

The role of glutathione in the detoxication of peroxides, arising from heated oils

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SUMMARY

Theory and empirical studies have demonstrated that arising temperature lead to form many toxic polymer compounds and peroxides. For instance, while the percentage of polymer compounds reaches 11.3 % in the continuously heated oil (C.H.O) and 37.8 % in the frying oil (F.O), polar compounds percentage reaches 25.6 % in the C.H.O % and 47.6 % in the F.O. With respect to peroxide concentrations, the percentage reaches 133.6 mM/kg and 157.1 mM/kg in the C.H.O and F.O, respectively. Thus, throughout this study we tried to examine the body ability in breaking these toxic compounds using the measurement of glutathione concentration (GSH).

Observing the ordinary oil (O.O) along the time of treatment, no changes have been appeared in the GSH level. This is a convincing evidence that O.O does not contain any toxic peroxides or metabolites. Whereas, after the treatment by a C.H.O for 10 hours at 220 C°, an important decrease in the GSH level had occurred along the time of treatment. This important decrease attained 23.3 %, 20.1 % and 21.2 % within three, five and seven weeks, respectively. On the other hand, the treatment by F.O leads also to a noticeable decrease in the GSH level. It reached 33 %, 16.6 % and 28.5 % after three, five and seven weeks, respectively. These results are very normal at least to the extent that the F.O and C.H.O contain large quantities of lipid peroxides and polymer compounds resulting from the heating process.

INTRODUCTION

The glutathione (GSH) is known as a free non protein thiol compound that has a main importance in the different vital system for living animals, plants and micro-organisms. But its concentration is different from one organ to another and also from one cell to another, (1, 2).

Chemically speaking, the glutathione is a tripeptide. It contains three amino acids: γ -glutamyl, cysteinyl, glycine which represent

reduced glutathione (GSH), (3, 4). The thiol group is considered as the most important active functional group in the GSH molecule, because of its growing biological and biochemical activity. Hence, it contributes in most of oxidoreduction reactions and may provide its proton (H^+) especially in the detoxication process of electrophilic compounds and peroxides, (4, 5).

The GSH molecule might be oxidised by losing its proton from thiol group (-SH) and thus formulate sulphur bridge with another

molecule to form the oxidised glutathione (GSSG), (3).

In the normal cases, most of the glutathione is in its reduced form. the ratio of GSH to GSSG may reach 100/1, but not in all the cases. In some physiological and illness cases, this ratio might be affected because of the GSH contribution in many oxidoreduction reactions, (1, 6, 7). The GSH enters in the different reactions of detoxication within the living cell through one of its quality reactions. Besides its contribution in the oxidoreduction reactions of toxic peroxides: organic or inorganic, and in the formation of non toxic primary alcohol in the presence of glutathione peroxidase enzyme (GPx), (1, 4). The GSH also contributes in the conjugation reactions with electrolytes, oestrogen, drugs and all foreign body compounds. All that in the presence of glutathione S-transferase enzymes (GSTs), (8, 9) and the production of related glutathione derivatives which were excreted as non toxic metabolic compounds in the urine and in the form of mercapturic acids, (10, 11).

MATERIAL AND WORK METHODS

Twelve groups each consisting of four Albino wistar male rats of 200 grams of weight, in average, have received in their diet 10 % of different types of oil, which consists of 40 % of colza oil and 60 % of sunflower oil, (SOGEDIA Company, ALGERIA).

These different types of oil are unheated ordinary oil (O.O), frying oil (F.O) and continuously heated oil (C.H.O) during 10 hours at 220 C°. After three, five and seven weeks from staring the treatment process, The animals have been killed by decapitation and taken their livers and the level of glutathione within their livers has been measured using the spectrophotometric method of Weckbecker and Cory (12), using Ellman reagent throughout optic density measurement at 412 nm wave length resulted from the 2-nitro-5-mercaptobenzoic acid formation through the reduction of the reagent 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) by the thiol group of the GSH.

Where as, the protein concentration was measured by the method of Bradford (13), while the quantity of peroxides was measured by the method of Aust (14), the ratio of polar compounds was measured by the method of Billek and al (15) and the ratio of polymers was measured by the method of Polled and al (16).

RESULTS AND DISCUSSION

When we analysed the different types of used oils along the time of treatment, the major findings from this empirical experience are showed in the table 1.

Table1. Peroxides, polymer and polar compounds concentrations in the food oil before and after heating.

Oil types Composition			
	O.O.	C.H.O.	F.O.
Peroxide - mM/kg	0	157.1	133.6
Polymer compounds %✓	0.9	11.3	37.8
Polar compounds %	2.1	25.6	47.6

It's evident from the above table that high temperature has a great effect on breaking some fatty acids leading to the formation of several free radicals, and thus the appearance of poisonous lipid peroxides and H_2O_2 (17, 18). Moreover, the obtained results, shown in table 1 confirm that, while the level of peroxides reaches 157.1 mM/kg and 133.6 mM/kg in C.H.O and F.O, respectively, the percentage of polymers reaches 11.3 % and 37.8 % in C.H.O and F.O, respectively, and only 0.9 % in the ordinary oil (O.O). On the other hand, the percentage of polar compounds arised up from 2.1 % in O.O to 25.6 % in C.H.O and 47.6 % in F.O.

The level of GSH within the rats liver under control which have been feeded by alipidic food, reaches 44.52 ± 1.22 nM/mg prot. Whereas, this level has changed in the animals feeded with heated oils during three weeks. More results are noted in table 2.

(GR) enzyme activity in order to rebuild the GSH and then preserve the ratio of GSH to

GSSG higher or increasing the GSH biosynthesis reactions.

Oil types Samples	Control	O.O	C.H.O	F.O
1	39.9	37.5	32.2	34.2
2	45.9	42.3	35.5	36.4
3	46.8	42.6	36.5	39.7
4	47.1	43.2	37.5	41.9
5	47.4	46.1	39.5	37.0
x ± SEM	45.42 ± 1.40	42.34 ± 1.39	** 36.30 ± 1.24	** 37.84 ± 1.34

** Compared with control (P<0.01)

Table3 The concentration of glutathione in rat’s livers after five weeks of treatment by

O.O, C.H.O and F.O (nM/mg prot.):

By extending the treatment duration to the seventh week, no changes have been appeared in the mechanical response of those rats towards these oils compared with the third and fifth week. (table 4).

Also, no change has been appeared in the GSH concentration level even after a treatment that lasted seven weeks with ordinary oil (O.O) . This concentration has reached 40.30 ± 1.51 nM/mg prot. Compared with the control, wich was 42.60 ± 1.38 nM/mg prot. This result is very obvious to the extent that the ordinary oil (O.O) does not contain any toxic compounds.

For both the heated and fried oils, the situation represents another case in which a significant decrease has been observed in the GSH level (P< 0.001). The concentration was 33.56 ± 1.82 nM/mg prot. and 30.44 ± 1.96 nM/mg prot., by a decrease of 21.2 % and 28.2 % regarding both the C.H.O and F.O, respectively. In addition, this observation represents another evidence that those two types

of oils contain a large quantities of toxic peroxides, cyclic monomers, electrolytes,...etc. Finally, this research does support the view that using the ordinary oil (O.O) as a part of a continuous treatment on rats, the GSH level should not change because this oil does not contain any toxic compounds, and so it may be considered as a fully consumption healthy oil used under a very strict healthy conditions.

Oil types Samples	Control	O.O	C.H.O	F.O
1	40.9	39.7	30.2	26.3
2	38.7	35.2	28.3	25.1
3	42.4	39.9	35.6	32.7
4	44.3	42.8	35.9	34.2
5	46.7	43.9	37.8	33.9
x ± SEM	42.60± 1.38	40.30± 1.51	** 33.56 ± 1.82	** 30.44 ± 1.96

** Compared with control (P<0.01)

Table 4 The concentration of glutathione in rat’s livers after seven weeks of treatment by O.O, C.HO and F.O (nM/mg prot.):

In addition, using the heated oil as a part of the treatment by C.H.O. until the fifth and seventh week, the observed decrease in the GSH level did not change, the percentages were 20.1 % and 21.1 %, respectively for the fifth and seventh week. This means that these animals have a very strong acclimation ability with the cumulative peroxides. The glutathione metabolising system has become suitable to the percentage of cumulative peroxides within those rats' body.

Whereas, continuing the treatment by using the F.O., the level of GSH in the fifth week has decreased to reach 16.7 % because of the animal acclimation with the new feeding system. But in the seventh week a rise was noticed in the GSH level to reach 28.5 % as a result of the continuation of peroxides formation and foreign body compounds to a degree that exceeds the glutathione enzymatic defensive system ability. In other words, the glutathione consumption in detoxication reactions exceeds the biosynthesis of GSH or its reformation by glutathione reductase (GR). Some researchers (27,28) have obtained a decrease in the GSH level, although the significant increase in the GR enzyme activity at the time when the rats' liver treated in vitro by t-butyl hydroperoxide.

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