# Preparation and evaluation of the hypocholesterolemic effect of fermented formula

Hamed I. M., Doha A. Mohamed, Mona M. Hussein and Soraya T. El-Damhugy.
Food Science and Nutrition Dept., National Research Centre, Dokki, Giza, Egypt.

imankam_2@yahoo.com

**Abstract**

The present research was postulated to prepare fermented formula, which is a mixture of yoghurt/cereals and vegetables. The aim also includes studying the hypocholesterolemic effect of the prepared fermented formula in hypercholesterolemic rats. The results of chemical analysis of the fermermented formula revealed that crude protein, crude fiber, calcium, zinc, iron and selenium presence in the sample by 21.9g, 2.5g, 250mg, 3.69mg, 3.93mg and 45.8ug, respectively. The level of total phenolic compounds of the fermented formula as determined by the Folin-Ciocalteu method was 1870 mg of gallic acid equivalents/ 100g dry weight. The pH of the fermented product was 6. The results of feeding hypercholesterolemic rats on balanced diet containing fermented formula showed significant reduction in plasma total lipids, total cholesterol, low density lipoprotein cholesterol and triglycerides. Diet containing fermented formula produced significant increase in high density lipoprotein cholesterol. Hypercholesterolemic rats fed on diet containing fermented formula showed significant decrease in plasma level of malondialdehyde as indicator of lipid peroxidation. These results reflect the possible beneficial use of fermented foods towards cardiovascular diseases. [Academia Arena, 2009;1(5):9-17]. ISSN 1553-992X.

**Key Words:** Fermentation, Fermented formula, hypercholesterolemic rats, plasma lipid profile.

## Introduction

Food fermentation is regarded as one of the oldest ways of food processing and preservation. More than anything else, man has known the use of microbes for preparation of food products for thousands of years and all over the world a wide range of fermented foods and beverages contributed significantly to the diets of many people (Achi, 2005). Fermented foods are food substrates that are invaded or overgrown by edible microorganism whose enzymes, particularly amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavors, aromas and textures pleasant and attractive to the human consumer (Steinkraus, 1997).

Fermentations involving production of lactic acid are generally safe. Lactic acid fermentations include those in which the fermentable sugars are converted to lactic acid by organisms such as *Lactobacillus brevis*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidus* etc. (Steinkraus, 1996). In the present research we prepared fermented formula, which is a modification of Egyptian Kishk. Egyptian Kishk is basically parboiled wheat/yoghurt mixtures that combine the high nutritional value of wheat and milks while attaining excellent keeping qualities. Our fermented formula besides wheat and yoghurt contain soybean and carrot to increase vitamins and phenolic compounds, which have antioxidant activity. So the studied fermented formula is yoghurt/cereal/vegetable mixture. It was reported that fermented foods especially fermented milk products such as Kefir have been shown to affect serum cholesterol concentration (St-Onge *et al*., 2002). So the objective of the present research was to prepare fermented formula. The aim also includes studying the hypocholesterolemic effect of fermented formula on hypercholesterolemic rats as functional fermented food product for cardiovascular diseases.

## Materials and Methods

### Materials

- All ingredients of the fermented formula (wheat, soybean, whey protein concentrate, carrot, milk, and vanilla essence as flavor was supplied from local market and Ministry of Agriculture.
- **Yeast** *Saccharomyces cervisia F-25* (260 mg Se/Kg powder and activity 550 $cm^3$ $CO_2$/hrs.) were obtained from NRC, Cairo, Egypt.
- **A commercial ABT-2** starter culture **(***Streptococcus Thermophilus, Bifidobacterium Bifidus* and *Lactobcillius acidophilus***)** was purchased from Chr. HANSEN Pty. Ltd., Bayswater, Australia.

- Male Sprague-Dawely rats of 112.7±1.029 g average body weight were used in the experiment. The animals were kept individually in stainless steal cages. Water and food were given ad-libitum.

**Preparation of bioactive ingredients**

- **Soybean.** Soybean seeds were cleaned by tap water, and soaked in tap water for 2 days at room temperature. Germination was carried out by spreading the soaked seeds in wet blotting paper and kept at 25 ºC for 72 h. It was boiled for 30 minutes in water with added 1% sodium bicarbonate. The soybean seeds were dried in air oven at 45 ºC then ground to powder and stored in polyethylene bags at 4 ºC to be used in the formulation.
- **Germinated wheat**. Whole wheat seeds were soaked in tap water (1:3 w/v) for 2 days. Germination was carried out by spreading the soaked seeds in wet blotting paper and kept at 25 ºC for 72 h. The seeds were kept wet throughout germination by spraying them with water every 12 h. The germinated wheat was then dried in air oven at 45 ºC, then ground into fine powder and kept at 4 ºC to be used in the formulation.
- **Cereal – soybean mixture.** The ground material of germinated wheat and soybean was mixed using a ratio of 70: 30 (w/w).
- **Carrots.** Carrot was washed with tap water and cut into small slices then dried in air oven at 45 ºC and ground into fine powder and kept at 4 ºC until used in the formulation.
- **Milk mixture.** One kilogram buffalo milk was heated at 85 ºC for 5 min. and rapidly cooled down to 4 ºC. After removing the butter film (which was formed on the milk surface) 300 g whey protein concentrate and 5 g turmeric rhizomes powder were mixed.
- **Yoghurt** was processing according to the method of Dave and Shah (1998).
- **Preparation of the fermented formula.** Cereal–soybean mixture (1 Kg), carrot (50 g), vanilla (5 g), sodium chloride (23 g) and yeast (10 g) were mixed with warm water 30-35 ºC (dilution 30% w/v). Fermentation was carried out for 20 h at 28-30 ºC, steamed for 20 minutes, dried in air oven at 45 ºC and ground into a fine powder. This fine dried powder was mixed with milk mixture (200 ml) and yoghurt (100 g) and fermented at 28-30 ºC for 20 h. After the fermentation the dough was dried in air oven at 40-50 ºC until the moisture content was reduced to 6%. The final product was stored in a glass jar and refrigerated until used for analysis.
- **Analytical procedure.** Moisture, protein, fat, crude fiber and ash contents were determined according to AOAC, (1995). Carbohydrates were calculated by difference. Dietary fiber was determined according to AOAC (1997). Minerals (Ca, Fe, Mg, Se and Zn) content of the fermented formula was determined by atomic absorption spectrophotometer (Varian spectr AA 220). Total phenolic content was determined in the fermented formula according to the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent. Absorbance was measured at 765 nm using UVPC spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100 gram dry material. The pH value of the fermented product was determined as described by Ibanoglu *et al*. (1999). Microbiological quality was done following the method of James (2000).
- **Preparation of diets for animal experiment.** Different experimental diets appeared in Table (1). Salt mixture and vitamin mixtures were prepared according to Briggs and Williams (1963) and Morcos (1967) respectively. Oil soluble vitamins were given orally in a dose of 0.1 ml/rat per week.

Table (1): Composition of different experimental diets. (g/100 g).

| Ingredients | Diets | | |
|---|---|---|---|
| | Balanced | Hyper-cholesterolemic | Fermented formula |
| **Casein** | 11.9* | 11.9* | - |
| **Corn oil** | 10 | - | 7.26 |
| **Butter** | - | 25 | - |
| **Sucrose** | 23.5 | 35 | 23.5 |
| **Starch** | 47.1 | 22.35 | 20.37 |
| **Salt mix.** | 3.5 | 3.5 | 1.31 |
| **Vit. mix.** | 1 | 1 | 1 |
| **Fiber** | 3 | - | 1.86 |
| **Cholesterol** | - | 1 | - |
| **Cholic acid** | - | 0.25 | - |
| **Fermented formula**[1] | - | - | 47 |

* 11.9 casein has been shown to contain 10 g protein (AOAC, 1995).

[1]An appropriate amount of fermented formula was added to the diet so diet would contain 10% protein, 10% fat, 23.5% sucrose, 3% fiber, 1% vitamin mix. 3.5% salt mix. and completed to 100% by starch.

**Design of animal experiment**

**The experiment was divided into two stages**

- **First stage.** Rats were assigned to two dietary groups. The first group (6 rats) received a balanced diet (CC group), while eighteen animals (HH the second group) were fed a hypercholesterolemic diet reported by Zulet *et al*. (1999). This stage continued for a month.
- **Second stage**. After development of hypercholesterolemia, hypercholesterolemic rats were divided into three sub-groups each of 6 rats. Rats of the first sub-group continued on the same hypercholesterolemic diet (HH group) and the remaining hypercholesterolemic 2 sub-groups of rats received the balanced diet (HB group), or balanced diet supplemented with fermented formula (HFF group) for four weeks. During this experimental period the control group continued on the same balanced diet (CC group). During the experiment, body weight and food intake were recorded weekly. At the end of first and second stages, total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. After an overnight fast blood samples were withdrawn from eye vein orbital from all rats at the end of first and second stages for the determination of plasma total lipids (Toro and Ackerman, 1975), total cholesterol (T-Ch) (Watson, 1960), high density lipoprotein cholesterol (HDL-Ch) (Burstein *et al*., 1980), low density lipoprotein cholesterol (LDL-Ch) (Gerard and Gerald, 1981) and triglycerides (TG) (Megraw *et al*., 1979). HDL-Ch / T-Ch ratio was calculated. Malondialdehyde (MDA) (Satoh**,** 1978**)** was determined in all plasma samples as indicator for lipid peroxidation.
- The results of animal experiment were expressed as the Mean ± SE and they were evaluated statistically using Student's t-test, $p < 0.05$ was used as the criterion of statistical significance.

**Results and Discussion**

The result of chemical analysis of the fermented formula sample is given in Table (2). Crude protein, crude fiber, calcium, zinc, iron and selenium concentration in the sample were 21.9g, 2.5g, 250mg, 3.69mg, 3.93mg and 45.8ug, respectively. The whole wheat grain was a good source of selenium and during germination several enzymes become active and brought about profound changes in the nutritive value of cereal (Subramanian, 1976). Also soybean was an excellent source of minerals including calcium, iron and copper. During fermentation bacterial enzymatic hydrolysis enhance the bioavailability of protein and increase the production of free amino acids and short chain fatty acid (Parvez *et al*., 2006). The result of chemical analysis showed that moisture of the fermented product was 6%; in another work this level was suitable for long term storage without deterioration for 2 and 3 years (Degirmencioglu *et al*., 2005). In the

present study the level of total phenolic compounds of the fermented formulae as determined by the Folin-Ciocalteu method (Table 2) was 1870 mg of gallic acid equivalents/ 100g dry weight. The pH of the fermented formula was 6. Ashraf (2006) found that the combination treatment of germination and fermentation brought an increase in nitrogen solubility both at acid and alkali pH.

Table (2): Chemical composition of the fermented formulae.

| Ingredients | Weight / 100 g sample |
|---|---|
| Moisture (g) | 6.20 |
| Energy (Kcal) | 400.8 |
| Total Protein (g) | 21.90 |
| Crude Fat (g) | 6.0 |
| Crude Fiber (g) | 2.50 |
| Dietary fiber (g) | 25.25 |
| Ash (g) | 4.80 |
| Total Carbohydrate (g) | 64.8 |
| PH | 5.80 |
| Total phenolic compounds (mg/100g sample as gallic acid equivalent) | 1870 |
| **Minerals** | |
| Zinc (mg) | 3.69 |
| Iron (mg) | 3.93 |
| Magnesium (mg) | 85.30 |
| Calcium (mg) | 250 |
| Selenium (μg) | 45.8 |

The results of microbilogical evaluation of the fermented formula are shown in Table (3). Microbiological analysis was done in the fermented formula and there is no indicator organisms found.

Table (3): Microbiological evaluation of the fermented formula.

| | Complete process of fermentation |
|---|---|
| Total bacterial count (CFU/g) | $4.7 \times 10^8$ |
| Streptococcus spp. | $< 10^6$ |
| Lactobacillus spp. | $< 10^6$ |
| Bifidobacteria spp. | $< 10^6$ |
| Yeast/mould count (CFU/g) | 0/0 |
| Coliform count (CFU/g) | Free |
| Escherichia coli | Free |
| Salmonella and shigella | Free |
| Bacillus cereus (CFU/g) | Nil (0) |
| Staphylococcus aureus (CFU/g) | Nil (0) |
| Listeria monocytogenes | Free |

**Evaluation of hypocholesterolemic effect of fermented product:**

It has been repeatedly reported that nutrition plays an important role in the eitology of hyperlipidemia and atherosclerosis. Several animals and human studies have confirmed the hypercholesterolemic properties of saturated fatty acids and cholesterol by increasing serum total cholesterol and altering the lipoprotein pattern (Bhadra *et al*., 1993 and Da-Silva *et al*., 1996). Moreover, nutritional recommendations have been given to prevent and treat some lipid metabolism disturbances (Dwyer, 1995).

Nutritional parameters of normal and hypercholesterolemic rats of first stage are shown in Table (4). The results revealed that non-significant change was found in final body weight, while body weight gain, total food intake and food intake/day were significantly lower in hypercholesterolemic rats in comparison with normal rats ($p < 0.025$, $<0.001$ and $< 0.001$ respectively).

Table (4): Nutritional parameters of normal and hypercholesterolemic rats (First stage).

| Parameters | Groups | |
|---|---|---|
| | Normal<br>Mean ± SE | Hypercholesterolemic<br>Mean ± SE |
| Initial body weight (g) | 122.83 ± 2.006 | 122.6 ± 1.152 |
| Final body weight (g) | 224.83 ± 8.049 | 205.4 ± 3.724 |
| Body weight gain (g) | 102 ± 6.596 | 82.833* ± 3.365 |
| Total food intake (g) | 418.27 ± 15.765 | 329.606** ± 7.637 |
| Food intake (g/day) | 13.942 ± 0.526 | 10.987** ± 0.255 |
| Food efficiency ratio | 0.2433 ± 0.011 | 0.251 ± 0.007 |

Values significantly differ from normal rats:
*: $p < 0.025$, **: $p < 0.001$.

Plasma lipid profiles of hypercholesterolemic rats (First stage) are shown in Table (5). The rats fed the hypercholesterolemic diet showed significant increase in the plasma levels of total lipids (+ 108 %, $p < 0.001$), Tch. (+ 82 %, $p < 0.001$), and LDL-ch (+ 274 %, $p < 0.001$), which was accompanied by a decrease in HDL-ch and HDL/T.ch ratio (- 32 %, - 62% respectively, $p < 0.001$) when compared to normal rats. Plasma TG level showed non-significant change. These results are in agreement with the results of Zulet *et al*. (1999) who reported significant increase in plasma levels of Tch and LDL-ch (+ 362 % and 2660%, $p < 0.001$ respectively) of rats fed similar hypercholesterolemic diet. The rats fed the hypercholesterolemic diet showed significant increase in the plasma levels of malondialdehyde (+ 119%, $p < 0.001$) when compared with normal rats, which indicate that lipidperoxidation elevates significantly in hypercholesterolemic rats.

The assessment of the lipid profile in plasma of rats fed a high-fat diet enriched in saturated fat and cholesterol revealed a situation of hypercholesterolemia, which was accompanied by a decrease in HDL-ch and an increase in LDL-ch. These alterations resembled a situation of type II hyperlipidemia in human (Tholstrup *et al*., 1995), which could be associated with a down-regulation in LDL receptors by the cholesterol and saturated fatty acids included in the diet (Stucchi *et al*., 1995).

Table (5): Plasma lipid profile of normal and hypercholesterolemic rats (First stage).

| Parameters | Groups | |
|---|---|---|
| | Normal<br>Mean ± SE | Hypercholesterolemic<br>Mean ± SE |
| Total lipids (g/dl)<br>% Change | 0.399 ± 0.009 | 0.829* ± 0.016<br>+ 108 |
| TCh (mg/dl)<br>%Change | 90.08 ± 1.345 | 163.71* ± 4.274<br>+ 82 |
| HDL-Ch (mg/dl)<br>% Change | 48.03 ± 0.705 | 32.49* ± 0.339<br>- 32 |
| HDL/TCh ratio<br>% Change | 0.534 ± 0.013 | 0.201* ± 0.006<br>- 62 |
| LDL-Ch (mg/dl)<br>% Change | 25.04 ± 0.312 | 93.59* ± 1.237<br>+ 274 |
| TG (mg/dl)<br>% Change | 93.52 ± 1.475 | 94.79 ± 0.930<br>+ 1 |
| MDA (nmol/ml)<br>% Change | 4.87 ± 0.307 | 10.670 ± 0.239<br>+ 119 |

Values significantly differ from normal rats: *: $p < 0.001$.

Nutritional parameters of hypercholesterolemic rats after feeding different dietary treatment (second stage) are shown in Table (6). Hypercholesterolemic rats fed on balanced diet (HB) showed significant

increased in body weight gain and food efficiency ratio ($p < 0.005$ and 0.001 respectively) when compared with hypercholesterolemic rats (HH) fed on hypercholesterolemic diet, while total food intake and food intake/day reduced significantly ($p < 0.005$). Hypercholesterolemic rats fed on balanced diet containing fermented formula (HFF) showed significant reduction in final body weight ($p < 0.001$), body weight gain ($p < 0.001$) and food efficiency ratio ($p < 0.001$) when compared with hypercholesterolemic rats (HB) fed on balanced diet.

Plasma lipids of hypercholesterolemic rats after feeding different dietary treatments in 2nd stage are shown in Table (7).

When comparing plasma lipids of different hypercholesterolemic rats that fed on balanced diet containing fermented formula (HFF) with rats continued fed on hypercholesterolemic diet (HH), as it was expected all plasma lipids were significantly improved. To exclude variation of plasma lipids that may occur as a result of changing the diet from hypercholesterolemic to balanced, we decided to compare the group of rats fed diet containing fermented product (HFF) with the group of hypercholesterolemic rats fed on balanced diet (HB) to know the actual change in plasma lipids. Hypercholesterolemic rats fed balanced diet (HB group) showed significant reduction in plasma levels of total lipids, T-Ch and LDL-Ch (-16%, -20%, -21%, respectively), while HDL-Ch and HDL/TCh ratio increased significantly (26%, 57% respectively $p < 0.001$), in comparison to hypercholesterolemic rats feeding the hypercholesterolemic diet (HH).

Table (6): Nutritional parameters of different experimental groups (2nd stage).

| Groups | Parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mean ± SE | Initial body weight (g) | Final body weight (g) | Body weight gain (g) | Total food intake (g) | Food intake (g/day) | Food efficiency ratio |
| CC | Mean | 224.8 | 276 | 51.2 | 343.1 | 11.437 | 0.149 |
| | ± SE | ± 5.365 | ± 7.722 | ± 2.509 | ± 8.746 | ± 0.292 | ± 0.006 |
| HH | Mean | 209.2 | 253.8 | 44.6 | 328.8 | 10.959 | 0.136 |
| | ± SE | ± 4.193 | ± 3.009 | ± 3.551 | ± 5.305 | ± 0.177 | ± 0.011 |
| HB | Mean | 206.8 | 259.8 | 53** | 305.4* | 10.2* | 0.174* |
| | ± SE | ± 3.734 | ± 5.164 | ± 2.118 | ± 5.242 | ± 0.175 | ± 0.007 |
| HFF | Mean | 200.3 | 214.2†† | 13.9†† | 319.7 | 10.66 | 0.043†† |
| | ± SE | ± 3.583 | ± 4.331 | ± 2.667 | ± 8.718 | ± 0.291 | ± 0.006 |

Values significantly differ from HH rats: *: $p < 0.005$, **$p < 0.001$

Values significantly differ from HB rats: ††: $p < 0.001$

Feeding hypercholesterolemic rats on diet containing fermented formula (HFF) showed significant increased in plasma level of HDL-Ch ($p < 0.025$). Hypercholesterolemic rats feeding on balanced diet containing fermented product showed improvement in all plasma lipid profile determined ranged from 4 to 11%. Fermented formula produced reduction in malondialdehye level in plasma as indicator of lipid peroxidation by 2% when compared with hypercholesterolemic rats feeding balanced diet. Our results are in agreement with the results of (Jang *et al*., 2007), who reported that fermented rice was very effective for improving the lipid metabolism and reducing stress by up-regulating the hepatic antioxidant enzymes in high-cholesterol-fed rats.

Table (7): Plasma lipids of hypercholesterolemic rats fed on different experimental diets (2nd stage).

| Parameters | Groups (Mean ± SE) | | | |
|---|---|---|---|---|
| | CC | HH | HB | HFF |
| Total lipids (g/dl)<br>% Change | 0.425 ± 0.017 | 0.897* ± 0.018<br>+ 112 | 0.749¨ ± 0.018<br>-16 | 0.689 ± 0.035<br>-8 |
| T-Ch (mg/dl)<br>%Change | 90.49 ± 2.589 | 191.6* ± 8.525<br>+112 | 153.9˙ ± 6.959<br>-20 | 146.6 ± 7.009<br>-5 |
| HDL-Ch (mg/dl)<br>% Change | 47.8 ± 0.525 | 29.7* ± 0.779<br>-38 | 37.4¨ ± 0.475<br>+26 | 39.7[a] ± 0.629<br>+ 6 |
| HDL/TCh ratio<br>% Change | 0.531 ± 0.021 | 0.157* ± 0.009<br>-71 | 0.245¨ ± 0.013<br>+57 | 0.273 ± 0.013<br>+11 |
| LDL-Ch (mg/dl)<br>% Change | 25.2 ± 0.364 | 110.2* ± 3.040<br>+338 | 87.22¨ ± 2.134<br>-21 | 83.5 ± 2.365<br>-4 |
| TG (mg/dl) | 94.57 ± 1.462 | 94.2 ± 1.467 | 90.2 ± 2.219 | 90.6 ± 2.221 |
| MDA (nmol/ml)<br>% Change | 5.2 ± 0.189 | 11.8* ± 0.530<br>+128 | 9.1¨ ± 0.226<br>-23 | 8.9 ± 0.327<br>-2 |

Values significantly differ from normal rats: *: $p < 0.001$.

Values significantly differ from HH rats: ˙ $p < 0.005$, ¨ $p < 0.001$

Values significantly differ from HB rats: a: $p<0.025$

The improvement in plasma lipid profile in hypercholesterolemic rats feeding on balanced diet containing fermented formula may be due to presence of probiotics and fibers, especially dietary fibers. It was reported that plasma cholesterol levels can be reduced by consumption of probiotic-containing dairy foods by people with elevated blood cholesterol (Parvez *et al*., 2006). Taranto *et al*. (1998) found that administration of low levels of *L. retueri* for 7 days decreased total cholesterol and triglyceride levels by 38% and 40% respectively, and increased the high-density lipid: LDL ratio by 20% in hypercholesterolemic mice.

The studied fermented formula contain high amount of dietary fiber 25.25g/100g sample as shown in Table (2). Dietary fiber plays an important role in lowering plasma cholesterol concentration through interference with bile acid absorption by binding or sequestering bile acids in the intestinal lumen (Anderson *et al*., 1990), dietary fiber reduces their active reabsorbtion in the lower small intestine, leading to fecal excretion. This leads to increased diversion of cholesterol to bile acid synthesis in the liver, up-regulation of lipoprotein receptors and depressed plasma cholesterol concentrations (Truswell and Beynen, 1992).

Feeding hypercholesterolemic rats on diets containing fermented formula showed significant reduction in malondialdehyde level as indicator of lipid peroxidation. This results indicate that studied formula possess antioxidant activity, which may be due to presence of phenolic compounds as shown in Table (2). Phenolic compounds possess antioxidant activity due to their redox properties which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Some phenolic compounds act as metal chelator and reduce lipid oxidation. It was reported that bioactive phenols can quench reactive oxygen species and protect from pro-oxidative damage (Wolfe and Liu, 2007 and Soobrattee *et al*., 2008).

**Conclusion** Fermented formula showed improvements in plasma lipid profile of hypercholesterolemic rats and reduced lipid peroxidation. Therefore fermented formula can be recommended as functional food to treat hypercholesterolemia or reduce the risk of atherosclerosis.

**References:**


- Achi O.K. The potential for upgrading traditional fermented foods through biotechnology. Afr. J. Biotechnol. 4 (5): 375-380 (2005).
- Anderson J.W., Deakins D.A. and Bridges S.R. Soluble fiber: hypocholesterolemic effects and proposed mechanisms. In: Dietary Fiber: Chemistry, Physiology and Health Effects (Kritchevsky D., Bonfield C. and Anderson J.W., eds.), pp. 339-363. Plenum Press, New York. NY (1990).

- AOAC. Official Methods of Analysis of the Association of official Agriculture Chemists 12th ed Washington DC (1995).
- AOAC Official methods of analysis of the Association of Official Agriculture Chemists 16th ed. Volume II, Section 45.4.07, Methods 985.29 (1997).
- Ashraf A. K. Nutritional improvement of an Egyptian breed of mung bean by probiotic lactobacilli. Afr. J. Biotechnol. 5: (2), 206-212 (2006).
- Bhadra S., Banavali S.D., Agrawal M. and Subbiah M.T. Cholesterol peroxidation potential as influenced by dietary fat type. Internat. J. Vit. Nutr. Res. 63: 223-228 (1993).
- Briggs G.M. and Williams M.A. A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. Fed. Proc. 22: 261 (1963).
- Burstein M., Scholnick HR. and Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Scand. J. Clin. Lab. Invest. 40: 583-595 (1980).
- Da-Silva M. H., Pithon T.C. and Do-Nascimento C.M. Effect of saturated and polyunsaturated fatty acids rich diets on hepatic and adipose tissue lipid metabolism in rats. Internat. J. Vit. Nutr. Res. 66: 258-262 (1996).
- Dave R.I. and Shah N.P. Ingredient Supplementation Effects on Viability of Probiotic Bacteria in Yogurt. J Dairy Sci. 81:2804–2816 (1998).
- Degirmencioglu N., Gocmen D., Dagdelen A. And Dagdelen F. Influence of tarhana herb (Echinophora Sibthorpiana) on fermentation of tarhana turki traditional fermented food. Food Technol. Biotechnol. 43 (2) 175-179 (2005).
- Dwyer J. Overview: dietary approaches for reducing cardiovascular disease risks. J. Nutr. 125: 656-665 (1995).
- Gerard T. and Gerald A.L. Process and reagents for the selective separation of low density lipoprotein (LDL) and for the quantification of their components. Eur. Path. (Appl.) E.P. 76: 211-221 (1981).
- Ibanoglu S., Ibanoglu E. and Ainsworth P. Effect of different ingredients on the fermentation activity in tarhana. Food Chem. 64: 103-106 (1999).
- James M.J. Modern food microbiology (6th Edition)- Chapman and Hall, Inc. p. 679, New York (2000).
- Jang YJ. Kim MH., Nam SH. and Kang MY. Effects of solid-state fermented rice on lipid metabolism and antioxidant status in high-cholesterol-fed rats. J Med. Food 10(4): 608-14 (2007).
- Megraw R., Dunn D. and Biggs H. Mannual and continuous flow colorimetry of triglycerols by a fully enzymaic method. Clin. Chem., 25: 273 (1979).
- Morcos S.R. The effect of protein value of the diet on the neurological manifestations produced in rats by β–immodipropionitrile. Br. J. Nutr. 21: 269 (1967).
- Parvez S., Malik K., Ah Kang S. and Kim H. Probiotics and their fermented food products are beneficial for health. J. of Appl. Microbiol. 100: 1171-1185 (2006).
- Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta 20: 37–43 (1978).
- Schram E., Moore S. and Bigwood E. Chromatographic determination of cystine as cysteic acid. Biochem. J. 57: 33-37 (1954).
- Singleton VL. And Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-158 (1965).
- Soobrattee MA., Bahorun T., Neergheen VS., Googoolye K. and Aruoma OI. Assessment of the content of phenolics and antioxidant actions of the Rubiaceae, Ebenaceae, Celastraceae, Erthoxylaceae and Sterculaceae families of Mautritian endemic plants. Toxicol. In Vitro. 22 (1): 45-56 (2008).
- Steinkraus K. H. Handbook of Indigenous fermented foods, Second edition. Marcel Dekker, New York (1996).
- Steinkraus K. H. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control 8 (5/6): 311-317 (1997).

- St-Onge MP., Farnworth E.D., Savard T., Mafu D.C. and Jones P.JH. Kefir consumption does not alter plasma lipid levels. BMC complement Alter. Med. 2: 1 (2002).
- Stucchi A.F., Terpstra A.H.M. and Nicolosi, R.J. LDL receptor activity is down-regulated similarly by a cholesterol-containing diet high in palmitic acid or high in lauric and myristic acids in cynomolgus monkeys. J. Nutr. 125: 2055-2063 (1995).
- Subramanian V., Manicham A. and Pathmanablan G. Biochemical changes during early germination of red gram (Cajanus Cajan L.) seeds. Indian Journal of Experimental Biology, 14: 736-737 (1976).
- Taranto M.P., Medici M., Perdigon C., Ruiz Holgado A.P. and Valdez G.F. Evidence for hypocholesterolemic effect of *Lactobacillus retueri* in hypocholesterolemic mice. J. Dairy Sci. 81: 2336-2340 (1998).
- Tholstrup T., Marckmann P., Vessby, B. and Sandstrom B. Effect of fats high in individual saturated fatty acids on plasma lipoprotein a levels in young healthy men. J. Lipid Res. 36: 1447-1452 (1995).
- Toro G. and Ackerman, P. G. Practical clinical chemistry 1st edition, printed by Little, Brown and Company, Boston USA. P. 352 (1975).
- Truswell A.S. and Beynen A.C. (1992): Dietary fiber and plasma lipids: potential for prevention and treatment of hyperlipidaemias. In: Dietary Fiber-A Component of Food (Schweizer T. F. and Edwards C. A., eds.), pp. 295-332. Springer, London, U. K.
- Watson D. A simple method for the determination of serum cholesterol. Clin. Chem. Acta., 5: 637 (1960).
- Wolfe KL. and Liu RH. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. J. Agric. Food Chem. 55 (22): 8896-906 (2007).
- Zulet M.A., Macarulla, M. T.; Portillo, M. P.; Noel-Suberville, C.; Higueret, P. and Matinez, J. A. Lipid and glucose utilization in hypercholesterolemic rats fed a diet containing heated chickpea (*Cicer Aretinum* L.): a potential functional food. Int. J. Vitam. Nutr. Res. 69 (6): 403-411 (1999).


3/22/2009