

Original Research Article

A comparative study of the effect of heating on antioxidant potential and lipid profile of two vegetable oils- sunflower oil and sesame oil- commonly used in culinary.

ABSTRACT

Aims:Vegetable oils are fats which are extracted from plant materials especially from the seeds which changes its chemical composition when heating. So the present study investigated the effect of heating of sunflower oil and sesame oil on the antioxidant status and the lipid profile in Wistar albino rats.

Place and duration of study:Department of Biological Sciences, University of Botswana. Botswana. The study was conducted from January 2012-December 2012.

Methodology:The animals were divided into five groups of six rats each and maintained as follows:Normal control received the normal diet and two positive controls treated with heated sunflower oil and sesame oil, two experimental groups-treated sunflower oil and sesame oil after frying the potato chips.At the end of the experimental period of 60 days the rats were sacrificed and the blood was collected for biochemical estimations. The weights of the animals were recorded every week.

Results:The results indicate that sesame oil is much better in keeping the antioxidant status and cause lesser lipid peroxidation as well as hepatotoxicity when compared to sunflower oil. Again the cardioprotective effect of sesame oil is higher than the sunflower oil, this may be due to its chemical composition with high levels of Omega-3

Conclusion: So in conclusion sesame oil showed a higher level of protective effect than sunflower oil and is a better choice for the culinary purposes.

KEY WORDS: *polyunsaturates, lipid peroxidation, oxidative stress, hepatotoxicity, cardioprotective.*

INTRODUCTION

Cooking oil is plant, animal, or synthetic fat which is edible. It is often used in frying, baking, seasoning, and other types of cooking. Biochemically, it is an edible triester of fatty acids and glycerol[1]. The appropriate amount of fat as a component of daily food consumption is a topic of controversy. The FDA recommends that 30% or fewer of calories consumed daily should come from fat. Other nutritionists recommend that no more than 10% of a person's daily calories should come from fat[2]. Heating oil changes its characteristics. Oils that are healthy at room

temperature can become unhealthy when heated above certain temperatures. When choosing cooking oil, it is important to match the oil's heat tolerance with cooking method. The smoke point of oil depends primarily on its free fatty acid content (FFA) and molecular weight. Through repeated use, as in deep fryer, the oil accumulates food residues or by-products of the cooking process that lower its smoke point further [3]. During cooking, the oils and fats are exposed to high temperature which may result in changes their chemical properties. Heating oil at a temperature above its smoke point results in breaking down its triglyceride to glycerol and fatty acids. Furthermore, heating breaks down the glycerol to acrolein which ends up in losing flavor and nutritional value of the specific oil. In addition, the heating of oils and fats without catalysts produces secondary lipid peroxidation products such as carbonyls and cyclic fatty acids which are toxic to human body [4]. Experts say that the consumption of dishes cooked in reheated oil may expose you to high levels of toxic aldehydes; chemicals that are known to cause neurodegenerative diseases and cancer. This finding does not exclude even popular cooking oils like soybean and sunflower oils that are also capable of generating these harmful chemicals when reheated. Researches on heated oils indicate the possibility of these to undergo thermal oxidation which results in the production of reactive oxygen species (ROS) which are harmful to the cells. In response to ROS production, the body activates its antioxidant system to defend against invasion of free radicals, which results in oxidative stress [5].

Sesame oil is obtained from seeds of *Sesamum indicum*, a plant that grows natively in India. It is an annual plant growing 50cm to 100 cm tall. It has oppositely arranged leaves which are 4cm to 14cm long with an entire margin [6]. The oil from the nutrient-rich seed is popular in alternative medicine, from traditional massages to the traditional Indian medical practice Ayurveda. It has been used in the treatment of several chronic disease processes, including hepatitis, diabetes and migraines. Research shows that sesame seed oil is a potent antioxidant [7]. It penetrates into the skin quickly and enters the blood stream through capillaries. Molecules of sesame seed oil maintain good cholesterol (HDL) and lower bad cholesterol (LDL). Sesame oil has a lowering effect on blood pressure and levels of sodium in the blood [8].

The sunflower, or *Helianthus annuus*, is a distinctive plant which produces large bright yellow flowers which resemble small suns, the flowers grow on tall stalks with simple leaves, and they have been known to reach the height of 3m in ideal growing conditions. Sunflower oil is one of

the most commonly and widely used cooking oil in the world[9].It is also low in saturated fats, it is a rich source of Vitamin E, A and D. As it has the ability to withstand high temperatures.Nevertheless, it is not devoid of side effects and drawbacks.In addition to vitamin E, the oil is also rich in Omega 6 fatty acid is a causative agent of colon and prostate cancer. As sunflower oil contains vitamin E, it can lead to decreased clotting of blood. This can elevate the risk of bleeding.[10].

Heating cooking oil or fats changes its chemical composition and reuse of such oils which are exposed to high temperatures can cause harmful effects. So the main objectives of this study were to establish the toxic effect of two different cooking oils commonly used in culinary which were exposed to high temperature during cooking. To evaluate the antioxidant status in the blood samples of albino rats after feeding with used cooking oils. To compare the extent to which each of these oils can produce the changes in the lipid profile of these rats fed with these cooking oils.

2. MATERIALS AND METHODS

2.1 Plant Material

Oils were purchased from the local supermarkets (Spar, and Pick 'n Pay in South Africa), was authenticated by Prof Dr. M. P. Setshogo of the Department of Biology, Faculty of Sciences, at the University of Botswana

2.2 Chemicals

All the chemicals used were of the analytical grade and bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.3 Animals

Male albino rats of the Wistar strain weighing approximately 200–250 g were used for all experiments. They were housed in colony cages at an ambient temperature of 25_28C with a 12-hour cycle of light=dark. Animals hadfree access to water ad libitum and were fed on commercial diet bought from Nola Food Corp. (Cape Town, South Africa). The experiments were conducted following internationallyaccepted principles for laboratory animal care at the Department of Biology, University of Botswana.

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95 **2.4 Oil samples preparation**

96 The oils were heated or fried separately; there were two groups of rats for each the oil. One
 97 group of rats received heated oil and the other group received fried oil. The oils were prepared in
 98 the following manner: cooking oil was heated for four minutes in the absence of any food items
 99 for the first group of rats and used as positive controls. After that the oil was allowed to cool and
 100 then it was mixed with normal rat food. The other group of rats was fed with oil that was used to
 101 fry thin strips of potatoes for four minutes as well. The potatoes were discarded and the oils were
 102 mixed with normal rat feed (EX groups). Oils or fats were mixed with feed at the rate of 10% of
 103 the feed weight.

104 **2.5 Experimental design**

105 The animals were divided into five groups of six rats each and maintained as follows:

106 Group 1-NC-Normal control- received normal diet without any oil supplemented.

107 Group 2-PC 1-Positive control- treated with heated sunflower oil (10mL/ Kg body weight)

108 Group 3- PC 2- Positive control- treated with heated sesame oil (10mL/ Kg body weight)

109 Group 4- EX- 1-Experimental group- treated with sunflower oil (10mL/ Kg body weight)

110 Group 5- EX- 2-Experimental group- treated with sesame oil (10mL/ Kg body weight)

111 The experiment was run for 60 days. At the end of the experiment the rats were sacrificed, and
 112 the blood was collected for various biochemical estimations.

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116 **2.6 Biochemical Analysis**

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118 TBARS in plasma was estimated by the method described by Niehaus and Samuelsson [11]

119 and the reduced glutathione was estimated by the method of Ellman [12]. Superoxide

120 dismutase was assayed by the method of Kakkar et al. [13]. The assay of SOD activity was

121 based on the principle of inhibitory effects of SOD on reduction of nitro blue tetrazolium dye

122 by superoxide radicals. A single unit of enzyme was expressed as 50% inhibition of NBT

(Nitro blue tetrazolium) reduction /min/mg protein. Catalase was estimated by the method of Hans Bisswagner [14]. AST ALT and ALP were estimated by using the kits from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA and the guidelines of the company were strictly followed. LDL, HDL Total cholesterol and triglycerides were estimated by using kits.

2.7 Statistical Analysis

All data were expressed as the mean $\pm$ S.E. mean of $n=6$. Analysis of variance was performed by one way ANOVA and the significant difference between the means were determined by Holm-Sidak method. p value $=0.05$ was regarded as significant. In all these cases, Statistical Software Sigma stat, 3.1 was used to analyze the data.

3. RESULTS

3.1 Percentage increase in weight

The average weights of these animals are shown in figure 1 and 2 which shows a gradual increase in weight from week one onwards, this may be due to lipids getting deposited in the adipose tissue. This was highest in PC 1 than in EX 1.

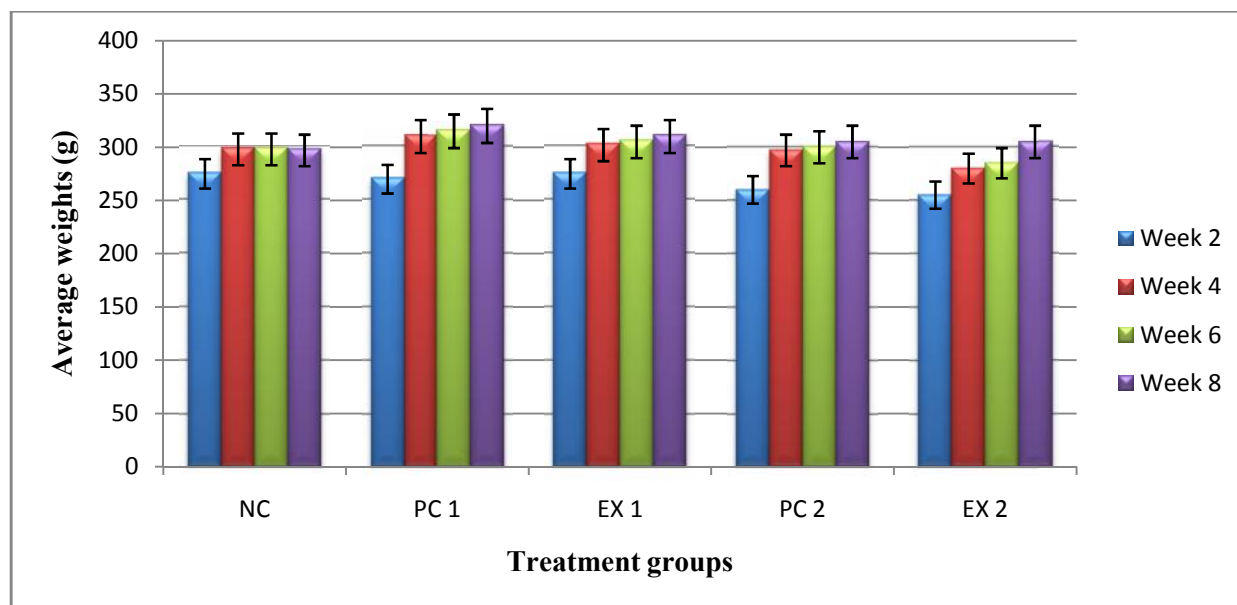


Figure 1: Average weights of experimental rats taken at an interval of two weeks over a period of eight weeks.

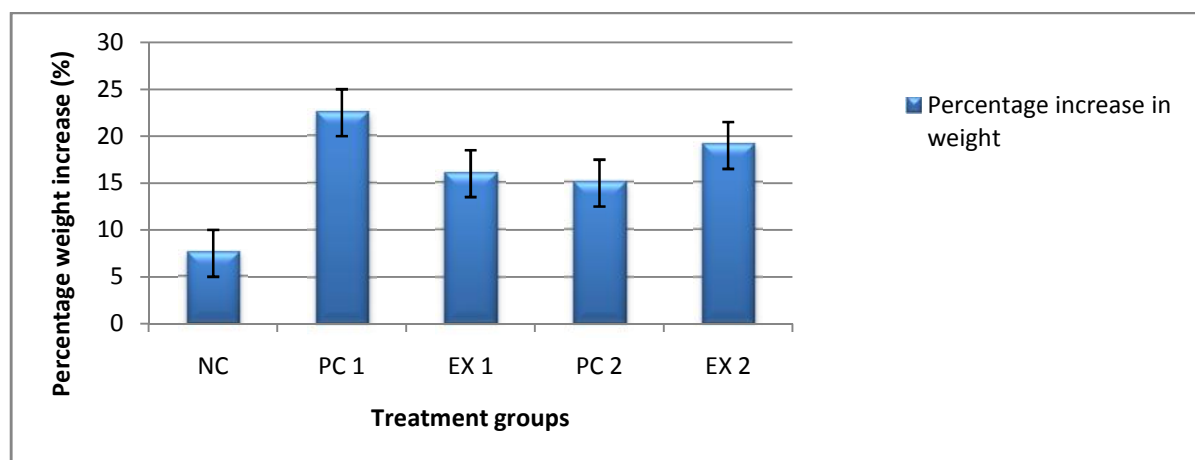


Figure 2: % weight increase in weight of rats fed with different oils.

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154 3.2 Lipid peroxidative indices and liver markers

155 Table 1. Lipid peroxidative indices

Groups	TBARS (nmol/L) PLASMA	AST (U/L) PLASMA	ALT (U/L) PLASMA	ALP (U/L) PLASMA
NC	1.28 ± 0.08	40.16 ± 0.23	38.98 ±0.09	45.76 ± 0.04
PC1	1.58 ± 0.21	46.95 ± 0.56	39.04 ±0.46	47.96 ± 0.34
PC2	1.05 ± 0.34	38.05 ± 0.91	34.08 ±0.05	42.76 ± 0.72
EX1	6.87 ±0.18 *	78.95 ± 0.41*	76.88 ±0.03*	88.56 ±0.17*
EX2	5.07 ±0.11 *	67.15 ± 0.71*	71.67 ±0.23*	83.76 ±0.67*

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157 TBARS: Thiobarbituric acid reactive substances, ALT: Alanine aminotransferase, AST:
158 Aspartate aminotransferase, ALP: Alkaline phosphatase,

159 * Significantly different

160 n= 6in each group and the results were given as mean ±SEM.

161 Therresults reflected in (Table 1) shows liver function indices of alanine aminotransferases
162 (ALT), aspartate amino transferases(AST), alkaline phosphatases (ALP), concentrations in the
163 serum after 60 days of oral administration of sunflower oil and sesame oil. There was a
164 significant ($P<0.05$) increase in the activities of all these liver marker enzymes (ALT, AST and
165 ALP) in the EX-groups when compared to control groups(NC).The PC groups did not show
166 any significant difference in the liver marker enzymes when compared with the NC group. The

167 levels of TBARS is a clear indication of the amount of lipid peroxidation also shows the same
168 type of effects as the liver markers.

169 3.3The antioxidant status in the blood

170 **Table2.The antioxidant levels in the blood**

Groups	GSH(mg/dl)	CAT (U/mg Hb)	SOD (U/mg Hb)
	PLASMA	HAEMOLYSATE	HAEMOLYSATE
NC	35.07± 1.09	48.75 ±1.32	2.65 ± 1.17
PC1	34.13± 0.12	44.15 ±1.01	2.15 ± 0.19
PC2	38.67± 1.11	49.78 ±0.92	2.81 ± 0.13
EX1	17.88 ±0.34*	28.48 ±1.19*	1.28 ±0.48*
EX2	19.09 ±0.84*	32.46 ±0.61*	1.88 ±0.98*

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172 **GSH: Reduced glutathione, SOD: Superoxide dismutase, CAT: Catalase,**
173 The antioxidant status in the blood included the non-enzymatic antioxidants such as GSH as well
174 as the enzymatic antioxidants such as CAT and SOD (Table 2). The results indicate that all the
175 parameters checked showed a significant decrease in Ex groups when compared to NC groups
176 ($P \leq 0.05$). The PC groups did not show any significant difference between the NC groups but
177 they showed slight a variation in different oils.

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3.4 Lipid profile in the plasma

Table3. Levels of different types of lipid in the plasma

Groups	TOTAL CHOLESTROL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TRIGLYCERIDES (mg/dL)
NC	10.20 ± 1.25	56.09 ± 1.32	15.00 ± 2.23	61.00 ± 4.21
PC1	18.28 ± 1.09	47.08 ± 1.52	36.08 ± 1.92	111.00 ± 0.28
PC2	12.02 ± 0.28	68.07 ± 0.39	16.68 ± 1.62	66.00 ± 1.53
EX1	124.40± 9.10*	12.80 ± 1.14*	150.87±3.1*	230.00 ± 9.31*
EX2	42.45± 1.18*	21.87 ± 0.17*	105.07±1.1*	201.80 ± 0.91*

n= 6in each group,* shows significant difference and the results were given as mean ±SEM. P≤0.05.Group 1- NC- Normal control, Group 2- PC 1 –Positive control –treated with heated sunflower oil, Group 3-PC 2- Positive control –treated with heated sesame oil, Group 4- EX-1- Experimental group-treated sunflower oil with oil fried, Group 5- EX-2-Experimental group-treated with sesame oil.

The results of the lipid profile lipid profile are presented in (Table 3). It shows that administration of the oils had significantly lowered HDL levels (p<0.001) in EX groups but the levels are different in different oils sesame oil is much higher when compared to the sunflower oil. All other parameters checked showed significant increase in EX groups when compared to NC group again the levels are slightly different for both the oils used.

4.DISCUSSION

Most of the lipids are in the form of triglycerides and they are the major fuel source in the body. Other lipids, including phospholipids, glycolipids and cholesterol are crucial constituents of the biological membrane. Fats are part of a normal, balanced, healthy diet and the body needs them for a variety of reasons. Fats provide a concentrated source of energy. One gram of fat provides 37kj (9kcal), more than double that provided by either protein or carbohydrate which provide 17kj/g (4kcal) and 16kj/g (3.75kcal), respectively [15]. Fat is a carrier for fat-soluble vitamins A, D, E and K. Some fats are essential to our well-being; these are known as the essential fatty acids (EFA's) often referred to as the omega fatty acids. Linoleic acid (omega 6) and alpha linoleic acid (omega 3) are the most common essential fatty acids found in vegetable oils. Individual fatty acids can either be saturated or unsaturated and the latter being further sub-divided into mono and polyunsaturated. Fatty acids are either saturated or unsaturated. Saturated fats have no double bonds. Saturated fatty acids are extremely stable, that is, they do not easily become rancid, and they can increase blood cholesterol levels, one of the major factors in heart disease [16].

Fig 1 and 2 showed the effect of these oils in the weight of these animals and result did not show any significant difference. The effects of sunflower oil and sesame oil on lipid peroxidation is presented in Table 1 indicate an increase in lipid peroxidation as measured by formation of TBARS or dyne conjugates induces shows mitochondrial dysfunction in rats subjected to the oxidative stress created by reused oils. This was much restricted in the control groups. The data obtained show that the reused oils in the experimental groups' administration in albino rats caused acute oxidative stress along with necrosis in the liver, which corresponds to the elevation of serum ALP, ALT and AST activity [17]. These pathological changes were accompanied with structural alterations of the hepatocytes, including glycogen and fat accumulation, organelle abnormality and cytoplasmic membrane degeneration due to lipid peroxidation causing the leakage of liver enzymes in to the blood stream. These conditions were not occurred in the control groups. Again the liver markers levels were higher in sunflower oils treated when compared with sesame oil treated group [18].

The antioxidant status is given in Table 2 which shows that the control groups have almost the same antioxidant status as the normal control but the experimental groups were significantly

different from the control groups. GSH is a non-enzymatic antioxidant which inhibits the generation of reactive oxygen species and hence the oxidative stress that damages the structural integrity of cell membrane and membrane organelles. The decrease in GSH levels in EX groups were due to the decreased availability of GSH caused by enhanced lipid peroxidation. In the present study, a significant decrease in the GSH content in the EX groups as compared to the entire PC and NC groups were also observed. A decrease in the SOD and Catalase activities was observed in EX 1 and EX 2 where reused oils were administered. It appears that the entire control group takes over the work of scavenging the free radicals and hence the normal levels of both enzymatic and non-enzymatic anti-oxidant were maintained. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, and it is an important antioxidant defense in all cells exposed to oxygen. Hydrogen peroxide is then converted to oxygen and water by glutathione peroxidase and CAT [19]. It has been reported that SOD, CAT, and GSH constitute a mutually supportive team of defense against reactive oxygen species. One of the secondary effects of ROS formation is mitochondrial dysfunction which results in ATP depletion and oxidative stress. The results prove that the extract worked in the way of scavenging the superoxide anion thus preventing the chain reactions that leads to the production of ROS.

Apart from oxidative stress generated in EX groups, there was also a significant reduction in the level plasma HDL along with an increment in the levels of triglyceride; LDL and total cholesterol were also noted. Free radicals enhance the oxidation of light density lipoprotein (LDL) and oxidized LDL affects many biological processes involved in atherogenesis. A recent study found that a toxin called 4-hydroxy-trans-2-nonenal (HNE) forms when the oils are reheated. Once absorbed in the body, HNE reacts with DNA and proteins affecting basic cellular processes. Frying foods at or above 375oF can lead to the accumulation of 4-hydroxy-trans-2-nonenal (HNE) in the oil. HNE is a toxic substance that has been associated with an increased risk of stroke, atherosclerosis, elevated levels of LDL cholesterol, Alzheimer's, Parkinson's, various liver diseases and cancer.[20]. It only takes one use to create HNE in the oil, and reusing oil at too high of a heat can cause even more HNE to build-up. Researchers found that HNE is more likely to build up in oils with high levels of linoleic acid. The oil with the highest percentage of linoleic acid is the sunflower oil compared to sesame oil[21].

260 There are two types of fatty acids that are termed ‘essential’ because the body cannot produce
 261 them. These are omega-3 and omega-6 fatty acids, when the balance of omega-6s and omega-3s
 262 in the cell is off, things can start to go terribly wrong. The more double bonds in a fatty acid, the
 263 more reactive it is. Polyunsaturated fats tend to react with oxygen, which can cause chain
 264 reactions, damaging other structures and perhaps even vital structures like DNA [22].

265 According to our findings we recommend that sesame oil is more advisable to use in cooking
 266 than the sunflower oil due to its high smoking point. Sesame seed oil is a potent antioxidant
 267 which will neutralize oxygen radicals by scavenging the ROS. In vitro, sesame seed oil has
 268 inhibited the growth of malignant melanoma so this is used as an anticancer agent. Sesame seed
 269 oil is a cell growth regulator and slows down cell growth and replication. Sesame seed oil
 270 absorbs quickly and penetrates through the tissues to the very marrow of the bone. It enters into
 271 the blood stream through the capillaries and circulates. The liver does not sweep sesame seed oil
 272 molecules from the blood, accepting those molecules as friendly. Sesame oil has a lowering
 273 effect on blood pressure and levels of sodium in the blood acting as a diuretic[23]. On the other-
 274 hand sunflower oil is rich in Omega-6 fatty acids. which are harmful in excess with many side
 275 effects[24].

276 **4. Conclusion** In conclusion the results of this study demonstrate that sesame seed oil have many
 277 protective characteristics when compared to sunflower oil. This is due to its antioxidant potential
 278 and rich in Omega-3 fatty acids. Again instead of sticking to one type of oil, try to use different
 279 oils in moderate amounts.

280 **CONSENT**

281 Not applicable.

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283 **ETHICAL APPROVAL**

284 The author hereby declares that "Principles of laboratory animal care" (published in 2005)
 285 were followed. All experiments have been examined and approved by the appropriate ethics
 286 committee.”

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