

## Antioxidant activity of a ginger extract (*Zingiber officinale*)

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Received 4 October 2005; received in revised form 18 April 2006; accepted 7 June 2006

### Abstract

The antioxidant effect and the total phenols of ginger extract were studied. The total phenols of the alcohol extract were found to be 870.1 mg/g dry extract. 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH) scavenging reached 90.1% and exceeded that of butylated hydroxytoluene (BHT), the IC<sub>50</sub> concentration for inhibition of DPPH was 0.64 µg/ml. The antioxidant activity in a linoleic acid/water emulsion system determined by means of thiobarbituric acid reactive substances (TBARS) was highest at 37 °C – 73.2%, and 71.6% when the formation of conjugated dienes was inhibited. At 80 °C the antioxidant activity at the highest concentration of a ginger extract was less efficient: 65.7% for conjugated dienes formation and 68.2% for TBARS. The ginger extract inhibited the hydroxyl radicals 79.6% at 37 °C and 74.8% at 80 °C, which showed a higher antioxidant activity than quercetin. The IC<sub>50</sub> concentration for inhibiting OH· at 37 °C was slower than that at 80 °C – 1.90 and 2.78 µg/ml, respectively. The ginger extract chelated Fe<sup>3+</sup> in the solution.

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**Keywords:** *Zingiber officinale*; Ginger extract; CO<sub>2</sub> extract; DPPH; OH·; Chelating capacity

### 1. Introduction

The auto-oxidation of fats is a big problem because of the deterioration in the quality of the foods in which they are contained and the reduction in their nutritional value (Esterbauer, 1993; Kubow, 1992). In addition, the oxidation of polyunsaturated fatty acids in biological membranes leads to serious damage such as coronary atherosclerosis, emphysemas, cancer and cirrhosis. Safeguarding fats against oxidation is normally done by restricting the access of oxygen or adding antioxidants. The most commonly applied antioxidants are synthetic phenols, such as, butylated hydroxytoluene and butylated hydroxyanisole (BHA). Their safety, however, is doubtful (Imadia et al., 1983). The initiation of the lipid peroxidation is by the superoxide radical or by hydroxyl radicals. For this reason, antioxidation is an extremely significant

activity which can be used as a preventive agent against a number of diseases (Aruoma, 1994; Basaga, 1990; Halliwell & Chirico, 1993). Therefore, attention is focused on natural antioxidants. These antioxidants are polyphenol compounds (Helle & Grete, 1995; Yen, Chang, & Su, 2003), which are found in all plants and in all parts of the plants (tree bark, stalks, leaves, fruits, roots, flowers, pods and seeds) (Aruoma et al., 1995; Kim, Kim, Kim, & Heo, 1997).

Ginger (*Zingiber officinale* (L.) Rosc) has been used as a spice for over 2000 years (Bartley & Jacobs, 2000). Its roots and the obtained extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen, Kuo, Wu, & Ho, 1986; Herrmann, 1994). Although the digestion stimulating effect of this spice became known a long time ago, the stimulating effect on peptic juices, such as gastric juice, bile, pancreatic and intestinal juices, was discovered later. Bile acids play a major role in the uptake of fats and each upset in the metabolism of fats would impede food digestion as a whole, because the fatty particles cover the other food

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elements and make them inaccessible for the action of the digestive enzymes. Lipase is the other key factor which plays a vital role in fat digestion. When ginger was included in animal diets, it was found that there was a considerable increase in the pancreatic and intestine lipase (Platel & Srinivasan, 2000).

Although various extracts are obtained from ginger (Chen et al., 1986; Rehman, Salariya, & Habib, 2003), it is the CO<sub>2</sub> extracts that are richest in polyphenol compounds and have a composition that is closest to that of the roots (Bartley & Jacobs, 2000; Chen et al., 1986). Methods for the characterization of antioxidants are presented and illustrated by their application to commercial ginger preparations, since it has been widely speculated that ginger might be beneficial to human health because it exerts ‘antioxidant activity’ (Aruoma et al., 1997). Ramanathan and Das (1993) also tested the antioxidant activity of some natural systems, including fresh spices in raw and cooked fish samples. Regardless of the numerous studies dedicated to this area, comprehensive investigations of the antioxidant properties of CO<sub>2</sub> ginger extracts are not available in the literature.

The purpose of this study was to assess the antioxidant activities of the CO<sub>2</sub> ginger extract with regard to the free radical 2,2-diphenyl-1-picryl hydrazyl, as well as with regard to the lipid peroxidation and pro-oxidant activity with regard to hydroxyl radicals at body temperature (37 °C) and at 80 °C.

## 2. Materials and methods

### 2.1. Preparation of extract

The air-dried roots of ginger from Vietnam were ground separately in an attrition mill to a size of 0.15–0.25 mm and the oleoresins obtained by a high-pressure CO<sub>2</sub>-extractor. As main compounds of the oleoresins,  $\alpha$ -zingiberene (36.9%),  $\beta$ -sesquiphellandrene (15.3%),  $\beta$ -bisabolene (8.8%), (E,E)- $\alpha$ -farnesene (7.0%),  $\alpha$ -curcumene (6.6%) and caphene (3.2%) were found (Jirovetz et al., 2005).

### 2.2. Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu reagents with analytical grade gallic acid as standard. One millimetre of extract or standard solutions (0–500 mg/l) was added to 10 ml deionized water and 1.0 ml of Folin–Ciocalteu phenol reagents. After 5 min, 2.0 ml of 20% sodium carbonate was added to the mixture. After 1 h in darkness the absorbance at 750 nm was measured. The concentration of total phenols was expressed as mg/g of dry extract (Kim, Jeond, & Lee, 2003).

### 2.3. Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

The radical scavenging ability was determined as described by Mensor et al. (2001). Briefly, one ml from

0.3 mM alcohol solution of DPPH was added to 2.5 ml from the samples with different concentrations of ginger extract. The samples were kept at room temperature in the dark and after 30 min the optic density was measured at 518 nm. The antiradical activity (AA) was determined by the following formula:

$$AA\% = 100 - \left\{ \frac{[(Abs_{\text{sample}} - Abs_{\text{empty sample}}) \times 100]}{Abs_{\text{control}}} \right\},$$

where empty samples – 1 ml ethanol + 2.5 ml from various concentrations of ginger extract; control sample – 1 ml 0.3 mM DPPH + 2.5 ml ethanol.

The optic density of the samples, the control and the empty samples were measured in comparison with ethanol. One synthetic antioxidant, butylhydroxytoluene, was used as positive control.

### 2.4. Evaluation of antioxidant activity in linoleic acid model system

Linoleic acid emulsions were prepared by mixing 0.285 g of linoleic acid, 0.289 g of Tween 20 as emulsifier and 50 ml phosphate buffer (pH 7.2). The mixture was homogenized for 5 min according to Yen et al. (2003). The antioxidant was added at the final concentrations of 0%, 0.005%, 0.01%, 0.02% and 0.05% wt/vol of dry extract, BHT 0.01% was used as control. The mixture was incubated in an oven at 37 and 80 °C for 12 d and 10 h, respectively. The course of oxidation was monitored by measuring the conjugated dienes formation (CD) and thiobarbituric acid reactive substances (TBARS).

The antioxidative activity at the end of assay time was expressed for each indicator as reduction percent of peroxidation (RP%) with a control containing no antioxidant being 0%.

$RP\% = \frac{[(\text{peroxidation indicator value without antioxidant}) - (\text{peroxidation indicator value with antioxidant})]}{(\text{peroxidation indicator value without antioxidant})} \times 100$ . A higher percentage indicates a higher antioxidant activity.

#### 2.4.1. Determination of conjugated dienes formation

Aliquots of 0.02 ml were taken at different intervals during incubation. After incubation, 2 ml of methanol in deionized water (60%) was added, and the absorbance of the mixture was measured at 233 nm. The conjugated dienes concentration was expressed in ml/mg in each sample. The results were calculated as  $CD = B \times \text{vol/wt}$ ; where  $B$  is the absorbance reading, vol denotes the volume (ml) of the sample and wt is the mass (mg) of emulsion measured (Zainol, Abd-Hamid, Yusof, & Muse, 2003).

#### 2.4.2. Determination of thiobarbituric acid reactive substances

A modified thiobarbituric acid reactive substances (TBARS) method was used to measure the antioxidant activity of dry extract in terms of inhibition on lipid

peroxidation. 0.1 ml of sample was taken every day, hour, respectively from the emulsion, the following were sequentially added: 2 ml of the TBA–TCA solution (20 mM TBA in 15% trichloroacetic acid (TCA)). The mixture was heated in a 100 °C water bath for 15 min and cooled at room temperature. After 2 ml of chloroform was added, the mixture was mixed and centrifuged at 2000 rpm for 15 min. The chloroform layer was separated and the absorbance of the supernatant was measured at 532 nm against a blank containing 2 ml TBA–TCA solution. Malonaldehyde standard curves were prepared by 1,1,3,3-tetramethoxypropane and TBARS were expressed as mg of malonaldehyde/kg dry matter.

### 2.5. Detection of hydroxyl radicals by deoxyribose assay

The assay was performed as described by Halliwell, Gutteridge, and Aruoma (1987) with minor changes. All solutions were freshly prepared. One millimetre of the reaction mixture contained 100 µl of 28 mM 2-deoxy-D-ribose (dissolved in  $\text{KH}_2\text{PO}_4$ – $\text{K}_2\text{HPO}_4$  buffer, pH 7.4), 500 µl solution of various concentrations of the ginger extract, 200 µl of 200 µM  $\text{FeCl}_3$  and 1.04 mM EDTA (1:1 v/v), 100 µl  $\text{H}_2\text{O}_2$  (1.0 mM) and 100 µl ascorbic acid (1.0 mM). After an incubation period of 1 h at 37 and 80 °C the extent of deoxyribose degradation was measured by the TBA reaction. 1.0 ml of TBA (1% in 50 mM NaOH) and 1.0 ml of TCA were added to the reaction mixture and the tubes were heated at 100 °C for 20 min. After cooling, the absorbance was read at 532 nm against a blank (containing only buffer and deoxyribose). The percentage inhibition was calculated by the formula:

$$I(\%) = 100 - (\text{Abs.}_{\text{sample}}/\text{Abs.}_{\text{control}}) \times 100.$$

The  $\text{IC}_{50}$  value represented the concentration of the compounds that caused 50% inhibition of radical formation. Quercetin was used as a positive control.

The data obtained at each point were the average of three measurements.

### 2.6. Statistical analysis

The statistical processing of the data obtained from all studies was implemented by means of dispersion analysis with the Sigma Plot 7.0 software. Data are expressed as means  $\pm$  standard deviation (SD). Statistical analysis was performed with Student's *t*-test. A difference was considered statistically significant when  $p \leq 0.01$ .

## 3. Results and discussion

### 3.1. Determination of total phenolic content

The determined total phenolic content of the extract amounted to 871 mg/g dry extract.

### 3.2. Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests used to prove the ability of the components of the ginger extract to act as donors of hydrogen atoms. The obtained results are shown in Fig. 1. The ginger extract showed a significant effect in inhibiting DPPH, reaching up to 90.1% at concentration 20 µg/ml and its  $\text{IC}_{50}$  was 0.64 µg/ml compared with the  $\text{IC}_{50}$  of BHT of 7.02 µg/ml.  $\text{IC}_{50}$  values were with statistical significance  $p \leq 0.01$  and had high regression coefficients of  $R^2 = 0.985$  and  $R^2 = 0.976$ , respectively.

### 3.3. Evaluation of antioxidant activity in linoleic acid model system

Polyunsaturated fatty acids, such as linoleic acid, are easily oxidized by the oxygen in the air. This auto-oxidation leads to the occurrence of chain reactions with the formation of coupled double bonds, and at a later stage also to obtaining secondary products, such as aldehydes, ketones, and alcohols. In order to assay the effect of temperature on the antioxidant effect of the ginger extract, the experiments for inhibiting the peroxidation of the linoleic acid were conducted at two temperatures –37 and 80 °C (Figs. 2 and 3). An intensive formation of conjugated dienes was observed on day 4 of incubation of the linoleic acid at 37 °C, and the maximum was reached on day 10. Of the three concentrations of the ginger extract that were used, the highest antioxidant activity was found at 0.02%, and it was the same as that of the inhibition of the synthetic antioxidant BHT, i.e. 62.9%. The concentrations of the ginger extract 0.05% and 0.01% showed a weaker effect in inhibiting lipid peroxidation.

At 80 °C, a peak in conjugated dienes formation was observed at 5 h of storage, although the level of conjugated dienes was lower than that at 37 °C. Most efficient was again the 0.02% concentration, whose inhibition after 5 h

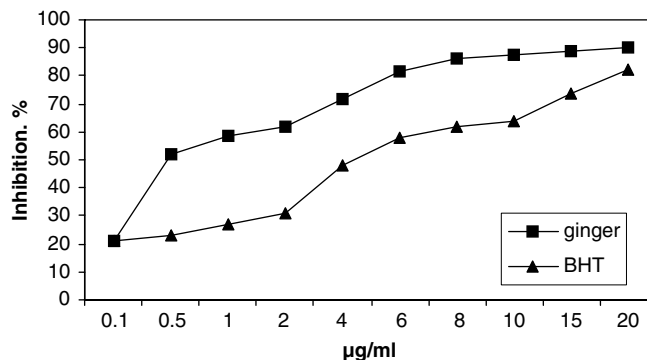


Fig. 1. Scavenging effects of ginger extract on DPPH radical.

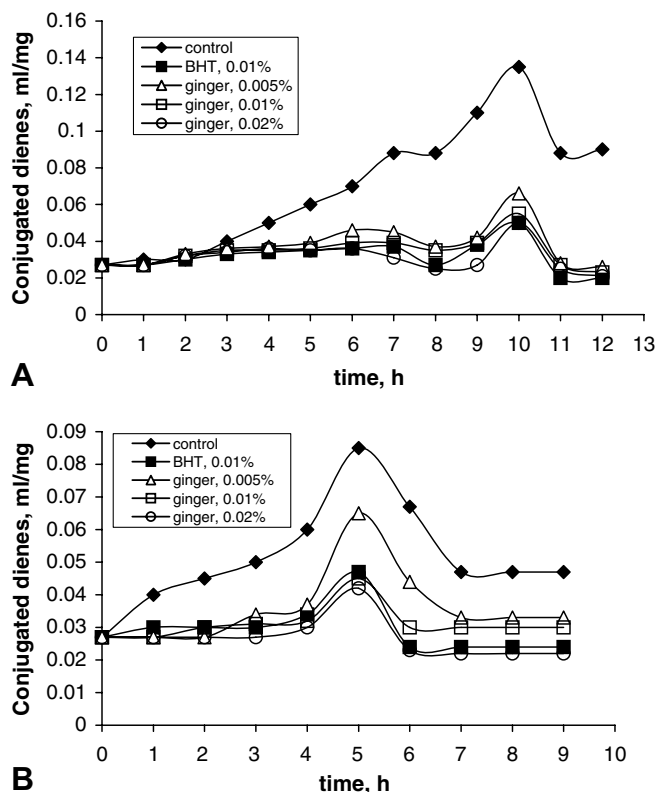


Fig. 2. Effect of ginger extract on conjugated dienes in a linoleic acid/water emulsion system at 37 °C (A) and 80 °C (B).

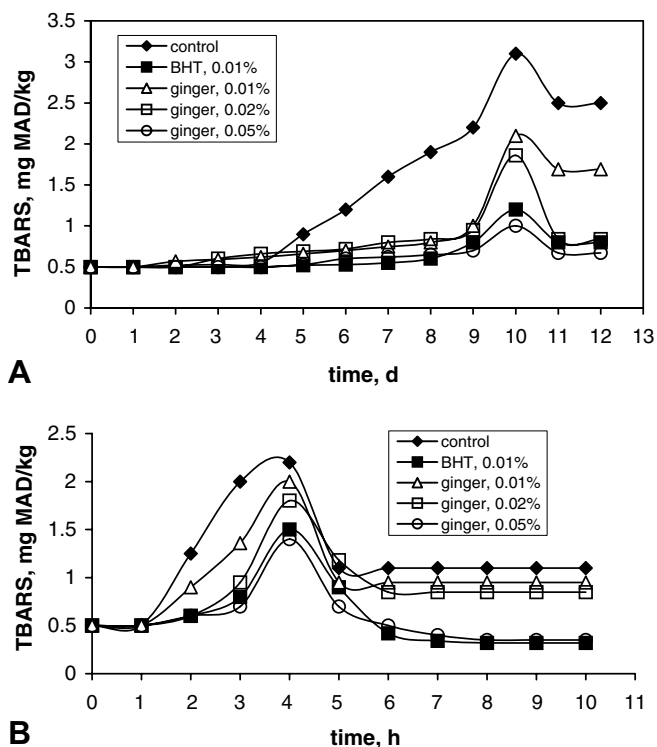


Fig. 3. Effect of ginger extract on TBARS in a linoleic acid/water emulsion system at 37 °C (A) and 80 °C (B).

was higher than that of BHT: 50.5% and 44.7%, respectively. The comparison of the antioxidant activity of the ginger extract for the two temperatures showed a greater value at a lower temperature reaching 71.6% as compared with 69.3% of BHT after 8 d of storage.

The second indicator used to identify lipid peroxidation was TBARS. The storage at two temperatures was conducted until reaching comparable concentrations of malonaldehyde. This assay also showed that the maximum formation of malonaldehyde was found after 10 d at 37 °C and after 4 h at 80 °C (compare Fig. 2 with Fig. 3), i.e. the process developed like the formation of coupled double bonds. It is worth noting that in order to obtain a level of process inhibition comparable with that of BHT, a higher concentration of the ginger extract was needed, viz. 0.05%, i.e. 2.5 times higher than at necessary for inhibiting the conjugated dienes formation. This is probably due to the difference in the activating power of the two processes and is yet another piece of evidence that the formation of coupled double bonds did not precede the formation of degradation products. Such a fact was not mentioned in the publication by Romero, Doval, Stura, and Judis (2004).

As for TBARS, the antioxidant activity of 0.05% ginger extract was higher than that of the control – 67.7% and 61.3%, respectively, after 10 d at 37 °C and 36.4% and 31.8%, respectively, at 80 °C after 4 h.

In both mechanisms of lipid peroxidation (conjugated dienes formation and TBARS), at 80 °C, the results showed lower antioxidant efficiency than those at 37 °C, but nevertheless, a good antioxidant potential was established comparable with that of BHT. In emulsion systems, it was shown by Wettasinghe and Shahidi (1999) that the hydrophobic antioxidants directed towards the fat–water interface prevent the fatty phase from oxidation. Naturally, however, there were also other factors at play that affected the efficiency of the process going on in dispersion systems. The data obtained for inhibiting the lipid peroxidation confirmed the results by Rehman et al. (2003).

### 3.4. Detection of hydroxyl radicals by deoxyribose assay

Ferric-EDTA was incubated with H<sub>2</sub>O<sub>2</sub> and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-D-ribose into fragments that on heating with TBA at low pH form a pink chromogen (Aruoma, Laughton, & Halliwell, 1989; Halliwell et al., 1987). The ginger extract added to the reaction mixture removed the hydroxyl radicals from the sugar and prevented it from degradation. These results are shown in Fig. 4. The effect from inhibiting the hydroxyl radicals was greater at 37 °C rather than at 80 °C – IC<sub>50</sub> at 37 °C was 1.90 µg/ml ( $R^2 = 0.967$ ), while at 80 °C a higher concentration of ginger extract was needed – IC<sub>50</sub> = 2.78 µg/ml ( $R^2 = 0.975$ ). The quercetin used as a control had IC<sub>50</sub> of 4.61 µg/ml ( $R^2 = 0.834$ ) at 37 °C and 7.41 µg/ml ( $R^2 = 0.984$ ) at 80 °C.



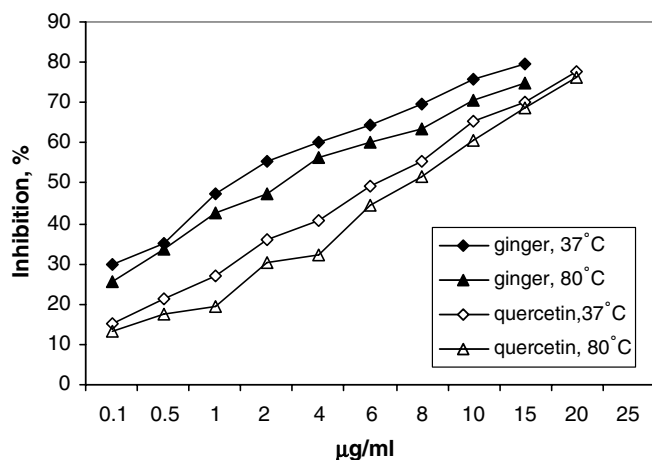


Fig. 4. Inhibition of the radical degradation of 2-deoxy-D-ribose of ginger extract and quercetin.

When a model system of  $\text{Fe}^{3+}$  and  $\text{H}_2\text{O}_2$  was used in the absence of ascorbate, a low rate of generation of hydroxyl radicals was observed. The addition of ascorbic acid considerably increased the rate of generation of  $\text{OH}^\cdot$  by converting  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$  (Table 1). The results showed that the ginger extract is a powerful  $\text{OH}^\cdot$  scavenger in competition with 2-deoxy-D-ribose. This analysis was also used to investigate the capacity of the ginger extract to inhibit the metal-ion dependent generation of  $\text{OH}^\cdot$ , and not only for the capacity to capture the released radicals. When  $\text{Fe}^{3+}$  ions were added to the reaction mixture, some of

Table 1  
Effect of ginger extract on deoxyribose degradation by  $\text{OH}^\cdot$

Additions	$A_{532} \pm \text{CD}$	% Inhibition
$\text{Fe}^{3+} + \text{H}_2\text{O}_2 + \text{DR}$	$0.816 \pm 0.007$	–
$\text{Fe}^{3+} + \text{ginger extract}^+ + \text{H}_2\text{O}_2 + \text{DR}$		
3.0 µg/ml	$0.762 \pm 0.006$	6.6
4.0 µg/ml	$0.737 \pm 0.005$	9.7
5.0 µg/ml	$0.688 \pm 0.002$	15.7
6.0 µg/ml	$0.677 \pm 0.003$	17.1
$\text{Fe}^{3+} + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$	$0.919 \pm 0.008$	–
$\text{Fe}^{3+} + \text{ascorbat} + \text{ginger extract} + \text{H}_2\text{O}_2 + \text{DR}$		
3.0 µg/ml	$0.691 \pm 0.005$	24.9
4.0 µg/ml	$0.651 \pm 0.004$	29.2
5.0 µg/ml	$0.615 \pm 0.002$	33.1
6.0 µg/ml	$0.568 \pm 0.001$	38.2
$\text{Fe}^{3+} + \text{EDTA}^* + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$	$0.848 \pm 0.007$	–
$\text{Fe}^{3+} + \text{EDTA}^* + \text{ginger extract} + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$		
5.0 µg/ml	$0.507 \pm 0.002$	40.4
6.0 µg/ml	$0.432 \pm 0.001$	49.1
$\text{Fe}^{3+} + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$	$0.919 \pm 0.008$	–
$^a\text{Fe}^{3+} + \text{ginger extract} + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$		
5.0 µg/ml	$0.511 \pm 0.002$	44.4
6.0 µg/ml	$0.430 \pm 0.001$	53.2
$^b\text{Fe}^{3+} + \text{EDTA} + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$	$1.51 \pm 0.008$	–
$\text{Fe}^{3+} + \text{EDTA} + \text{ginger extract} + \text{ascorbat} + \text{H}_2\text{O}_2 + \text{DR}$		
5.0 µg/ml	$0.573 \pm 0.003$	62.1
6.0 µg/ml	$0.537 \pm 0.002$	64.4

EDTA\* was present in the reaction mixture before  $\text{Fe}^{3+}$  was added.

<sup>a</sup>  $\text{Fe}^{3+}$  – 5 min after the mixing of  $\text{Fe}^{3+}$  and ginger extract, the rest of the components were added.

<sup>b</sup>  $\text{Fe}^{3+}$  and EDTA were previously mixed.

them formed a complex with deoxyribose. Only those molecules which could chelate Fe ions and render them inactive could inhibit the degradation of deoxyribose. The results obtained clearly showed that when  $\text{Fe}^{3+}$  was complexly bound with EDTA, the deoxyribose degradation was not changed, evidence of which was the higher degree of inhibition, which was due to the metal chelating properties of the ginger extract.

In this case, the ginger extract had not only scavenging, but also a higher chelating capacity as compared with other similar systems earlier mentioned in the literature (Basaga, Poli, Tekkaya, & Aras, 1997).

The conducted in vitro tests show that the ginger extract can control the quantity of free radicals and the peroxidation of lipids. Thus, it could prevent or decrease the damage in a human body caused by free radicals, which, according to Aruoma et al. (1997) and Valko et al. (2004), attack biological macromolecules such as lipids, proteins and DNA. By averting the oxidation of the phospholipids of cell membranes, the specific cell permeability is preserved and the cell metabolism is not disturbed. Protecting the deoxyribose from aggressive  $\text{OH}^\cdot$  radicals ginger extract could prevent from DNA damage.

Although all organisms possess antioxidant systems for protection from oxidative damage, these systems are insufficient to prevent all possible damage. That is why the interest towards the inclusion of nontoxic antioxidant flavanoids and polyphenols in the human diet has become greater. Apart from the studied antiradical activity, ginger extract has antibacterial activity (Jirovetz et al., 2005) and can promote the increase of the pancreatic lipase (Platel & Srinivasan, 2000). The combination of nutritional and medicinal benefits can determine ginger extract as a functional food. Consumption of ginger extract may be proven beneficial in attenuation of atherosclerosis development, since it is associated with reduced macrophage-mediated oxidation of LDL, reduced uptake of oxidized LDL by macrophages, reduced oxidative state of LDL and reduced LDL aggregation (Fuhrman, Rosenblat, Hayek, Coleman, & Aviram, 2000). All these effects lead to a reduced cellular cholesterol accumulation and foam cell formation, the hallmark of early atherosclerosis.

Ginger is an indispensable component of many food additives. With regard to antioxidant properties of ginger extract established in this work, it can be successfully used as a component of curry powder, sauces, ginger bread and ginger flavoured carbonated drinks and in preparation of dietaries for its aroma and flavour. Many authors discuss the chemical composition of ginger (Bartley & Jacobs, 2000; Nishimura, 1995; Sakamura & Hayashi, 1978; Smith & Robinson, 1981), but no toxic effect was found.

Natural antioxidants have great impact on the safety and acceptability of the food system and will continue to do so. Not only do they keep the food stable against oxidation but can also be effective in controlling microbial growth. By increasing the inherent levels of the antioxidants in animal products through dietary supple-

mentation, we are providing a more consumer acceptable product. This area of research is exploding in the literature. However, the traditional practice of adding antioxidants during processing can still play a very important role since the added compounds have the potential for enhancing the activity of the inherent antioxidants systems. More work is needed to define the optimum dietary combinations and/or the minimum levels of the compound in the food necessary for obtaining the greatest stability in the resultant product. This may involve defining interactions of dietary components on the uptake on the desired compounds; this will eventually require more sophisticated feed formulations and a better understanding of the nutrient impact of the by-products that are traditionally used as food.

#### 4. Conclusion

The CO<sub>2</sub> extract from ginger has high polyphenol content. It manifested a very good scavenging of DPPH and reduced its reducing capacity. The extract can be used as an antioxidant at an earlier stage of fat oxidation.

The ginger extract showed an antioxidant activity comparable with that of BHT in inhibiting the lipid peroxidation both at 37 °C, and at a high temperature of 80 °C. Most inhibited was the stage of formation of secondary products of the auto-oxidation of fats.

The ginger extract also showed an inhibiting effect with regard to the hydroxyl radicals, better than that of quercetin at both temperatures in the study. Under the conditions of conducted experiments the polyphenols in the ginger extract also demonstrated a higher chelatoforming capacity with regard to Fe<sup>3+</sup>, leading to the prevention of the initiation of hydroxyl radicals which are known inducers of lipid peroxidation.

The properties of the ginger extract under study, compared with the synthetic antioxidant, determine its potential as a natural preservative, applicable in the food and pharmaceutical industries.

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