

Effect of Broccoli on the Antioxidant Activity of Experimental Rats Ingested Thermally Oxidized Oil

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Abstract: The effect of broccoli powder, aqueous and methanolic extracts on antioxidant activity in rats fed on thermally oxidized oil was studied. Chemical analysis of broccoli was investigated. Five groups of rats were used; group (1) was used as a negative control, while rats of the other groups were fed thermally oxidized oil in diet. Group (2) was left as a positive control, while groups (3), (4) and (5) were administered broccoli powder, aqueous and methanolic extracts for 60 days. The obtained results revealed that broccoli contains carbohydrate, protein, fiber and ash, and also high amount of potassium, calcium, phosphorous, total phenol and flavonoids. Positive control group which consumed oxidizing frying oil in diet showed a significant decrease in body weight, Food efficiency ratio (FER) and food intake. Also, it showed a significant increase in serum cholesterol (CHO), triglycerides (TG), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and CHO/ HDLc, malodialdehyde (MDA); and liver cholesterol and total lipid but a significant decrease in serum high density lipoprotein cholesterol (HDLc), superoxide dismutase (SOD), catalase, glutathione transferase (GST), and glutathione peroxidase (GPX); and liver triglyceride in comparing to negative control group. Broccoli powder, aqueous and methanolic extract rat groups showed that the values of liver cholesterol, total lipid and triglyceride appeared within values of negative control. However, they showed a significant decrease in liver parameters and serum MDA and significant increase in serum antioxidant enzymes activity comparing to positive control group. This study concluded that broccoli have ability to increase antioxidant activity in rats ingested thermally oxidized oil. Broccoli methanolic extract give the most favorable results, therefore this study recommends to intake broccoli when consuming fried foods.

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1. Introduction

Oils and fats are important part of the human diet as food or as ingredients in food products which affects both growth and health. Vegetable oils are the main source of dietary fat for almost people and play important functional and sensory roles in food products and they act as carriers of fat-soluble vitamins (A, D, E and K). Degree of saturation of oil is an important factor determining the quality of cooking oils. Unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids and for this reason they are main source of free radicals (Bakkali et al., 2008).

Frying is one of the most popular culinary processes worldwide, for both industrial and domestic food preparation procedures. During deep frying, the cooking oil is heated at high temperature with exposure to air and moisture, resulting in lipid peroxidation. This thermal deterioration generates harmful oxygen reactive species which might be deleterious to the cardiovascular system. Several studies have also demonstrated the detrimental effects of oxidized oil, including alterations in platelet function, liver dysfunction, and endothelial impairment (Owu et al., 1989, Naz et al., 2005 and Driss et al., 2009). However, the tocopherols

(vitamin E) are largely destroyed during frying as well. They are usually completely destroyed before the point at which the frying oil should be replaced based on the content of polymerised triacylglycerols or polar compounds (Reblova et al., 2009).

Evidence suggests that fruit and vegetables are the major antioxidant sources in our daily diet. Major antioxidants present in fruit and vegetables are: vitamin C, vitamin E, carotenoids and polyphenols, especially flavonoids, which all provide protection against free radicals that minimizes some of these harmful effects (Abd El-Ghany et al., 2009 and Monero et al., 2010). Broccoli (*Brassica oleracea*) belongs to the *Brassica* genus and is a green vegetable from the cabbage family. It is generally sold in heads, which have multiple florets branching off a central stem, and sometimes have leaves still attached. Packed with nutrients, it is best briefly steamed, stir-fried, or eaten raw. A high intake of cruciferous vegetables is associated with a reduced risk of cancer, particularly lung and those of the gastrointestinal tract (Davies, 2000 and Piao et al., 2005). Broccoli is rich in vitamin C, as well as dietary fiber and also contains multiple nutrients with potential anti cancer properties such as di-indolylmethane. It contains many bioactive, including

vitamins C and E, quercetin and kaempferol glycosides. Broccoli consumption has been also shown to be beneficial in the prevention of heart disease (**Elizabeth and Marcela 2009**).

Therefore, the main aim of the present study was to investigate the effects of broccoli on the antioxidant activity of experimental rats ingested thermally oxidized oil for a period of 60 days.

2. Materials and methods

2.1. Plant material:

Broccoli, one kilogram of sliced sweet potatoes, and commercial food oil for ideal frying performance (Mazola) were obtained from local market in Riyadh.

2.2. Chemicals:

All the materials used for this experiment were of analytical grade. BioMerieux Kits were purchased from Alkan Co. for Chemicals and Biodiagnostics.

2.3. Test animals:

35 adult male of white albino rats (Sprague dawley strain) weighing between 150-157g, provided from experimental animals' center in Medicine collage of King Saudi University in Riyadh. Rats were housed as groups in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaption period. Food and water were provided *ad-libitum*. Weekly body weight gain and daily food intake were recorded.

2.4. The basal diet:

The basal experimental diet was composed of corn starch(598),casein(200),corn oil(100), vitamins mixture(10),salts mixture (40),cellulose (50) and choline chloride (2) in g/ kg diet according to **Second Report of American Institute Of Nutrition(1980)**.

2.5. Preparation of thermally oxidized frying oil:

1 kg sliced sweet potatoes was frying in 3 L of oil in a stain-less-steel wok at 200°C for 20 min. The heated oil was then allowed to cool for at least five hours then oils were heated three hours per day for five days without any addition of new fresh oil to get oxidized frying oil as described earlier by **Adam et al., 2008** with some modification. Oxidized frying oil was added to basal diet in substitution of corn oil to form oxidative diet.

2.6- Preparation of broccoli powder:

The broccoli was cut into small pieces, dried at 60°C in hot oven and crushed to a fine powder. Broccoli powder was added as 10% of basal diet.

2.7- Preparation of aqueous and methanolic broccoli extract:

100 mL deionised water was added to 100 grams of broccoli powdered and left at room temperature for 12 h to allow complete hydrolysis then centrifuged at 5000g for 15 min to and finally

filtrated to obtain aqueous broccoli extract according to the method of **Bertelli et al., 1998**. Methanolic broccoli extract was prepared by soaking 100g of broccoli powdered in 600ml of 80% methanol with constant stirring by a magnetic stirrer for 48 hr then filtered followed by removal of the solvent on the rotatory evaporator. The rat received aqueous and methanolic broccoli extract in 100 mg kg/body weight by stomach tube (**WHO 1983**).

2.8-Chemical analysis of broccoli:

Moisture, protein, fat, fiber and ash contents of broccoli were determined according to **A.O.A.C. (2007)**. Carbohydrate content was calculated by difference as described by **Ceirwyn 1995**. Mineral contents (Ca, Fe, Mn, P&K) were determined as described by **Pearson 1993** using atomic absorption spectrophotometer. Total carotenoids and antioxidant activity were determined by the DPPH radical scavenging method of **Zhang and Hamauzu (2004)**. The phenolic and total flavonoid content of the obtained extracts was estimated by a colorimetric assay based on procedures by **Singleton and Rossi (1965) and Heimler et al., (2005)**, respectively.

2.9. Treatment schedule:

Animals were divided into five groups as follows:

Group (1): Served as a negative control group which fed on basal diet.

Group (2): Kept as positive control group which fed on oxidative diet only.

Group (3): Kept as broccoli powder group which fed on oxidative diet with 10% of broccoli.

Group (4): Kept as broccoli aqueous extract group which fed on oxidative diet and broccoli aqueous extract 100mg/kg body weight orally by stomach tube.

Group (5): Kept as broccoli methanolic extract group which fed on oxidative diet and broccoli methanolic extract 100mg/kg body weight orally by stomach tube.

The food intake was recorded daily and body weight of the rats recorded weekly. After completion of experimental period (60 days), rats were fasted overnight and sacrificed and blood was collected for various estimations. After 24 h of last dose, rats were sacrificed for obtain blood and liver for biochemical estimations.

2.10. Serum biochemical estimations:

Serum cholesterol (CHO), triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods (**Abell et al., 1952, Buccolo and David 1973, and Kostener 1977**). Activity of superoxide dismutase(SOD), catalase, glutathione peroxidase (GPX),glutathione transferase (GST) enzymes and malodialdehyde (MDA) were determined using

commercial kits according to the methods described by **Kakkor et al.,(1984)**, **Sinha (1972)**, **Habig et al., (1974)**, **Ellman (1958)** and **Uchiyama and Mihara (1978)**, respectively.

2.11. Liver biochemical estimations:

Liver cholesterol (CHO), total lipids and triglyceride were determined according to **Richmond (1973)**, **Folch et al., (1957)** and **Scheletter and Nussel (1975)**, respectively.

2.12. Calculation of some parameters:

Food efficiency ratio (FER) was determined by **Chapman et al., (1950)**. Low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and CHO/ HDL-c were calculated according to **Fruchart (1982)** and **Castelli and levitar (1977)**.

2.13-Statistical analysis:

Collected data were presented as mean \pm SD and statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance according to **Artimage and Berry (1987)**.

3. Results

Gross chemical composition of broccoli showed 41.35, 25.1, 12.77, and 10.11% of carbohydrate, protein, fiber and ash, respectively in ascending manner. Data showed that broccoli contains low level of fat as the value was 3.25 % as represented in table (1).

The obtained data in table (2) showed that broccoli contains high amount of potassium, calcium and phosphorous that the values reached to 501.16, 181.11 and 165.66 mg/100g, respectively. The values of iron and manganese reached to 4.12 and 1.61 mg/100g, respectively.

Estimation of total carotenoids, phenol, flavonoids and antioxidant activity of broccoli was illustrated in table (3). Broccoli had high value of total phenol and flavonoids as the value were 87.38 and 19.96 mg/100 g, respectively. Total carotenoids in Broccoli were 7.01 mg/100 g. The antioxidant activity of broccoli was 0.201 μ m.

Positive control group which consumed oxidizing frying oil in diet showed a significant decrease in body weight, FER and food intake at $p < 0.001$ & 0.05 as the values 45.61 ± 1.11 , 0.049 ± 0.002 and 15.41 ± 1.10 in comparing to negative control group (91.41 ± 4.61 , 0.085 ± 0.001 and 17.81 ± 1.20).

Broccoli powder and aqueous extract rat groups showed significant lower values of body weight and FER in comparing to negative control group ($p < 0.01$ & 0.05) but significant higher in comparing to positive control. Broccoli methanolic extract rat group showed a significant lower value of FER in comparing to negative control group ($p < 0.01$) but significant higher in comparing to positive control group. Food intake in broccoli powder, aqueous and methanolic extract rat groups was within the value of negative control as recorded in table (4).

Positive control group showed a significant increase in serum CHO, TG, LDLc, VLDLc and CHO/ HDLc and significant decrease in HDLc at $p < 0.001$ in comparing to negative control group. Broccoli powder and aqueous extract rat groups showed a significant increase in serum CHO, TG, LDLc and VLDLc at $p < 0.5$ & 0.01 in comparing to negative control group. Broccoli methanolic extract rat group showed only significant increase in value of LDLc ($p < 0.01$) comparing to negative control group but the other serum lipid profiles were within normal. CHO/ HDLc was within normal value in broccoli aqueous and methanolic extract rat groups as shown in table (5).

Positive control group showed a significant increase in liver cholesterol, and total lipid ($p < 0.001$) and significantly decrease in liver triglyceride ($p < 0.05$) in comparing to negative control group. Broccoli powder, aqueous and methanolic extract rat groups showed non significant difference in the liver parameters and appear within values of negative control but showed significant decrease in comparing to positive control group as shown in table (6).

Positive control group showed a significant decrease in serum SOD, catalase, GST, and GPX and significant increase in MDA at $p < 0.001$ in comparing to negative control group. Broccoli powder rat group showed a significant decrease in serum catalase and GST at $p < 0.01$ & 0.05 , respectively and significant increase in MDA at $p < 0.05$ while aqueous and methanolic extract rat groups showed a significant decrease in serum catalase and GST at $p < 0.05$ in comparing to negative control group. Broccoli powder, aqueous and methanolic extract rat groups showed a significant increase in serum SOD, catalase, GST, and GPX and significant decrease in MDA in comparing to positive control group as shown in table (7).

Table (1): Gross chemical composition of broccoli

Protein	Fat	Fiber	Moisture	Ash	Carbohydrate
25.21%	3.25%	12.77%	7.31%	10.11%	41.35%

Table (2): Some mineral content of broccoli (mg/100g)

Ca	Fe	Mn	P	K
181.11	4.12	1.61	165.66	501.16

Table (3): Total carotenoids, phenol, flavonoids and antioxidant activity of broccoli

Total carotenoids (mg/100g)	Total. phenol (mg/100g)	Total flavonoids (mg/100g)	Antioxidant activity (μm)
7.01	87.38	19.96	0.201

Table (4): Effect of oral administration of broccoli powder, aqueous and methanolic extract on body weight gain, food intake and FER of the experimental rat groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
Body weight(g)	91.41 \pm 4.61 ^a	45.61 \pm 1.11 ^{d***}	66.47 \pm 3.61 ^{c**}	83.60 \pm 3.71 ^{b**}	85.11 \pm 3.61 ^{ab}
Food intake(g/w)	17.81 \pm 1.20 ^a	15.41 \pm 1.10 ^{b*}	16.21 \pm 1.01 ^a	17.71 \pm 1.27 ^a	18.11 \pm 1.21 ^a
FER	0.085 \pm 0.001 ^a	0.049 \pm 0.002 ^{d***}	0.068 \pm 0.004 ^{c**}	0.078 \pm 0.005 ^{b**}	0.078 \pm 0.004 ^{b**}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (5): Effect of oral administration of Broccoli powder, aqueous and methanolic on serum lipid profiles of the experimental rat groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
CHO (mg/dl)	120.61 \pm 12.61 ^d	247.41 \pm 29.61 ^{d***}	175.31 \pm 15.61 ^{b**}	150.71 \pm 13.21 ^{bc*}	145.61 \pm 14.31 ^{cd}
TG (mg/dl)	75.61 \pm 7.21 ^c	129.65 \pm 12.14 ^{d***}	96.67 \pm 10.12 ^{b**}	91.21 \pm 8.14 ^{b*}	85.11 \pm 7.13 ^{bc}
HDLc (mg/dl)	39.81 \pm 3.61 ^a	25.11 \pm 3.21 ^{c***}	31.16 \pm 4.17 ^{ab}	35.21 \pm 5.19 ^a	36.41 \pm 6.01 ^a
LDLc (mg/dl)	65.68 \pm 6.22 ^d	196.37 \pm 19.21 ^{d***}	124.82 \pm 11.14 ^{b**}	97.26 \pm 8.99 ^{c**}	92.18 \pm 9.61 ^{c**}
VLDLc (mg/dl)	15.12 \pm 1.19 ^c	25.93 \pm 2.17 ^{d***}	19.33 \pm 1.61 ^{b*}	18.24 \pm 1.51 ^{b*}	17.02 \pm 1.41 ^{bc}
CHO/ HDLc	3.02 \pm 0.66 ^c	9.85 \pm 1.67 ^{d***}	5.62 \pm 0.88 ^{b**}	4.28 \pm 0.57 ^{bc}	3.99 \pm 0.53 ^c

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (6): Effect of oral administration of broccoli powder, aqueous and methanolic on liver cholesterol, total lipid and triglyceride (mg/g) of the experimental rat groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
Cholesterol	4.22 \pm 0.45 ^b	6.17 \pm 1.31 ^{d***}	4.99 \pm 0.55 ^b	4.66 \pm 0.46 ^b	4.51 \pm 0.37 ^b
Total lipids	35.60 \pm 3.66 ^b	48.91 \pm 4.81 ^{d***}	40.35 \pm 4.21 ^b	37.16 \pm 3.50 ^b	39.61 \pm 3.14 ^b
Triglyceride	3.41 \pm 0.22 ^a	2.17 \pm 0.18 ^{c**}	2.85 \pm 0.21 ^{ab}	3.21 \pm 0.34 ^a	3.30 \pm 0.33 ^a

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (7): Effect of oral administration of Broccoli powder, aqueous and methanolic serum SOD, catalase, GST, GPX, and MDA of the experimental groups

Groups Variables	Negative control	Positive control	Broccoli		
			Powder	Aqueous Extract	Methanolic Extract
SOD (mmol/l)	40.21 \pm 5.33 ^a	15.41 \pm 1.77 ^{c***}	33.21 \pm 4.29 ^{ab}	36.17 \pm 6.11 ^a	35.14 \pm 5.10 ^a
Catalase (μ /l)	270.11 \pm 27.41 ^a	125.71 \pm 12.71 ^{d***}	199.51 \pm 19.61 ^{bc**}	210.14 \pm 21.61 ^{b*}	212.21 \pm 22.21 ^{b*}
GST (mmol/l)	125.77 \pm 13.13 ^a	65.14 \pm 7.21 ^{c***}	97.11 \pm 9.67 ^{b*}	95.40 \pm 9.61 ^{b*}	99.61 \pm 10.21 ^{b*}
GPX (mmol/l)	63.71 \pm 7.20 ^a	27.61 \pm 3.66 ^{b***}	55.19 \pm 6.60 ^a	59.61 \pm 6.09 ^a	61.11 \pm 7.01 ^a
MDA (mmol/l)	7.20 \pm 1.31 ^c	13.71 \pm 1.66 ^{d***}	9.14 \pm 1.33 ^{b*}	8.21 \pm 1.21 ^{bc}	8.11 \pm 1.10 ^{bc}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

4. Discussion

The obtained results of chemical analysis of broccoli were in accordance with that of other authors, who had shown that broccoli is high in fiber, which aids in digestion. A tablespoon of broccoli powder has as much protein as a cup of rice or corn with half the calories. It is a good source of energy-producing vitamin B1, vitamin B3, vitamin B5, protein, and iron; bone-healthy magnesium, phosphorus and calcium. Broccoli is rich in both nutritional antioxidants; vitamins C and E, and non-nutritional antioxidants; carotenoids, and phenolic compounds, particularly flavanoids (**Lin and Chang 2005 and Faller and Fialho 2009**). According to several studies, reported that broccoli is also rich in polyphenols, a large group of phytochemicals that are often considered the most abundant antioxidants in the diet. There is a linear correlation between polyphenol content and antioxidant function (**Borowski et al., 2008**).

Research has shown that the heating of the oil at high temperatures produces structural chemical, physical, changes which lead to compositional diversities as a result of degradation reactions that take place such as auto-oxidation, thermal polymerization, cyclisation and hydrolysis (**Warner 1999**). During deep frying, oxidative and thermal effects result in the formation of many volatile and nonvolatile products, some of which are potentially toxic. Results of this study showed reduction in body weight gain, food intake and FER in positive control. These results were agreed with **Alexander (1981)** who reported that consumption of the thermally oxidized oils results in decreased food consumption, growth retardation, and weight gain in organs such as liver and kidneys, as well as mutagenicity and cellular damage in various organs of laboratory animals.

It is known that elevated levels of LDL cholesterol constitute a major risk factor for atherosclerotic diseases, whereas elevated levels of HDL have beneficial effects. Atherosclerosis is increased through intake of heated oil. However, heating of fats leads to decomposition of poly unsaturated fatty acids and therefore to changes in the fatty acid composition. The dietary fatty acids influence the fatty acid composition of the LDL and therefore the susceptibility of LDL to oxidation (**Cohn 2002 and Kratz et al., 2002**). Plasma total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol with decrease in HDL-cholesterol content were found to be significantly increased in the heated oil fed groups compared to corresponding fresh oil fed groups. Since LDL particles have atherogenic properties, the presence of elevated levels of these particles may have role in hyperlipidemia and hemostatic changes associated with atherosclerosis. The oxidation of oil produces lipid peroxides, which

may be one of the reasons for the elevation of total cholesterol and influence platelet alterations that play crucial role in cardiovascular events (**Siti et al., 2008 and Chinu and Thankappan 2011**). The exposure to the high heat repeatedly for many times can lead to deterioration of the precursor of vitamin E and destroys all the vitamins and increase in free radicals formation. Thus can injury of renal and liver tissues. Broccoli is rich in dietary fiber which can lower levels of LDL, cholesterol. This lowering of cholesterol helps protect the arteries and prevents the onset of heart disease (**Totani and Ojiri 2007, Adam et al., 2008 and Bahadoran and Tohidi 2012**). Broccoli contain Vitamin C which protects against cell death, directly scavenges superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radicals, and acts as a lipid peroxidation chain breaking agent. Vitamin C also co-operates with vitamin E to regenerate membrane-bound oxidized α -tocopherol, creating an antioxidant network (**Gliszczynska-Swiglo et al., 2006**).

Data in this study showed an increased in serum MDA concentration which may be related to the increased in free radicals. This finding was consistent with the observation that the free radicals reduced the activity of the endogenous antioxidant enzyme SOD (**Conner and Grisham 1996**).

Broccoli provides many antioxidants such flavonoids, carotenoids lutein, zeaxanthin, and beta-carotene in significant amounts. Other antioxidants provided by broccoli in beneficial amounts include vitamin E and the minerals as manganese and zinc. Proanthocyanidin compounds and flavonoids in broccoli extract can increase antioxidative defense that caused significant increases in the levels of GPX, SOD and catalase as well as improved liver structure (**Wargovich et al., 2001, Piao et al., 2005 and Podsedek 2007**).

Conclusion

As a conclusion, the broccoli consumption could have a protective effect against thermally oxidized food oil. Broccoli extract showed the best results in improving lipid profiles in serum and liver and enhancement of serum antioxidant enzymes

Recommendation

Consumption of vegetables especially broccoli is recommended when eating fried food. Further research is needed to increase knowledge regarding the bioavailability of antioxidant compounds from broccoli, and to confirm effects of different cooking methods of broccoli on the concentration of the antioxidant.

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