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STUDY ON THE INFLUENCE OF HEAT TREATMENTS ON THE ANTIOXIDANT PROPERTIES OF GINGER

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Abstract: *The purpose of this paper is to study the influence of thermal treatments such as refrigeration, freezing, boiling, and drying on the antioxidant properties of ginger. The determination of antioxidant activity was performed by the spectrophotometric method with the DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) and the photometric dosing of the polyphenols by the FOLIN-CIOCALTEU method. The vitamin C concentrations were also determined by HPLC as well as the spectrophotometric β-carotene content. The analyses performed showed that thermal processes have a significant influence on the antioxidant capacity of ginger. In the drying process there occurs an increase in antioxidant capacity, and in the case of frozen ginger the polyphenol content is higher than in the fresh form or aqueous extract. Also, the content of β-carotene and vitamin C was higher in the case of dried ginger than in the refrigerated one.*

Key words: *ginger, antioxidant capacity, β-caroten, C vitamin*

1. Introduction

A quarter of the antioxidants discovered to date are found in ginger. The major source of antioxidants in ginger is represented by: flavonoids, polyphenols (gingerols, shogaols, and catechins), tannins, vitamin C, and beta-carotene. The chemical composition of fresh ginger varies depending on the cultivation mode, the location where it is cultivated, the extraction methods, and the processing methods [1]. A proximate composition of fresh ginger rhizome is: moisture 80.9%, crude protein 2.3%, fat 0.9%, mineral 1.2%, crude fibre 2.4%, and carbohydrates 12.3%. With such a composition, ginger root is an excellent source of nutrients the body needs and active principles with remarkable therapeutic properties for health [2], [3]. Ginger active compounds are volatile oils, 1% -3% (bisabolen, zingiberene, zingiberol), non-volatile oils represented by spicy oleoresin components [4], and phenolic compounds (gingerol and (6)-shogaol), and curcuma, geraniol, gingersulfonic acid, [5] etc.

The compounds that give the specific taste are gingerol, (6)-shogaol and zingerone. Gingerol is the active ingredient of fresh ginger, with many therapeutic effects. Gingerol has got anti-inflammatory, anti-platelet, antibacterial and anti-tumor properties which are remarkable [6]. Thus, by administering an amount of about 500mg / kg body of ginger, the level of prostaglandins was significantly lower, these results suggesting that ginger could be used as an anti-thrombotic and anti-inflammatory agent [7], [8] and as a reducer for the platelets deposition on the blood vessels. Ginger exerts many direct

and indirect effects on blood pressure and heart rate [9]. Among the beneficial effects on body health, the most appreciated and highlighted ones are: it is a powerful vasodilator antioxidant-by attenuating or preventing free radical formation, immuno-stimulating by increasing thymus and spleen activity, and production of phagocytes, stimulating the digestive system by increasing the secretion of protective mucus of the gastric mucosa that prevents the occurrence of gastric or duodenal ulcers. It has also antiviral and antifungal properties, while being considered one of the most rewarding cancer enemies [10], even considered to have anti-tumoral effect in leukemia [11], [12].In vitro studies have shown that gingerol present in the root of ginger inhibits the development of *Helicobacter pylori*, the major cause of ulcer, gastric and colon cancer [13]. The ginger root has been shown to have antiemetic effect, reduce nausea and post-operative vomiting or chemotherapy effects due to the presence of gingerol [14]. It has effects of calming and reducing migraines [15]. The mechanism of ginger action in the control of diabetes is due to the inhibition of carbohydrate-metabolizing enzymes, alpha-amylase and alpha-glucosidase, inhibition produced by gingerol, with an effect in reducing glucose absorption [16] [17]. An important study highlighted the antimicrobial action of ginger against *E. coli, Salmonella typhi* and *Bacillus subtilis* [18], [19], [20] and the ethanolic ginger extract showed the largest inhibition zone against *Salmonella typhi*, it showed antibacterial activity against periodontal bacteria such as *Candida albicans* [21], [22], [23], [24] and inhibition of bacteria of the genus *Mycobacterium avium* and *Mycobacterium tuberculosis* in vitro. Ginger"s root has a strong antibacterial activity against Gram-negative and Gram-positive bacteria such as Klebsiella *pneumoniae, Proteus vulgaris, Streptococcus pyogenes* and *Staphylococcus aureus* that can be compared with antibiotics such as trimethoprim, chloromphenicol, gentamicin and erythromycin [25]. Like other antioxidants and β-carotene shows sanogenic characteristics for the body in the prevention of certain forms of cancer, heart disease, cataracts, Alzheimer's disease, depression, epilepsy, migraines, stomach burns, hypertension, infertility, rheumatoid arthritis, schizophrenia and skin disorders[26]. The antioxidant properties of ascorbic acid are explained by the fact that vitamin C acts as an electron donor. Thus, it donates electrons to a series of enzymes that act in the body, part of them participate in the hydrolysis of collagen, others interact in the metabolism of tyrosine, and another category is needed in norepinephrine biosynthesis [27]. Stability studies of the active compounds with antioxidant properties in the various stages of raw material processing are required to ensure the effectiveness of the finished product. Processing techniques involving extraction solvent, a certain pH, light and heat, can significantly influence the levels and efficacy of bioactive compounds in dietary supplements [28].

2. Materials and Methods

Ginger was purchased from local market. All reagents used were of analytical grade from the Sigma Aldrich and Merck. All analysis were done in triplicate for each of the samples, fresh ginger, oven dried, refrigerated ginger and frozen ginger.

Determination of antioxidant activity of ginger by DPPH method

DPPH (2,2-diphenyl-1-picrylhydrazyl) is one of the most stable organic nitrogen radicals with a maximum absorption in UV-VIS at 517 nm. 2.5 g of each sample

to be tested was brought into a 50 ml volumetric flask, which was added over a concentration of 99.8% methanol. Samples were stirred for 15 minutes, after which they were filtered. The absorbance of the 0.004% DPPH standard solution in methanol was read and then the absorbance values of the samples. Percent inhibition of free radicals (I%) is calculated by comparing with the DPPH solution, with the relation(1):

$$\mathbf{I\%} = \left[\frac{\mathbf{A_{etalon}} - \mathbf{A_{probă}}}{\mathbf{A_{etalon}}}\right] \times \mathbf{100}$$

(1)

I% - inhibition percentage of radicals;
A_{etalon} - absorbance of the standard sample;
$A_{probă}$ - absorbance of the analyzed sample.

Total polyphenol content.

The total phenolic content of the extract was determined by the Folin–Ciocalteu method.

The principle of this method is to reduce the mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid($H_3PMo_{12}O_{40}$), in the presence of phenols in a basic medium, to yield the blue oxides of tungsten (W_8O_{23}) and molybdenum($Mo_{12}O_{23}$). Polyphenols, due to their redox properties, can be oxidized by the Folin-Ciocalteu reagent, resulting in a blue color whose maximum absorption is 750nm. 1 mL of the liquid sample (obtained by ethanol extraction of 2.5 g sample), diluted 1/5, 50 mL distilled water, is mixed with 5 mL of Folin-Cobalt reagent with stirring and 20 mL of 20% Na_2CO_3 20%solution. After standing for 30 minutes at room temperature, in the dark, the absorbance of the mixture was measured at 750 nm using a double beam UV-VIS-NIR Spectrometer 3600 Shimadzu. In order to obtain the calibration curve, gallic acid solutions were used in the following concentrations: 0; 0.2; 0.4; 0.6; 0.8; 1 g / L. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

The Folin-Ciocâlteu (Fc) index is calculated by the relation (2):

$$F_c = A_{750} \times d \times 100$$

(2)

were:

F_c –Folin-Ciocâlteu index;
A_{750} – the absorbance at 750 nm;
d – sample dilution;

Determination of Vitamin C by High Performance Liquid Chromatography - HPLC.

4 grams of ginger were milled with 12 ml of acid solution (10% perchloric acid + 1%, a o-phosphoric acid). The preparation obtained was transferred into a volumetric flask of 50 ml, the mixture is stirred vigorously for 2 minutes and subsequently filled to the mark with mobile phase, phosphate buffer solution (TFA) to a pH of 3.5. The standard solution of ascorbic acid has the concentration of 1000 mg / l, and for calibration curve plotting standard solutions were prepared at concentrations of 300, 200, 100, 75, 50 and 25 mg / l ascorbic acid respectively HPLC-LC-10ADVP– Shimadzu. is provided with automatic injector and UV-VIS detection system (DAD) and an analytical column with a reverse phase C18 type, length of 250 mm and diameter of 4.6 mm filled with particles of 5 μm diameter.

Determination of carotenoids content

7.5 g of ginger were milled, over which cyclohexane is added to 25 ml. After extraction the resulting liquid is used to determine the carotenoids, by measuring the absorbance at UV-VIS spectrophotometer at a wavelength of 470

nm. The carotene concentration is calculated using the relationship (3):

$$\left[carotenoids \ \frac{mg}{kg} \right] = \frac{A_{470} \times 10^3 \times 25}{2000 \times 7.5} \quad (3)$$

were :

A_{470} – sample absorbance measured at 470 nm.

3. Results and discussion

Quantities of 210, 150, 90, 30, 10 g of frozen, refrigerated, boiled, tea and dried ginger were analyzed. The analyzed samples have the composition shown in Table 1.

Table 1.
The component of samples to be analyzed

Composition (µl)	**1**	**2**	**3**	**4**	**5**
Analyzed sample	210	150	90	30	10
Methanol	2790	2850	2910	2970	2990
DPPH 0,004 %	500	500	500	500	500

From the point of view of the heat treatment of the ginger rhizome, it is observed that the ginger subjected to the drying has the greatest inhibition capacity of radicals, followed by the refrigerated, boiled, frozen one and ginger in the form of the tea with the smallest percent of inhibition rates, as shown in the Table 2.

Table 2.
Percent inhibition of ginger samples

Sample/% inhibition	I_{210}	I_{150}	I_{90}	I_{30}	I_{10}
Refrigerated	93.76	91.72	91.18	89.42	88.33
Frozen	91.59	91.11	90.91	90.64	89.83
Boiled	93.01	92.61	90.50	89.55	89.15
Tea	89.01	88.61	88.61	88.60	88.54
Dried	98.77	98.57	98.30	97.96	96.61

The high antioxidant activity in the case of dried ginger is due to the concentration process by which water is removed, which determines the concentration of the active substance. This high activity for the dried ginger was shown in the study conducted by R. Offei - Okyne according to which, ginger dehydrated by oven had the highest antioxidant power to reduce iron. Also, some of the ginger antioxidants are heat-resistant, which is also explained by the rather high antioxidant capacity of boiled ginger.

According to the results, it can be noticed that thermal treatments influence the phenolic compounds content as shown in Figure 1.

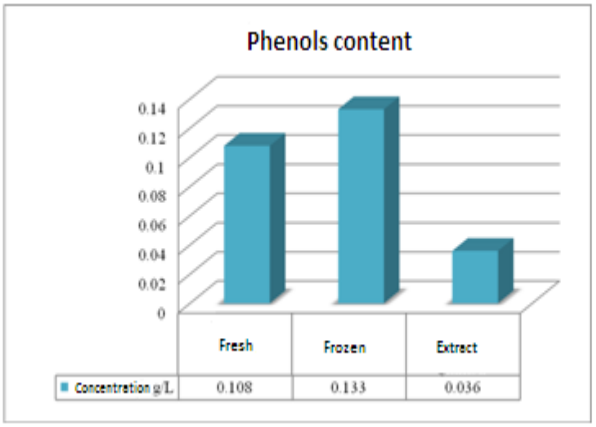

Fig. 1. Polyphenol content in ginger

It is noticed that the content of phenols is higher in frozen ginger than in the fresh one. This has been demonstrated by R. Offe-Okyne in his study, according to which frozen ginger presented the highest content of phenols and flavonoids as compared to dehydration in the oven ginger, dried ginger, ginger under the influence of solar heat and fresh ginger. Also, in the liquid in which the ginger has been boiled, the phenolic compounds pass in a very small amount, due to their reduced solubility in water.

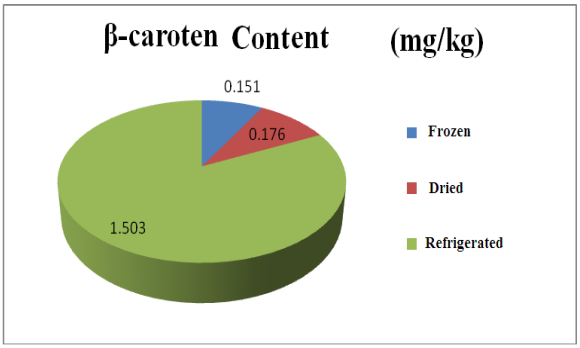

Fig. 2. β-caroten content in ginger

The largest share of β-carotene, as shown in the Figure 2, shows dried ginger, while the difference between refrigerated and frozen ginger is insignificant. The high content of β-carotene for dried ginger, as compared to the refrigerated and fresh one is due to the dehydration process of ginger, whereby water is removed from the product, this one being concentrated in the active substances. The variation in the amount of ascorbic acid for the three types of ginger: refrigerated, frozen and dried ginger is shown in Figure 3.

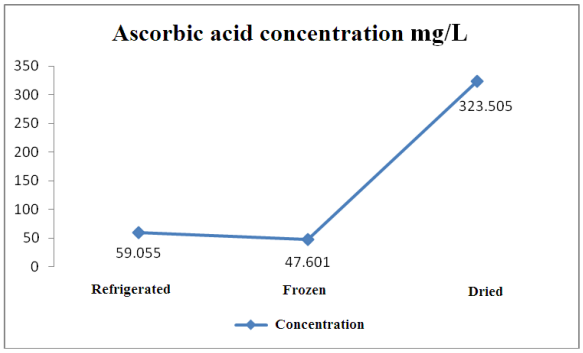

Fig. 3 . Variation of ascorbic acid concentration in the analyzed samples

As can be seen from Figure 3, the ginger dried up has the highest amount of ascorbic acid, while the difference between the refrigerated and the frozen one is very small. The destruction of vitamin C during the freezing depends very much on the freezing time and freezing temperature, freezing in this case was carried out in a short period of time of about 29 days.

4. Conclusions

According to the performed analyses, it can be appreciated that the thermal processes have a significant influence on the antioxidant capacity of ginger. Thus, the drying process by dehydration, produces an increase in antioxidant capacity, and the frozen ginger has a higher polyphenols content than the fresh or aqueous extract. Also, the content of β-carotene and vitamin C was much superior in the case of dried ginger than the refrigerated one.

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