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## CHAPTER 1

# Strategies for reducing postharvest losses of vegetables through integral assessment of antioxidant status

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### Abstract

The global food system is facing a challenge due to high total food losses and waste, with the problem exacerbated by unpredictable events like pandemics and conflicts. The loss of fruit and vegetable products, particularly during storage, becomes a critical issue demanding attention and technological advancement. Reducing such losses will not only ensure a sustainable food resource, but also contribute to the reduction of greenhouse gas emissions and efficient use of resources. Long-term and efficient storage of vegetable products is, however, a difficult task, since many vegetables have a short production and marketing cycle and perish quickly. After separation from the mother plant, vegetables are exposed to various stress factors that lead to the generation of reactive oxygen species, which are harmful to cells, but also act as signal messengers at low concentrations. The plant's antioxidant system, comprising both low-molecular and high-molecular antioxidants, plays a crucial role in regulating the level of reactive oxygen species (ROS) and maintaining redox homeostasis. A well-functioning antioxidant system is important for preserving the quality of vegetables during storage and preventing postharvest disorders. The use of edible coatings with antioxidant properties is an effective strategy for maintaining the quality of vegetables during storage. However, it is important to note that high doses of antioxidants can potentially have a toxic effect, and their efficacy may vary depending on the concentration and type of vegetables. To strengthen the endogenous antioxidant system, it's crucial to determine the concentrations of exogenous antioxidants that align with the endogenous pool of antioxidants in plant tissues and ensure the maintenance of the antioxidant status and the preservation of the quality of vegetables during the postharvest period. To assess the antioxidant status, we propose employing the method of analyzing hierarchies (AHP). The main drawback of AHP is its susceptibility to subjective evaluation judgments. This subjectivity

can be eliminated by relying on experimental or analytical information about the quantitative indicators of the chemical composition, correlations between the components of the antioxidant system and with markers of oxidative stress. This study introduces the method of integral assessment of the antioxidant status of vegetables using the hierarchy analysis method. The integral assessment was conducted on three varieties of asparagus with different colors. We suggest adjusting the concentration of antioxidants in the composition of edible coatings based on the determined antioxidant status of vegetables. This approach ensures the prevention of product losses during an extended shelf life.

### **Keywords**

Postharvest loss and waste, storage, vegetables, antioxidants, antioxidant system, edible coatings, hierarchy analysis method.

## **1.1 Introduction**

To date, the global food system exhibits a considerable fragility. Russia's war in Ukraine has notably exacerbated the negative trends within the current state of the world food system, leading to breaches in guaranteeing obligations to ensure food security [1]. Undoubtedly, the consequences of Russian aggression, such as the blockade of Ukrainian seaports (which serve as the primary logistical route for grain export), the looting of crops in occupied territories, the destruction of arable land in combat zones, and the demolition of the Kakhovka Dam, will have lasting repercussions not only for Ukraine's food system but also for global food security. The food system includes all stages from the production (growing) of food raw materials to the consumption of ready-made food. Mandatory links of the food system are cultivation, harvesting, postharvest processing, pre-processing storage, raw material transformation into finished products, storage of the finished products, their delivery to distribution centers, distribution, and sale to the final consumer. Naturally, transportation involving loading and unloading occurs between these stages, thus leading to losses of food raw materials and products at each link of the food chain.

Food losses and waste are symptomatic of the inefficient functioning of the food system. After all, the production of food requires significant resources, such as water, soil, energy and fertilizers. When part of the products turns into waste, these resources are irretrievably lost and become an additional source of greenhouse gas emissions. This leads to the use of non-renewable resources to produce food that will not be used (for example, about 25 % of the water resources used by

agriculture annually and 23 % of arable land, which generates about 8 % of annual global greenhouse gas emissions) [2]. Concerned with the problem of food loss reduction, Food and Agriculture Organization (FAO) introduced the global initiatives "Safe food" and "Technical Platform on the Measurement and Reduction of Food Loss and Waste". Fruits and vegetables occupy leading positions in the list of food losses and waste (40–50 % of their total production). Reducing the loss of fruit and vegetable products thus is not only vital for sustainable food resources but also aids in mitigating the environmental impact by curbing greenhouse gas emissions and promoting more efficient use of valuable resources within the food industry. This endeavor necessitates involvement across the entire food chain, from agricultural producers to consumers, in order to effectively reduce postharvest losses of vegetables.

According to the analysis of the food waste market, in 2022 the fruit and vegetable segment dominated the market and accounted for 20.3 % of the total revenue share. This dominance is attributed to factors such as improper handling, storage, processing, and cultivation practices of these products [3]. Losses of fruit and vegetable products occur at all stages, including cultivation, harvesting, processing, storage, logistics and distribution to consumers. The later the losses occur, the more damage these losses will cause to the global food system. It is known that in countries with imperfect collection, processing, and storage technologies, the greatest losses occur at the initial stages. In countries with high-tech systems of agriculture with a well-developed cold chain, the largest share of products is thrown away at the stage of retail trade and consumption of products. In both cases, a certain share of fruit and vegetable products is lost at the stage of storage. One of the solutions to this problem is the improvement of storage technologies and methods that allow to extend the shelf life of vegetables and reduce their losses.

On the other hand, according to the FAO recommendations, the share of fruit and vegetable products in the population's diet should constantly increase with the transition to sustainable consumption patterns [4]. Fruits and vegetables contribute to health by providing essential phytonutrients such as phenolic compounds, carotenoids, vitamins, mineral compounds including potassium, calcium and magnesium, iron, iodine, zinc and fiber. They are important for the prevention of "hidden hunger". The spectrum of biologically active compounds is, however, a feature specific to species and variety. Bioactive substances in fruits and vegetables with redox modulator properties may also mitigate the risk of chronic diseases such as diabetes, vision disorders, as well as asthma and viral infections. Numerous studies have shown a direct correlation between the consumption of vegetables and the reduction of cardiovascular diseases (Mediterranean, flexitarian diets). Vegetables contain more protein and fiber and less carbohydrates than fruits.

In general, FAO recommends consuming at least 400 g (5 portions of 80 g) of fruits and vegetables per day. It is believed that at least three portions (240 g/day) should consist of vegetables [5]. The general concept is that including various vegetables in the diet is a key element for a balanced diet. Hence, significant attention should be devoted to addressing the issue of reducing vegetable losses, given their importance as a valuable food resource.

## **1.2 Problems of reducing losses during storage of vegetables**

Effectively storing vegetable products poses a significant challenge due to several issues. The production of vegetables is seasonal and their production and marketing cycle is quite short. In general, for many vegetable crops, this cycle lasts only for 2–3 months. Some cruciferous vegetables – cauliflower, kohlrabi, broccoli can be stored for only 2–3 weeks. However, there are very perishable vegetables (for example, leafy and fruit vegetables), which shelf life is measured not even in weeks, but only in days. This leads to oversaturation of the market during the production season followed by further large losses and waste. Almost all types of vegetables, with the exception of some varieties of pumpkins, possess thin covering tissues that are susceptible to mechanical damage during the loading and unloading processes, leading to potential injuries. These damages, as a rule, become evident already in storage, which leads to increased losses. After all, injured tissues become an easy target for pathogens that multiply quickly and attack intact vegetables. High free water content in tissues can cause wilting and weight loss as a result of natural shedding. Vegetables with low turgor become more vulnerable to pathogens, quickly undergo microbiological spoilage and lose their quality.

The preservation of vegetables is affected by a combination of various factors, the main of which are: botanical and biological properties of raw materials, climatic and soil growing conditions, agrotechnical measures during the growing season, conditions of collection, transportation, postharvest processing and storage.

The main purpose of storage (long-term or short-term) of fruit and vegetable products is to maintain the initial quality of products during a certain period. After harvesting, fresh vegetables remain living biological objects and continue metabolic activity. Temperature, humidity, lighting affect the life processes of the fruit almost as much as before separation from the mother plant. The speed of metabolic reactions, including respiration, slows down by 2...3 times with a decrease in temperature for every 10 °C and, accordingly, accelerates with its increase. Respiration is considered the main physiological process of the postharvest period, which performs certain

functions in the plant organism. First of all, the energy released during the oxidation of biological substrates (acids, sugars) is transformed into convertible forms of cellular energy and is used to maintain vital processes. When biological substrates undergo oxidation, they produce metabolites that are utilized in various biosynthetic pathways. Due to metabolic processes and transpiration, moisture loss occurs. In the postharvest period, as photosynthesis ceases, replenishing reserve substances and moisture becomes impossible. Accumulated substances are constantly spent on maintaining metabolism, which leads to the loss of nutritional value, decline in organoleptic indicators, weight loss, and a decrease in quality. Therefore, quality losses of fruit and vegetable products during storage is a natural and irreversible process. Since it is already impossible to improve the quality of vegetables in the postharvest period, the main task is to maintain the proper quality of products for as long as possible.

Primarily, normal metabolism is inhibited by altering physical factors such as temperature, relative humidity, or gas composition within the storage atmosphere. The maximal preservation of food, retention of vitamin value, maintenance of quality parameters, and ensuring safety of fruit raw materials, along with minimizing production losses, can only be achieved through the application of artificial refrigeration. It is known that a decrease in storage temperature is directly correlated with the intensity of respiration, production of ethylene, inhibition of metabolism, as a result of which the shelf life is extended. Different types and varieties of vegetables require different modes not only for storage, but also for pre-cooling and subsequent heating after storage. The difficulty of choosing the optimal storage regimes also lies in the fact that the recommendations developed for products grown in different regions and agro-climatic conditions may differ.

Traditional methods of vegetables storage, based on artificial refrigeration, fail to comprehensively address the challenge of long-term storage and loss prevention. Low positive temperatures only slow down, but do not stop, oxidation-reduction processes and the development of microflora, and therefore, during vegetables storage in ordinary refrigerating chambers a relatively high rate of aging processes and significant losses from microbiological diseases and physiological disorders are noted. Control of relative air humidity along with the temperature control is important for reducing mass loss. Increase in relative humidity in storage leads to stimulation of the development of fungal pathogens. As a supplement to the influence of temperature and relative air humidity, other technological methods can be used during storage. Supplementing the cold chain with a regulated storage atmosphere further slows postharvest metabolism and extends shelf life. However, controlled atmosphere storage can be beneficial, ineffective, or even harmful depending on the type of product. There is great variability in the tolerance of fruit and vegetable products to a regulated

atmosphere, and genetic factors determine the product's ability to withstand stress from changes in the composition of the atmosphere [6]. In addition, there are questions about the environmental friendliness of this method of storage. Nowadays, it is important to develop and implement vegetable storage technologies that not only reduce losses, but also minimize the negative impact on the environment.

In any case, preventing the natural process of aging and deterioration of fruits and vegetables during storage is a fundamental challenge from a technical point of view.

### **1.3 Endogenous mechanisms of maintaining normal metabolism in the postharvest period**

After separation from the mother plant, vegetables undergo various stress factors during postharvest processing and storage, including mechanical shocks and damage, compromise of covering tissue integrity, fluctuations and changes in temperature regimes, and conditions leading to increased water loss. These stress factors cause intensive generation of partially reduced reactive oxygen species (ROS), such as singlet oxygen ( $^1\text{O}_2$ ), superoxide anion ( $\text{O}_2^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydroxyl radical ( $\text{OH}^\cdot$ ), peroxyxynitrite ( $\text{ONOO}^-$ ). Free radicals and other oxygen derivatives are inevitable side products of biological redox reactions, as well as a consequence of aerobic metabolism in plants [7]. They are formed in the process of respiration, photosynthesis, oxidation of fatty acids. Depending on their concentration in the cell, ROS can be both harmful and beneficial. At high concentrations, ROS can damage various types of biomolecules, whereas at low or moderate concentrations, they serve as messengers in intracellular signaling pathways [8]. ROS signaling is important during plant vegetation. However, in the postharvest period, excess ROS generation leads to the loss of the body's ability to maintain cellular redox homeostasis. The duration of reactive oxygen species (ROS) activity within tissues is determined by the antioxidant system or the antioxidant status of the cell (AOS), comprising a collection of protective mechanisms within cells, tissues, organs, and systems aimed at preserving and maintaining homeostasis. Endogenous antioxidants help maintain a low steady-state level of ROS, thereby preventing oxidative damage during the postharvest period.

The antioxidant system of plant tissues is formed basing on the complex of non-enzymatic (low-molecular) and enzymatic (high-molecular) antioxidants. Low-molecular-weight antioxidants (AO) are most important in the early stages of activation of increased ROS formation. These substances donate their hydrogen atom, transform free radicals into stable molecules and prevent the development of a chain reaction of peroxide oxidation. However, with time their number is quickly exhausted and

depends on the activity of enzymes that restore them. Low-molecular non-enzymatic antioxidants are present in all plant organs and include ascorbic acid, carotenoids, phenolic compounds, glutathione, etc. [8].

Ascorbic acid (AA) has several antioxidant properties: it acts as a primary substrate in the cyclic pathway of enzymatic detoxification of ROS ( $H_2O_2$ ), has the ability to directly neutralize superoxide radicals, singlet oxygen, and hydroxyl radicals. AA also serves as a cofactor for many enzymes and promotes ROS detoxification. In addition, the endogenous level of AA plays an important role in the regulation of aging processes and protection against pathogens [9]. Certain vegetables contain large amounts of AA. However, AA content tends to decrease during storage. Losses of AA during storage of plant products are associated with enzymatic metabolism and oxidation by ascorbate oxidase localized in the cell wall. Plant AA is also oxidized by peroxidase.

Plant carotenoids (CAR) belong to the group of lipophilic antioxidants and are able to neutralize various forms of ROS. Carotenoids are the main utilizers of singlet oxygen [10]. They protect cellular structures from the influence of ROS, not only by extinguishing singlet oxygen, but also prevent peroxidation of lipid components of cell membranes by neutralizing peroxide radicals and interrupting the chain reactions of free radical oxidation of unsaturated carboxylic acids. The ability of carotenoids to utilize excess ROS and prevent or minimize the formation of triplet chlorophyll is defined by the specificity of their chemical structure. CARs have a chain of isoprene residues with multiple double bonds that allow easy absorption of energy from excited molecules and dissipation of excess energy as heat. Carotenoids also serve as precursors of signaling molecules that influence the development of plant responses to biotic and abiotic stresses [10].

Phenolic compounds (PC) are various secondary metabolites (flavonoids, tannins, hydrocinnamic esters and lignin) that have antioxidant properties. Polyphenols contain an aromatic ring with several hydroxyl groups, which determines their biological activity, including antioxidant action. In terms of antioxidant activity, phenolic substances are not less efficient than ascorbic acid or  $\alpha$ -tocopherol. Polyphenols can chelate metal ions with the help of phenolic OH groups. Metals with variable valence are often involved in the generation of free radicals through the decomposition of hydrogen peroxide and lipid hydroperoxides, with the formation of hydroxyl or alkyl radicals, respectively. Flavonoids, by chelating the metal, can isolate these ions, and thus prevent the generation of free radicals. In addition, flavonoids and phenylpropanoids are oxidized by peroxidase, and hydrogen peroxide is utilized through the PC/AA/peroxidase system [10]. Total phenolic content (TPC) in plant products is strongly correlated with their antioxidant activity.

Studies of the last decade prove that simple carbohydrates in plant cells also perform the functions of antioxidants and signaling molecules [11]. Thus, on the one hand, an increase in the content of sugars (SAC) can be the cause of changes in the ROS generation by mitochondria, on the other hand, the activation of the pentose phosphate oxidation pathway can be a source of antioxidants. As a new concept, a theory is proposed, according to which soluble carbohydrates can participate in vacuolar antioxidant processes under stress [12]. According to this point of view, sugars that accumulate in significant amounts in vacuoles can act as scavengers of ROS, acting together with vacuolar phenolic compounds. It is believed that any saccharide in close proximity to any cell membrane has the potential to act as a ROS acceptor and contribute to membrane stability under stress conditions. During the storage of fruit and vegetable products, simple saccharides are formed during the degradation of polysaccharide components and at the same time are used to maintain the postharvest metabolism, which is the reason for the change in the concentration of soluble saccharides during the storage of vegetable products.

Three enzymes are mainly responsible for the enzymatic system of protecting the body against oxidative damage: superoxide dismutase, catalase, and peroxidase [8].

Superoxide dismutase (SOD) is one of the most important components of the system of protecting cells and tissues from oxidative destruction. Superoxide dismutase plays a central role in protection against oxidative stress in all aerobic organisms. SOD exists in four isoforms (CuZn-SOD, Mn-SOD, Fe-SOD, Ni-SOD) [7]. SOD is present in plant cells where redox processes involving oxygen occur. A comparison of data on the localization of different forms of SOD shows that CuZn-SOD is most abundant in plant cells. All isoforms of SOD are united by a single function – dismutation of superoxide radicals. Superoxide radicals are a source of formation of other ROS, including more reactive ones. Because hydroxyl radicals, singlet oxygen, and peroxynitrite actively oxidize protein molecules, there are no specific deactivator enzymes for these reactive oxygen species (ROS). Instead, their levels in the cell are indirectly regulated by SOD through the utilization of superoxide radicals, which are the source of their formation. Hence, SOD serves as the primary line of defense against oxidative damage by interrupting the oxidation of cellular macromolecules at the initiation stage.

The result of dismutation of superoxide anions is hydrogen peroxide, therefore, utilizing hydrogen peroxide is a necessary link in plant antioxidant protection. In the cell like this, it is provided by such enzymes as catalase and peroxidase – part of the second line of defense against ROS. Catalase (CAT) catalyzes the conversion of  $H_2O_2$  into water and  $O_2$ . It is believed that catalase does not have a high affinity for  $H_2O_2$  and cannot efficiently neutralize this compound at the low concentrations present

in the cytosol. In peroxisomes, where the concentration of hydrogen peroxide is high, catalase actively destroys it. However, catalase is practically absent in some cell compartments, so there is a need for the functioning of other enzymes involved in the detoxification of hydrogen peroxide.

Peroxidases (PODs) catalyze hydrogen peroxide reduction reactions involving various substrates. In dependence of the substrate, peroxidases are divided into three groups. Guaiacol peroxidase is present in cell walls and vacuoles, where it reduces hydrogen peroxide due to the oxidation of phenolic compounds. Ascorbate peroxidase is involved in the  $H_2O_2$  detoxification in the cell due to the oxidation of ascorbic acid. In addition, glutathione peroxidase is present in plant tissues. This enzyme can potentially use glutathione to reduce hydrogen peroxide. In general, peroxidases, reacting with hydrogen peroxide, form substrate oxidation products and water. Some scientists single out the vacuolar ascorbate/phenol/peroxidase system as an important component of the antioxidant complex [13].

Endogenous antioxidants contained in vegetables create an inner circle of anti-radical protection, which contributes to the preservation of their natural quality and nutritional properties. As a result of the disruption in the synthesis pathways of substances essential for normal metabolism, the system of antioxidant control over the generation of reactive oxygen species (ROS) functions properly only for a limited duration. When irreversible aging processes develop, the ROS level increases dramatically [14] and the antioxidant defense capabilities exhaust, which leads to a number of metabolic disorders and cell death.

A well-functioning antioxidant system is necessary to protect against postharvest stresses, maintain the quality of vegetables during storage, and prevent postharvest physiological disorders. A reliable relationship between the endogenous pool of antioxidants and the preservation of fruit and vegetable products has not been established. However, the formation of a powerful system of antioxidant protection can contribute to increasing the preservation of vegetables.

#### **1.4 Regulation of postharvest metabolism by exogenous substances**

Application of coatings on the surface of fruit and vegetable products has been actively used since the beginning of the 2000s. In contrast to synthetic polymer packaging, biodegradable coatings offer a more environmentally friendly solution. The use of edible coatings can be an effective strategy for maintaining the endogenous system of vegetables and ensuring their quality during a long period of storage. Such coatings can also affect the shelf life, reducing losses and helping to preserve

the valuable properties of vegetables. Edible coatings act as an additional layer that covers the stomates. The main function of edible coatings is to limit respiratory gas exchange and transpiration, hence slowing down the ripening and aging process of the fruit. Such coatings can be used as an alternative method of protection against oxidative stress and food spoilage. However, the gas permeability of the coating could prevent the development of anaerobic fermentation and undesirable changes in taste qualities. From a practical point of view, achieving such an effect can be noticeably difficult.

Edible coatings can be produced from various biopolymers. Among the most widely used are various natural polysaccharides (chitosans, alginates, pectins, starches, cellulose derivatives, carrageenans and gums), protein polymers (caseinates, milk protein concentrate, whey protein, gelatins, zein, gluten) and lipid components (waxes, paraffin, essential oils, resins, actoglycerides, emulsifiers and plasticizers) [15].

Ideal coatings should meet many requirements, namely:

- be generally recognized as safe;
- do not grant vegetables an extraneous smell and taste;
- be transparent and not affect the natural color of the fruit;
- ensure the slowing down of breathing and evaporation of moisture;
- maintain a normal level of oxygen in the tissues, preventing the creation of anaerobic conditions;
- possess antimicrobial properties;
- have a melting point above 40 °C;
- have low viscosity and high plasticity;
- dry well without additional measures;
- be non-sticky and non-brittle after drying.

Nowadays, however, edible coatings still have certain disadvantages. For example, natural polysaccharides, as a rule, are hydrophilic compounds, have low water resistance and unsatisfactory mechanical properties. At the same time, chitosan coatings have a good antimicrobial effect. Protein coatings significantly affect moisture and gas exchange, slowing down metabolism, but do not have bactericidal properties and can cause allergic reactions. Lipid coatings have hydrophobic properties, so they are an excellent barrier to moisture loss. Still, these coatings have unsatisfactory mechanical characteristics and are highly brittle. The mechanical properties of coatings are improved with the help of low-molecular plasticizers (glycerin, sorbitol, polyethylene glycol). On the other hand, such compounds change the organoleptic properties of products, so their use is undesirable.

Several recent scientific studies consider the possibility of obtaining edible and biodegradable films by combining different polysaccharides, proteins and lipids.

Their goal is to leverage the properties of each component effectively and attain synergy among them. The mechanical and barrier characteristics of these coatings depend not only on the compounds used in the polymer matrix, but also on their interaction and compatibility. Improving the composition of edible coatings is recognized as one of the key problems of scientific research in this area. This task requires careful formulation of the components of the films so that they correspond to the properties of the specific fruits and vegetables to which their application is planned.

A new trend in edible coatings is the introduction of components with high biological activity to obtain desired properties and expand their functionality. Most often this applies to antimicrobial and antioxidant substances. After all, the constant increase in the amount of ROS due to the aging processes must be balanced by a pool of antioxidants. This concept, therefore, is utilized by introducing exogenous antioxidants into the composition of edible coatings. Using synthetic antioxidants for this purpose is currently limited because of their potential toxic effects. Moreover, consumers perceive use of the natural antioxidants as an advantage, although they possess weaker antioxidant activity. The addition of such antioxidants as ascorbic acid, citric acid, resveratrol or tocopherol to the composition of edible coatings was demonstrated [16]. Essential oils and natural phenolic compounds are also often used. Extensive research on natural antioxidants for preserving fruit and vegetable raw materials is driven by their additional properties. In particular, flavonoids were shown to cause anti-carcinogenic, antibacterial, anti-allergic and antiviral effect.

The efficiency of storage significantly varies depending on the concentration of processing substances, storage conditions, and the type of fruit along with its characteristics. The effect of exogenous antioxidants is also dose-dependent. For example, in case of agave storage, a combined coating based on sodium caseinate and gum arabic with cinnamon and lemongrass oils in different concentrations was used. The use of cinnamon oil and lemongrass oil at a concentration of 1 % made it possible to obtain good color characteristics of guava ( $L^*$  value varied between 63–72). However, when both oils were used at a concentration of 2 %, the color characteristics were significantly degraded ( $L^*$  value was 39). In addition, at higher concentrations of essential oils, the content of ascorbic acid and the overall antioxidant activity of guava decreased [17], which is evidence of a pro-oxidant effect.

Maintaining the pro-antioxidant balance in plant tissues is crucial for preserving the quality of vegetables in the postharvest period. High doses of certain antioxidant compounds can be toxic, due to their pro-oxidant effects or the ability to react with physiological concentrations of ROS, which are necessary for the optimal functioning of the cell [18]. Such an extreme dependency of antioxidant effectiveness on concentration poses a significant obstacle to their widespread utilization. Namely, when

using antioxidants in edible coatings for a different type or variety of vegetables, it is necessary to check the effectiveness of the selected concentrations each time experimentally. As a consequence, when conducting research, the selection of effective concentrations of exogenous antioxidants takes a lot of time and labor resources.

To bolster the potency of the endogenous antioxidant system, it's logical to set concentrations of exogenous biologically active substances based on the evaluation of the plant organism's antioxidant status. In other words, concentrations of exogenous antioxidants should be inversely correlated with the endogenous pool of antioxidants.

### **1.5 Integral assessment of the antioxidant status of vegetables**

Antioxidant status can be defined as the overall ability of a system or organism to neutralize free radicals and prevent oxidative stress. In contrast, "antioxidant activity" is a specific indicator or measure of the ability of a particular antioxidant or group of antioxidants to neutralize free radicals. Antioxidant activity is measured in percentages or other units of measurement and indicates how effectively a particular antioxidant is able to prevent oxidation.

The integral assessment of the antioxidant status of the system is a challenging task. Laboratory methods for assessing total antioxidant activity have a number of features that limit the possibilities of their application. Neither method measures all the antioxidants present in the system. The tests are limited to estimation of the effect of oxidatively active antioxidants, and therefore do not measure the catalytic effect of high molecular weight antioxidants. Some methods can be less specific and determine content of not only antioxidants, but also other compounds. Number of biologically active substances in plants do not necessarily have a pronounced antioxidant effect, but can still cause interference during analysis, leading to inaccurate results. Some processes in sample preparation (grinding, stabilization) before analysis itself might alter antioxidant properties of the sample, which makes the results less accurate. Today, there is no universally accepted "standard" method for determining antioxidant status, and even with the same method, reaction conditions can vary greatly in different laboratories, thus creating difficulties for interpretation and operation with results obtained by other researchers. Some variability in results, available in the literature, may arise from differences in the chosen measurement methods or from individual differences between samples. It is worth noting that laboratory methods of research require specialized equipment and are labor intensive, which makes them less reasonable to use on a mass scale or in settings where rapid assessment is required.

The obtained information is not always direct, which additionally complicates interpretation of results and determination of the exact relationships.

In such cases, it is advantageous to ensure the accuracy of judgments using mathematical methods. While there are numerous methods for tackling complex multi-criteria problems, most of them come with significant limitations and shortcomings that restrict their applicability. Previously [19] we suggested to use the Analytic Hierarchy Process (AHP), developed by T. Saaty [20] for the mathematical assessment of the antioxidant status of fruits and vegetables. Hierarchy analysis method is widely used in project management, decision-making, strategic planning and other areas. It allows to systematize and coordinate various aspects of decision-making and determine their importance in a hierarchical structure.

The primary drawback of AHP is arguably the subjectivity of evaluation judgments. However, an undeniable advantage of AHP is its capability to accommodate the variation in measurement units of the components within the antioxidant system. This method enables the comparison and assessment of the antioxidant status of any type of product. The subjectivity of the assessment can be mitigated by relying on experimental or analytical data regarding the quantitative indicators of the chemical composition.

The basic idea behind AHP is to break down a complex solution into smaller, more manageable steps. The process includes the following stages:

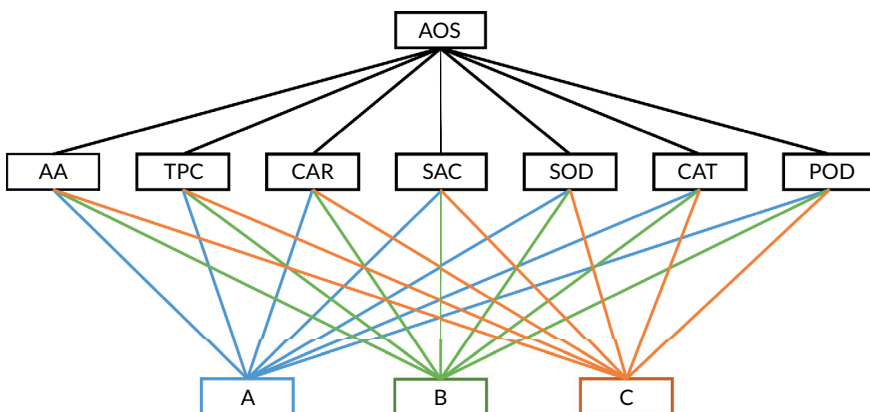
1. Hierarchy creation: breaking down the problem on the levels of criteria and alternatives to form a hierarchical structure.
2. Pairwise comparison analysis: evaluating the importance of each element of the hierarchy by means of pairwise comparisons. A matrix of pairwise comparisons is usually used to obtain numerical values of importance.
3. Element importance calculation: calculating the importance of each element using mathematical operations such as generalized eigenvalues.
4. Decision synthesis: making decisions and comparing alternatives based on the calculated importance.
5. Sensitivity to changes: providing assessment of the impact of changes in the input data or decisions made on the final result.

The step of creating hierarchies in AHP is considered as the initial step in solving a complex problem or making decisions. This stage includes defining the goal and creating a hierarchical structure by breaking down the problem into component parts. For example, here the AOS evaluation of asparagus of three different color varieties (green Prius, green-purple Rosalie and purple Erasmus) is demonstrated. Experimental data of the asparagus biochemical composition were obtained by our group under the identical laboratory conditions and averaged (**Table 1.1**).

**Table 1.1** The content of antioxidants in asparagus

Antioxidants	Prius	Rosalie	Erasmus
AA, mg·100 g <sup>-1</sup> FW	18.04	22.68	13.64
TPC, mg·100 g <sup>-1</sup> FW	93.90	94.15	98.82
CAR, mg·100 g <sup>-1</sup> FW	3.76	4.39	4.12
SAC, g·100 g <sup>-1</sup> FW	2.63	2.89	2.95
SOD, % inhibition of adrenaline auto-oxidation	108.55	101.26	119.32
CAT, μmol H <sub>2</sub> O <sub>2</sub> ·g <sup>-1</sup> ·min <sup>-1</sup>	43.27	59.83	62.13
POD, μmol H <sub>2</sub> O <sub>2</sub> ·g <sup>-1</sup> ·min <sup>-1</sup>	59.94	24.62	68.97

The built structure includes three levels: a global criterion (general goal – integral assessment of AOS), a level of criteria (individual antioxidants of the system) and a level of alternatives (species or varieties of vegetables) (Fig. 1.1).

**Fig. 1.1** Hierarchical structure of antioxidant status assessment of three vegetable varieties

Depending on the available experimental or analytical data on the components of the antioxidant protection system of a specific species or variety of vegetables, the criteria of the hierarchy can be supplemented with other antioxidants or changed, as well as sub-criteria can be evaluated. For example, it is possible to base evaluation not on the total content of phenolic substances, but on content of phenolic acids or flavonoids, or to consider them as sub-criteria of the hierarchy.

At each level of the hierarchy, the importance of elements relative to each other is determined. Experts rank each criterion and alternative relative to the global criterion. For this, the procedure of pairwise comparisons is used, where for each pair of elements their relative influence or importance is evaluated. When assessing the importance of each antioxidant, it's essential to consider their individual contributions to the antioxidant system (AOS), as well as correlations between components and with markers of oxidative stress like malondialdehyde. This approach helps mitigate subjectivity in the assessment process. This approach allows to systematize and take into account various aspects of antioxidant activity ensuring objectivity in decision-making.

For each level, a matrix of pairwise comparisons is created, where elements relative to each other receive numerical values, reflecting the degree of their importance. To present the results of assessments in quantitative terms, T. Saaty introduces a pairwise comparison scale [20] (Table 1.2).

**Table 1.2 The pairwise comparison scale by T. Saaty**

Relative importance (score)	Definition	Explanation
1	equal importance	both elements contribute equally
3	one element is slightly more important than another	experience and judgement slightly favour one element over another
5	strong advantage	experience and judgement strongly favour one over the other
7	very strong advantage	the dominance if one element is efficiently demonstrated in practice
9	absolute superiority of one over the other	the evidence favouring one element over another is of the highest possible order of affirmation
2, 4, 6, 8	intermediate scores between adjacent statements	a compromise decision
Reciprocal values of the above-mentioned scores	if one element is assigned a number between 1 and 9 when comparing it with another, then when comparing the second element with the first, the reciprocal value is obtained	a reasonable assumption

According to this scale, the difference in units of measurement does not matter. The scale involves a pairwise comparison of the weight (importance) of each element

with the weight of other elements of the set, which is carried out using expert judgments that are quantified. The primary advantage of the method is its dimensionless nature, eliminating issues when converting to the same units of measurement (Table 1.3).

**Table 1.3** Pairwise comparison matrix for the set of criteria

Antioxidant	AA	TPC	CAR	SAC	SOD	CAT	POD	Eigenvector	Priority vector
AA	1	3	3	5	1	1	1	1.7226	0.1833
TPC	1/3	1	2	4	1/5	1/5	1/4	0.5959	0.0634
CAR	1/3	1/2	1	4	1/5	1/5	1/4	0.4888	0.0520
SAC	1/5	1/4	1/4	1	1/7	1/7	1/5	0.2437	0.0259
SOD	1	5	5	7	1	3	4	2.9827	0.3174
CAT	1	5	5	7	1/3	1	2	1.9737	0.2100
POD	1	4	4	5	1/4	1/2	1	1.3895	0.1480
$\Sigma$								9.3967	1.0000
$\lambda_{\max}$									7.4982
C.I.									0.0830
C.R.									0.0629

To obtain priority estimation from the matrix, an algorithm is employed, which follows a schematic form like the following:

1. According to the approximate formula, the main eigenvector of the matrix is determined as the geometric mean of the corresponding row:

$$w_i = \sqrt[n]{\prod_{j=1}^n a_{ij}}, \quad (1.1)$$

where  $w_i$  – components of the eigenvector;  $n$  – matrix dimension (7 in the current example);  $a_{ij}$  – components of the matrix,  $i \in \{1 \dots n\}$ ,  $j \in \{1 \dots n\}$ .

Hence:  $w_1 = \sqrt[7]{1 \cdot 3 \cdot 3 \cdot 5 \cdot 1 \cdot 1 \cdot 1} = \sqrt[7]{45} = 1.7226$ , etc. (Table 1.2).

2. The found components of the eigenvector are normalized:

$$v_i = \frac{w_i}{\sum_{i=1}^n w_i}, \quad (1.2)$$

where  $v_i$  – components of the normalized vector.

$$\sum_{i=1}^7 w_i = 9.3967, \text{ thus:}$$

$$v_1 = \frac{1.7226}{9.3967} = 0.1833; v_2 = \frac{0.5959}{9.3967} = 0.0634; \text{ etc. (Table 1.2).}$$

The consistency of the inversely symmetric source matrix of pairwise comparisons is equivalent to the condition of equality between its maximum eigenvalue  $\lambda_{\max}$  and the number of compared objects  $n$ , i.e.  $\lambda_{\max} = n$ . Therefore, as a measure of inconsistency, it is customary to consider the normalized deviation from  $n$ , called the consistency index. Consistency of priorities is calculated as a matrix consistency index:

$$C.I. = \frac{\lambda_{\max} - n}{n - 1}, \tag{1.3}$$

where  $C.I.$  – consistency index;  $\lambda_{\max}$  – the largest eigenvalue of the matrix, which is found according to the standard algorithm available in online calculators.

The  $\lambda_{\max}$  of the matrix of pairwise comparisons for the criteria level was calculated as 7.50. Then:

$$C.I. = \frac{7.5 - 7}{7 - 1} = 0.083.$$

To assess the degree of consistency of judgments, the index of consistency  $C.I.$  is compared with a random index. A random index is a consistency index calculated for a square  $n$ -dimensional positive inversely symmetric matrix, the elements of which are generated by a random number sensor for the range of values from 1 to 9 (Table 1.4).

**Table 1.4** Random consistency index ( $R.I.$ )

Matrix size	1	2	3	4	5	6	7	8	9	10
$R.I.$	0	0	0.58	0.9	1.12	1.24	1.32	1.41	1.45	1.49

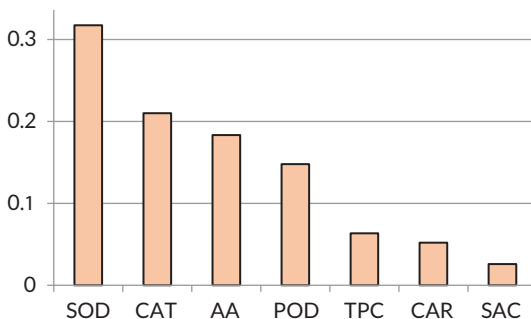
Having the consistency index and choosing from the Table 1.3 random index for the given order of the matrix, the consistency ratio can be calculated:

$$C.R. = \frac{C.I.}{R.I.}, \tag{1.4}$$

where  $C.R.$  – consistency ratio;  $R.I.$  – random consistency index.

$$C.R. = \frac{0.083}{1.32} = 0.063.$$

The acceptable value of *C.R.* must be about 10 % or less. If *C.R.* exceeds these limits, the judgment in the matrix have to be checked. In our case, *C.R.*=0.063, i.e. the received priorities are completely consistent. The ranking of AOs according to the calculated priority estimations is presented in the **Fig. 1.2**.



**Fig. 1.2** Ranking of endogenous AOs in vegetable tissues

Based on **Fig. 1.2**, it's evident that SOD makes the maximum contribution to AOS, while sugars play a minimal role.

The next step involves comparing asparagus varieties based on second-level criteria. For each criterion, we compare the asparagus varieties by compiling 3×3 judgment matrices. According to the algorithm described earlier (formulas (1.1)–(1.4)), priority ratings and consistency of the matrix are calculated for each criterion. The matrix of pairwise comparisons for AA is characterized by an acceptable consistency of about 9 % (**Table 1.5**).

**Table 1.5** Comparison matrix for AA

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	1/4	6	1.1447	0.2430
Rosalie	4	1	9	3.3019	0.7008
Erasmus	1/6	1/9	1	0.2646	0.0562
$\Sigma$				4.7112	1.0000
$\lambda_{\max}$					3.1080
<i>C.I.</i>					0.0540
<i>C.R.</i>					0.0931

Rosalie has the highest priority for AA, and Erasmus has the lowest.

The matrix of pairwise comparisons for TPC allows to obtain an estimate of priority with a consistency of the matrix of about 1.5 % (**Table 1.6**).

**Table 1.6 Comparison matrix for TPC**

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	1/2	1/4	0.5000	0.1365
Rosalie	2	1	1/3	0.8736	0.2385
Erasmus	4	3	1	2.2894	0.6250
$\Sigma$				3.6630	1.0000
$\lambda_{\max}$					3.0180
C.I.					0.0090
C.R.					0.0155

The maximum priority of PC is for Erasmus, and the minimum for Prius.

For carotenoids, the matrix consistency ratio is only 0.3 %. However, such a high degree of agreement can be a disadvantage and may indicate excessive confidence of experts in their judgments (**Table 1.7**).

**Table 1.7 Comparison matrix for CAR**

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	1/5	1/3	0.4055	0.1094
Rosalie	5	1	2	2.1544	0.5816
Erasmus	3	1/2	1	1.1447	0.3090
$\Sigma$				3.7046	1.0000
$\lambda_{\max}$					3.0040
C.I.					0.0020
C.R.					0.0034

The consistency of the matrix for sugars is identical to the matrix for phenolic compounds (**Table 1.8**).

As can be seen from the **Table 1.8**, Erasmus asparagus has the highest priority in terms of sugar content.

According to SOD activity, the highest priority is typical for Erasmus. The constructed matrix has a consistency ratio of 4.7 % (**Table 1.9**).

**Table 1.8** Comparison matrix for SAC

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	1/2	1/3	0.5503	0.1692
Rosalie	2	1	1	1.2599	0.3874
Erasmus	3	1	1	1.4422	0.4434
$\Sigma$				3.2525	1.0000
$\lambda_{\max}$					3.0180
C.I.					0.0090
C.R.					0.0155

**Table 1.9** Comparison matrix for SOD

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	3	1/4	0.9086	0.2176
Rosalie	1/3	1	1/6	0.3816	0.0914
Erasmus	4	6	1	2.8845	0.6910
$\Sigma$				4.1746	1.0000
$\lambda_{\max}$					3.0540
C.I.					0.0270
C.R.					0.0465

The matrix of pairwise comparisons of asparagus by catalase activity allows to obtain estimations of priority with a consistency of about 2 % (**Table 1.10**).

**Table 1.10** Comparison matrix for CAT

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	1/4	1/5	0.3684	0.0974
Rosalie	4	1	1/2	1.2599	0.3331
Erasmus	5	2	1	2.1544	0.5695
$\Sigma$				3.7828	1.0000
$\lambda_{\max}$					3.0250
C.I.					0.0125
C.R.					0.0215

The largest catalase activity priority vector is typical for Erasmus, and the smallest for Prius. The matrix of paired comparisons of fruit and vegetables by peroxidase activity has a consistency ratio of 0.0560 (Table 1.11).

**Table 1.11 Comparison matrix for POD**

Asparagus	Prius	Rosalie	Erasmus	Eigenvector	Priority vector
Prius	1	5	1/3	1.1856	0.2789
Rosalie	1/5	1	1/7	0.3057	0.0719
Erasmus	3	7	1	2.7589	0.6491
$\Sigma$				4.2503	1.0000
$\lambda_{\max}$					3.0650
C.I.					0.0325
C.R.					0.0560

Peroxidase activity has the highest priority for Erasmus and the lowest for Rosalie.

After determining the importance of all elements and constructing matrices of pairwise comparisons, an analysis is carried out to obtain the importance (weight) of each element. A synthesis of the hierarchy is carried out, which allows to consider all aspects and make the decision.

Global priorities, which will be integral assessments of the antioxidant status of asparagus varieties, are calculated using the following formula:

$$I_{AOS} = P_1^2 \cdot P_1^3 + P_2^2 \cdot P_2^3 + \dots + P_n^2 \cdot P_n^3, \quad (1.5)$$

where  $I_{AOS}$  – integral assessment of antioxidant status;  $P_1^2 \dots P_n^2$  – priority evaluations of the matrix of criteria;  $P_1^3 \dots P_n^3$  – priority evaluations of the matrix of alternatives.

For asparagus of Prius variety:

$$\begin{aligned} I_{AOS} &= 0.1833 \cdot 0.2430 + 0.0634 \cdot 0.1365 + 0.0520 \cdot 0.1094 + \\ &+ 0.0259 \cdot 0.1692 + 0.3174 \cdot 0.2176 + 0.2100 \cdot 0.0974 + 0.1480 \cdot 0.2789 = \\ &= 0.1940 \approx 0.19 \text{ (Tables 1.3, 1.5–1.11)}. \end{aligned}$$

By similar calculations, we get:

- for Rosalie  $I_{AOS} = 0.2934 \approx 0.29$ ;
- for Erasmus  $I_{AOS} = 0.51245 \approx 0.51$ .

The calculated integral evaluation shows that, among the studied varieties, the highest antioxidant status is in asparagus of the Erasmus variety, and the lowest

in asparagus of the Prius variety. Therefore, when applying exogenous edible coatings, the concentration of antioxidants in the composition can be adjusted according to the established antioxidant status. Such approach ensures the prevention of product losses during extended shelf life.

## **Conclusions**

Reducing losses of fruit and vegetable products, particularly during storage, is a pressing issue that requires attention and technological advancement. Addressing these losses not only ensures a sustainable food resource but also aids in reducing greenhouse gas emissions and optimizing resource utilization.

Maintaining a well-functioning antioxidant system is crucial for preserving vegetable quality during storage and preventing postharvest disorders. Utilizing edible coatings with antioxidant properties emerges as an effective strategy for maintaining vegetable quality throughout the storage period. However, it is important to note that excessive antioxidant doses can potentially have toxic effects, and their efficacy is influenced by the concentration and type of vegetables.

To enhance the potency of the endogenous antioxidant system, it is vital to establish concentrations of exogenous antioxidants that correlate with the endogenous antioxidant pool in plant tissues. This approach ensures the maintenance of antioxidant status and quality preservation during the postharvest period. Here we propose a method that employs hierarchical analysis for objective assessment of vegetable antioxidant status.

While the hierarchical analysis method offers a systematic approach, it has drawbacks related to the subjectivity of the evaluation judgments. They can be omitted by integrating into calculations experimental or analytical data on chemical composition, as well as correlations between antioxidant system components and oxidative stress markers. The proposed integrated approach provides objectivity and aids decision-making in determining vegetable antioxidant status, thereby contributing to the prevention of product losses during extended storage.

## **Conflict of interest**

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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