

AN EXTENSIVE SURVEY OF THE IMPACT OF TROPOSPHERIC OZONE ON THE BIOCHEMICAL PROPERTIES OF EDIBLE PLANTS

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Abstract: - Tropospheric ozone is an important atmospheric pollutant, which affects significantly the growth and yield of cultivated plants. The phytotoxic impact of ozone has serious economic repercussions on farming. In the area of Eastern Mediterranean, tropospheric ozone values are significantly increased during the vegetation period. However, ozone causes variations on the quantities of nutrients of edible plants, thus influencing their nutritional value to man. In the present paper, an overview of the acquired knowledge on the impact of ozone on nutritional value of edible plants is presented. More particularly, ozone impact on the nutritional value of potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), wheat (*Triticum* spp), spinach (*Spinacia oleracea*) and lettuce (*Lactuca sativa*) is presented and analyzed. In leafy vegetables are presented reductions in micronutrient components (vitamins, inorganic constitutive), while in the fruits and in the tubers are presented fluctuations mainly in the makronutrient components (proteins, carbohydrates). Changes in other components as antioxydant, antinutritional factors are also observed. The consequences in the Mediterranean diet when using these plants is discussed.

Key-Words: - Tropospheric ozone, Edible plants, *Solanum tuberosum*, *Triticum* spp, *Lactuca sativa*, *Spinacia oleracea*, *Lycopersicon esculentum*, Biochemical properties, Nutritional value

1 Introduction

Since the second half of the 19th century, ozone concentrations have been constantly rising [1],[3]. Tropospheric ozone levels have more than doubled in the last one hundred years and they are expected to continue to rise at an even faster rate in the future.

Ozone is a secondary pollutant with lifetime of about one month and therefore high concentrations of ozone are considered a cross-border problem with serious repercussions on living organisms.

In the Mediterranean area, values appear particularly high (40-80 ppbv); mean monthly values in the Eastern Mediterranean area (Finokalia station, Crete, Greece) are among the highest in Europe. They remain very high almost throughout the year, obviously due to high sunshine in this area [4], [7], [8], [9].

The negative results of the ozone on grains have been known for fifty years, but in the last decades, ozone has become a major cause for concern for grains in Europe as well as in the United States.

The impacts of ambient ozone pollution on crops in the Mediterranean countries have been recorded

regularly in the so-called "grey literature" of UN/ECE Workshop Reports for the Convention on Long-range Transboundary Air Pollution, and less frequently in the peer-reviewed literature. This short communication reviews such records and shows that ambient ozone episodes have been reported to cause visible injury on 24 agricultural and horticultural crops grown in commercial fields including three of the most important crops in the region (wheat, maize, and grapevine) [40].

There is clear evidence that present concentrations of the pollutant are high enough to cause a drop in the production of cultivated plants and to affect the composition and variety of ecosystems [2], [6], [10], [11],[33] [38],[39].

Ozone influence has been particularly studied on edible vegetative species, given the fact that the nutrition of human depends on the yield of these plants and therefore their importance for the economy is high. [6],[35].

However, there are very few studies on the quality of products with relation to the influence of ozone on them. Furthermore, there are no studies in international bibliography with sole object the

changes in the nutritional value of vegetative products due to the exposure of edible plants to high levels of tropospheric ozone. [2], [6], [12], [15], [17], [38],[39].

Except the attribution of cultures important role for the humans' diet has also the alimentary value of the produced foods.

In this article, solid evidence regarding the influence of ozone on edible cultivated plants (mostly of the Mediterranean area) is presented and it is clearly showed its impact on their nutritional value.

The focus on the Mediterranean area was chosen due the high (40-80 ppbv) values of ozone recorded almost throughout the whole year [4],[8],[9].

The article presents data that have been gathered and analysed from the experimental work that has been conducted mostly during the last ten years on plants (potato, tomato, wheat, lettuce, spinach).

2 The potato (*Solanum tuberosum*)

The potato is the second most important agricultural product in Europe (production in tons) after the wheat, and the fourth in the world, after wheat, rice and corn.

It is a rich source of hydrocarbons (5% of daily requirements in energy) for Europeans and the main source of vitamin C for the peoples of Northern Europe. It also contributes significantly to the protein uptake of humans since the protein in its tubers, expressed in dry weight, is as high as in wheat, and its composition in amino acids is valuable from a physiological point of view.

The potato plant is a species with great storage capacity in hydrocarbons and its system of apoplasts for loading substances of phloem transport is based on the transport of sucrose [6], [10].

The potato is also largely used in industry for the production of chips and pre-fried potatoes. In these uses, quality is of the utmost importance and the content of dry matter must be high in order to avoid absorbing a large quantity of fat during the process.

On the other hand, since monosaccharides (fructose, glucose) determine the colour of fried potatoes, it is better to keep them at low levels, because when their concentration is high they give fried products with dark undesirable colour.

Another element that determines the quality of tubers is their content in glycoalkaloids (usually toxic substances) of the Solanaceae family. A low concentration in solanine enhances the good taste of

the potato whereas a high concentration results in a sour taste. Solanine has been reported to cause gastroenteritis and even cancer after chronic consumption [6].

The ozone seems to differentiate quantitatively the nutrients -as well as other substances related with their quality- of potato tubers by affecting their production and growth processes.

2.1 Ozone impact on the assimilation and redistribution of inorganic nutrients in potatoes

Fangmeier et al. [11] make a detailed analysis of the data from the CHIP project [14] (Changing Climate and Potential Impacts on Potato Yield and Quality), from which useful information can be obtained concerning the influence of O₃ in nutrient intake, availability and redistribution in potatoes. Furthermore, the correlation of CO₂ and O₃ in the assimilation and redistribution of the above nutrients is also studied.

The statistical analysis of the data (ANOVA) has shown the significance of CO₂/O₃ correlation in the concentration of Fe in the tubers and in the concentration of N in tubers as well as in the concentrations of Ca and Mg in surface organs. Fe concentration in tubers is reduced by 12% with increased CO₂ and low O₃.

Fe concentration is increased by 7.8% when the concentrations of both CO₂ and O₃ are high. (CO₂680 ppm, O₃>40ppb)

The decrease in N concentration in tubers is more evident when the concentrations of CO₂ and O₃ are increased rather than when the concentration of CO₂ is increased and the concentration of O₃ is reduced in the air.

On the other hand, O₃ is more effective in increasing the N concentration in the tubers under reduced(360ppm,520ppm) rather than under high(680ppm) concentrations of carbon dioxide. The N concentration in the tubers is directly linked to the protein quantity in them and by extension, to the quality of the tubers and their nutritional value.

The ozone also has a small impact on the tuber biomass in the final stage. The loss of biomass in the tubers balances the increase in N and Mn that is observed.

A long exposure in increased amounts of O₃ increases the concentrations of Mn and N in the tubers by 5.3% and 5.2% respectively.

2.2 Glycoalkaloids and other toxic substances in potatoes

One qualitative aspect of potatoes is the toxic substances that might be present in the edible parts of the plant.

The glycoalkaloids are natural toxic substances, present in all the parts of the plant. A-solanine and a-chaconine constitute the major glycoalkaloids (95% of the total amount) in cultivated potatoes.

A low concentration of glycoalkaloids is considered to enhance the taste of potatoes while a high concentration of glycoalkaloids can give a bitter taste or even cause gastroenteritis [12].

A quantity of 3 to 6mg per kilo of body weight is lethally toxic for man. 20mg per 100g (total glycoalkaloids) is considered a generally accepted safe high limit for glycoalkaloids in the tubers.

The analysis of the results from the CHIP experiments [14] revealed that the concentrations of alkaloids in the tubers do not seem to be significantly affected by ozone.

Speroni et al. [12] (1981) report a reduction in glycoalkaloids and total solids in plants that have been exposed to ozone.(AOT40) However, because the differences were not exhibited in total alkaloids based on dry weight, it can be considered that the differences in fresh weight are due to the changes of humidity.

Contrary to the reports of Donnelly et al (2001) [10], an increase in glycoalkaloids was observed under elevated ozone. (60 ppb O₃, 8h per day , 5 days in the week).

The relationship between a-chaconine and a-solanine was not transformed considerably. However, an increase in a-solanine was observed in increased amount of O₃.

2.3 Nitrates

High concentrations of nitrates, which constitute initial forms of nitrites is not desirable in the potato tubers. The high uptake of nitrites by human organisms through nutrition is considered to increase the risk for stomach cancer, as they react with amines in the gastrointestinal system and are transformed into nitro-amines.

Leafy vegetables are the most important source of nitrates but potatoes are particularly important because they are consumed in large numbers all over the world.

The data from the CHIP project [11], [12], [14] show a small increase in nitrates in the tubers under the influence of ozone. The differences are not considered significant.

2.4 Ozone impact in the accumulation of Carbohydrates in potato tubers

Potato components such as starch, simple sugars and organic acids are important to the quality and nutritional value of the potato.

Starch is the most important substance of potato tubers as it constitutes 65-80% of their dry weight (dw).

In the CHIP experiments, it has been observed that O₃ and CO₂ accelerate the aging process of the potato leaves, which might have an effect on the metabolism of carbohydrates in the tubers. [12]

From a nutritional point of view, the reduction of starch and simple sugars is not desirable since the nutritional value of the food is also reduced.

However, from the point of view of the potato processing industry, some of these reductions can be desirable.

Thus, high concentration (65%-80% dw) of starch in the tubers is generally desirable so that excessive absorption of fat can be avoided during frying.

Contrary, low(<1,5 mgr/gr) concentration of simple sugars is desirable so that fried products can maintain their golden colour. When the concentration of simple sugars is high, fried potatoes and chips have a dark brown colour that is generally not desirable.

The maximum accepted limit of concentration of simple sugars is less than 1,5mgr/gr measured in fresh weight.

The combined exposure to CO₂ and O₃ showed a correlation to the concentration of simple sugars and the concentration of starch. Thus, the simultaneous exposure to high concentration of CO₂ and O₃ shows a balance of the reduction of the simple sugars by increased CO₂, which derives from the exposure to ozone.

The ozone also reduces the concentration of starch in the tubers with negative effects in the quality of the tubers but the exposure to increased concentration of CO₂ improves their quality by increasing the concentration of starch.

Furthermore, Donnelly et al (2001) observed that in simple sugars O₃ tended to reduce the content of fructose under increased CO₂ but not under environmental CO₂, whereas the content in glucose tended to increase under increased CO₂ but not under environmental CO₂ and the total sugar content did not seem to be affected under increase O₃. Also, Kollner & Krause (2000)(13) report that when similar doses of O₃ were applied (110 nmol/mol (110ppb) for 8 hours per day and 35 nmol/mol (35ppb) for 24 hours per day) the highest

concentrations of O₃ for short periods caused a higher decrease of the content in sucrose in the tubers than the long-term exposure to lower concentrations.

Contrary to the results of the statistical analysis of data from the CHIP project, where starch reduction is reported due to the exposure to ozone, the content of the bulbs in starch did not seem to be affected in the studies of Donnelly et al [10] in the exposure conditions of ozone (60nmol/mol for 8h) (60ppb) that were used.

Also, Kollner and Krause (2000) [13] report that the content of starch was clearly reduced in the tubers following a more intensive exposure to ozone.

2.5 Impact of ozone to ascorbic acid in potatoes

Significant increases in the concentration of apoplasmatic ascorbate have been reported in studies that have conducted regarding the impact of ozone on various vegetative species and the potato. [10], [12], [32].

Vorne et al (2002) [12] reported that the concentration of ascorbic acid (vitamin C) in potato tubers increased under the influence of high concentrations of ozone (>40ppm) During the period from the highest foliage surface until harvest, a 15% increase was reported. Similar results are given by the experiments the experiments of Donnelly et al in Bonington, UK, [10].

Potatoes are an important source of ascorbic acid and their nutritional value would be improved with higher concentrations of ascorbic acid.

3 Impact of ozone on the level of free radicals and antioxidants in tomato grains (*Lycopersicon esculentum*)

Tomatoes are considered a necessary nutritional product in the Mediterranean, although they originate from the New World.

They belong to the Solanaceae family and they are short-lived perennial plants.

In Southern Europe, tomatoes are cultivated in gardens during the summer and in hothouses during the winter.

Tomatoes have significant economic value for Greece and constitute an exportable product.

Its nutritional value lies mainly in the fact that it contains large quantities of vitamin C, potassium and dietary fibres. Also, it contains pro-vitamins A

in the form of b-carotene and lycopene, which have anti-oxidant action.

During the last few years, many studies have been conducted on the antioxidants of grain and tomatoes have been recognized as agents of powerful anti-oxidants which bind free radicals (15).

The main anti-oxidant of tomatoes is carotene lycopene, which acts as a binder of free radicals, which come from oxygen such as peroxide anions (O₂⁻) and prevents the hyperoxidosis of lipids.

It is considered that these phytochemicals protect the health of people based on these qualities. Lycopene has been linked to the protection from certain forms of cancer [15].

Exposure to ozone can differentiate the quantities of anti-oxidant vitamins and provitamins and possibly reduce the nutritional value of tomatoes. [37]

In 2003, Pirker and Reichenauer et al [15] published their work, in which they studied tomato plants in OTCs (open – top champers) under conditions of increased ambient ozone, calculating the creation of free radicals in tomato grains when the plants are under conditions of stress resulting from increased ozone or water shortage.

The effect of ozone treatment at the level of free radicals in tomato grains was considered by the researchers as much clearer than the effect of drought.

During the first harvest, the level of free radicals in tomatoes from the plants exposed to ozone was 60-70% higher than in the plants that had not been treated with ozone.

The antioxidative content of these grains was reduced with increased concentrations of ozone. The differences in free radical levels between the samples were reduced with consecutive harvests and there was no significant difference in the third and fourth harvests. It is worth noting that the signal of free radicals increased stable with consecutive harvests for grains from plants that had not received ozone, which is related to age results in these samples. On the other hand, the signal of free radicals from the grains of plants exposed to ambient air was reduced with consecutive harvests, which is probably related to the development of anti-oxidative mechanisms [15].

4 Wheat (*Triticum spp*)

The wheat grain is mainly used for human consumption (at 75-78%). Secondly, it is used as forage but also for industrial use (16-17%) and propagation material (9-10%).

4.1 Composition and nutritional value of the grain

The grain is starchy, with high percentages of protein. All the starch is located in the endosperm, dissolvable sugars mainly in the embryo and the other polysaccharides (cellulose, semi-cellulose) in the husk. Proteins, fats and ash are mainly in the embryo and the scutellum and in smaller percentages in the endosperm.

The starch is mainly in the form of amylose and amylopectin, and dissolvable sugars such as glucose, fructose, maltose, sucrose and various pentosans (rafinose, meliviose).

The endosperm proteins (apart from aleurone) consist of equal proportions of gliadine and glutelina, which form a colloid substance in the water called gluten, which plays a decisive role in the bread making quality of the grain. Wheat proteins also include amino acids, which are necessary for human organisms (lysine, methionine, tryptofan).

From all lipo-soluble vitamins, wheat is an excellent source of vitamin E. From the water-soluble vitamins, there are significant amounts of vitamins of the B complex, particularly thiamine, riboflavin, niacin and less pyridoxine, biotin and pantothenic acid.

The wheat grain also includes K (0.48), P (0.40), S (0.18), Mg (0.17), Ca (0.05) % k.b and smaller amounts of Fe, Na (16).

4.2 Wheat as a sensitive species to ozone exposure

Wheat is considered to be a sensitive species to ozone exposure. Indeed, it is considered the most sensitive species from grains and particularly the new and improved varieties [17],[35].

It is broadly known that tropospheric ozone causes loss of production in wheat and reduces the harvest index [18].

This reduction represents a translocation in the biomass distribution inside the plant, which is possible to occur after a disruption in the balance between the creation location and the final destination of the photosynthesis products.

An ozone-induced reduction of the green foliage surface due to premature aging or a reduction in the percentage of clear assimilation of CO₂ might lead to a lower production of carbohydrates. Ozone may also affect the possibility of transport of the assimilation products [18].

The results of ozone on wheat depend on the time of the exposure in relation to the development stage of the plant. Thus, it has been established that there is a greater sensitivity and larger reductions in production when exposure is taking place during the period between anthesis and seed maturity than in the period before anthesis [18].

The increase of the grain of wheat depends on the production of carbohydrates, the possibility of transport of the assimilation products from the production organs to the grains, as well as from the ability of the grains to store them.

Young grains during filling are the main receivers of the produced carbohydrates and proteins. Most of the carbohydrates that are produced during this stage of development are immediately transported to the grains. If the activity of the source exceeds the storage activity of the grains, the surplus of carbohydrates is stored in the stem. When the production rate is reduced to a level under the storage capacity, the stored carbohydrates in the stem or the leaves can be transported to the grains [18].

In this way, the percentage in the grains can remain quite stable. The production of carbohydrates (the activity of source during time) can be affected by a change in the photosynthesis rate (source activity) and the duration of the photosynthesis activity (source duration). The possibility of grain storage depends on the creation percentage of storage carbohydrates from imported sucrose (e.g. starch synthesis) and the storage ability of starch.

The storage ability is then affected by the number of grains per spikelet and the number of cells of endosperms per grain.

The activities of source and storage organs in the grains are not independent the one from the other and both contribute in grain yield.

Given the fact that ozone has been proven to affect the percentage and the duration of photosynthesis, as well as the transport of assimilation products from the source to storage, the ozone exposure will probably cause a reduced supply of the developing grains.

The abovementioned result is more evident in a plant, in which the increase of the grain is already limited by the source [18].

4.3 The impact of ozone on non-structural carbohydrates of wheat

Sild et al in 2002 [17] gathered the results of experiments that studied the effect of ozone on non-structural carbohydrates in the wheat plants.

Knowing that increased tropospheric ozone can reduce the assimilation of CO₂, accelerate the aging of the plant and affect the grain yield, it was necessary to define the role of the ozone in the production, transport and storage of non-structural carbohydrates.

Fuhrer et al in 1990, 1992 [19] showed that higher than ambient ozone concentrations reduced the concentration of starch in the wheat grains.

However, this reduction was not present in all later experiments. This is possibly connected to the development stage of the plant and the exposure as well as the role of the stored soluble sugars in the stem.

Stems can store soluble sugars during anthesis when the foliage surface is maximum and the shoot and root increase is minimum. These sugars can be transported toward the grains during filling [16].

In non-increased tropospheric ozone conditions, the contribution of these sugars does not exceed 10% of the final weight of the grains.

In increased(>40ppb) tropospheric ozone conditions, especially during the period of anthesis and later on, when the activity of production sources of photosynthesis products is reduced, the transport of sugars from the stem becomes more definitive and contributes to the balance of the grain content in non-structural carbohydrates. [17].

In the same experiments, there were no statistically significant differences in the concentrations of carbohydrates in the grains.

There were reductions in starch and in whole non-structural carbohydrates, especially in large doses(>2000ppbh) of ozone after anthesis.

More particularly, whereas starch is considered to constitute 75-85% of dry weight of the grain, under the impact of ozone, this percentage fell to 55%. It is worth mentioning however, that in the specific experiment, the largest concentration of starch in the grain that was recorded in the treatment without extra ozone did not exceed 61%.

Similar reductions were recorded in whole carbohydrates (TNC), where the maximum concentration of 65% in the treatment without ozone was reduced in the treatment NF3(10900ppbh) to 58%.

On the other hand, the differences in TNC percentages to 1000 grains were significant and reduced at the increase of the ozone concentration [17].

Studying the dependence of the sensitivity of wheat on ozone with relation to the translocation of the ratio of activity of assimilation sources / storage areas, Gelang et al [18] produced data concerning

the changes in the content of carbohydrates and nitrogen in the grains under exposure to ozone.

In the shoots where the "source" was treated, the percentage of malto-dextrine was reduced in the grains as a result of its exposure to ozone whereas it was increased in the plants where the source was treated.

Also, the percentages of soluble carbohydrates (WSC: glucose, fructose and sucrose) were lower in the grains.

The results of the exposure to ozone were more intense when the exposure occurred after anthesis rather than before.

A decrease(>10%) in starch and the totality of non-structural carbohydrates (TNC) was observed in the grains. The amount of soluble carbohydrates, starch and malto-dextrine per wheat was significantly reduced by the exposure to ozone.

4.4 Impact of tropospheric ozone on protein content in the wheat grains in combination with the impact of increased carbon dioxide and sufficient humidity

Pleijel et al [20] report the results from experiments that were conducted in four different countries in 16 open-top chambers.

The combination of these experimental results has provided a clear reverse relationship between the protein concentrations in the grains (GPC) and the grain yield (production) (GY). This phenomenon is called "growth dilution" and it is also provided for other nutrients apart from nitrogen.

If nitrogen availability is stable and the grain yield is reduced due to certain external factors, the protein concentration in the grains increases.

In several studies, it has been established that the reduction in yield (GY) that is caused by increased(>40ppb) ozone concentrations in the atmosphere is combined with an increase in protein concentration in the grains (GPC) (Fuhrer et al., 1992 [19], Pleijel et al. [21], 1991, 1998 [22], Gelang [18]). On the other hand, increased CO₂ concentrations lead to the intensification of the development and the increase in carbon in the tissues in relation to the nitrogen. These reductions in grain protein affect the quality of the flour (raising of leaven) and differentiate the nutritional value of the product (bread).

If the conditions for filling (duration of filling) are favourable, the protein concentration in the grains will be reduced due to high percentages of carbohydrates that will be transferred to the grains.

The impact of ozone affects the assimilative ability, especially the one of higher leaves, and reduces their life span and activity. As a result, it reduces the filling period of the grains and contributes to the increase in protein content of the grains.

A longer life span of the leaves that is favoured by increased concentrations of CO₂ leads to an increase in GY.

However, although the increase in protein amount in the grains is linked to the ozone-induced reduction in GY, the protein production per surface unit tends to decrease with the impact of ozone. The opposite happens with the impact of increased CO₂.

Therefore, when the conditions are favourable for the assimilation of carbon dioxide (increased dioxide) the carbohydrates increase but also the protein per surface unit, whereas they decrease when the assimilative process is weakened due to the ozone.

A restraining factor in this process is the nitrogen. Initially, nitrogen allows for large carbon dioxide assimilation ability. After and during the filling process, nitrogen is redistributed in the grains. The total protein per surface unit can not continue to increase indefinitely. Nitrogen will start becoming the restraining agent as the production increases.

The loss of grain protein per surface unit, which is mainly due to ozone-induced loss, is larger than the protein earned per surface unit due to increased CO₂. Thus, the changes in grain protein from agents such as ozone and carbon dioxide can be largely explained by the relationship between the grain protein and GY at a defined nitrogen availability level.

The gain of the farmers is linked to the quality of the grains which in turn is directly linked to the protein concentration in the grains. The effect of increased carbon dioxide levels in GPC (protein concentration in grains) may lead to increased fertilization on the part of farmers (20).

4.5 The effect of ozone exposure during the development process of the wheat plant on the quantity of free radicals in flour

Electron paramagnetic resonance (EPR) spectrum in experiments conducted by Reichenauer et al (23) in wheat flour has established the presence of Fe (III), Mn (II) and free radicals (FR). Signals concerning metals were more intense (higher quantities) in flours that were produced from plants that had been exposed to increased levels of ozone.

Signals from free radicals increased during the storage period of the flour (4-5 months after milling). This was even more intense in flours that were produced from wheat plants that had been exposed to increased levels of ozone (80ppb) during their development [23].

In non-photosynthesing plant tissues such as seeds, the mitochondria constitute the main areas for the generation of radicals, where reactive oxygen species (ROS) are produced in aerobic metabolism.

Under stress conditions, such as elevated ozone levels, ROS generation is increased as a result of an imbalance in the concentrations of the sub layers (in the electron transport chain). The main initial ROS are the peroxide radicals, which is transformed into hydrogen peroxide by the superoxide dismutase enzyme (MnSOD). Thus, a small increase in the concentration of Mn in the flour due to the ozone impact on the developing grain may result in an increase in the production of MnSOD. Although hydrogen peroxide is detoxified by a number of enzymes in the plant mitochondria, powerful hydroxyl radicals may develop in the presence of oxidized sub layers. Thus, elevated superoxide levels lead to a decrease in the levels of anti-oxidants that bind free radicals. Such anti-oxidants are the ascorbic acid and glutathione. The decrease can occur either directly through their reaction with hydrogen superoxide or indirectly through the reaction with reaction products of hydroxyl radicals.

Therefore, the highest production of free radicals due to ozone exposure of the developing grains results in lower levels of anti-oxidants in the produced flour, which lowers their quality and nutritional value.

4.6 Other changes in wheat tissues related to elevated concentrations of tropospheric ozone and with possible effect on the grain's nutritional value

Fangmeier et al (1997) [24], 1999 [25] report that there is no significant statistical change in the concentration of nutrients in wheat seeds when plants are exposed to elevated ozone concentrations, especially when at the same time there is a simultaneous impact of elevated carbon dioxide.

More particularly, the concentrations of macro-elements (N, P, K, S, Ca, Mg) and trace elements were studied in different parts of the plant as well as the grains at different ozone concentrations.

Herbinger et al in 2002 [26] studied the changes in the concentrations of anti-oxidant substances on higher wheat leaves, with the effect of elevated

concentrations of tropospheric ozone combined with sufficiency or shortage of humidity.

Concentrations of ascorbic acid were reduced but there was an increase in the concentration of glutathione, tokopheroles and certain carotenoids.

Naito et al [27] reported decreases in thiamine and riboflavin in flours that had been produced by grains that had been previously exposed to ozone for their protection from fungi and bacteria.

5 Leafy vegetables

This category includes plants, cultivated or wild, the leaves of which are consumed by people as food.

The consumption of cultivated and wild vegetables and herbs in Mediterranean dates back more than 2500 years. Leafy vegetables and herbs are an important source of carbohydrates, inorganic elements, anti-oxidants and dietary fibres.

The importance of anti-oxidants, which include ascorbic acid, carotenoids, phenols and other compounds, to our health has been established in numerous studies during the last ten years.

Anti-oxidants are substances which prevent or hinder the oxidation of other molecules, by terminating the chain of oxidative reactions. Natural anti-oxidants show a large number of biological effects, including anti-bacterial, anti-toxic, anti-inflammatory, anti-allergic, anti-thrombosis and vascular diastolic action. The anti-oxidative action is a basic quality, very important to life. Protective mechanisms against mutations, carcinogenesis and tissue aging process are based on the action of anti-oxidants.

The existence of anti-oxidants in leafy vegetables is known and their connection to the protection against chronic diseases has been established to a significant degree during the last years [28].

The presence of high concentrations of ozone in the troposphere leads to a decrease in the anti-oxidative substances in leafy vegetables, by reducing the beneficial presence for human health. [34],[36].

Apart from their nutritional value, leafy vegetables constitute a significant economic value for Greece and the Mediterranean in general, as they constitute exportable goods.

In the year 2000, the gross worth of lettuce, cabbage, spinach and radish production in Greece exceeded 150.000 euros [29].

The negative effect of ozone on the production and yield of vegetables harms financially a large part of the population, who are involved in vegetable farming but also affects the economy of the countries of the Mediterranean.

5.1 Lettuce (*Lactuca sativa*)

Calatayud, Ramirez et al in 2002 [30] investigated the results of the ozone exposure on lettuce leaves with the help of gas exchange, chlorophyll fluorescence and anti-oxidative systems.

Visual symptoms of injuries and necrotic spots mostly in young leaves, the assimilation of CO₂ decreased, stomatal conductivity and total evaporation rate was affected, photosynthetic process was reduced.

The effect on the level of ascorbic acid on the leaves was also important as well as on the activity of anti-oxidative enzymes.

The concentration of endogenous ascorbic acid at the end of the increase period (picture 7) was higher in the leaves from CFA plants compared to leaves from NFA and NFA+O₃ plants. The decrease in NFA+O₃ plants was approximately 35% compared to control plants (CFA). (30)

The changes in the activity of anti-oxidative enzymes that occurred at the end of the increase period in lettuce leaves that were exposed to ozone are as follows:

More particularly, there was a decrease in the activity of ascorbate peroxidase (ARX), especially in NFA + O₃ plants. The activity of glutathione reductase (GR) was slightly affected in all the treatments, whereas the activity of superoxide dismutase (SOD) was particularly affected. In NFA treatment, there was an increase in its activity by 23% whereas in NFA+O₃ the increase exceeded 130% [30].

The exposure to ozone has led to superoxidation of lipids. In the study conducted by Calatayud and Ramirez (30), the index used for the superoxidation on lettuce leaves was the malondialdehyde (MDA – dialdehyde of malonic acid). At the end of the increase period, the MDA count showed a significant increase in NFA, NFA+O₃ treatments. In the last case, superoxidation increased by 56% compared to CFA plants.

Ascorbic acid is a significant factor of the anti-oxidative mechanism of cells. Thus, the decrease observed in plants that have been cultivated in conditions of elevated ozone exposure (NFA + O₃) can be related to the existence of oxidative stress on them.

The concentration of MDA, which shows the status of the membrane superoxidation and has been linked to the degree of ozone exposure, is shown clearly higher in NFA+O₃ thus confirming the oxidative stress.

ARX enzymes, which use ascorbic acid as a sub layer, are substances of vital importance in the metabolic defence against oxidative stress. Thus, the higher ARX activity is linked to a higher ability of ascorbic biosynthesis.

ARX uses two molecules of ascorbic acid to reduce H₂O₂ into water with simultaneous generation of two molecules of monohydroascorbate. In NFA+O₃ plants, ARX activity was smaller than in any other treatment and this is linked to the fact of smaller ascorbic concentration on leaves.

Superoxides that are created in different parts of the plant under normal conditions quickly turn into H₂O₂ with the help of SOD. In oxidative stress conditions, superoxide radicals increase and can intensify the SOD activity, which was established in the above mentioned experiment.

5.2 Spinach (*Spinacia oleracea*)

Similar effects were reported in spinach (*Spinacia oleracea*) in the study conducted by A. Calatayud and D. Iglesias [31] in elevated levels of tropospheric ozone.

Spinach is a widely spread leafy vegetable, cultivated and consumed in the countries of the Mediterranean.

Spinach is a rich source of iron and carbohydrates and offers human organisms with anti-oxidants such as carotenes and ascorbic acid.

The effect of ozone disrupts the functions of photosynthesis and CO₂ assimilation, reduces the activity of anti-oxidative enzymes as well as the concentration of anti-oxidants on spinach leaves, which constitute the edible part of the plant.

6 Discussion and Conclusions

6.1 Potatoes

The results of increased ozone concentrations in the troposphere, in the production as well as in the quality of potatoes, should be examined in conjunction with the impact of increased concentrations of atmospheric carbon dioxide, since increased concentrations of atmospheric CO₂ and ozone, have been proven to have reverse results on the development and physiology of the potato plants as well as of other plants. Increased CO₂ in general increased pure photosynthesis and the yield of C₃ in plants, whereas increased O₃ has a negative effect on the development, the yield and the photosynthesis activity [10].

The potato that belongs to type C₃ plants, which follow the photosynthesis path of the creation of initial products with three atoms of carbon, is a species with large storage organs and areas of carbohydrate consumption.

Also, the transport system of photosynthesis products, which is based on sucrose, is quite effective. In this way, it can respond very well to the increased concentration of atmospheric carbon dioxide and lessen the possible negative results of the increased atmospheric ozone [6].

However, it has been reported that ozone, alone or in combination with increased atmospheric carbon dioxide, affects potato plants.

The impact is initially presented in the photosynthesis and respiration rate and then at a second stage they are presented in the grain yield and in the quality of the tubers [14].

An important factor to this is the concentration of gases (CO₂, O₃) in the atmosphere, the time of exposure as well as the existence of exposure peaks to specific stages of plant development.

The interesting part with reference to the quality of edible parts in potatoes can be focused on their value with regard to industrial use.

The quality of the tubers in relation to their immediate nutritional value and the effect that the increase of tropospheric ozone can have on it, have not been examined so far.

From the experimental work conducted so far, it is possible to draw conclusions regarding the changes that can take place to the quantities of nutrients in the tubers due to the increased concentration of tropospheric ozone and the correlation with carbon dioxide.

The following important remarks can be drawn:

-Increase of the inorganic substances in the tubers with the effect of increased ozone particularly at long-term exposure of the plants. More specifically, an increase in the content of N and Mn in the tubers was observed and the decrease of other inorganic elements such as iron due to increased carbon dioxide was lessened. Increased quantity of N is related to the content of the tubers in protein [11].

-Decrease of simple sugars (glucose, fructose) as well as compound carbohydrates (starch) with the increase of ozone concentration. Combined to the exposure to increased levels of carbon dioxide, a compensation of the loss of simple sugars was observed [12].

-Increase of vitamin C in the tubers at increased levels of O₃ [12].

-Increase of antinutritional agents (glycoalkaloids, nitrates) [10].

Most of the above conclusions have been established in more than one experimental process, in different ambiances and different potato varieties [10], [11], [12], [13].

However, some deviations with regard to the above observations have been recorded, such as increase in certain simple sugars, decrease or non-effect of the alkaloid concentration etc.

These deviations however, should always be examined and comparisons should be made taking into consideration all the factors that can be related to them.

Such factors may include: Ozone and carbon dioxide concentration levels, exposure period, humidity, soil-air temperature, sufficient uptake of nutrients from the plants, potato species.

6.2 Wheat

The grain of wheat and its components are affected secondarily by ozone as this affects the quantities of the nutrients that will be transported to the grains from the photosynthesing organs.

- It is also possible that the transport system of nutrients is affected.

- The experiments carried out in open-top chambers reported a decrease in starch and other non-structural carbohydrates in the grains and a decrease in whole carbohydrates.

- When nitrogen is sufficient, there is an increase in the concentration of protein in the grains in conditions of elevated ambient ozone since there is a decrease in the production of grains and total yield.

- Also, a higher production of free radicals has been established due to ozone exposure of the developing grains, which results in lower levels of anti-oxidants in the produced flour, which in turn lowers their quality and nutritional value.

- Decreases in thiamine and riboflavin have been reported in flours that have been produced from grains exposed to ozone for protection from fungi and bacteria.

- Increased CO₂ favours a larger assimilation and mediates the effect of ozone.

In order to cope with oxidative stress, plants consume vitamins and other anti-oxidants. The detoxification processes take place mainly in the apoplasts of the leaves at the expense of anti-oxidative substances.

6.3 Leafy vegetables

Leafy vegetables present decreases in the concentration of ascorbic acid due to elevated ozone levels. Thus, the quantities of nutrients and anti-oxidants that would have been available for human

nutrition and which are valuable for humans and the good function of their organisms are reduced.

Recent research has established that leafy vegetables widely consumed in the countries of the Mediterranean contain significant amounts of ω -3 fats. The consumption of such vegetables contributes in maintaining the correct ratio of ω -3 ω -6 for human organisms. These unsaturated fats that are found in vegetative tissues may suffer changes due to the existence of free radicals and H₂O₂ under the effect of elevated levels of ambient ozone. In this way, the nutritional value of vegetables exposed to high ozone levels is further undermined. It is necessary to conduct further research so that the impact of ozone on these vegetative fats can be known.

6.4 Future research and perspectives

It is important to be stressed, that the systematic research with regard to the nutritional value of edible plants is necessary in the next future, under the new atmospheric conditions that have been created and any forecasts would be rehabilitated in still superior levels of ozone and dioxide of coal in the close future.

The dynamic cultivations in the Mediterranean area (olive, vine, horticultural) have not been studied sufficiently concerning the effects of ozone in their growth, their nutritional value and the impact of the actual environmental conditions, thus much of attention must be paid in the next future on these issues given their importance and the perspectives they present for the nutrition science.

Finally, it is desirable in future research the effect of ozone on the plants of the Mediterranean area to be extracted according to the approach of critical levels II while the other implicated environmental conditions would also be taken into consideration at the same time.

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