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SHORT COMMUNICATION

THE IMPACT OF PESTICIDES ON BIOACTIVE MOLECULES IN VEGETABLES

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Abstract: Organic agriculture is a production system that, due to the very low or absent use of pesticides, has as its main advantage the absence of the risk of transmitting toxic substances to the population. Another advantage of organic agriculture over conventional agriculture is the reduction of the impact of pesticides on the environment.

In this work, the metabolic changes presented by three selected organic and conventional vegetables were analyzed (pepper, parsley and ginger) using redox titration and enzymatic methods, with a focus on fresh products. The results show increased accumulations of reactive oxygen species (ROS) and, implicitly, a very high activity against oxidative stress, in the case of the three vegetables from conventional agriculture.

Therefore, increasing organic farming practices can largely eliminate both the risks posed by dietary exposure to pesticides and the negative impact on the environment. Encouraging farmers to adopt organic farming in parallel with developing policies to support and supply supermarkets with ecological food.

Keywords: *ascorbate oxidase, catalase, iodometry, oxidative stress, permanganatometry, vitamin C*

INTRODUCTION

Global vegetable production has increased in recent years, reaching 1,186,682,489 tons in 2023, 1.31 % more than in the previous year [1]. In Europe, production was 62.2 million tons, an increase of 6 % compared to the previous year, with Romania taking 10th place in vegetable production [2]. Although intense debates are taking place at the European Union level and regulations have been adopted [3, 4] regarding the marketing of pesticides, as well as the maximum level of residues that can be found in vegetables, pesticide sales have not decreased significantly, registering a value of 350,000 tons per year [5].

From a legal point of view [1] pesticides are considered any substance that acts as a herbicide, insecticide or fungicide. They can enter the human body both through ingestion of food and drinks, approximately 80 - 85 % [6, 7], and through breathing or absorption through the skin. The accumulation of such substances in the human body following the consumption of food from conventional agriculture can be the basis for the occurrence of various diseases, which has led to the application of organic agriculture on increasing areas. Production yields in organic systems are lower, on the other hand, vegetables have a very low or absent content of synthetic pesticides compared to those from conventional agriculture. In recent years, there has been a strong preference for the consumption of foods from "organic agriculture", especially in the diets of children, people with certain conditions, but also other consumers in general [8].

Based on the trend of vegetable consumption, the present study aimed to analyze some vegetables recognized for their vitamin C content, a vitamin also known as the "anti-oxygen vitamin" due to its behavior towards free radicals.

Thus, antioxidants, such as vitamin C, protect the body by capturing free radicals and reactive oxygen species, thus preventing disruption of the chemical stability of cells [9, 10]. However, oxidative stress is produced by prolonged exposure to pesticides, an exposure that produces an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacities of a biological system [11, 12]. Basically, oxygen species are converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), and from there into H_2O and O_2 by ascorbate oxidase (AO) and catalase (CAT) [13].

Thus, pepper (*Capsicum annuum L.*), parsley (*Petroselinum crispum L.*) and ginger rhizome (*Zingiber officinale L.*) were taken into the analysis. These vegetables represent important sources of bioactive molecules with a role in maintaining a healthy metabolic function due to high levels of vitamin C, antioxidants, dietary fiber and minerals [14 – 17]. This study evaluates the compositional differences of pepper, parsley and ginger from conventional and organic crops, from the perspective of antioxidant activity.

MATERIALS AND METHODS

Sample collection

Vegetable samples were collected from supermarkets, markets, fields (vegetable farms and small vegetable producers) taking into account the type of crop they come from, conventional or organic. Pepper and parsley from both organic and conventional farming were collected from farmers in Bacau County, Romania (pepper and parsley) and from

supermarkets, and ginger was purchased only from supermarkets because it is not grown in Romania. In this regard, the label marking with “organic product” was followed.
For vegetables harvested from the field, two to four samples were collected from each field, packaged, labeled and placed in a container in the shade, and subsequently kept cold until analyzed, and vegetables from supermarkets were collected from the main markets in Bacau County, with samples being collected from 4 different areas of the stall. In this case too, the inscription on the label as a product from conventional or organic agriculture was taken into account.

Sample preparation

Samples collected from the field or supermarket were sampled using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method as indicated in the AOAC Official Method 2023 [18] and adapted. Thus, equal samples of each type of vegetable (0.2 - 0.5 kg) were thoroughly chopped and homogenized using a high-speed multifunctional chopper. Freshly prepared samples were used for each determination.

Determination of vitamin C

The concentration of ascorbic acid in vegetables was determined by the AOAC iodometric method [7, 19]. A solution of meta-phosphoric acid (30 %) and acetic acid (7.98 %) was added to the vegetable sample and centrifuged at 4000 rpm, and the supernatant was used for titration. The titration was performed with a sodium thiosulfate solution (0.05 mM) and a starch indicator.

$$\text{Concentration of vitamin C (mg/100g)} = 25y/b$$

where b is the volume (mL) from the titration of the standard ascorbic acid solution. y = volume (mL) from the titration of the sample mixture.

Antioxidative Metabolism

Catalase activity (CAT) was determined by recording the disappearance of H_2O_2 from one gram of product by the permanganometry method. The sample was shaken and then 5 mL of 1.5 $mol \cdot L^{-1}$ H_2SO_4 were added. After that, the solution was filtered and titrated using 0.05 $mol \cdot L^{-1}$ KMnO4.One enzyme unit was calculated as the amount of enzyme that catalyzed the consumption of 1 µmol of H_2O_2 per g of vegetable per minute [20].
Ascorbate oxidase (AO) is an enzyme that catalyzes the oxidation of ascorbic acid to dehydroascorbic acid. Ascorbate oxidase activity is determined by the iodometric method [21]. The extract was treated with ascorbic acid for 30 minutes after which the enzyme was inactivated. Subsequently the sample was treated with, titrated with potassium iodate, and the excess iodate was titrated with 0.001 N sodium thiosulfate. The same procedure was applied to a blank vial, where the enzyme was inactivated. Ascorbate oxidase activity was expressed in mg of oxidized ascorbic acid from one gram of vegetable product.
Analyzes were performed in triplicate.
All reagents used were analytical purity.

RESULTS AND DISCUSSION

Vitamin C content in conventional and organic vegetables

The vitamin C content of vegetables varies in the order: pepper < ginger < parsley. The vitamin C content increases in the same order in conventional vegetables compared to organic ones (Figure 1). Thus, accumulations of 20 % for pepper, 10 % for parsley and 8 % for ginger were found in conventional versus organic farming. The determinations were performed in triplicate and the standard deviation (SD) was calculated for them. A SD value below 1 % represents a very precise result, and between 1 - 5 % represents a superior, good precision. The vast majority of vitamin C determinations fell within the SD below 1 %.

The increase in vitamin C content in vegetables grown in a conventional system, through the use of synthetic pesticides and intensive fertilization, is a metabolic response to the toxin found in the applied pesticide [22].

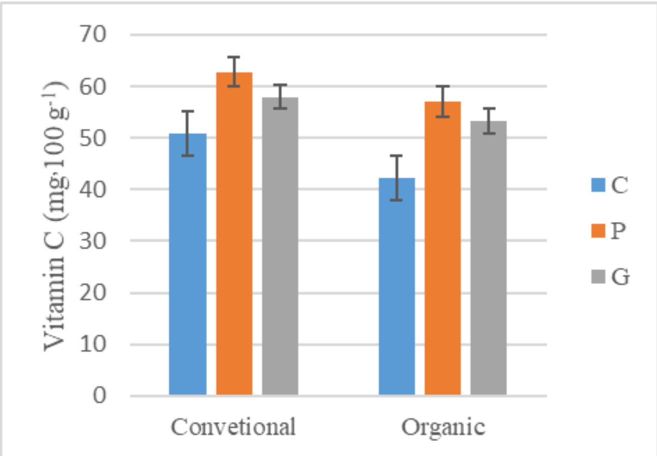

***Figure 1**. Vitamin C content in vegetables from conventional and organic farming*

Specialized literature highlights the additional accumulation of metabolites such as proteins, carbohydrates and other compounds such as vitamin C under stress conditions [7, 23], with the role of reducing the harmful effects of pesticides. Having a role in the capture of reactive oxygen species (ROS), which are formed in large quantities in the presence of pesticides, it can explain the increased level of vitamin C in the case of vegetables from conventional agriculture.

Basically, vitamin C acts together with CAT and AO in combating oxidative stress, reducing lipid peroxidation caused by pesticides and can also participate in a redox cycle with vitamin E, reducing lipid peroxidation radicals and attenuating oxidative stress through the key antioxidant system of the plant cell, the glutathione-ascorbate cycle [22,24].

Antioxidative Metabolism

At the metabolic level, pesticides, due to their toxicity, produce oxidative stress, which leads to the accumulation of ROS in plant tissue, such as O^{2-}, O_2, OH^- and H_2O_2 [23].

The accumulation of ROS results in the damage of proteins, lipids and other substances, causing biochemical and physiological damage.
In this situation, the plant activates enzymatic antioxidant defense mechanisms, through superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and catalase (CAT) [26,27].
The use of pesticides in conventional agriculture has the effect of increasing catalase activity by 22 % for pepper, 16.3 % for ginger and 11 % for parsley compared to vegetables from organic systems (Figure 2). The differences in catalase activity are due to both the type of vegetable and the pesticide [22].

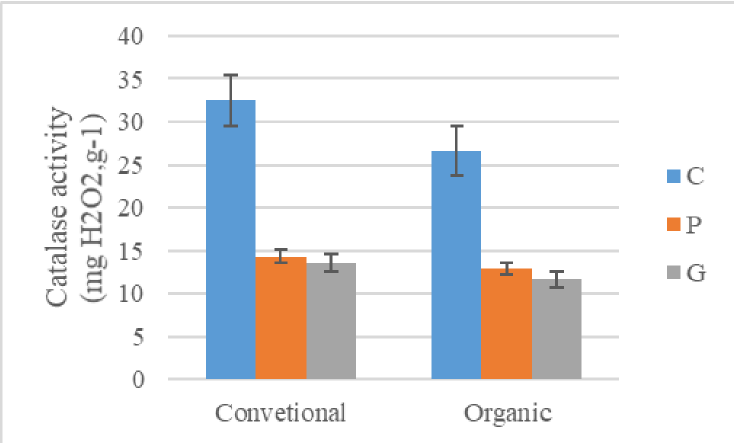

Figure 2. *Catalase activity in vegetables from conventional and organic farming*

Similarly, increased ROS production in response to exposure to pesticides and intensive fertilization to increase agricultural productivity has led to increases in AO activity.
AO activity is directly related to the amount of ascorbic acid in the tissue. A low level of ascorbic acid causes AO inactivation, which is a self-regulating mechanism against oxidative damage [28]. It can be seen (Figure 3) that AO is much more intense in the case of parsley from the conventional system (46.3 %) compared to pepper and ginger which show activities approximately 30 % higher than the organic ones.

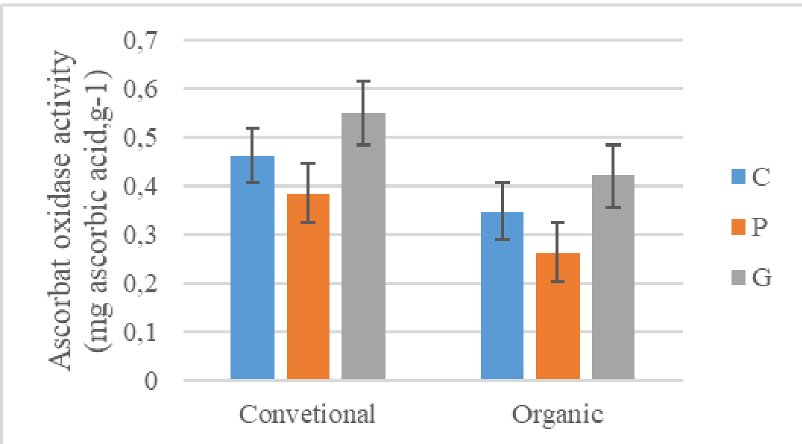

Figure 3. *Ascorbate oxidase activity in vegetables from conventional and organic farming*

The response of ascorbate peroxidase activity correlates with the vitamin C content of the corresponding plants (Figure 1), confirming that there is a constant balance between ROS and the antioxidant system [29]. This balance consists of the production of metabolites of the oxidant system to eliminate ROS from plant cells to prevent cellular damage.

CONCLUSIONS

AO can be considered an enzyme sensitive to the presence of ROS and a trigger factor of the defense system against oxidative stress.

Increased concentrations of vitamin C cause the activation of AO which recognizes free radicals more quickly, as proven by the much higher enzymatic activities of AO than of CAT in vegetables from conventional agriculture. Moreover, although the vegetables came from the EU where the legislation is very strict regarding the use of pesticides, considerable differences were highlighted regarding oxidative stress and implicitly on the physiological and biochemical deterioration of vegetables from conventional agriculture. The results obtained highlight once again the development of organic cultivation techniques in order to increase productivity and reduce prices, as well as the continuous encouragement of farmers to resort to organic agriculture at the European level.

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