



Effects of Oxidized Tallow on the Rabbit Serum Lipids and Antioxidant Activity of the *In-vitro* Lipids

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This paper describes the effects of thermally oxidized tallow on the serum lipids profile and radical scavenging activity (RSA) of the lipids extracted from the different tissues of the rabbits. Tallow was thermally oxidized at 130°C for 9, 18, 27, 36 and 45 h respectively. Thermally oxidized tallow was fed to the local strain of Himalayan rabbits for one week. Results show that oxidation increases the formation of hydroperoxides and decrease the level of radical scavenging activity of the tallow. The rabbit serum lipids profile showed a dose dependent increase in triglyceride, total cholesterol and LDL-cholesterol. However, no statistically significant increase was observed in the HDL-cholesterol with an increase of oxidation time. Serum glucose and rabbits body weight decrease significantly ($p < 0.05$) and was highly correlated with the serum lipids profile. The percent RSA of the lipids extracted from the liver, brain and muscles tissues showed a significant decrease with respect to 0.5, 1.0 and 1.5 g/body weight as well as oxidation time. Data suggests that thermal oxidation and use of thermally oxidized beef tallow is harmful and therefore an alternative way of cooking should be used.

Key words: Tallow, Thermal oxidation, Rabbit, Serum lipids profile, Radical scavenging activity

INTRODUCTION

Tallow is a form of animal fats obtained from the rendered animal bones and soft tissues. The major composition of tallow consists mainly of triglycerides which are made of fatty acids such as oleic, palmitic and stearic acids. The composition of the fatty acid depends on the type of raw material used. The beef tallow contains 45.38% of unsaturated fatty acids, and 52.59% of total saturated fatty acids. The stearic acid (C18:0) contents in beef tallow is about 16.40%. Tallow also contains 0.14~4% cholesterol (Ryan and Gray, 1984; Sabir *et al.*, 2003). Animal fats or tallow has a negative impact on human health by increasing LDL-cholesterol, obesity and enhance the risk of coronary heart diseases and other diseases like cancer (Yuan *et al.*, 1999; Jurek *et al.*, 2005; Zeb and Ali, 2008). Leplaix-Charlat *et al.* (1996) showed that by the addition of cholesterol to diets favoured accumulation of low density lipopro-

teins in plasma and the net apparent secretion of these particles by the liver, especially in the diet containing soybean oil with cholesterol. Hypercholesterolemia and greater severity of atherosclerotic plaques were observed in birds fed with high cholesterol diets of the beef tallow (Yuan *et al.*, 1999).

During frying or thermal stress, tallow has been found to be oxidized significantly (Zeb and Ali, 2008). The determination of peroxide contents can be used as a standard factor in the determination of oxidation of tallow (Ali *et al.*, 2009). These thermally oxidized fats are toxic. Yang *et al.* (1998) showed a correlation between the thermally oxidized tallow and colon cancer. Experiments showed that when rats were fed with diet containing the oxidized fats, significant negative effects were observed in the concentrations of triglycerides in liver, plasma, and VLDL than the rats whose diet contained fresh fat. The study also suggests that thermally oxidized fats contain substances that suppress gene expression of lipogenic enzymes in the liver.

In Pakistan and most of the South East Asia, tallow is one of the important frying medium for preparing foods. Tallow is used in a broad range of applications from shortenings, frying fats and margarine to animal and pet feeds, and in the manufacture of oleo-chemical products for a very wide variety of applications including food, feed, cosmetics, medicinal and pharmaceutical products. However, there is a lack of literature regarding the toxic effects of ther-

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Abbreviations: HDL: high density lipoprotein, LDL: low density lipoprotein, PUFA: polyunsaturated fatty acids, RSA: radical scavenging assay, POV: peroxide value, ACS: American chemical society, DPPH: 2,2-diphenyl-1-picrylhydrazyl

mally oxidized tallow on the serum lipids and antioxidant potentials of the lipids present in different tissue. This paper describes the possible toxic properties and effects of thermally oxidized tallow with the special emphasis on the serum lipids profile and *in-vitro* lipids radical scavenging activity.

MATERIALS AND METHODS

Materials. Beef tallow was obtained from the local market. Standard glucose and cholesterol were from Sigma Aldrich (Germany). All other chemicals and reagents were of ACS grade from Sigma Aldrich USA or otherwise mentioned.

Thermal oxidation. Thermally oxidation of the tallow was carried out on a hot plate at 130°C, for five consecutive days for 9 h each. At the end of each day, tallow sample was taken and stored in refrigerator at -20°C.

Peroxide value. The peroxide value (PV) of control and oxidized tallow samples were determined using the standard AOCS official method (method Cd 8b-90) (AOCS, 1998) and expressed as meq O₂/kg of fat. All samples were measured in triplicate or otherwise mentioned.

Experimental animals. Rabbits of the local Himalayan strain were selected for the study because easily availability of these animals for our experiments. Rabbits were grouped randomly into five groups and each group contains nine animals. All animals were placed in same approved animal house facility and were supplied fresh diets daily. These rabbits had free access to food and water throughout the study. Before starting the treatments, all animals were placed for one week in order to acclimatize. The experiments were carried out according to the approved guidelines for the care and proper use of the animals of the Department of Biotechnology, University of Malakand.

Animal feeding. The five samples of oxidized tallow were fed to the rabbits classified into five groups. Group 1, 2, 3, 4 and 5 were fed on oxidized tallow samples of 9, 18, 27, 36 and 45 h respectively. Each single group was classified further to three doses designated as A, B and C corresponds with 0.5, 1.0 and 1.5 g/body weight of daily doses of the oxidized tallow. Feeding was continued for one week and the animals were sacrificed two days later. The blood samples were collected and serum was collected for further analysis.

Weight change. The change in the weight of the rabbit was measured with digital platform scale analytical balance (Marino, China) with weighing capacity of 30 kg.

Extraction of lipids. Lipids were extracted from the selected tissues (liver, brain & muscles) according to the procedure of Folch *et al.* (1957) with little modification. Briefly after homogenization of tissues, chloroform-methanol (2 : 1) mixture was added. The sample was kept on shaker for 98 h at 30 rpm. To the extracted mixture 10% KCl were added, washed and filtered. The solvent was evaporated using rotary evaporator.

Biochemical analyses. Biochemical parameters like cholesterol, HDL-cholesterol, LDL-cholesterol, and glucose were measured using HUMAN (Germany) kits, while total triglycerides were measured with DiaSys (Germany) kit. The quantification of glucose, triglycerides, and cholesterol were carried out using a six point standard calibration curves with the help of Shimadzu UVvis-1700 spectrophotometer (Shimadzu, Japan).

Radical scavenging assay. Radical scavenging assay (RSA) of the tallow, serum and lipids samples extracted from the tissues were carried out according to the procedure of Lee *et al.* (2007) with some modifications. Briefly, a solution of DPPH radicals was freshly prepared in ethyl acetate at a concentration of 0.1 mM. Five millilitres of DPPH solution were mixed with 56 µl oil samples in a 30 ml serum bottle and kept them in the dark for 30 min. The absorbance of the sample mixture was measured at 515 nm using UVvis-1700 spectrophotometer (Shimadzu, Japan). The RSA toward DPPH radicals was estimated from the differences in absorbance of the DPPH solution with or without sample (control), and the percentage of RSA was calculated from the following equation:

$$\text{Radical scavenging activity (\%)} = (\text{Ac} - \text{As}/\text{Ac}) \times 100$$

Statistical analysis. All samples were measured in triplicate or otherwise mentioned. Data were analysed by one-way analysis of variance (ANOVA) and Holm-Sidak method of multiple comparison method at $\alpha = 0.05$ using SigmaPlot for windows version 11.0 (Systat Software, Inc., 2008).

RESULTS AND DISCUSSION

Generally in kitchens, restaurants, fast food chains and in the food industries, fat frying is the main traditional and popular cooking method for most of the foods (Ramadan *et al.*, 2006). Tallow is one of the important fats used in such frying. However, it was found that un-oxidized tallow produce undesirable effects in animals. Tallow was found to increase the fat content of broiler fed for 7 days (Pour-Reza and Edriss, 1997). Similarly beef tallow diet had decreased digestibility and adversely affected energy and bone metabolisms in growing healthy male rats (Segura *et al.*, 2011).

Thus due to the well-known unsafe properties and wide uses of un-oxidized tallow, it was necessary to look for the effects of oxidized tallow in animal models.

In Pakistan, tallow is used for the frying of traditional *chapli* and *shami* kebab as well as processed food products from the street foods to the restaurants. During frying, the beef tallow is exposed continuously or repeatedly to uncontrolled high temperatures, air, moisture and foods. The tallow is thus oxidized and the oxidation increases with increasing oxidation time (Zeb and Ali, 2008). The oxidized tallow thus enters the food matrix and produce toxic effects (Yang *et al.*, 1998; Kamal-Eldin *et al.*, 2003). Due to the absence of relevant literature and scientific knowledge, these tallows fried foods are becoming one of the most serious health risk factor. This study shows the effects of thermally oxidized tallow on the serum lipids and antioxidant activity of the lipids in different tissues and will serve the prime source of knowledge in this regard.

Composition of tallow. It is well known that tallow contain saturated fatty acids and high amounts of cholesterol. The composition of beef tallow is relatively similar as reported in literature. Table 1 shows the relative composition of fatty acids and cholesterol. Segura *et al.* (2011) observed that palmitic acid, stearic acid and oleic acid were the major fatty acids amounting 25.7%, 26.7% and 37.9% respectively. The cholesterol contents were about 4% percent. The values of the composition are close to the values reported by Sabir *et al.* (2003).

Hydroperoxides in tallow. Fats and oils are usually oxidized by thermal oxidation to form hydroperoxide. Further oxidation or degradation of hydroperoxides produces secondary oxidation products of various chain lengths (Kamal-Eldin *et al.*, 2003; Zeb and Murkovic, 2010). Peroxide value (PV) is the important analytical parameter for the measurement of the hydroperoxides formed in fats and oils. Fig. 1A shows that PV increase with oxidation time increase in comparison to control (5.1 meq/kg). The oxidation is statistically significant at $P < 0.05$ and reached a

Table 1. Relative composition of beef tallow measured using GC (Segura *et al.*, 2011)

S. No	Component	Relative composition
1	Fatty acids (%)	
	C14:0	2.8
	C16:0	25.7
	C16:1	2.8
	C17:0	1.4
	C18:0	26.7
	C18:1	37.9
2	C18:2	0.8
	Cholesterol (%)	4.1

value of 410.3 meq/kg. When pure triacylglycerols mixture is thermally oxidized under controlled conditions, the oxidation is highly dependent on the time of oxidation (Zeb and Murkovic, 2010). A similar observation was also observed in vanaspati ghee thermally oxidized relatively under similar conditions (Zeb and Mehmood, 2012). However, it is interesting that vanaspati ghee which had been manufactured from palm oil produces less hydroperoxides than tallow. The main reason may be the presence of added synthetic antioxidants present in the gas. The radical scavenging assay of the tallow oxidized at different timings was carried out using DPPH assay as shown in Fig. 1B. It was observed that radical scavenging activity of the tallow decrease with increasing oxidation time. This decrease was statistically significant ($P < 0.05$), with a correlation coefficient of 0.967. Peroxide value and radical scavenging assay are considered as quality indicators (Zullo and Ciafardini, 2008). Based on this concept, it was found that the quality

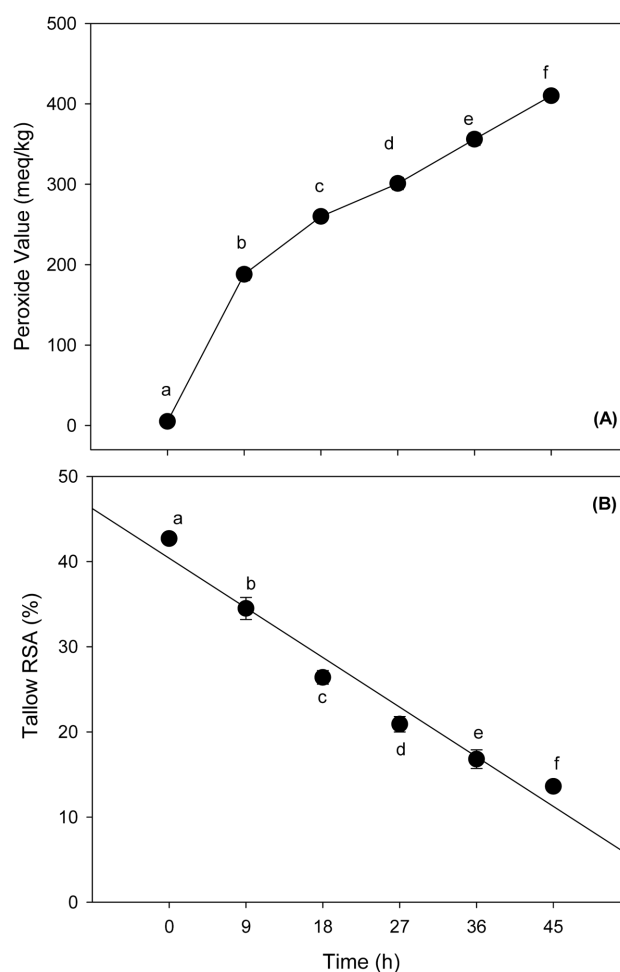


Fig. 1. Characteristics of the thermally oxidized tallow, (A) Changes in peroxide value (meq/kg) of the tallow, and (B) Changes in the radical scavenging activity (%) of tallow. Different letters (a-f) represents significant at $p < 0.05$.

of the tallow decreased with increasing oxidation time. The peroxide value and radical scavenging assay were highly correlated.

Effects on serum lipids profile. The thermally oxidized tallow was fed to the rabbits classified in to five groups based on the five different oxidized tallow samples. The weights of the rabbits were measured before and after the feeding of the respective tallow samples. It was found that weight of the control animals increased after one week of control feeding. In comparison to control, the 9 h oxidized samples showed a tremendous decrease in weight. By increasing the quantity (0.5 to 1.5 g/weight) of oxidized tallow, a decrease was observed as shown in Fig. 2A. The decrease in the weight as a response to the other oxidized samples is not as much higher as in the 9 h. However, the decrease was significant beyond 9 h time points. In rats, high-fat feeding leads to an increase in basal and hormone-stimulated lipolytic activity which leads to body weight loss (Berger and Barnard, 1999). In the present study; highly oxidized fats were found to decrease the weight which might also be due to the lipolysis of body fats and increase oxidation of other metabolites for the sake of obtaining energy. Fig. 2B shows the effects of thermally oxidized tallow on glucose content. A significant decrease was observed in all cases. The change in the glucose highly correlated with the change in the body weight of the experimental animals. This shows that oxidation enhances the chance of hypoglycaemic condition and thus weight loss occurs. It may be due to the enhancement of the activity of insulin to increase the oxidation of glucose by the oxidized lipids.

Triglycerides (TG) are the most important components of biological lipids. Table 2 shows a significant (100%) increase (122.1 mg/dl) at the dose A of 9 hours compared to control

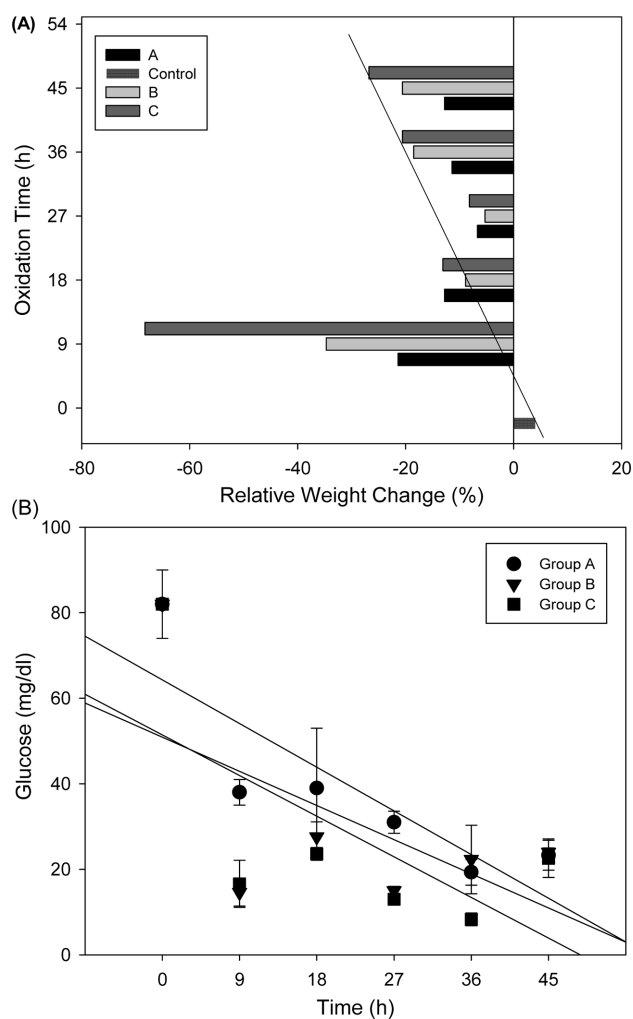


Fig. 2. Effects of oxidized tallow, (A) Effects of change in the body weight, and (B) Effects of change in the serum glucose contents.

Table 2. Effects of different doses of thermally oxidized tallow on the serum lipids profile of Rabbit

Parameter	Dose	Concentration (mg %)					
		Oxidation time (h)					
		Control	9	18	27	36	45
Triglyceride	A		122.1 ± 9.2	28.6 ± 7.0	67.0 ± 15.1	72.2 ± 8.3	94.3 ± 4.2
	B	59.5 ± 3.1	78.3 ± 7.1	63.0 ± 3.0	63.0 ± 6.2	65.5 ± 5.1	117.2 ± 2.2
	C		100.2 ± 4.5	88.3 ± 13.2	75.0 ± 3.0	86.3 ± 14.6	141.9 ± 4.1
Total cholesterol	A		134.7 ± 10.1	152.9 ± 9.2	137.6 ± 15.4	153.3 ± 11.1	140.5 ± 7.3
	B	66.8 ± 4.1	132.0 ± 6.7	113.0 ± 18.2	170.4 ± 12.2	153.8 ± 16.2	140.3 ± 6.4
	C		83.5 ± 17.5	97.3 ± 6.3	123.6 ± 7.2	112.3 ± 7.5	97.2 ± 18.2
HDL-cholesterol	A		62.2 ± 10.2	69.0 ± 6.5	53.2 ± 2.1	65.3 ± 5.7	62.5 ± 3.2
	B	45.3 ± 2.3	61.1 ± 10.0	56.2 ± 2.1	55.5 ± 1.9	72.7 ± 3.0	53.2 ± 2.3
	C		77.2 ± 7.1	70.5 ± 8.3	57.2 ± 5.4	68.7 ± 4.0	54.4 ± 1.3
LDL-cholesterol	A		71.2 ± 10.2	83.0 ± 15.5	84.2 ± 10.1	75.3 ± 14.7	78.5 ± 3.2
	B	21.5 ± 2.1	71.1 ± 10.0	45.2 ± 12.1	114.5 ± 7.9	81.7 ± 0.0	86.2 ± 2.3
	C		13.4 ± 0.0	43.5 ± 9.3	62.2 ± 6.4	65.7 ± 0.0	42.4 ± 4.3

Dose A, B, and C represent 0.5, 1.0 and 1.5 g/body wt of the experimental animals. Values are the means ± standard deviation (n = 3).

(59.5 mg/dl). Similar is the case in doses B and C. By feeding further oxidized samples (18 to 45 h), an initial decrease and then successive significant increase were observed in all three doses (A~C). The overall increase in TG may be due to the significant oxidation of glucose, the produced acetyl CoA contribute to the synthesis of Fatty acids and consequently TG. Fig. 3 shows that by increasing the oxidation time, an increases in TG and % RSA of the tallow was observed. This means that the % RSA as an indicator of decrease of quality of tallow highly correlated with the increase of TG. When rats were fed with the diet containing beef tallow, the concentration of TG increased and was influenced by lecithin (Lechowski *et al.*, 1999). This means that if beef tallow is used in protected as well as oxidized form, it will increase TG contents in the body.

Initially the total cholesterol contents increase significantly ($P < 0.05$) and then further increase is not significant at the doses of A and B by increasing the oxidation time. While for dose C a significant increase was observed till 27 h, and then further decrease was observed till 45 h treatment. Beef tallow with plant sterol esters were significantly more effective in reducing liver and plasma cholesterol concentrations than plant sterol and saturated fatty acid consumed individually by the hamsters (Guderian *et al.*, 2007). There were initial increases in the total amounts of HDL-cholesterol but the increase is not significant in all three doses. While for LDL-cholesterol, an initial increase was observed at dose A at 9 h, and further no significant increase was observed. However in case of doses B or C, there was significant ($P < 0.05$) increase due to the increase of oxidation time. Fig. 3 shows that the hydroperoxides levels highly correlated with the LDL-cholesterol and HDL-cholesterol. Thus

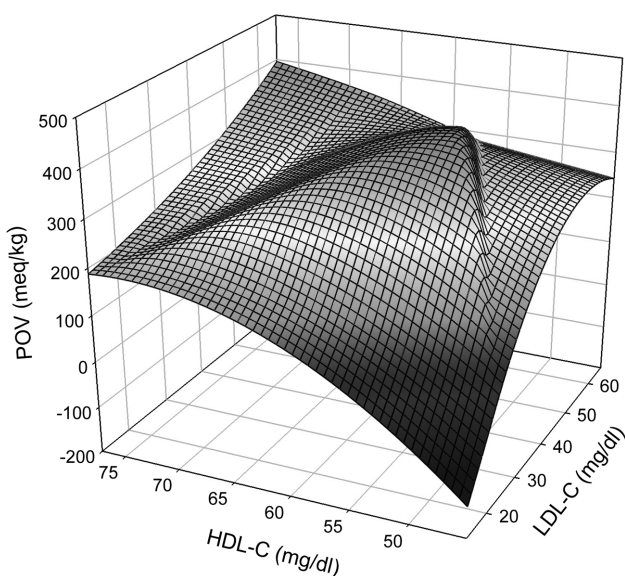


Fig. 3. Correlation of peroxide value (POV) of oxidized tallow, serum HDL-cholesterol and LDL-cholesterol values.

the increase of LDL-cholesterol can be positively attributed to the increase availability of hydroperoxides in the body.

Effects on RSA of in-vitro lipids. Free radicals and or reactive oxygen species are usually generated by exogenous chemicals or endogenous metabolic processes in food systems. These radicals cause oxidative degradation of lipids and other antioxidants and consequently play a significant role in some chronic diseases like atherosclerosis, cancer and arthritis (Kehrer, 1993; Zullo and Ciafardini, 2008). Ingestion of foods containing antioxidants such as ascorbic acid, tocopherols and carotenoids reduce the oxidative damage and thus overcome the free radicals. Recent results show that thermal oxidation degrades significantly the antioxidants present in the food system and thus decreasing the radical scavenging activity (Karabulut, 2010; Achir *et al.*, 2010; Zeb, 2012). Table 3 shows the radical scavenging assay of the lipids extracted from the liver, brain

Table 3. Radical scavenging assay (RSA) of the lipids extracted from the liver, brain and muscles of the Rabbit

Sample	Dose	Radical scavenging assay (%)					
		Oxidation time (h)					
		Control	9	18	27	36	45
Liver	A		30.8 ^b	31.1 ^b	28.7 ^c	28.7 ^c	20.7 ^d
	B	42.7 ^a	34.3 ^b	28.6 ^c	26.2 ^c	26.9 ^c	18.7 ^d
	C		34.6 ^b	29.3 ^c	28.6 ^c	21.4 ^d	14.9 ^e
Brain	A		36.7 ^b	33.5 ^c	30.5 ^d	31.2 ^d	21.5 ^e
	B	44.4 ^a	38.4 ^b	34.5 ^c	27.7 ^d	29.6 ^d	20.3 ^e
	C		39.6 ^b	34.1 ^c	28.0 ^d	25.5 ^e	18.7 ^f
Muscles	A		42.3 ^a	37.2 ^b	32.8 ^c	27.6 ^d	22.0 ^e
	B	45.8 ^a	40.7 ^b	36.8 ^c	30.5 ^d	25.9 ^e	23.7 ^e
	C		38.3 ^b	38.0 ^b	32.5 ^c	24.7 ^d	21.1 ^e

Different letters (a~f) in the row represent significance at $p < 0.05$.

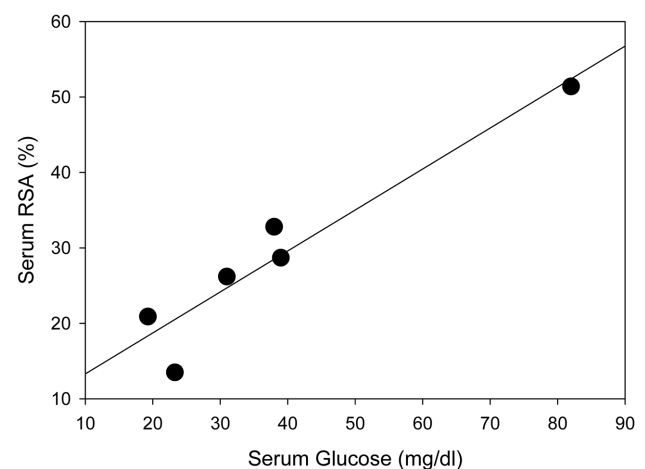


Fig. 4. Correlation of serum glucose (mg/dl) and radical scavenging activity of tallow.

and muscles. Significant degradation was observed in all extracted lipid samples. Increased feeding of oxidized samples also shows a decrease in RSA value. In case of muscles, the initial decrease was not significant as compared to other tissues. Fig. 4 shows that the serum glucose level has a high correlation ($R^2 = 0.906$) with serum RSA value. This means that the increase of oxidation compounds in the body consequently decreases the serum glucose level by enhancing glucose oxidation pathways. The oxidation of glucose thus leads to increase in the synthesis of fats in the body.

In this paper, the effects of thermally oxidized beef tallow on the serum lipids profile and antioxidant activity of the lipids extracted from the different tissues were observed. Tallow was thermally oxidized for 9 to 45 h. It was found that thermal oxidation enhances the formation of hydroperoxides in tallow and decreases the level of radical scavenging activity. The feeding of oxidized tallow significantly changes in the lipids profile, in the rabbits. Triglycerides, cholesterol and LDL-cholesterol were increased with increasing oxidation time. The HDL-cholesterol shows no significant increase with increase of oxidation time. Serum glucose level and body weight of the rabbits decrease significantly. Radical scavenging assay of the lipids extracted from the liver, brain and muscle tissues shows a significant decrease with respect to increase of oxidation time and dose. Therefore, other safe way avoiding frying should be used like microwave, which does not change the fatty acid pattern nor cause isomerisation of the unsaturated fatty acids in the beef tallow and other fats (Mai *et al.*, 1980). In conclusion, the uses of uncontrolled thermal oxidation and thermally oxidized tallow are harmful and longer oxidation and continuous usage should be avoided.

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