (KRITCHEVSKY, 1979; CARROL and HAMILTON, 1975). According to views of specialists the hypocholesterolemic effect of plant proteins arises from the presence of saponins (OAKENFULL and FENWICK, 1978) or from their amino acid compositions per se (KRITCHEVSKY, 1979).

Since chickpea flour contains a high proportion of internal saponin (around 5% (FENWICK and OAKENFULL, 1983) it is expected to have a hypocholesterolemic effect. Hence the high content of cholesterol in the cake originating from the egg ingredient will not have adverse biological

effects in the proposed experimental cake. So, the purpose of this study was to prepare experimental cakes by replacing different proportions of their wheat flour ingredients, 0, 25, 50, 75 and 100%, by chickpea flour. All cakes were composed, prepared and baked according to the ordinary procedure followed in the Egyptian house. Prepared cakes were ingested to Albino rats for biochemical evaluation.

## Materials and Methods

### *Materials*

Chickpea was obtained from a local market, dried and finely milled by a laboratory mill. Cake ingredients were also obtained from the local market.

Albino rats were purchased from the small animal unit, Veterinary Medicine Faculty, Zagazig University.

*Preparation of the cakes*

The ingredients of the Egyptian cakes enough for one big tray are:

| | |
|---|---|
| 800 g | wheat flour (ash content 0.6%) |
| 8 eggs | (400 g) |
| 500 g | sugar |
| 200 g | butter |
| 150 g | milk powder |

The experimental cakes were made with the same ingredients but wheat flour was replaced by 0, 25, 50, 75, 100% of chickpea flour.

The ingredients were mixed thoroughly before baking for around 1 hour.

*Preparation of the diet*

The prepared cakes were used as the main diet of the rats by correcting the protein content to be 10% in the final food by adding either casein or starch. Also the fat content was corrected to be 10% by adding maize germ oil. Vitamin and salt mixture were added at a ratio of 1% and 4% according to CAMPBELL (1961) and HEGESTED et al. (1951), respectively.

### *Methods*

Fifty Albino rats, 30 days old weighing 40–50 g were kept under healthy conditions and offered the basal experimental diet according to CAMPBELL (1961) for one week adaptation period before changing to the experimental diet as shown before. Rats received the experimental cake diet for 5 weeks.

After 2 and 5 weeks from the start of the treatment five rats of each group were killed by decapitation. Blood samples were collected and the organs (liver, heart and kidney) were separated and freezed until analysis.

Total serum proteins were determined according to LOWRY et al. (1951). Serum albumins and globulins were determined using the method described by PETER (1966). Serum glucose was analyzed using the method according to SOMOGYI (1952). Creatinine and uric acid were estimated according to CHASSON et al. (1961) and CARAWAY (1963), respectively. Serum glutamate- oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) were assayed according to REITMAN and FRANKEL (1957). Alkaline and acid phosphatases were estimated using the method described by KIND and KING (1954). The methods of KNIGHT et al. (1972); CHAURCHAMI et al. (1959), FLETCHER (1968) and BARTLETT (1969) were applied for the determination of total lipids, total cholesterol, triglicerides and phospholipids, respectively, in all the samples studied. Statistical analysis (T-test) was conducted according to SNEDECOR and COCHRAN (1967).

## Results and Discussion

The data presented in *Table 1* show the levels of serum proteins of Albino rats administered different chickpea cakes. It is seen that all changes in total serum proteins, albumins or globulins are within normal limits. Hence, replacement different proportions of cake wheat flour by chickpea flour did not have any effect on the contents of serum proteins as the differences between the treatments were insignificant. This might suggest that replacement wheat gluten by chickpea protein in the cake diet did not induce a specific effect on the protein metabolism. Moreover, rats extracted their protein and amino acid requirements without any problems arising from the difference in protein quality. The presence of egg ingredient in each cake might have corrected for the deficient limiting amino acid in each protein. So, the different cakes afforded nearly the nitrogen requirement to the rats to retain a normal serum protein content.

Since the levels of serum proteins is a measure of the equilibrium between anabolic and catabolic processes (PATTERSON et al., 1967), it can

**Table 1**
Serum proteins concentration of rats administered different chickpea cakes

| Treatments | Total proteins gm/100 ml | | Albumins gm/100 ml | | Globulins gm/100 ml | |
|---|---|---|---|---|---|---|
| | Time in weeks | | | | | |
| | 2 | 5 | 2 | 5 | 2 | 5 |
| 0% Chickpea cake | 6.25 ±0.40 | 6.37 ±0.51 | 3.74 ±0.27 | 3.88 ±0.22 | 2.51 ±0.19 | 2.49 ±0.12 |
| 25% Chickpea cake | 6.39 ±0.38 | 6.48 ±0.46 | 3.75 ±0.24 | 3.95 ±0.24 | 2.64 ±0.19 | 2.53 ±0.15 |
| 50% Chickpea cake | 6.39 ±0.48 | 6.78 ±0.40 | 3.64 ±0.28 | 4.23 ±0.31 | 2.75 ±0.18 | 2.55 ±0.18 |
| 75% Chickpea cake | 6.16 ±0.41 | 6.60 ±0.39 | 3.65 ±0.32 | 4.30 ±0.25 | 2.51 ±0.19 | 2.30 ±0.21 |
| 100% Chickpea cake | 6.27 ±0.46 | 6.41 ±0.52 | 3.95 ±0.28 | 3.95 ±0.31 | 2.32 ±0.20 | 2.46 ±0.18 |

be said that the administered cakes did not affect this equilibrium and kept protein metabolism within stable normal limits.

The results summarized in *Table 2* show the effect of administering different chickpea cakes on the concentration of glucose, creatinine and uric acid in blood.

The glucose content in the serum of rats fed different chickpea cakes responded negatively to the replacement of wheat flour by chickpea flour. This response reached a maximum at a replacing ratio of 75% chickpea. This effect was equal after 2 and 5 weeks of administration. This effect might be due to the expected improvement in protein quality of the cakes supplemented by chickpea flour, since there is a complementary effect between wheat protein and chickpea protein. The first protein is limiting in lysine but rich in methionine (KENT, 1975 and NIERLE, 1985) and the second one is limiting in methionine but rich in lysine (KHAN et al., 1979 and ROSSI et al., 1984).

This improved protein quality of the chickpea supplemented cake would result in enhanced protein assimilation. So, the losses in nitrogen components during protein metabolism in the case of the cakes of high quality protein will be lower than that of the low quality protein ones. Wasting nitrogen components during protein metabolism of that derived from a low quality protein may deviate the general metabolism towards carbohydrate metabolism and result in relatively high levels of serum glucose. Conversely administration of high quality protein may lessen the ratio of

**Table 2**
Serum glucose, creatinine and uric acid contents of rats administered different chickpea cakes

| Treatments | Glucose mg/100 ml | | Creatinine mg/100 ml | | Uric acid mg/100 ml | |
|---|---|---|---|---|---|---|
| | Time in weeks | | | | | |
| | 2 | 5 | 2 | 5 | 2 | 5 |
| 0% Chickpea cake | 84.3 ±6.3 | 88.5 ±5.6 | 0.83 ±0.07 | 0.86 ±0.05 | 5.37 ±0.24 | 5.42 ±0.31 |
| 25% Chickpea cake | 79.6 ±4.6 | 80.1 ±7.2 | 0.85 ±0.07 | 0.85 ±0.05 | 5.50 ±0.32 | 5.08 ±0.42 |
| 50% Chickpea cake | 78.1 ±5.8 | 78.2 ±5.1 | 0.84 ±0.07 | 0.86 ±0.05 | 4.88 ±0.40 | 4.79 ±0.31 |
| 75% Chickpea cake | 73.4 ±4.9 | 72.9 ±5.1 | 0.84 ±0.05 | 0.82 ±0.06 | 4.62 ±0.40 | 4.63 ±0.29 |
| 100% Chickpea cake | 75.0 ±3.9 | 73.9 ±4.4 | 0.83 ±0.05 | 0.83 ±0.04 | 4.62 ±0.35 | 4.67 ±0.25 |

wasted nitrogen and consequently the chance to afford the stuff required for carbohydrate metabolism is limited. And hence serum glucose concentration is limited. Other possible causes of the lowered serum glucose concentration may be the improved liver function or low serum cholesterol (TSAI, 1977).

The serum creatinine levels in rat groups did not show significant differences. This may indicate that replacement of wheat flour by chickpea flour did not have any adverse effect on the renal function.

The values of serum uric acid in the rats administered different chickpea cakes were within normal levels. However, some reduction in serum uric acid levels was associated with the ingestion of chickpea supplemented cakes. This uric acid reducing effect of chickpea supplementation appeared after 5 weeks when the ratio of chickpea replacement was 25% but appeared after 2 weeks when this ratio was 50–100%. A ratio of 75% chickpea replacement induced the highest reduction in serum uric acid level. The reduced uric acid values might refer to improved renal function or enhanced nucleoprotein anabolism reflect the improvement of protein metabolism. This may support the previous conclusion that supplementing cakes with chickpea improved their protein quality which enhanced the protein metabolism in the rat.

The activities of both transaminases, glutamic-oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and phosphatases:

alkaline phosphatase and acidic phosphatase in the serum of rat groups receiving different chickpea cakes are within normal levels and show no significant differences at any experimental period (*Table 3*). Hence, chickpea flour may be void from effective contents of antinutritional factors, chickpea saponins did not cause any adverse effects since they were deactivated by the high cholesterol content present in the cake ingredients. The deactivation of saponins by cholesterol was previously stated by DAGHIR and ZAATARI (1983).

**Table 3**
Activities of transaminases and phosphatases of rats fed different chickpea cakes

| Treatments | Acid phosph.* I.U./I. | | Alk. phosph.** I.U./I. | | G.O.T.*** U./ml | | G.P.T.**** U./ml | |
|---|---|---|---|---|---|---|---|---|
| | Time in weeks | | | | | | | |
| | 2 | 5 | 2 | 5 | 2 | 5 | 2 | 5 |
| 0% Chickpea cake | 6.25 ±0.52 | 6.30 ±0.46 | 9.9 ±0.82 | 10.0 ±0.64 | 63.2 ±2.9 | 64.4 ±5.2 | 43.3 ±2.9 | 41.6 ±4.0 |
| 25% Chickpea cake | 6.5 ±0.45 | 6.50 ±0.38 | 10.8 ±0.81 | 10.8 ±1.1 | 65.0 ±6.5 | 65.2 ±4.3 | 42.4 ±3.12 | 41.5 ±2.5 |
| 50% Chickpea cake | 6.87 ±0.62 | 6.4 ±0.48 | 10.9 ±0.62 | 10.90 ±0.55 | 63.0 ±5.2 | 64.4 ±3.7 | 44.1 ±3.6 | 44.5 ±2.8 |
| 75% Chickpea cake | 6.4 ±0.39 | 6.9 ±0.42 | 10.7 ±0.64 | 10.6 ±0.92 | 65.6 ±4.3 | 65.1 ±5.3 | 44.9 ±3.2 | 47.2 ±3.1 |
| 100% Chickpea cake | 6.8 ±0.62 | 6.8 ±0.62 | 9.4 ±0.89 | 9.9 ±0.92 | 67.4 ±5.1 | 65.8 ±3.9 | 47.4 ±4.2 | 47.5 ±3.5 |

* Acid phosphatase.
** Alkaline phosphatase.
*** Glutamate oxaloacetate transaminase.
**** Glutamate pyruvate transaminase.

The data in *Table 4* indicate clearly that serum cholesterol level was reduced by replacing wheat flour by chickpea in the cakes administered to Albino rats. The most evident reduction in serum cholesterol level was associated with the 100% chickpea cake. The cholesterol reducing effect appeared early after 2 weeks of chickpea cake administration but did not increase with extending time of administration to 5 weeks. The decreased levels of serum total cholesterol in the rats administered chickpea cakes were accompanied by increased ones of serum phospholipids. This inversed relationship was interpreted by SITOHY et al. (1990) in terms of the common lipid transporting role of each of these two lipid fractions; phospholipids and cholesterol.

Table 4
Some lipid fractions in the serum or liver of rats administered different chickpea cakes

| Organs | Treatments | Total lipids mg/100 ml | | Cholesterol mg/100 ml | | Phospholipids mg/100 ml | | Triglycerides mg/100 ml | |
|---|---|---|---|---|---|---|---|---|---|
| | | Time in weeks | | | | | | | |
| | | 2 | 5 | 2 | 5 | 2 | 5 | 2 | 5 |
| Serum | 0% Chickpea cake | 564 ±32.4 | 582 ±41.6 | 217.2 ±13.7 | 223.8 ±11.5 | 185 ±12.5 | 194 ±13.4 | 90.5 ±7.3 | 92.6 ±6.2 |
| | 25% Chickpea cake | 520.2 ±30.6 | 528 ±29.4 | 191.3 ±12.7 | 212.3 ±13.5 | 198 ±16.2 | 216 ±15.1 | 81.2 ±6.2 | 91.6 ±6.4 |
| | 50% Chickpea cake | 540 ±41.2 | 602.9* ±32.7 | 194.7 ±10.9 | 195.4 ±11.8 | 196 ±10.5 | 205 ±11.6 | 85.2 ±6.4 | 102.4 ±5.9 |
| | 75% Chickpea cake | 540 ±36.4 | 603.3* ±35.2 | 184.4* ±11.2 | 188.5* ±13.7 | 192 ±14.5 | 203 ±13.2 | 86.4 ±4.8 | 100.0 ±5.9 |
| | 100% Chickpea cake | 523 ±29.5 | 642* ±36.7 | 180.0* ±9.5 | 186.2* ±10.2 | 196 ±12.7 | 198 ±11.8 | 86.2 ±5.4 | 105.1 ±7.2 |
| Liver** | 0% Chickpea cake | 5.28 ±0.39 | 5.61 ±0.32 | 248 ±22.4 | 293 ±18.5 | 350 ±20.5 | 365 ±21.4 | 3.98 ±0.29 | 4.27 ±0.36 |
| | 25% Chickpea cake | 5.05 ±0.31 | 5.36 ±0.19 | 245 ±21.6 | 278 ±19.5 | 346 ±21.4 | 352 ±22.6 | 3.90 ±0.25 | 4.12 ±0.28 |
| | 50% Chickpea cake | 4.82 ±0.26 | 4.94 ±0.30 | 226 ±18.6 | 267 ±19.6 | 348 ±28.4 | 348 ±2.48 | 3.89 ±0.27 | 3.92 ±0.32 |
| | 75% Chickpea cake | 4.96 ±0.35 | 4.85 ±0.37 | 218 ±18.5 | 260 ±21.6 | 356 ±25.2 | 350 ±31.2 | 3.95 ±0.19 | 3.86 ±0.21 |
| | 100% Chickpea cake | 4.79 ±0.32 | 4.80* ±0.25 | 209 ±16.4 | 221* ±19.2 | 368 ±26.5 | 379 ±19.7 | 3.76 ±0.25 | 3.66 ±0.24 |

* Differences are significant relatively to 0% chickpea cake ($P < 0.01$).
** Total lipids and triglycerides are expressed as g/100 g fresh wt., cholesterol and phospholipids as mg/100 gm in liver.

There was also a direct proportionality between the serum cholesterol and each of total lipids and triglycerides in accordance with the results of PATHIRANA et al. (1981) and TOPPING et al. (1980). However, this relationship held true only after 2 weeks.

The rate of cholesterol deposition on the rat liver was reduced by the administration of chickpea cakes (25–100%) in accordance with the cholesterol trend in serum. The phospholipid content in rat liver did not show a clear trend. Both total lipid and triglyceride contents in rat liver showed some reduction in parallel with that of liver cholesterol. So, administration of chickpea cakes not only reduces total lipids, triglycerides and cholesterol in the serum but also prevents their deposition on the liver.

These hypocholesterolemic and hypolipidaemic effects of chickpea cakes might be due to the amino acid composition of chickpea proteins per se as suggested by KRITCHEVSKY (1979) or to the presence of saponins in chickpea flour as proposed by OAKENFULL and FENWICK (1978). The saponins' lowering effect on liver cholesterol was observed by OAKENFULL et al. (1979).

**Table 5**
Some lipid fractions in the kidney and heart of rats fed different chickpea cakes

| Organs | Treatments | Total lipids gm/100 ml | | Cholesterol gm/100 ml | | Phospholipids gm/100 ml | | Triglycerides gm/100 ml | |
|---|---|---|---|---|---|---|---|---|---|
| | | Time in weeks | | | | | | | |
| | | 2 | 5 | 2 | 5 | 2 | 5 | 2 | 5 |
| Kideny | 0% Chickpea cake | 7.25 ±0.37 | 7.34 ±0.62 | 446 ±34.6 | 472 ±32.7 | 1186 ±64.4 | 1214 ±71.2 | 5.42 ±0.41 | 5.81 ±0.32 |
| | 25% Chickpea cake | 6.92 ±0.41 | 7.16 ±0.52 | 435 ±37.5 | 436 ±39.5 | 1195 ±72.6 | 1200 ±65.1 | 5.24 ±0.29 | 5.36 ±0.32 |
| | 50% Chickpea cake | 6.82 ±0.51 | 7.19 ±0.42 | 441 ±34.7 | 425 ±33.8 | 1208 ±81.6 | 1216 ±78.6 | 5.16 ±0.38 | 5.42 ±0.26 |
| | 75% Chickpea cake | 7.12 ±0.45 | 7.16 ±0.61 | 440 ±36.5 | 436 ±31.2 | 1210 ±71.6 | 1196 ±68.4 | 5.20 ±0.29 | 5.32 ±0.41 |
| | 100% Chickpea cake | 7.25 ±0.48 | 7.26 ±0.42 | 422 ±28.4 | 428 ±32.6 | 1218 ±71.6 | 1224 ±78.7 | 5.18 ±0.32 | 5.18 ±0.42 |
| Heart | 0% Chickpea cake | 1.96 ±0.12 | 1.92 ±0.11 | 186.2 ±13.2 | 196.4 ±11.2 | 346 ±31.2 | 365 ±21.4 | 1.50 ±0.14 | 1.62 ±0.12 |
| | 25% Chickpea cake | 1.85 ±0.15 | 1.90 ±0.13 | 172.4 ±9.5 | 186.4 ±12.4 | 349 ±30.4 | 395 ±26.5 | 1.56 ±0.13 | 1.48 ±0.11 |
| | 50% Chickpea cake | 1.62 ±0.09 | 1.74 ±0.12 | 170.5 ±14.2 | 192.5 ±12.4 | 354 ±25.4 | 348 ±23.2 | 1.42 ±0.09 | 1.46 ±0.08 |
| | 75% Chickpea cake | 1.96 ±0.14 | 1.85 ±0.09 | 176.5 ±11.9 | 180.4 ±11.4 | 386 ±18.6 | 382 ±19.5 | 1.40 ±0.08 | 1.41 ±0.09 |
| | 100% Chickpea cake | 1.78 ±0.13 | 1.90 ±0.16 | 184.2 ±16.2 | 192.4 ±13.5 | 358 ±29.5 | 376 ±30.6 | 1.56 ±0.08 | 1.38 ±0.13 |

The deposition rate of cholesterol in the kidney and heart of rats showed some reduction in response to the administration of chickpea cakes (*Table 5*). This reduction was more evident after 5 weeks especially in the kidney. Some parallel reductions occurred in both the total lipid and triglyceride contents but they were either slight or irregular. Simultaneously some irregular increase in the phospholipids content of both the kidney and heart was associated with the administration of chickpea cakes

and the observed reduction in the cholesterol content in these organs. So the internal relationship of lipid classes observed in the serum applies also to kidney and heart but to a limited extent.

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