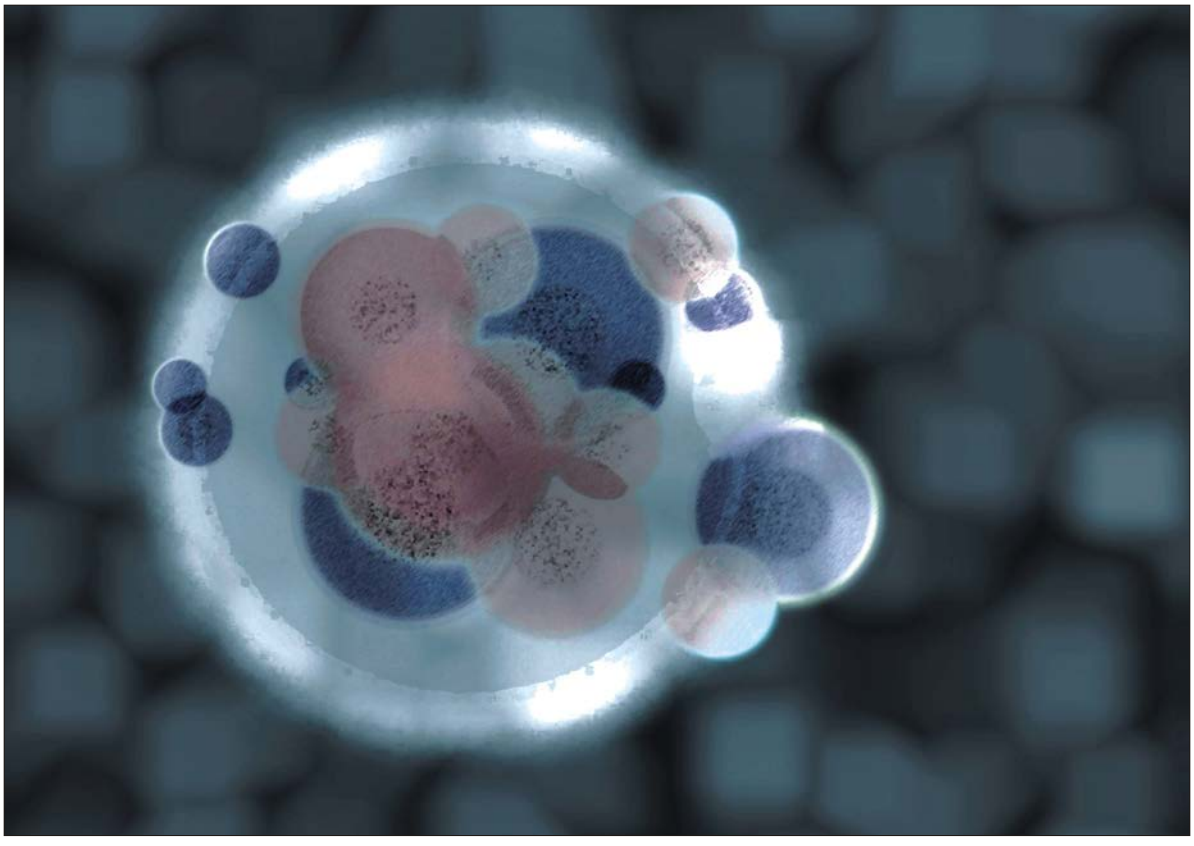

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Research Article
Antagonizing the Hazardous Impact of Increased Oxidative Stress in Wistar Rats by Biscuits with Dried Orange Peel

[1]Maha Hanafy Mahmoud, [1]Hany Mohamed Ahmed Wahba, [2]Marwa Hanafy Mahmoud and [2]Ferial Mohamed Abu-Salem

[1]Department of Nutrition and Food Science, National Research Centre, 33 El-Bohouth St., P.O. 12622, Dokki, Giza, Egypt
[2]Department of Food Technology, National Research Centre, 33 El-Bohouth St., P.O. 12622, Dokki, Giza, Egypt

Abstract

Background and Objective: It is settled nowadays that oxidative stress is a causative factor for many chronic diseases. Increasing intake of natural antioxidants is believed to reduce the negative effect of oxidative stress and decrease the risk for diseases. The present study was designed to evaluate the biological effect of feeding rats on dried orange peel as a rich source for natural antioxidants when being incorporated in biscuits along with sodium nitrite as an inducer for increased oxidative stress. **Materials and Methods:** The optimum method for orange peel drying without remarkable loss in its antioxidant potency was determined. Polyphenol content for orange peel and antioxidant activity with four different methods were assessed. A biological experiment was done on 24 male Wistar albino rats as four groups which lasted for 5 weeks. The first group was a control negative group fed on control diet. The second was another control group but was fed on 200 g of biscuits with dried orange peel kg^{-1} diet. The third and fourth groups were fed on a diet containing 7 g sodium nitrite kg^{-1} diet but 200 g of biscuits with dried orange peel kg^{-1} diet was mixed with the diet of the fourth group. **Results:** Evaluation of antioxidant activity and polyphenol content for the three drying methods of orange peel revealed that solar energy was the best drying method. Biochemical analysis revealed that nitrite fed group recorded a significant increase in malondialdehyde, G6PDH, ALT and a non-significant decrease in blood hemoglobin, the activity of catalase and SOD. Also, a significant decrease in body weight gain, feed intake and feed efficiency ratio was noticed for the same group. These parameters were restored in the group of rats that was fed on biscuits with dried orange peel along with sodium nitrite. **Conclusion:** Feeding rats on dried orange peel which was dried with solar energy and included in biscuits with their diet was able to antagonize the hazardous effect of increased oxidative stress due to sodium nitrite. So, it is recommended to incorporate orange peel in our food like biscuits to protect us from the increased oxidative stress.

Key words: Oxidative stress, dried orange peel, polyphenols, SOD, catalase, G6PDH, malondialdehyde

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Corresponding Author: Maha Hanafy Mahmoud, Department of Nutrition and Food Science, National Research Centre, Dokki, Giza, Egypt
Tel:+201225757319

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Increased oxidative stress is believed to be one of the causative factors for many chronic diseases[1] such as neurodegenerative diseases[2], cancer, coronary heart disease, Parkinson's disease and possibly Alzheimer's disease[3], diabetes mellitus and inflammatory diseases[4]. Also, it is one of the leading causes for aging. Production of free radicals occurs normally in all cells which is a part of normal cellular function. But, excess production of these free radicals either from endogenous or exogenous sources plays an important role in the etiology of many diseases[5]. Oxidative stress is the disturbance in the equilibrium status between generation of free radicals and the cellular antioxidant defense system[6]. Natural antioxidants can prevent tissue damage induced by free radicals either by inhibiting free radical chain initiation or breaking their chain propagation by scavenging them or by promoting their decomposition[7]. Thus, antioxidants fight free radicals and protect us from various diseases. The human body has its own antioxidant defense system and natural antioxidants are believed to strengthen this system[8]. Many reports have indicated that the regular consumption of fruits and vegetables was correlated with the decreased risks of some diseases. The 2015 report for healthy people mentioned that fruit consumption increasing by 75% per day may increase life longevity by 2 years or more[9].

Citrus fruits and juices are important sources of bioactive compounds including antioxidants such as ascorbic acid, carotenoids, flavonoids, phenolic compounds and pectin[10]. Epidemiological studies showed that citrus flavonoids are capable of reducing the risk of coronary heart diseases[11], neurodegenerative diseases[2] and also have an anti-inflammatory effect and anticarcinogenic effect[12]. Recently, a number of studies have suggested that by-products of some fruits and vegetables could be good source of natural antioxidants and it must be reused in order to utilize their benefits[13,14]. Orange peel is considered as a by-product in food industry and it constitutes about 50% of the whole fruit. It is a very rich source for bioactive compounds among which are polyphenolic compounds, β-carotene, vitamin C and other antioxidants[15,16]. It contains ascorbic acid even with more concentrations than that of the fresh juice and it contains the highest concentrations of flavonoids in the orange fruit[17,18]. Orange peel has also been utilized for the recovery of flavonoids (i.e., hesperidin), essential oils and carotenoids[19]. Orange peel because of its high content of antioxidants can be used in food industries as antioxidant to inhibit lipid peroxidation and it can replace the synthetic antioxidants which are currently used like butylated hydroxytoluene (BHT) with its documented hazardous effects on human health[20]. Moreover, orange peel with its high content of antioxidants was reported before to have many health benefits among which minimizing central nervous system disorders[21].

Sodium nitrite is an oxidizing agent. It is used as food additive for preservation of meat and fish products and as a color fixative for them[22,23]. Also, it could be found in fresh water and in fruits and vegetables as residual portions from fertilizers used during cultivation of these plants. Although, toxicity and carcinogenicity of sodium nitrite has been reported by the FDA, yet it is still of widespread use in food industry, since no alternatives have been introduced. Moreover, sodium nitrite is assumed to be an etiologic factor for the increased risk of gastrointestinal cancer in adults, increased incidence of brain tumors, nasopharyngeal tumors and leukemia in children and increased methemoglobinemia in infants[24,25].

Since, there are huge amounts of food by-products which may be a source for contamination constituting environmental and health hazards, these food by-products have to be reused in such a way to make use of their benefits and avoid their hazards. To do that, first we have to think about drying these by-products using the suitable techniques to avoid any loss in the bioactive components of these by-products. Thermal treatments for drying like hot-air drying, vacuum drying and sun-drying are used in food industry mainly for preservation to inactivate the enzymes, get rid of the microorganisms and to reduce water content. However, high temperatures and long drying periods usually reduce the quality of the final product[26]. It was reported previously that different drying methods can affect the bioactive compounds found in the orange peel in particular[26]. So, searching for the most suitable technique is very important to keep the bioactivity of the orange peel antioxidants.

The main objective of the present study is to use a bakery product which is biscuits (known to be of a wide and public use) after adding dried orange peel, with its high content of natural antioxidants, to antagonize the deleterious effect of increased oxidative stress in Wistar rats. Different drying methods for orange peel were trailed and the method that gave the least loss in antioxidant activity was chosen. Sodium nitrite was chosen to be the inducer for the increased oxidative stress in this study as it is one of the widely used food additives in the food industry.

MATERIALS AND METHODS

The present study was conducted in January, 2016 at the Department of Food Technology and the Department of Nutrition and Food Science, National Research Centre, Egypt.

Materials

Materials used for preparation of biscuits and analysis of orange peel antioxidant activity and polyphenolic content: Orange peel from *Citrus valencia* (*C. valencia*) was purchased in its ripening season from the local market. Wheat flour (72% extraction), sugar powder, baking powder, vanilla-flavored sugar, eggs and margarine were purchased from the local market, Cairo, Egypt.

Chemicals used for chemical analysis of antioxidant activity of orange peel were of analytical grade and obtained from Sigma-Aldrich Chemical Co. (St. Ouis, Mo, 63103, USA). Folin-ciocalteu reagent was obtained from Merck Company, Germany. Gallic acid was obtained from Sigma-Aldrich Chemical Co., USA.

Materials used for the animal experiment: Most of the constituents of the diet formulated and introduced to the rats were purchased from the local market except for casein which was obtained from Scerma Co., France. Also, the salt and vitamin mixtures used were of analytical grade and obtained from Fluka (Germany) and BDH (England) chemical companies. Sodium nitrite was obtained from Al-Nasr Co., Egypt.

Animals used in this experiment were adult male Wistar albino rats of Sprague Dawley strain with a body weight ranging from 140-170 g. They were obtained from the central animal house of National Research Centre (Egypt). The animal experiment was done in compliance with the Guidelines for Animal Care and Ethics Committee of the NRC (Egypt).

Materials used for biochemical analysis: Diagnostic kits used for the determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were obtained from Salucea Co., Netherlands. Kit used for determination of G6PDH was obtained from BEN Biochemical Enterprise Co., Milano, Italy. Kits used for determination of blood hemoglobin concentration and the activities of plasma catalase, erythrocyte SOD and erythrocyte GPx were obtained from Biodiagnostic Co., Egypt. Kits used for determination of urea and creatinine were obtained from Chronolab Systems, Barcelona, Spain. Chemicals used for the determination of lipid peroxide product (malondialdehyde, MDA), the thiobarbituric acid (TBA) and the trichloroacetic acid (TCA) were obtained from Merck (Germany) and BDH (England) Companies, respectively.

Methods

Preparation of the dried orange peel: Orange (*C. valencia*) was washed with distilled water, then the peel was separated from the whole fruit carefully by manual peeler. Then, orange peel was sliced into small pieces and allowed to dry till complete dryness by either of the following three methods. The first method of drying was done by air oven drying at a temperature of 40°C. The second method was by microwave drying in a Samsung Microwave, Model MF245 with a power source of: 230 V-50 Hz and nominal microwave power outputting of 900 Ws at air temperature of 40°C/6 min. The third drying method was the solar drying for 48 h at 40°C with ambient air temperature of 19.4°C, relative humidity of 57%, relative humidity inside the dryer of 10% and global solar radiation of 845 W m^{-2}. The control for these drying methods was the fresh citrus peel. Then, the dried peel for each treatment was ground into fine powder by a mechanical grinder (Braun, KMM 30 mill, type 3045, CombiMax, Germany) to pass through 100 mesh screen sieves. This powder was subjected to chemical analysis for determination of total polyphenolic compounds and antioxidant activity for each treatment after being extracted by methanol prior to the feeding experiment for evaluation of the effect of drying technique on the antioxidant activity.

Extraction and determination of total polyphenols: The powdered formula was extracted with 80% methanol according to the method of Hayat *et al.*[27]. The moisture content of the sample was determined in order to calculate the concentrations on a dry weight basis.

Total polyphenol content was determined by the folin-ciocalteu assay, according to Ramful *et al.*[28].

Determination of antioxidant activity of the dried orange peel: Antioxidant activity of the dried orange peel by each of the three methods was determined by four different methods as follows, (1) The DPPH radical scavenging activity (DPPH) which was carried out according to the procedure of Hayat *et al.*[27], (2) Ferric reducing power (FRAP) was assessed according to De Moraes Barros *et al.*[29], (3) β-carotene bleaching test was determined as described by Moulehi *et al.*[30] and (4) The hydroxyl radical (OH) scavenging activity as described by Hayat *et al.*[27].

Table 1: Composition of diet of control rats (g/100 g) diet

Ingredients	Amount (g/100 g diet)
Casein*	22.00
Sucrose	10.00
Cellulose	5.00
Corn oil	4.00
Salt mixture (AIN-93)**	3.50
Vitamin mixture (AIN-93)***	1.00
Choline bitartrate	0.25
L- Cystine	0.18
Corn starch	54.07

*Protein content of casein was estimated as 54.6%, **Salt and ***Vitamin mixtures were prepared according to Reeves *et al.*[31]

Preparation of the biscuits: The dough of the biscuits was prepared and baked as previously described by Mahmoud *et al.*[16]. The amount of orange peel powder that was added to the dough was selected as 15% which gave the best rheological testing for the biscuits and the best score for biscuits sensory evaluation at the same study after trying different percentages as previously mentioned by Mahmoud *et al.*[16].

Formulation of the diet for the feeding experiment: The standard control diet was prepared according to Reeves *et al.*[31] as shown in Table 1. Then biscuits were added to the diet of the selected groups on the expense of starch.

Animal experiment: Twenty four male adult Wistar albino rats of Sprague Dawley strain with a body weight range of 140-170 g were used. Animals were kept in controlled temperature 25°C for 1 week as an accommodation period prior to the animal experiment. Food and water were allowed ad-libitum to each rat. Animals were divided into 4 groups, each comprising 6 rats. Animals were kept individually in stainless steel cages in a temperature controlled room at 25°C. Light was adjusted day and night. Food and water were allowed *ad libitum* to the animals during the whole experimental period which lasted for 5 weeks. Increased oxidative stress was induced to the rats by introducing sodium nitrite to rats with their diet as 7 g for each Kg diet according to the dose mentioned in a previous study by Mahmoud[32]. Biscuits were added to the diet of rats on the expense of starch as 20% of the diet for groups 2 and 4. Then, the feeding experiment started as follows:

Group 1: It was given the standard control diet and served as a control negative

Group 2: It was given the standard control diet+20% biscuits

Group 3: It was given the standard control diet+7 g sodium nitrite and served as a control positive

Group 4: It was given the standard control diet+7 g sodium nitrite+200 g biscuits for each kg diet

During the experiment, the daily feed intake and the body weight were followed and recorded, then the feed efficiency ratio was calculated at the end of the experimental period as the body weight gain (final-initial)/the feed intake of the whole experimental period.

At the end of the experiment, fasting blood samples were collected from each rat after overnight fasting from suborbital vein and divided into two portions, one over heparin to determine parameters of the whole blood and that of the RBCs immediately. The other was also over heparin but subjected for separation of plasma after centrifugation at 4000 rpm for 15 min. All biological samples were chilled in crushed ice mixture till handled or for the next step. Plasma was kept in deep freeze at -20°C until analysis, while RBCs hemolysate was kept at -70°C after washing with normal saline for three consecutive times until analysis of antioxidant enzymes.

Biochemical analysis: Blood hemoglobin was determined according to Betke and Savelsberg[33]. The activities of both ALT and AST were measured according to the method of Henry *et al.*[34]. Plasma urea and creatinine each was determined according to Fawcett and Scott[35] and Bartels *et al.*[36], respectively. The activity of the enzyme G6PDH was determined in whole blood as described previously by Beutler *et al.*[37]. The antioxidant enzymes namely RBCs SOD, plasma catalase and RBCs GPx were estimated according to the methods of Nishikimi *et al.*[38], Aebi[39] and Paglia and Valentine[40], respectively. Plasma malondialdehyde (MDA) was measured as lipid peroxide product by the thiobarbituric acid assay (TBA) according to the method of Draper and Hadley[41].

Statistical analysis: Data were analyzed statistically using the computerized program SPSS software, version "20" for Windows. The one-way ANOVA test was done followed by Duncan's test. Data were represented as Mean±SE. The level of significance $p<0.05$ was considered significant, otherwise was non-significant.

RESULTS AND DISCUSSION

Total phenolic content of orange peel for each drying method: Data obtained in the present study for the polyphenolic content of orange peel as affected by the drying method (Fig. 1) revealed that the highest polyphenolic content was that for both the solar energy drying method followed by the microwave drying method compared to the fresh peel as control. Sun *et al.*[42] reported that the drying method affected significantly the total polyphenolic content when he studied the drying methods of freeze-drying, hot air

drying and solar energy drying. It is worth mentioning that the polyphenolic content of biscuits with dried orange peel as an ingredient was determined in a previous study for our team Mahmoud *et al.*[16]. It was found to be 27.81±0.15 mg polyphenols/100 g biscuits (for the 15% orange peel in the biscuits), thus biscuits with 15% dried orange peel was considered as a rich source for polyphenols.

Antioxidant activity of orange peel for each drying method: Drying orange peel with any of the selected methods either microwave or solar energy or oven (with the conditions stated above for each drying method) was able to keep the activity of the antioxidants with different varying degrees. The best of them was the solar energy technique which was able to yield a dried orange peel with the highest antioxidant activity compared to the fresh peel. Microwave and solar drying methods had better antioxidant activities than oven drying methods as seen in Fig. 2. It may be due to direct effect of heat on degradation of bioactive compounds (polyphenoles, vitamin C and β-carotene). Garau *et al.*[43] reported that drying citrus peel at relatively high temperatures resulted in a decline in its antioxidant capacity.

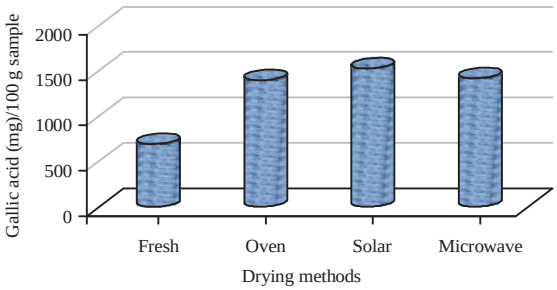

Fig. 1: Total polyphenol content of the dried orange peel with different drying methods

Biological evaluation of the biscuits containing orange peel

Antioxidant enzymes and malondialdehyde: According to the obtained data from the biochemical analysis, it is obvious that a state of typical increased oxidative stress was achieved successfully when feeding rats on sodium nitrite which is a strong oxidizing agent and that seems to be in accordance with previous studies. It was reported before in many studies that exposure to nitrite enhances the formation of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS) which in turn generates a state of increased oxidative stress[44-46]. This was evidenced in the present study from the results recorded for the antioxidant status represented by the peroxidation product, the malondialdehyde (Fig. 3a) and the antioxidant enzymes namely, glucose-6-phosphate dehydrogenase (G6PDH) in Fig. 3b, catalase (Fig. 3c),

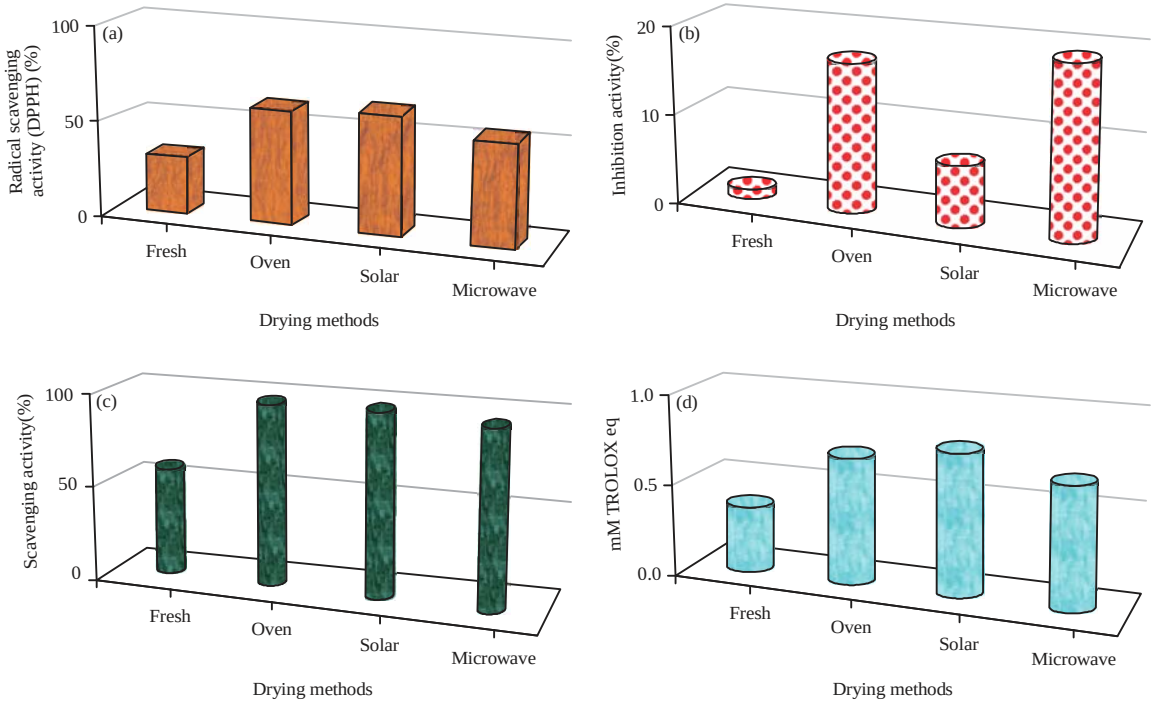

Fig. 2(a-d): (a) DPPH radical scavenging activity, (b) β-carotene bleaching test, (c) Hydroxyl radical (OH) scavenging activity and (d) Ferric reducing power (FRAP) assay

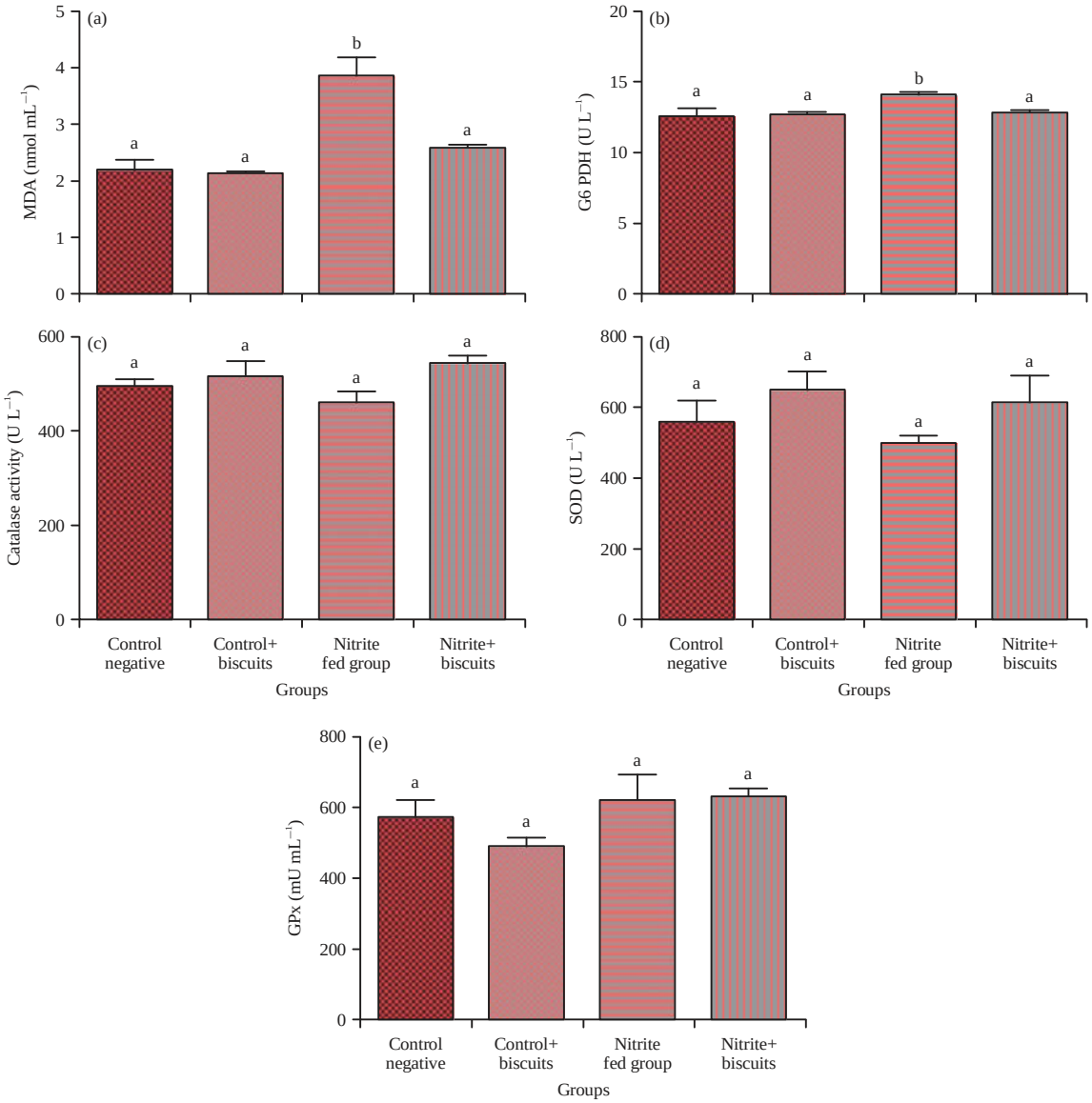

Fig. 3(a-e): (a) Concentration of malondialdehyde (MDA), (b) Activity of whole blood G6PDH, (c) Activity of plasma catalase, (d) Activity of superoxide dismutase (SOD) and (e) Activity of glutathione peroxidase (Gpx)

Bars represent Mean ± SE and the mean difference is significant at $p<0.05$ level. Bars that share the same letter are not significant, while those that share different letters are significant

superoxide dismutase (SOD) in Fig. 3d and glutathione peroxidase (GPx) in Fig. 3e. A significant increase in the concentration of malondialdehyde (MDA) was noticed for the nitrite fed group compared to the control negative group. Ansari *et al.*[47] reported similar results of elevated lipid peroxide when giving mice sodium nitrite. Also, Nooman *et al.*[48] reported an increased concentration of plasma MDA of rats due to increased oxidative stress as a result of feeding rats on high fat and cholesterol diet. The elevation of lipid peroxide product can be explained on the basis of the ability of nitrite to induce free radicals that attack the polyunsaturated fatty acids of the cellular membrane liberating lipid peroxides which in turn lead to impaired membrane function and structural integrity[49]. Introducing a diet containing biscuits with dried orange peel in addition to nitrite was able to antagonize the deleterious oxidizing effect of nitrite. This can be attributed to the strong antioxidant potency of the orange peel dried with the solar energy as illustrated in Fig. 2, also to its high polyphenolic content (Fig. 1) and in particular the high flavonoid content as mentioned in many previous studies[50].

Table 2: Blood hemoglobin concentration, liver function and kidney function of all groups

	Parameters				
Groups	Hb (g dL^{-1})	AST (U L^{-1})	ALT (U L^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Control negative	13.88±0.53^{ab}	50.50±4.69^{a}	17.75±0.65^{a}	32.35±3.22^{a}	0.60±0.09^{a}
Control+biscuits	14.78±0.29^{a}	49.00±2.37^{a}	19.80±2.52^{a}	30.41±2.90^{a}	0.63±0.08^{a}
Nitrite fed group	12.91±0.15^{b}	58.00±10.43^{a}	29.20±1.66^{b}	29.93±3.03^{a}	0.68±0.05^{a}
Nitrite+biscuits	13.61±0.13^{b}	50.25±5.26^{a}	25.50±2.20^{ab}	37.63±3.28^{a}	0.70±0.02^{a}

Values are expressed as Mean±SE and the mean difference is significant at $p<0.05$. Values that share the same letter at the same column are not significant while, values that share different letters at the same column are significant

Nitrite induced free radicals (ROS and RNS) are reported before to have the ability to attack the antioxidant defense system of the body causing a consequent loss of antioxidant elements of the body which are represented by SOD, GPx and catalase[51,52]. More or less, the same finding was noticed in the present study whereas a non-significant decrease in the activity of catalase and SOD was recorded for the nitrite fed group compared to the control negative group (Fig. 3c, d, respectively), while there was a non-significant increase in the activity of GPx (Fig. 3e). An explanation for the decrease in the activity of catalase and SOD may be due to the down regulation effect of the increased oxidative stress on the gene expression of the antioxidant enzymes as previously reported by Matsuzawa *et al.*[53]. A remarkable improvement of these enzymes occurred for the group of rats that was fed on biscuits with the orange peel added to the nitrite containing diet. The antagonizing effect of biscuits with orange peel is due to the high antioxidant activity of orange peel.

A significant increase in the activity of glucose-6-phosphate dehydrogenase (Fig. 3b) was noticed for the nitrite fed group compared to either the control negative group or the control group that received biscuits with its control diet. This increase was more or less counteracted in the nitrite fed group that received biscuits with its diet. The increased activity of G6PDH is due to the increased demand for the NADPH whose origin is the reaction catalyzed by the enzyme G6PDH in the phosphate pentose pathway. Increasing demand for the NADPH is a consequent result for increased activity of the enzyme methemoglobin reductase which is mainly activated to clean up the toxic methemoglobin formed due to hemoglobin oxidation by the action of the oxidizing nitrite. Elevated methemoglobin concentration in blood is a well-known sign for nitrite intoxication in human[54,55]. The obtained results for the antagonistic effects of the orange peel against the oxidizing and toxic effect of nitrite was in accordance with a previous study conducted by Mahmoud[32], in which feeding diet containing different antioxidants of natural sources was able to normalize the elevated blood methemoglobin concentration due to nitrite ingestion with the diet. This may be attributed to the antioxidant components of which the orange peel is very rich as documented from the obtained data for the antioxidant activity and polyphenolic content in this study and the high flavonoid and vitamin content of orange peel in a previous study for our team[15].

Blood hemoglobin, liver function and kidney function: Data obtained for hemoglobin concentration as illustrated in Table 2 showed that there was a decrease in hemoglobin concentration for the nitrite fed group, although insignificant when compared to the control negative group, but a correction for this decrease was observed for the group that was fed biscuits with nitrite in their diet. It seems that, orange peel antioxidants were able to counteract the oxidizing and toxic effect of the nitrite so as to restore the hemoglobin concentration to near the value of the control negative group. In addition, orange peel contains considerable amount of iron as reported by Mahmoud *et al.*[56], which is essential for hemoglobin synthesis in the body.

An increase of the liver transaminases was noticed for the nitrite fed group (Table 2). This increase was significant in case of ALT while it was insignificant in AST. Similar results were previously reported for the elevation in liver transaminases by nitrite intoxication by Salama *et al.*[57]. Feeding rats on biscuits including orange peel with the nitrite containing diet resulted in restoring AST completely, but ALT was restored to some extent. This positive effect on liver transaminases may be attributed to the high polyphenolic content and the high antioxidant potency of orange peel dried with solar energy as shown in Fig. 1 and 2, respectively. The high antioxidant potency of orange peel is due to its high content of antioxidants like vitamin C, β-carotene and flavonoids as reported before in a study conducted by Abou-Arab *et al.*[15], in addition to the total polyphenolic compounds recorded in the present study. It is assumed that the orange peel antioxidants antagonized the oxidizing effect of sodium nitrite and consequently the liver transaminases were improved. On the other hand, there was no significant change in case of urea and creatinine.

Body weight gain, feed intake, feed efficiency ratio and percentage of organ weight: Data recorded for body weight gain, feed intake and feed efficiency ratio (FER) in the present study (Fig. 4a-c, respectively) revealed a very highly significant

decrease in each of the aforementioned parameters for the group of rats that received sodium nitrite with their diet when being compared to either of the control negative group or the control group that received biscuits with their diet. Introducing biscuits fortified with the dried orange peel with the nitrite containing diet resulted in a slight but insignificant improvement in either of the body weight gain or feed intake or FER. Toxicity of sodium nitrite may explain the decreased feed intake and the consequent decrease in body weight gain for the group of rats that received sodium nitrite. In fact, the decrease in body weight is not just a consequent result for the decreased feed intake, but it is beyond that. The inefficient utilization of different food components as a result of nitrite toxicity shared in this observed phenomenon and this is documented by the very low FER compared to that of the control negative group. Although, the improvement in FER was considerable: From 0.006 for the nitrite fed group to 0.025 for the group that received biscuits with the nitrite containing diet, yet the increase in feed intake that corresponding to each of the theses two groups was not detectable (from 434.17-443.33 g, respectively), meaning that there was a better and more efficient utilization of food components rather than general increase in food consumption or increased appetite. This observation may reinforce our suggested explanation for the decrease in body weight gain. This improvement in feed intake, body weight gain and FER that was noticed due to introduction of the biscuits containing the dried orange peel with the nitrite containing diet may be explained on the basis of the high antioxidant potency of the dried orange peel as documented from the obtained results for antioxidant analysis of dried orange peel (Fig. 2) which revealed that the optimum method for drying was the solar energy drying method which is used for drying in the present study. These orange peel antioxidants antagonized the toxic effect of the oxidizing nitrite but to a low extent. May be the amount of orange peel is not enough to overwhelm the strong oxidizing effect of nitrite and an extra amount is needed to neutralize this nitrite oxidizing effect to obtain a better improvement in those parameters. The negative effect of sodium nitrite on body weight gain and feed intake was previously reported by Majchrzak and Gronowska-Senger[58].

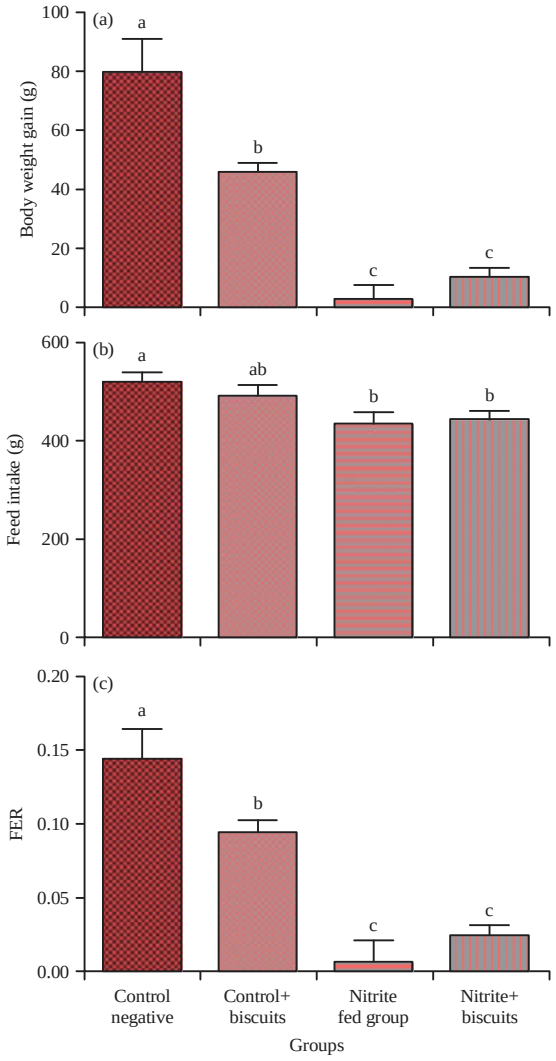

Fig. 4(a-c): (a) Body weight gain, (b) Feed intake and (c) Feed efficiency ratio (FER) of all groups
Bars represent Mean±SE and the mean difference is significant at $p<0.05$ level. Bars that share the same letter are not significant, while those that share different letters are significant

Percentage of organ weight: The percentage of organ weight per final body weight as illustrated in Table 3 showed nearly no significant change in all organs, except for a significant increase in kidney weight % for the nitrite fed group which can be attributed to the toxic effect of nitrite.

Table 3: Percentage of organ weight of all groups

	Organ weight (%)			
Groups	Liver wt. %	Kidney wt. %	Heart wt. %	Spleen wt. %
Control negative	2.95±0.095[a]	0.61±0.026[a]	0.39±0.018[a]	0.33±0.037[a]
Control+biscuits	2.90±0.218[a]	0.67±0.033[ab]	0.38±0.025[a]	0.33±0.035[a]
Nitrite fed group	2.91±0.213[a]	0.76±0.062[b]	0.44±0.033[a]	0.39±0.048[a]
Nitrite+biscuits	2.65±0.211[a]	0.73±0.018[b]	0.40±0.027[a]	0.38±0.021[a]

Values are expressed as Mean±SE and the mean difference is significant at $p<0.05$. Values that share the same letter at the same column are not significant while, values that share different letters at the same column are significant

CONCLUSION

From the obtained results, it can be concluded that solar energy was the most optimum drying method for orange peel. Feeding rats on nitrite containing diet induced a state of increased oxidative stress which in turn resulted in some metabolic disorders. When using dried orange peel as an ingredient in biscuits for feeding rats along with nitrite, a considerable improvement was noticed in most of the studied nutritional parameters particularly the FER and the biochemical parameters especially those of the antioxidant status like lipid peroxidation product (MDA) and G6PDH. Consequently, a precise recommendation can be given in this respect to those who are exposed to nitrite toxicity either from the frequent use of the preserved food products or as an environmental contaminant. They are advised to ingest food products that contain dried orange peel like biscuits produced in this study. Also, for industries of preserved foods, it is better to add dried orange peel to the food products to minimize the hazardous effect of food additives like sodium nitrite.

SIGNIFICANCE STATEMENT

This study discovered that feeding rats with dried orange peel biscuits which contain potent antioxidants can be beneficial for antagonizing the deleterious effect of increased oxidative stress when being exposed to strong oxidizing agents such as the widely used food additive, sodium nitrite by improving most of the studied nutritional and biochemical parameters provided that solar energy is the most optimum method for drying orange peel. This will help the researchers to combat the increased oxidative stress with a widely used food product, biscuits when adding dried orange peel to it and to solve the problem of maintaining most of the bioactive components of the food ingredients after being processed with different industrial techniques for production of healthy and tasty food not only tasty food.

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