

## **VARIOUS COMBINATIONS OF ANTIOXIDANT SOURCES AND CHOLESTEROLEMIA IN RATS**

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### **ABSTRACT**

Low thermal processed semimodified foods (SMF) are of great nutritional and healthful sources for antioxidants (AO). To test this qualification, SMF has to be examined facing an upnormal or over optimal cholesterol challenge. A number of 60 aged rats have been grouped to fulfill this estimation. The ten animal groups were arranged to test a sort of building up multi various combination of antioxidant sources. The results showed that rats make an increment in final body weight during 45 days at that age according to dietary pattern used. Dietary intervention concurrently appeared with fat challenge. The best food ingredient intervention in controlling FBW was accomplished by SSFTI followed by SSFTh.

Moreover, more positive changes in serum total cholesterol level were achieved at the end of trail for SSFC and SSFTh followed by SSFTI. Blood cholesterol reduction by either S, SS or SSF may in part due to higher plant protein content with high-quality amino acids. The total cholesterol level was decreased with supplementation of vitamin E and C, the food formulas mentioned before most probably have more vitamin C than the others.

All treatment groups showed significant reduction in triglycerides levels through that period relative to the positive control. The parsley food fragment is devoted as the most effective potential agent against cholesterolemia followed in this concern with tomatos. Dietary protein level has also been shown to influence lipid metabolism.

In brief, according to data, SS can be considered a good food fragment in HDL-C dietary therapy that may further improved by parsley, flaxseed and tomato addition. Lastly, the strongest action against elevation of glucose in blood was recorded for SSF group followed by SSFTh group, assuming a powerful action in this concern to the combinations of soybean, sunflower and flaxseed in the presence of tomato. It is, to be always in mind that, lipidemia in most cases conjugates several degenerative diseases especially glycemia.

**Keywords:** Semimodified foods, Antioxidant sources, Lipoproteins, Cholesterolemia, Glycemia.

### **INTRODUCTION**

Antioxidants (AO) are becoming the most important nutritional issue nowadays. They are a group of nutrients and substances that distributed in various food sources. They are all acting against free radicals in different ways. Ou *et al.* (2002) in a comparative study analysis antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays.

Likewise, Gorinstein *et al.* (2004) compare the main antioxidant compounds content and the antioxidant activity of white grapefruit. Trans-hydroxycinnamic acids (caffeic, p-coumaric, ferulic and sinapic acids) were more abundant in white grapefruit than in his hybrid. However, grapefruit hybrid has a higher total phenol content as well as a higher antioxidant capacity in comparison with white grapefruit. A linear relationship has been existed between total antioxidant activity and anthocyanins, flavonoids and total phenols.

It is widely accepted that fruits and vegetables have many healthful properties. Consumption of fruits is beneficial to health and contributes to decrease of the mortality rate of cardiovascular and other diseases. A high intake of fruits also reduces blood pressure. (Ascherio *et al.* 1996 and Joshipura *et al.* 1999).

Moreover, Larrauri *et al.* (1996) studied a high dietary pre-powders from orange and lime peels associated with polyphenols and antioxidant. Antioxidant and prooxidant behaviors of flavonoids, structure-activity and relationships with free radicals in biology and medicine were estimated by Cao *et al.* (1997). However, the antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds were estimated (Chen and Ho, 1997). Antioxidant activity and phenolic composition of citrus peels or the chlorogenic acids and other cinnamates nature, occurrence, dietary burden, absorption and metabolism were studied by Bocco *et al.* (1998) and Clifford (2000).

The AO health aspects are accounted beyond most degenerative and pathological diseases. They are believed to be the crucial elements behind the nutritional factors correlated to hypertension among men. In this regard, Hollman *et al.* (1995) studied the relation between flavonoid intake and long-term risk of coronary heart disease and cancer. As well, Miller *et al.* (1998) observed the effect of dietary patterns on measures of lipidoxidation as a results from a randomized clinical trial. Finally, Leontowicz *et al.* (2002) have investigated the comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. The hypercholesterolemia in rats is a practical biochemical nutritional theme to evaluate the role of AO sources as a group of metabolic protective agents.

## **MATERIALS AND METHODS**

### **Materials and processing:**

All materials and chemicals used were obtained from corresponding local markets. Soy low fat extrudates are available at Soy Pilot Project, Food Technology Research Institute (FTRI), Agriculture Research Center (ARC), Cairo, this was reextruded with sunflower whole seeds from the FTRI, ARC, to produce SS of 15% sunflower. Flaxseeds, which partechased from fiber crop department at the FTRI, ARC, were added at the same step at level of 5% to SS to get the SSF using the reextrusion as shown in Table (1).

Both parsley curly and garden rocket are regular leafy plants in the Egyptian market. The later, separately or together, have been used at a level of 30% extractability to be added to the texturized SSF. Table (1) shows the stepwise incorporation of food ingredients in diet formulations comparing to the basal diet. The greatest attention was devoted to the extracts of the green vegetables, that processed at low thermal condition, which were prepared similar to that method used by Ibrahim and Ahmed (1990), while texturized SSF was a modification soy protein process conducted by Ahmed *et al.* (1999). The combinations of these prepared food materials in different semi modified foods (SMF) diets were carried out according to the procedure outlined by Ahmed *et al.* (2005) to be employed in the biological evaluation.

**Table (1): The stepwise preparation of semi modified foods (SMF) as dietary sources for various antioxidants**

Groups	Diets	Descriptions
1	NC	Negative control (Casein basal diet without cholesterol)
2	PC	Positive control (Casein basal diet + 1% cholesterol)
3	S	PC + 50% source for v.E, S (100% soybean extrudates)
4	SS	PC + 50% of 15%whole sunflower in S
5	SSF	Same of variable sources+5%whole flaxseeds(source for legnan)
6	SSFG	Same PC + v.C and carotene (1:1w/v SSF and 30 % extract of G)
7	SSFC	Same PC + v.C and carotene (1:1w/v SSF and 30 % extract of C)
8	SSFCG	Same PC + v.C and carotene( 1:1w/v SSF and 30 % extract of CG)
9	SSFTl	Same PC +v.C and lycopene ( 1:1w/v SSF and 30 % low thermal processed tomato)
10	SSFT <sub>h</sub>	Same PC + v.C and lycopene ( 1:1w/v SSF and 30 % high thermal processed tomato)

Where: NC= Negative control, PC= Positive control, S= Defatted soybean extrudate, SS= Re-extrusion of extruded-expelled defatted soybean + sunflower 20%, SSF= Re-extrusion of extruded-expelled defatted soybean + sunflower 20% + flaxseed 20%, SSFG= 30% extractable garden rocket (G), SSFC= 30% extractable parsley curly (C), SSFCG= Both leafy vegetables at half amount addition, SSFTl= SSF with addition of tomato concentrate by ratio (1:10) w/w at low thermal cooking and SSFT<sub>h</sub>= SSF with the addition of tomato juice by ratio (2:1) and cooked in microwave oven for 1 min at full power (high thermal cooking).

#### **Biological experiment:**

Male albino rats of western strains were housed in the Ophthalmology Research Institute, Giza, Egypt. Animal groups were 6 x 10 (60 aged animals of around 250 gm). To test the biological effect of these food formulations, the groups listed in Table (1) were investigated based on the SMF formulas proposed. The animals were fed on basal diet for 10 days for adaptation, then divided into those groups taking casein + 1% cholesterol as positive control (PC) and casein alone as a negative control (NC) as described by Bowman *et al.* (1990). The basal diet composed of casein (12%), cellulose (5%), vitamin mixture (1%), salt mixture (4%), corn oil (5%) and corn starch (73%). The ingredients of basal diet formulation were performed according to A.O.A.C.(2000). The hypercholesterolemic diet composed of the same basal diet plus cholesterol (1%), choline chloride (0.25%) and completed to 100 gm with corn starch. Rats blood samples were taken at 2, 4 and 6 weeks intervals during the experimental period and animals were anethetized. The organs were separated and weighed when the trial has been terminated.

#### **Analytical methods:**

Triglycerides were determined by an enzymatic method described by Lowell *et al.* (1973). Total cholesterol was determined according to the method of Allain *et al.* (1974). Determination of high density lipoprotein (HDL) was carried out according to the method described by Havel *et al.* (1955). In the determination of low density lipoprotein (LDL), the method described by Hatch and Lees (1968) was used. Lastly, Glucose was enzymatically determined according to the method of Tinder (1969).

#### **Statistical analysis:**

The LSD (0.05) was used to compare the main differences between treatments. Percentages were transformed to the arcsine. The arcsine percentages were subjected to the proper analysis of variance (Clark and

Kempson, 1997). In some cases, a value of average of 6 samples was calculated to be recorded.

## RESULTS AND DISCUSSION

As may extracted from Table (2), final body weight/initial body weight (FBW/IBW) in all treatments were coming in between negative control (NC) and positive control (PC) assuming strong regulatory effect in controlling FBW for this type of antioxidant combinations. In some details, all animals under the present work gave positive body weight with insignificant differences if compared to the negative control. The positive control was an exception. In this regard, Chi-Myung *et al.* (1982) reported that rats fed on cholesterol diets showed increases in food intake and weight gain compared to the negative control group. On the other hand, María de Regil *et al.* (2004) found that rats fed on casein had significant higher values in weight gain than rats fed on soy protein + methionine. However, there were no differences in body weight gain between rats fed on soy protein diet and casein in some other cases (Pilvi *et al.* 2006).

**Table (2): Initial and final body weight (gm) as affected by antioxidants in rats diets.**

Groups	Diets	IBW	percent NC	FBW	percent NC	FBW/IBW
1	NC	246.2 ± 3.2 <sup>B</sup>	100	294.3 ± 1.0 <sup>D</sup>	100	1.11
2	PC	248.8 ± 7.8 <sup>AB</sup>	101.1	341.4 ± 3.3 <sup>A</sup>	116.0	1.38
3	S	247.0 ± 4.9 <sup>AB</sup>	100.3	304.2 ± 2.1 <sup>F</sup>	103.4	1.23
4	SS	247.7 ± 5.0 <sup>AB</sup>	100.6	305.3 ± 2.3 <sup>F</sup>	103.7	1.23
5	SSF	246.6 ± 4.9 <sup>AB</sup>	100.2	306.0 ± 3.1 <sup>F</sup>	104.0	1.24
6	SSFG	247.5 ± 7.0 <sup>AB</sup>	100.5	308.0 ± 2.9 <sup>F</sup>	104.7	1.24
7	SSFC	246.9 ± 6.3 <sup>AB</sup>	100.3	304.0 ± 3.5 <sup>F</sup>	103.3	1.24
8	SSFCG	246.9 ± 6.3 <sup>AB</sup>	100.3	307.0 ± 3.2 <sup>F</sup>	104.4	1.25
9	SSFTI	247.8 ± 6.5 <sup>AB</sup>	100.6	290.2 ± 2.6 <sup>D</sup>	98.6	1.17
10	SSFTh	246.4 ± 5.6 <sup>AB</sup>	100.1	293.6 ± 3.6 <sup>CD</sup>	99.8	1.19

Mean ± standard error of the mean, Means within the same column followed by the same letter(s) are not significantly different

The fact that must be kept in mind as concluded from Table (2) is that, excess fat in diet is the main causative agent for relatively massive body weight gain (BWG) as well as lipidemia. Normal rats (NC) make 1.11 FBW/IBW increment in a month and half at that age, meanwhile, a range of increase to almost 1.40 for PC or sized from 1.17 to 1.25 according to dietary pattern used are existed. It is true that dietary intervention concurrently appeared with fat challenge. The best food ingredients intervention in controlling FBW was accomplished by SSFTI followed by SSFTh. Table (2) also confirmed the unique role of tomato in general and the idea that dietary therapy possess its effect at longer run.

Organs weight changes are tabulated in Table (3). All organs weight in grams was in between those of NC and PC. The nearest one to NC or even smaller was SSFCG for heart and spleen and SS for brain, lungs and tests.

**Table (3): Organs weight (gm) as affected by antioxidant in rats diets.**

Groups	Diets	Liver	Heart	Spleen	Kidney	Brain	Lungs	Tests
1	NC	7.790 ± 0.032 <sup>E</sup>	0.788 ± 0.020 <sup>D</sup>	0.788 ± 0.014 <sup>C</sup>	1.838 ± 0.031 <sup>D</sup>	1.770 ± 0.017 <sup>BC</sup>	1.352 ± 0.020 <sup>BC</sup>	2.098 ± 0.099 <sup>E</sup>
2	PC	11.982 ± 0.329 <sup>A</sup>	1.168 ± 0.019 <sup>A</sup>	0.880 ± 0.011 <sup>BC</sup>	2.902 ± 0.055 <sup>A</sup>	2.430 ± 0.008 <sup>A</sup>	1.532 ± 0.046 <sup>A</sup>	3.560 ± 0.076 <sup>A</sup>
3	S	8.560 ± 0.030 <sup>CD</sup>	0.970 ± 0.034 <sup>BC</sup>	0.870 ± 0.022 <sup>BC</sup>	1.490 ± 0.013 <sup>CD</sup>	1.660 ± 0.056 <sup>C</sup>	1.590 ± 0.062 <sup>A</sup>	2.590 ± 0.047 <sup>CD</sup>
4	SS	9.080 ± 0.030 <sup>CD</sup>	0.960 ± 0.034 <sup>BC</sup>	0.720 ± 0.013 <sup>C</sup>	1.490 ± 0.013 <sup>CD</sup>	1.680 ± 0.056 <sup>C</sup>	1.300 ± 0.020 <sup>BC</sup>	2.020 ± 0.099 <sup>E</sup>
5	SSF	10.980 ± 0.030 <sup>CD</sup>	0.780 ± 0.034 <sup>BC</sup>	0.880 ± 0.014 <sup>C</sup>	1.770 ± 0.013 <sup>CD</sup>	1.660 ± 0.056 <sup>C</sup>	1.480 ± 0.020 <sup>BC</sup>	1.920 ± 0.099 <sup>E</sup>
6	SSFG	11.350 ± 0.030 <sup>CD</sup>	0.830 ± 0.034 <sup>BC</sup>	0.680 ± 0.014 <sup>C</sup>	1.950 ± 0.013 <sup>CD</sup>	1.500 ± 0.056 <sup>C</sup>	1.580 ± 0.020 <sup>BC</sup>	2.650 ± 0.099 <sup>E</sup>
7	SSFC	9.870 ± 0.030 <sup>CD</sup>	0.940 ± 0.034 <sup>BC</sup>	0.590 ± 0.014 <sup>C</sup>	1.680 ± 0.013 <sup>CD</sup>	1.400 ± 0.056 <sup>C</sup>	1.320 ± 0.020 <sup>BC</sup>	2.920 ± 0.099 <sup>E</sup>
8	SSFCG	10.900 ± 0.030 <sup>CD</sup>	0.780 ± 0.034 <sup>D</sup>	0.776 ± 0.014 <sup>C</sup>	1.760 ± 0.013 <sup>CD</sup>	1.590 ± 0.056 <sup>C</sup>	1.380 ± 0.020 <sup>BC</sup>	2.380 ± 0.099 <sup>E</sup>
9	SSFTI	8.852 ± 0.430 <sup>B</sup>	0.960 ± 0.022 <sup>B</sup>	0.880 ± 0.020 <sup>BC</sup>	1.968 ± 0.008 <sup>B</sup>	1.790 ± 0.035 <sup>B</sup>	1.500 ± 0.072 <sup>AB</sup>	3.038 ± 0.189 <sup>B</sup>
10	SSFTh	8.682 ± 0.019 <sup>D</sup>	0.840 ± 0.060 <sup>C</sup>	0.870 ± 0.053 <sup>B</sup>	1.584 ± 0.049 <sup>C</sup>	1.480 ± 0.059 <sup>D</sup>	1.450 ± 0.047 <sup>AB</sup>	2.830 ± 0.099 <sup>BC</sup>

Mean ± standard error of the mean, Means within the same column followed by the same letter(s) are not significantly different.

The effect of AO addition on both total cholesterol (TC) and triglycerides (TG) is shown in Table (4). Positive changes in serum total cholesterol level were achieved at the end of trail for SSFC and SSFTh followed by SSFTI. It can be noticed that initial values of serum TC were not significantly different in all groups except negative control, where the value was lower than other groups. The serum TC value remained significantly unchanged in the negative control through the experimental period but increased markedly in the positive control. There were significant reductions in all groups at the end in relative to initial time or positive control. From these data, it is clear that SSFTh diet had a great effect as hypocholesterolemic agent, but the greatest effect as dietary therapy may obtained for food combinations containing SSFC with SSFTh. This is in agreement with the observations of Hermansen *et al.* (2001) who found that, the lowering cholesterol effect of experimental diets may vary according to the amount, type and preparation of diets, in addition to pre-existing level of serum lipids. The components in soy, sunflower, flaxseed, parsley and tomato responsible for their salutary effects on plasma lipids are not been fully identified. The reduction of blood cholesterol by either S, SS or SSF may in part due to higher plant protein content with high-quality amino acids (Prasad *et al.* 1998 and Sirtori *et al.* 2002).

It was proposed by Bhatena *et al.* (2003) that the cholesterol-lowering effects of flaxseed meal and soy protein concentrate are more likely due to lignan in the case of flaxseed and to the type of protein and isoflavones in the case of soy protein. The combined effect of various food sources is highly accepted. Ratnayake *et al.* (1992) found that, 10% flaxseed diet did not affect serum lipids but 20% and 30% flaxseed diets lowered plasma TC by 21% and 33%, respectively.

**Table (4): Total cholesterol and triglycerides as affected by antioxidants in rats diets.**

Groups	Diets	Total cholesterol (mg/dl)			Triglycerides (mg/dl)		
		2weeks	4weeks	6weeks	2weeks	4weeks	6weeks
1	NC	64.43 ± 0.480 <sup>J</sup>	63.48 ± 0.393 <sup>J</sup>	61.60 ± 0.455 <sup>J</sup>	71.17 ± 0.393 <sup>F</sup>	69.75 ± 1.509 <sup>F</sup>	65.99 ± 0.402 <sup>F</sup>
2	PC	148.18 ± 0.099 <sup>E</sup>	170.73 ± 0.099 <sup>E</sup>	195.36 ± 0.980 <sup>E</sup>	93.42 ± 0.393 <sup>J</sup>	94.10 ± 0.099 <sup>J</sup>	102.02 ± 1.609 <sup>J</sup>
3	S	130.72 ± 0.104 <sup>E</sup>	103.22 ± 0.728 <sup>G</sup>	95.81 ± 0.099 <sup>HI</sup>	89.59 ± 0.393 <sup>J</sup>	87.95 ± 0.368 <sup>E</sup>	86.76 ± 0.099 <sup>E</sup>
4	SS	129.00 ± 0.099 <sup>E</sup>	120.0 ± 0.099 <sup>F</sup>	110.0 ± 0.099 <sup>HI</sup>	88.30 ± 0.393 <sup>J</sup>	86.49 ± 0.099 <sup>E</sup>	78.32 ± 0.099 <sup>E</sup>
5	SSF	135.0 ± 0.099 <sup>E</sup>	131.01 ± 0.099 <sup>F</sup>	121.01 ± 0.099 <sup>HI</sup>	94.22 ± 0.099 <sup>J</sup>	90.17 ± 0.099 <sup>E</sup>	84.06 ± 0.393 <sup>E</sup>
6	SSFG	130.06 ± 0.099 <sup>E</sup>	128.0 ± 0.099 <sup>F</sup>	108.03 ± 0.099 <sup>HI</sup>	84.61 ± 0.099 <sup>J</sup>	79.75 ± 0.099 <sup>E</sup>	74.20 ± 0.393 <sup>E</sup>
7	SSFC	93.0 ± 0.099 <sup>E</sup>	89.11 ± 0.099 <sup>HI</sup>	88.43 ± 0.099 <sup>HI</sup>	84.50 ± 0.393 <sup>J</sup>	80.36 ± 0.099 <sup>E</sup>	72.06 ± 0.099 <sup>E</sup>
8	SSFCG	134.0 ± 0.099 <sup>E</sup>	130.01 ± 0.099 <sup>F</sup>	127.12 ± 0.099 <sup>HI</sup>	96.33 ± 0.099 <sup>J</sup>	75.63 ± 0.099 <sup>E</sup>	84.30 ± 0.393 <sup>E</sup>
9	SSFTI	138.59 ± 0.125 <sup>E</sup>	117.47 ± 0.803 <sup>F</sup>	92.80 ± 2.900 <sup>HI</sup>	95.62 ± 0.393 <sup>J</sup>	91.65 ± 0.247 <sup>AB</sup>	88.42 ± 1.398 <sup>E</sup>
10	SSFTh	129.45 ± 0.089 <sup>E</sup>	116.53 ± 2.464 <sup>F</sup>	89.09 ± 1.600 <sup>I</sup>	91.44 ± 0.247 <sup>J</sup>	88.44 ± 0.247 <sup>AB</sup>	87.64 ± 1.157 <sup>E</sup>

Mean ± standard error of the mean, Means within the same column followed by the same letter(s) are not significantly different. LSD at 0.05 level.

Pilvi *et al.* (2006) found that total and LDL cholesterol levels were 18% lower in rats of soy group compared to casein group. Bobek (1999) found that the addition of 15% tomato pomace to the cholesterol diet (0.3%) for male wistar rats reduced serum cholesterol levels including TC and LDL from 4.4 mmol/L to 2.5 mmol/L. Hamilton *et al.* (2000) reported that total cholesterol level was decreased with supplementation of vitamin E and C, the food formulas mentioned before most probably have more vitamin C than the others. It is true that a strong effective single AO needs specific accompying nutrients for full action. Rats fed on the lycopene-rich diet showed an acute decrease in cholesterol content in the liver (Andrei *et al.* 2003).

From the aforementioned data it could be observed that the initial values of serum triglycerides were not significantly different in all groups except negative control, which was lower than other groups. At the end of feeding period, the TG levels in the positive control were significantly higher than those in the negative control and all treatment groups. The present study revealed that, soybean protein compared with casein lowered plasma triglycerides as seen in Table (4). There, triglyceride levels and related VLDL value were less in rats fed soybean protein than that fed casein. The higher VLDL uptake could be responsible for the hypotriglyceridemia in rats fed soybean protein. Here, there is no doubt that dietary protein affect plasma cholesterol concentration, this effect is somewhat variable but is generally greater in hypercholesterolemic than in normocholesterolemic subjects. The TG levels differ significantly from the negative control through the feeding period. All treatment groups showed significant reduction in TG levels through that period in relative to the positive control or comparing to the initial time.

Likewise, Friedman *et al.* (2000) found that when Hamsters fed on diets containing both green and red freeze-dried tomato powders, the plasma triglyceride concentrations reduced by 47% and 31%, respectively. Also, Nishimukai and Hara (2004) reported that soybean phosphatidylcholine enhances the lymphatic absorption of triglyceride and lycopene. Furthermore, Ratnayake *et al.* (1992) found that plasma triglycerides of rats fed on 20% and 30% flaxseed diets were lowered by 23 % and 32 %, respectively.

Aoyama *et al.* (2001) reported that the plasma triglyceride level was significantly lower in the soy protein isolate (SPI) and beta-conglycinin groups than that in the casein group. Also, these results are in agreement with Bhathena *et al.* (2003) who found that plasma triglyceride concentration in obese rats was reduced by 37% in rats fed on flaxseed meal and by 19% in rats fed on soy protein compared to those fed on casein. Chen *et al.* (2005) reported that soy protein intake significantly decreased triglyceride levels by 43.1% in hyperlipidemic subjects. Serum and hepatic cholesterol and triglyceride levels as well as VLDL-cholesterol and LDL-cholesterol were significantly lower in rats fed on soy protein than in rats fed on casein diet up to 160 days (Tovar *et al.* 2005).

The low density lipoprotein (LDL) and high density lipoprotein (HDL) were significantly changed with time and dietary cholesterol as illustrated in Table (5). The increase due to dietary cholesterol was several times the normal of LDL, which was only around one and half in case of HDL. The highest food interventions for lowering LDL were SSFTh followed by SSFTI, which seemed to be the best when dietary therapy is administered for long time. In case of HDL, the best values were that of SS and SSFC.

**Table (5): Low and high density lipoproteins as affected by AO in rats diets.**

Groups	Diets	Low density lipoprotein (mg/dl)			High density lipoprotein (mg/dl)		
		2weeks	4weeks	6weeks	2weeks	4weeks	6weeks
1	NC	27.68 ± 1.323 <sup>I</sup>	27.28 ± 0.742 <sup>I</sup>	25.46 ± 0.729 <sup>I</sup>	64.95 ± 0.302 <sup>F</sup>	63.19 ± 0.080 <sup>F</sup>	65.36 ± 0.139 <sup>F</sup>
2	PC	90.65 ± 1.322 <sup>C</sup>	121.56 ± 2.775 <sup>C</sup>	164.85 ± 2.008 <sup>C</sup>	33.78 ± 0.218 <sup>I</sup>	31.79 ± 0.641 <sup>I</sup>	26.75 ± 0.993 <sup>I</sup>
3	S	92.46 ± 0.491 <sup>C</sup>	90.92 ± 0.742 <sup>I</sup> 1.902 <sup>G</sup>	73.88 ± 0.697 <sup>F</sup>	40.26 ± 0.729 <sup>I</sup>	55.55 ± 0.729 <sup>H</sup>	76.23 ± 0.729 <sup>H</sup>
4	SS	92.12 ± 0.729 <sup>C</sup>	117.05 ± 0.742 <sup>B</sup>	82.22 ± 0.491 <sup>G</sup>	40.96 ± 0.729 <sup>I</sup>	58.02 ± 0.729 <sup>H</sup>	97.34 ± 0.729 <sup>E</sup>
5	SSF	91.13 ± 0.491 <sup>C</sup>	137.36 ± 0.742 <sup>B</sup>	88.05 ± 0.729 <sup>G</sup>	44.01 ± 0.729 <sup>I</sup>	64.33 ± 0.729 <sup>H</sup>	76.11 ± 0.729 <sup>H</sup>
6	SSFG	85.15 ± 0.491 <sup>C</sup>	130.11 ± 0.742 <sup>B</sup>	76.33 ± 0.729 <sup>G</sup>	46.00 ± 0.729 <sup>I</sup>	70.21 ± 0.729 <sup>H</sup>	88.38 ± 0.729 <sup>H</sup>
7	SSFC	94.99 ± 0.491 <sup>C</sup>	90.96 ± 0.742 <sup>G</sup>	88.98 ± 0.729 <sup>G</sup>	47.02 ± 0.729 <sup>I</sup>	74.25 ± 0.729 <sup>H</sup>	91.31 ± 0.729 <sup>E</sup>
8	SSFCG	89.37 ± 0.729 <sup>C</sup>	121.12 ± 0.742 <sup>B</sup>	86.35 ± 0.491 <sup>G</sup>	41.00 ± 0.729 <sup>I</sup>	75.00 ± 0.729 <sup>H</sup>	82.11 ± 0.729 <sup>H</sup>
9	SSFTI	91.636 ± 1.475 <sup>C</sup>	64.76 ± 1.357 <sup>F</sup>	36.76 ± 3.657 <sup>I</sup>	49.62 ± 1.563 <sup>I</sup>	78.82 ± 0.980 <sup>H</sup>	89.39 ± 1.549 <sup>H</sup>
10	SSFTh	89.433 ± 0.996 <sup>C</sup>	62.39 ± 1.790 <sup>F</sup>	35.32 ± 0.729 <sup>I</sup>	48.72 ± 0.972 <sup>I</sup>	75.60 ± 0.865 <sup>H</sup>	88.55 ± 1.020 <sup>H</sup>

Mean ± standard error of the mean, Means within the same column followed by the same letter(s) are not significantly different.

This data show that dietary therapy is facilitated by some sorts of carotenes and vitamins, However, all tested lipoproteins have been sized in rats upon feeding on other foods that rich in carotenes, vitamins, polyphenols, sulfur substances and etc.

In some other words, parsley food fragment is devoted as the most effective potential agent against cholesterolemia followed in this concern with tomato. It seems also that the SS is basically important, hence the studies clearly demonstrate that soybean protein compared with casein accelerates LDL uptake by liver. Dietary protein level has also been shown to influence lipid metabolism.

In brief, according to data, SS can be considered a good food fragment in HDL-C dietary therapy that may further improved by parsley, flaxseed and tomato addition. Higher blood levels of the phytoestrogen, daidzein, were associated with lower triglycerides, higher HDL-C levels and a beneficial total cholesterol to HDL-C ratio in women with low estrogen levels (Bailey Merz *et al.* 2006). These data are confirmed by the results of Zhuo *et al.* (2004) and Chen *et al.* (2005) who found that, with identical soy protein intake, high isoflavones intake led to greater decreases in serum LDL cholesterol than low isoflavones intake, demonstrating that isoflavones have LDL cholesterol-lowering effects independent of soy protein. On the other side, Cunnane *et al.* (1994) showed that consumption of 50 grams of flaxseeds/day for four weeks resulted in a small but significant reduction in LDL cholesterol in young healthy humans.

Table (6) illustrated the changes in blood glucose level of rats as a result of feeding on cholesterol-enriched. AO diets. At the end of the experimental period, blood glucose level of the positive control was significantly higher than the negative control. Blood glucose levels of all treatment groups were significantly decreased as compared with the positive control. There were no significant differences in blood glucose levels among the negative control group and all treatment groups. However, the strongest action against this elevation of blood glucose was recorded for SSF group followed by SSFTh group, assuming a powerful action in this concern to the combinations of soybean and flaxseeds in the presence of tomato. However, Lemay *et al.* (2002) found that flaxseed decreased glucose and insulin levels in hypercholesterolemic women.

It could be observed from table (6) that, blood total lipids of the positive control were significantly higher than the negative control. Blood total lipids of all treatment groups were significantly decreased as compared with the positive control. The best results for lowering blood total lipids were obtained with SSF and SSFTh, which were similar to that of blood glucose levels. Thus, it could be concluded that glucose in blood is most probably correlated with total lipids and lipidemia in most cases conjugates several degenerative diseases especially glycemia.



**Table (6): Blood total lipids and glucose levels as affected by antioxidants in rats diets.**

Groups	Diets	Blood total lipids (mg/dl)			Blood glucose level (mg/dl)		
		2weeks	4weeks	6weeks	2weeks	4weeks	6weeks
1	NC	81.431 ± 0.059 <sup>H</sup>	81.807 ± 0.088 <sup>H</sup>	86.400 ± 0.589 <sup>H</sup>	108.295 ± 2.039 <sup>C</sup>	106.961 ± 2.750 <sup>C</sup>	96.257 ± 1.236 <sup>C</sup>
2	PC	167.950 ± 0.302 <sup>F</sup>	191.198 ± 0.080 <sup>F</sup>	203.362 ± 0.139 <sup>F</sup>	179.410 ± 1.196 <sup>E</sup>	177.872 ± 0.667 <sup>E</sup>	166.168 ± 2.348 <sup>E</sup>
3	S	117.738 ± 0.437 <sup>F</sup>	118.367 ± 0.074 <sup>A</sup>	101.754 ± 0.269 <sup>A</sup>	125.878 ± 1.385 <sup>E</sup>	122.202 ± 2.703 <sup>A</sup>	100.360 ± 1.298 <sup>CD</sup>
4	SS	117.990 ± 0.074 <sup>F</sup>	117.752 ± 0.564 <sup>A</sup>	95.690 ± 0.351 <sup>BC</sup>	116.548 ± 1.843 <sup>E</sup>	113.53 ± 1.908 <sup>A</sup>	99.460 ± 2.256 <sup>C</sup>
5	SSF	117.990 ± 0.074 <sup>F</sup>	117.752 ± 0.564 <sup>A</sup>	89.752 ± 0.564 <sup>H</sup>	106.18 ± 1.843 <sup>E</sup>	93.55 ± 1.908 <sup>A</sup>	93.218 ± 2.256 <sup>C</sup>
6	SSFG	117.890 ± 0.074 <sup>F</sup>	117.752 ± 0.564 <sup>A</sup>	98.752 ± 0.564 <sup>A</sup>	106.54 ± 1.843 <sup>E</sup>	100.62 ± 1.908 <sup>A</sup>	97.560 ± 2.256 <sup>C</sup>
7	SSFC	117.970 ± 0.074 <sup>F</sup>	115.752 ± 0.564 <sup>A</sup>	93.752 ± 0.564 <sup>BC</sup>	126.48 ± 1.843 <sup>E</sup>	96.32 ± 1.908 <sup>A</sup>	93.466 ± 2.256 <sup>C</sup>
8	SSFCG	117.790 ± 0.074 <sup>F</sup>	116.752 ± 0.564 <sup>A</sup>	102.752 ± 0.564 <sup>A</sup>	126.52 ± 1.843 <sup>E</sup>	100.22 ± 1.908 <sup>A</sup>	101.450 ± 2.256 <sup>C</sup>
9	SSFTI	117.690 ± 0.074 <sup>F</sup>	116.002 ± 0.564 <sup>A</sup>	90.752 ± 0.564 <sup>BC</sup>	126.68 ± 1.843 <sup>E</sup>	95.62 ± 1.908 <sup>A</sup>	90.440 ± 2.256 <sup>C</sup>
10	SSFTh	118.332 ± 0.049 <sup>F</sup>	117.884 ± 0.280 <sup>A</sup>	88.650 ± 0.183 <sup>H</sup>	127.150 ± 2.557 <sup>E</sup>	114.694 ± 0.524 <sup>A</sup>	93.660 ± 0.517 <sup>C</sup>

Mean ± standard error of the mean, Means within the same column followed by the same letter(s) are not significantly different.

## REFERENCES

- Ahmed, A. I. S.; M. A. H. Saleh and N. T. Saleh (1999). Chemical characteristic of processed soybean. Arab Conf. Food Sci., march. Cairo, 520-530.
- Ahmed, A. I. S.; G. M. Habib; Soheir, A.; M. Saad and F. El-Deeb (2005). Relation of natural antioxidants to hormonal balance and immune system. *Egy. J. Biomed. Sci.*, 17, 294-305.
- Allain, C. C.; Poon, L. S. and Chan, C. S. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.*;20:470-475.
- Andrei, S.; Pinteau, A. and Sevastre, B. (2003). Influence of lycopene administration on fatty acids and cholesterol content in rats I.P treated with asbestos. *Bulentinu-Universitatii-de-Stiinte-Agricole-si-Medicina-Veterinara-Cluj-Napoca,-Seria-Medicina Veterinara*; 60: 24-27.
- A.O.A.C. (2000). Official Methods of Analysis of the Association of Official Agriculture Chemists, 17 th ed., Washington D.C., USA.
- Aoyama, T.; Kohno, M.; Saito, T.; Fukui, K.; Takamatsu, K.; Yamamoto, T.; Hashimoto, Y.; Hirotsuka, M. and Kito, M. (2001). Reduction by phytate-reduced soybean beta-conglycinin of plasma triglyceride level of young and adult rats. *Biosci. Biotechnol. Biochem.*; 65(5):1071-1075.
- Ascherio, A.; Rimm, E. B.; Giovannucci, E. L.; Dspiegelman, M. S. and Willett, W. C. (1996). Dietary fat and risk of coronary heart disease in men: cohort follows up study in the United States. *Brit. Med. J.*; 313: 84-90.

- Bairey Merz, C. N.; Johnson, B. D.; Braunstein, G. D.; Pepine, C. J.; Reis, S. E.; Paul-Labrador, M.; Hale, G.; Sharaf, B. L.; Bittner, V.; Sopko, G. and Kelsey, S.F. (2006). For the women's ischemia syndrome evaluation study. Phytoestrogens and lipoproteins in women. *J. Clin. Endocrinol. Metab.*; 91: 2209-2213.
- Bhathena, S. J.; Ali, A. A. and Haudenschild, C. (2003). Dietary flaxseed meal is more protective than soy protein concentrate against hypertriglyceridemia and steatosis of the liver in an animal model of obesity. *J. Am. Coll. Nutr.*; 22: 157 –164.
- Bobek, P. (1999). Dietary tomato and grape pomace in rats: Effect on lipids in serum and liver, and on antioxidant status. *Br. J. Biomed. Sci.*; 56(2):109-113.
- Bocco, A.; Cuvelier, M. E.; Richard, H. And Berset, C. (1998). Antioxidant activity and phenolic composition of citrus peel and seedextracts. *J. Agric. Food Chem.*;4:320-325.
- Bowman, T. A.; M. Gonewardene and C. E. Taylor. (1990). VA deficiency decrease NK cell activity. *J. Nutr.*,120:1264-1273.
- Cao, G., So, C. E. And Prior, L. R. (1997). Antioxidant and prooxidant behaviors of flavonoids:Structure-activity, relationships and free radicals in *Biology and Medicine*,22,749 -760.
- Chen, J. H. And Ho, J. (1997). Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.*,45,2374 -2378.
- Chen, S. T.; Ferng, S. H.; Yang, C. S.; Peng, S. J.; Lee, H. R. and Chen, J. R. (2005). Variable effects of soy protein on plasma lipids in hyperlipidemic and normolipidemic hemodialysis patients. *Am. J. Kidney Dis.*; 46(6): 1099-1106.
- Chi-Myung, S.; KOH, E. T. and Stewart, T. J. (1982). Effects of garlic on lipid metabolism in rats fed cholesterol or lard. *J. Nutr.*,112: 241-246.
- Clark, E. J. and Kempson, J. (1997). Development in plant breeding for improved nutritional quality of soybean: Antinutritional factors. *J. Agric. Sci.*, 134: 125-136.
- Clifford, M. N. (2000). Chlorogenic acids and other cinnamates nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food and Agric.*,80:1033 -1042.
- Cunnane, S. C.; Ganguli, S.; Menard, C.; Liede, A. C.; Hamadeh, M. J.; Chen, Z. Y.; Wolever, T. M. and Jenkins, D. J. (1994). High alpha-linolenic acid flaxseed (*Linum usitatissimum*): Some nutritional properties in humans. *Br. J. Nutr.*; 69: 443 –453.
- Friedman, M.; Fitch, T. E.; Levin, C. E. and Yokoyama, W. H. (2000). Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. *J. Food Sci.*, 65 (5): 897-900.
- Gorinstein, S.; Zo Z.; Elena K.; Elke P.; Ratiporn H.; Simon T. and Olga Martin, B. (2004). Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. *Lebensm. Wiss. Technol.*,37:337-343.

- Hamilton, I. M. J.; Gilmore, W. S.; Benzie, I. F. F.; Mulholland, C. W. and Strain, J. J. (2000). Interactions between vitamins C and E in human subjects. *Brit. J. of Nutr.*, 84(3): 261-267.
- Hatch, F. T. and Lees, R. S. (1968). Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.*, 6:1- 68.
- Havel, R. J.; Eder, H. A. and Bragdon, J. H. (1955). The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*,34:1345-1353.
- Hermansen, K.; Sondergaard, M.; Hoie, L.; Carstensen, M. and Brock, B. (2001). Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care*; 24:228–233.
- Hollman, E. J. M.; Hollman, P. C. H. And Katan, M. B. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine*,155:1115-1122.
- Ibrahim, E. M. and A. I. S. Ahmed (1990). Economic nonpolluting yeast production. *Agric. Res. Rev.*, 68 (2):379-387.
- Joshiyura, K. J.; Ascherio, A.; Manson, J. E.; Stampfer, M. J.; Rimm, E. B.; Speizer, F. E.; Hennekens, C. H.; Spiegelman, D. And Willett, W. C. (1999). Fruit and vegetable intake in relation to risk of ischemic stroke. *J. Amer. Med. Association*,282:1233 -1239.
- Larrauri, J. A.; Ruperez, P.; Bravo, L. And Saura-Calixto, F. (1996). High dietary pre-powders from orange and lime peels:Associated polyphenols and antioxidant capacity. *Food Res. Inter.*,29:757 -762.
- Lemay, A.; Dodin, S.; Kadri, N.; Jacques, H. and Forest, J. C. (2002). Flaxseed dietary supplement versus hormone replacement therapy in hypercholesterolemic menopausal women. *Obstetrics and Gynecology*; 100: 495-504.
- Leontowicz, H.; Gorinstein, S.; Lojek, A.; Leontowicz, M. C.; Soliva-Fortuny, R.; Park, Y. S.; Jung, S. T.; Trakhtenberg, S. and Martin-Belloso, O. (2002). Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. *J. Nutr. Biochem.*,13:603-610.
- Lowell, R.; Rosebrough, A.; Fan, J. and Randall, A. (1973). A simplification of protein assay method. *Ann. Biochem.*, 83:346-349.
- María de Regil, L. and Calderon de la Barca (2004). Nutritional and technological evaluation of an enzymatically methionine enriched soy protein for infant enteral formulas. *Inter. J. food sci. and nutr.*,55(2): 91-99.
- Miller, E. R.; Appel, L. J. And Risby, T. H. (1998). Effect of dietary patterns on measures of lipidoxidation: Results from a randomized clinical trial. *Circulation*,98:2390 -2395.
- Nishimukai, M. and Hara, H. (2004). Enteral administration of soybean phosphatidylcholine enhances the lymphatic absorption of lycopene but reduces that of  $\alpha$ -tocopherol in rats. *J. Nutr.*;134: 1862-1866.
- Ou, B.; Huang, D.; Hampsch-Woodill, M.; Flanagan, J. And Deemer, E. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric

- reducing antioxidant power (FRAP) assays: A comparative study. J. Agric. Food Chem.,50:3122 -3128.
- Pilvi, T. K.; Jauhiainen, T.; Cheng, Z. J.; Mervaala, E. M.; Vapaatalo, H. and Korpela, R. (2006). Lupin protein attenuates the development of hypertension and the vascular function of NaCl-Loaded Goto-Kakizaki rats. Inst. of Biomed. Pharma., 15:112-118.
- Prasad, K.; Mantha, S. V.; Muir, A. D. and Westcott, N. D. (1998). Reduction of hypercholesterolemic atherosclerosis by CDC-flaxseed with very low alpha linolenic acid. Atherosclerosis; 36:367–375.
- Ratnayake, W. M. N.; Behrens, W. and Fischer, P. (1992). Chemical and nutritional studies of flaxseed (variety Linott) in rats. J. Nutr. Biochem.; 3: 232-240.
- Sirtori, C. R.; Bosio, R.; Pazzucconi, F.; Bondioli, A.; Gatti, E.; Lovati, M. R. and Murphy, P. (2002). Soy milk with high glycitein content does not reduce low-density lipoprotein cholesterol in type II hypercholesterolemic patients. Ann. Nutr. Metab.; 46: 88-92.
- Tinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6:24-28.
- Tovar, R. A.; Torre-Villalvazo, I.; Ochoa, M.; Elías A. L.; Ortíz, V.; Aguilar-Salinas, C. A. and Torres, N. (2005). Soy protein reduces hepatic lipotoxicity in hyperinsulinemic obese Zucker fa/fa rats. J. of Lipid Res.,46: 1823-1832.
- Zhuo, G.; Etherton, T. D.; Martin, K. R.; West, S. G.; Gillies, P. J. and Kris-Etherton, P. M. (2004). Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J. Nutr.; 134: 2991-2997.

**علاقة مضادات الأكسدة من مصادر غذائية متنوعة بكوليسترول الدم في الفئران  
مها محمود ، خالد محمد نعمة الله ، محمود عبد الله صالح و أحمد محمد جعفر  
قسم الأغذية الخاصة والتغذية – معهد بحوث تكنولوجيا الأغذية- مركز البحوث الزراعية بالجيزة**

تعتبر الأغذية النصف مصنعة ذات قيمة غذائية عالية ومصادر غنية بمضادات الأكسدة وقد تم دراسة علاقة مضادات الأكسدة من مصادر غذائية متنوعة تشمل كل من فول الصويا وبذور عباد الشمس والكتان والأوراق الخضراء للبقدونس والجرجير وكذلك مركزات وعصير الطماطم بكوليسترول الدم في الفئران عن طريق إضافة مضادات الأكسدة الطبيعية بالتدرج في غذاء الفئران و متابعة بعض مكونات الدم. ومن الواضح ان الغذاء يلعب دورا هاما في ضبط ليبيدات الدم والحفاظ على الأعضاء الهامة بالجسم. وقد ظهر من نتائج هذا البحث أن البقدونس يعتبر أقوى الأغذية تحت الدراسة في خفض مستوى الكوليستيرول ليلية الطماطم كما أن الخلطات المحتوية على فول الصويا وبذور عباد الشمس والكتان في وجود مركزات وعصير الطماطم لها دور فعال في تحسين ليبيدات الدم. وترتكز أهمية هذا البحث في الإشارة إلى الأدوار المتميزة لبعض مضادات الأكسدة مع أهمية وجود العناصر الأخرى مثل البروتينات للوصول إلى التأثير البيولوجي الأمثل على كوليستيرول الدم و من ثم على الأمراض الباثولوجية الأخرى المترتبة على وجودها ومنها مرض السكر حيث خفضت الخلطات المستخدمة تحت الدراسة من مستوي جلوكوز الدم بالمقارنة مع الفئران المغذاة علي الكوليستيرول ومن ثم فان التنوع الغذائي والاعتماد على الأغذية النصف مصنعة من مصادر متنوعة قد تفيد كثيرا في منع خطر الإصابة بالأمراض.