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THE ROLE OF CAROTENOIDS IN PREVENTING OXIDATIVE STRESS: EXPLORING THE ANTIOXIDANT POTENTIAL OF PUMPKIN AS A FUNCTIONAL CROP

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Abstract: This review paper aims to explore the antioxidant properties of carotenoids and their role in mitigating oxidative stress caused by reactive oxygen species (ROS). Excessive ROS levels are linked to cellular damage, contributing to chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. Carotenoids, natural pigments in various fruits and vegetables, exhibit significant antioxidant activity by neutralizing ROS and protecting cellular components. This paper highlights how carotenoids combat oxidative stress, emphasizing their ability to scavenge free radicals and prevent further lipid, protein, and DNA damage. Pumpkin (*Cucurbita* spp.) stands out as a valuable alternative crop due to its high carotenoid content, potential for sustainable cultivation, and versatility in developing functional foods and nutraceuticals. Among carotenoid-rich sources, pumpkins are distinguished by their high β-carotene content and additional carotenoids like lutein and zeaxanthin. These compounds not only enhance antioxidant defenses but also provide provitamin A activity, contributing to overall health. The review further discusses factors influencing carotenoid content in pumpkins, including cultivation practices, and post-harvest storage conditions. It also examines the impact of processing methods on carotenoid bioavailability, highlighting techniques such as steaming and freeze-drying that optimize nutrient retention. Key findings underscore the relevance of pumpkins as a sustainable and cost-effective source of carotenoids, suitable for functional food development. Promoting the inclusion of pumpkin-based products in diets is proposed as a practical strategy to combat oxidative stress and support public health.

Key words: *antioxidants, carotenoids, Cucurbita, oxidative stress, Reactive Oxygen Species (ROS)*

INTRODUCTION

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. While ROS play essential roles in cellular signaling and defense mechanisms, their overproduction or inadequate removal can lead to oxidative stress, damaging biomolecules and disrupting cellular homeostasis (Shehzad & Mustafa, 2023). ROS are naturally generated in cells during metabolic and physiological processes, but environmental and lifestyle factors can also

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contribute to ROS production (Martemucci et al., 2022). Under normal physiological conditions, ROS are balanced by antioxidant systems, including enzymatic defenses and non-enzymatic molecules. However, when ROS levels exceed the cellular antioxidant capacity, oxidative stress occurs, leading to DNA damage, protein oxidation, and lipid peroxidation (Juan, Pérez de la Lastra, Plou & Pérez-Lebeña, 2021). This imbalance contributes to the pathogenesis of numerous diseases, including cancer, neurodegenerative disorders, cardiovascular diseases, and aging, hence understanding ROS and mitigating oxidative stress are crucial for maintaining cellular health and preventing disease (Jomova et al., 2023).

Carotenoids are a large group of naturally occurring pigments found in plants, algae, and some bacteria. They are responsible for the bright yellow, orange, and red colors in many fruits, vegetables, and flowers (Swami et al., 2020). They are known for their provitamin A and antioxidant activity (Merhan, 2017). Carotenoids act as potent antioxidants by neutrallizing ROS through different mechanisms, particularly singlet oxygen ($^1O_2$) and other free radicals (Ahmad, 2024). Their polyene structures allow them to stabilize reactive species, thereby preventing oxidative damage to lipids, proteins, and DNA (Krinsky & Yeum, 2003; Stahl & Sies, 2003). The protective effects of carotenoids extend beyond their antioxidant activity. For example, some studies highlight their role in modulating oxidative stress-related pathways and enhancing cellular resilience to oxidative insults (Kaulmann & Bohn, 2014).

This topic is particularly significant in the context of increasing oxidative stress due to environmental pollution, unhealthy diets, and stress-related factors. Promoting the intake of carotenoid-rich foods such as carrots, pumpkins, spinach, and tomatoes, represents a practical strategy for improving the body’s natural defense mechanisms and reducing the impact of ROS. Moreover, carotenoids are being explored in nutraceuticals and functional foods for their therapeutic potential, further emphasizing their importance in preventive health and disease management (Fiedor & Burda, 2014; Tan & Norhaizan, 2019).

This review explores the relationship between carotenoids and reactive oxygen species (ROS), emphasizing the critical role of carotenoids in mitigating oxidative stress and promoting cellular health. The emphasis is on pumpkins, a carotenoid-rich raw material, and exploring the potential of carotenoids from this crop as natural antioxidants. By examining the mechanisms through which carotenoids neutralize ROS and their implications for human health, this review aims to highlight the value of pumpkin in addressing oxidative stress-related challenges.

CAROTENOIDS: STRUCTURE, CLASSIFICATION, AND FUNCTIONS

Carotenoids are a diverse group of naturally occurring pigments synthesized by plants, algae, and certain bacteria. They are characterized by their long, conjugated polyene chains, which consist of alternating double and single bonds. They are composed of eight isoprene units and are classified as tetraterpenes in terms of their chemical structure (Lajšić & Grujić-Injac, 1998). This conjugated system is responsible for the vivid yellow, orange, and red color of these molecules, and their antioxidant properties, as it allows carotenoids to absorb light and neutralize ROS (Britton, 2022).

Carotenoids can be classified into two main categories based on their chemical composition: *carotenes* which are purely hydrocarbon-based carotenoids, containing no oxygen atoms in their structure (e.g. β-carotene, lycopene), and *xanthophylls* which are oxygenated derivatives of carotenes, containing functional groups such as hydroxyl (-OH) or keto (=O) groups (e.g. lutein, zeaxanthin) (Maoka, 2020).

Carotenoids play vital roles in plant life, particularly in photosynthesis and photoprotection. They absorb light in the blue-green spectrum (400–500 nm) and transfer energy to chlorophyll molecules, optimizing photosynthetic efficiency (Demmig-Adams, Stewart, López-Pozo, Polutchko & Adams, 2020). Carotenoids also protect plants from photo-oxidative damage by quenching singlet oxygen ($^1O_2$), a harmful by-product of photosynthesis (Havaux, 2023). Regarding human health, carotenoids act as potent antioxidants by scavenging ROS and protecting cells from oxidative stress. Some carotenoids, such as α-carotene, β-carotene, and β-cryptoxanthin, can exhibit provitamin A activity (Kadian & Garg, 2012). Under the action of oxygenases, they can be converted into vitamin A (retinol), which also possesses antioxidant properties. This characteristic is associated with the presence of an unsubstituted ring at the end of the molecule (the β-ionone ring). Thus, one molecule of β-carotene, which contains two β-ionone rings, is converted by the enzyme carotene dioxygenase into two molecules of vitamin A.

Carotenoids influence immune responses by enhancing the production of immune cells and reducing inflammation. For instance, β-carotene has been shown to stimulate the activity of natural killer cells and T-lymphocytes, contributing to enhanced pathogen defense (Chew & Park, 2004). Among the 600 known carotenoids, those that stand out from a nutritional perspective include: β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin (Meléndez-Martínez et al., 2022).

The carotenoid content in plants is influenced by a combination of genetic and environmental factors, as well as the applied agro-technical procedures and post-harvest processing. The genetic structure of a plant determines its capacity to synthesize and accumulate specific carotenoids. Variations in carotenoid biosynthesis genes lead to differences in both the type (e.g., β-carotene, lutein) and quantity of carotenoids among plant species and cultivars. For example, pumpkin varieties with darker orange flesh typically have higher carotenoid concentrations (Provesi, Dias & Amante, 2011). Concerning environmental conditions, exposure to sunlight enhances carotenoid biosynthesis by activating enzymes in the carotenoid pathway. Higher light intensity often correlates with increased carotenoid accumulation (Rodriguez-Amaya, 2019). Moderate temperatures are optimal for carotenoid production, whereas extreme heat or cold can disrupt biosynthesis. Adequate nitrogen, potassium, and micronutrients such as magnesium are essential for carotenoid synthesis and stability in plants. Controlled water stress can increase carotenoid content, as it triggers stress-response pathways that enhance secondary metabolite production (Akula & Ravishankar, 2011).

Furthermore, post-harvest processing signifycantly affects carotenoid stability. Cooking or thermal treatments can both degrade and enhance carotenoid bioavailability, depending on the method and duration. For example, steaming may help release carotenoids bound to plant cell walls, while at the same time, excessive heat can lead to their degradation (Adadi, Barakova & Krivoshapkina, 2018). Moreover, prolonged exposure to oxygen, light, or high temperatures during storage can lead to carotenoid oxidation and degradation. Proper storage conditions, such as cool and dark environments, help preserve carotenoid content (Dias, Camões & Oliveira, 2014).

REACTIVE OXYGEN SPECIES (ROS) AND OXIDATIVE STRESS

Aerobic oxidation is a cellular process that constitutes a part of the metabolic pathways responsible for energy production in the form of adenosine triphosphate (ATP). ATP and similar molecules are fundamental energy sources for all living systems. However, despite being essential, oxidation can lead to numerous changes and damage within the organism, as it generates compounds known as ROS (Lobo, Patil, Phatak & Chandra, 2010). These include free radicals, such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$), as well as non-radical molecules, like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and ozone (O_3).

A free radical is defined as an atom or a molecule with one or more unpaired electrons in its outer orbital, capable of existing independently. The presence of an unpaired electron makes it unstable, highly reactive, and short-lived. Due to their high reactivity, free radicals tend to capture electrons from other molecules, turning those molecules into free radicals themselves. This initiates a chain reaction that ultimately leads to cellular damage. The half-life of free radicals varies, ranging from a few nanoseconds to several seconds or even minutes for more stable radicals (Phaniendra, Jestadi & Periyasamy, 2015). Free radicals are formed through three distinct steps: initiation - the breaking of a covalent bond, resulting in the formation of two free radicals, thereby increasing the number of free radicals; propagation - the chain reaction progresses as existing free radicals interact to form a new radical and a stable product, maintaining a constant number of free radicals; and termination - two radicals combine to form a stable, non-radical product, reducing the overall number of free radicals (Foret, Lincoln, Do Carmo, Cuello & Cosa, 2020).

Oxygen is a small molecule capable of penetrating biomembranes and is essential for all living organisms to produce energy via the electron transport chain. In this chain, O_2 serves as the terminal electron acceptor, while the electron donor is most often nicotinamide adenine dinucleotide (NAD+). The electron transport chain is located within the mitochondrial membrane, where mitochondria utilize over 80% of the available O_2 in aerobic organisms during energy production, making these organelles the primary source of free radical species. The oxygen molecule itself is a radical due to its π* antibonding orbitals, each containing one electron with parallel spin. This is the most stable state of oxygen, commonly found in the atmosphere. Oxygen acts as an oxidizing agent and tends to accept electron pairs to fill its orbitals. However, these electrons must have parallel spins; if they originate from the same molecule, this violates Pauli's exclusion principle. Consequently, oxygen captures electrons individually, reacting slowly with non-radicals (Halliwell & Gutteridge, 2015).

Besides free radicals and oxygen, ROS include compounds like hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2). Free radicals are generally more unstable than non-radicals, though their reactivity can vary. The simplest example of a free radical is the hydrogen atom, consisting of one proton and one unpaired electron. Free radicals of biological importance include: superoxide anion radical ($O_2^{\bullet -}$), nitric oxide radical ($NO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), and peroxyl radical ($ROO^{\bullet}$) (Ifeanyi, 2018). During the reduction of an oxygen molecule to two water molecules, ROS are formed as intermediates, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Mailloux, 2015). The most toxic effects of the superoxide anion radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) stem from their involvement in the formation of hydroxyl radicals (•OH). Hydroxyl radicals are products of incomplete oxygen reduction and are among the most reactive radicals, with a half-life on the order of nanoseconds. The Haber-Weiss reaction, which integrates the Fenton reaction, is one of the most common mechanisms for generating hydroxyl radicals in biological systems (Kehrer, 2000).

Sources of free radical formation can be classified as either exogenous or endogenous. Exogenous sources include ionizing radiation, tobacco smoke, metals, alcohol, drugs, smog, pesticides, ultraviolet (UV) light, and certain medications, such as halothane and paracetamol, and endogenous sources include mitochondrial respiration, peroxisomes, enzymatic reactions, and the endoplasmic reticulum (Phaniendra et al., 2015). While ROS are often associated with cellular damage, at low to moderate concentrations, they play essential roles in signaling and maintaining cellular homeostasis. Although beneficial at physiological levels, excessive ROS disrupt signaling pathways and homeostasis, causing oxidative stress (de Almeida et al., 2022). This leads to molecular damage, mitochondrial dysfunction, and inflammatory cascades, contributing to the development of chronic diseases such as cancer, cardiovascular conditions, and neurodegenerative disorders (Simpson & Oliver, 2020). Understanding the dual nature of ROS, both as signaling molecules and potential threats, highlights the importance of maintaining redox balance for optimal cellular function and organismal health (Sies et al., 2022).

Antioxidant defenses are vital for maintaining redox homeostasis by neutralizing ROS and preventing oxidative stress. These defenses include endogenous systems, which are naturally produced by the body (enzymatic and non-enzymatic endogenous antioxidants), and dietary antioxidants (carotenoids, vitamins, polyphenols, minerals), derived from external sources like food (Mirończuk-Chodakowska, Witkowska & Zujko, 2018). Dietary antioxidants complement endogenous defenses by neutralizing ROS, preventing lipid peroxidation, and protecting biomolecules (Liu et al., 2018).

CAROTENOIDS AS ANTIOXIDANTS AGAINST ROS

Due to the recognition of the harmful effects of ROS and their role in the pathogenesis of numerous diseases, scientists are increasingly focusing on studying these compounds and exploring methods to prevent their formation and neutralize their effects. An antioxidant is defined as a substance that slows, prevents, or removes oxidative damage to a target molecule. Antioxidants can be synthesized *in vivo* or obtained through diet (Halliwell, 2024). They are commonly classified into enzymatic and non-enzymatic types. Enzymatic antioxidants include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which actively detoxify ROS. Non-enzymatic antioxidants involve small molecules like vitamins C and E, glutathione, carotenoids, and polyphenols, which scavenge free radicals and protect cellular components (Irato & Santovito, 2021). This classification underscores the diversity of the antioxidant defense system, illustrating the synergy between endogenous mechanisms and dietary contributions in maintaining redox balance and preventing oxidative stress (Halliwell, 2024).

The central part of the carotenoid molecules, composed of alternating double and single bonds, constitutes the key characteristic for their antioxidant activity, chemical reactivity, light absorption capacity, and molecular shape (Singh, 2007). The primary role of these compounds lies in their antioxidant capacity. In addition to their antioxidant activity, these compounds are considered to have a positive effect on the human body by enhancing immune competence and inhibiting premalignant lesions. Some of the antioxidant mechanisms of carotenoid action include: singlet oxygen scavenging, peroxyl radical scavenging, reduction of cell proliferation, and stimulation of intercellular communication (Padovani, Amaya-Farfán, Colugnati, & Domene, 2006).

Carotenoids have the ability to physically and chemically "scavenge" singlet oxygen ($^1O_2$). In physical scavenging, an interaction occurs between the carotenoid and singlet oxygen, transferring the energy of singlet oxygen to the carotenoid molecule, which results in the singlet oxygen transitioning to a stable oxygen state, while the carotenoid molecule moves to an excited state. The carotenoid molecule then returns to its ground state by dissipating its energy to the solvent molecules. Due to the cycle of returning the carotenoid molecule from the excited to the ground state and vice versa, carotenoids can participate multiple times in cycles of free radical scavenging (Stahl & Sies, 2003). Carotenoids also react efficiently with peroxyl (ROO•) radicals, which are produced as a result of lipid peroxidation. By scavenging these radicals, the reaction leading to the damage of lipid structures, such as cell membranes and lipoproteins, is interrupted. As a product of this reaction, a resonance-stabilized, carbon-centered carotenoid radical is formed (Stahl & Sies, 2003). In addition to this mechanism, carotenoids can react with peroxyl radicals by electron transfer or by hydrogen atom transfer (Martinez, Vargas & Galano, 2009). It is also known that carotenoids have synergistic effects with other antioxidants, particularly vitamins C and E, thereby providing an effective barrier against oxidation (Shi, Kakuda & Yeung, 2004).

The oxidative damage is associated with aging and several chronic diseases, including cardiovascular disease and cancer. Carotenoids, particularly β-carotene, lutein, and zeaxanthin, help mitigate these effects. Wang et al. (2014) conducted a large cohort study that found a significant inverse relationship between carotenoid intake and the risk of cardiovascular disease (CVD). This study indicated that higher intake of carotenoids, particularly β-carotene, was associated with a reduced risk of CVD, likely due to their antioxidant properties that protect against oxidative damage to lipids in the bloodstream. The association between carotenoid intake and cancer risk, particularly lung and prostate cancer was also explored. The results suggested that individuals with higher levels of carotenoids, particularly β-carotene and lycopene, had a lower incidence of these cancers (Peters et al., 2007, Gallicchio et al., 2008). The studies proposed that carotenoids help prevent DNA damage and inhibit cancer cell proliferation through their antioxidant and immune-modulating properties. Mozaffarieh, Sacu & Wedrich (2003) reviewed the role of carotenoids like lutein and zeaxanthin in protecting the eyes from oxidative stress, specifically in preventing age-related macular degeneration (AMD). Carotenoids, which are present in high concentrations in the retina, play a crucial role in protecting the eyes from light-induced damage and oxidative injury, thereby reducing the risk of AMD. As fat-soluble antioxidants, they are particularly effective in safeguarding lipid-rich tissues, such as cell membranes, skin, and the retina, against oxidative damage. This property makes them essential for maintaining eye health and preventing retinal damage caused by light exposure. Pechinskii and Kuregyan (2014) discussed how carotenoids such as β-carotene, lutein, and zeaxanthin enhance immune function. The study suggested that these carotenoids promote the production of cytokines and stimulate lymphocyte proliferation, thereby strengthening the body's immune response and potentially reducing the risk of chronic diseases related to immune dysfunction, such as cancer and autoimmune disorders. In comparison, polyphenols and vitamin C are water-soluble antioxidants that function primarily in aqueous environments like blood and tissues (Mrowicka, Mrowicki, Kucharska & Majsterek, 2022).

PUMPKIN AND ALTERNATIVE RAW MATERIALS AS SOURCES OF CAROTENOIDS

Pumpkin (*Cucurbita*) is an important vegetable crop cultivated globally, primarily because of its good price and high nutritional value (Batool et al., 2022). The advantage is that pumpkins can be stored for a few months, even under ambient temperature and humidity, without changes in nutritional composition (Mohammed, 2014).

Pumpkin owes its sweet taste to many polysaccharides, which also show antidiabetic effects (protein-bound polysaccharides can increase the level of insulin and reduce the sugar blood level) (Dar, Sofi & Rafiq, 2017).

Pumpkins and other vegetables, such as sweet potatoes and carrots, are excellent sources of carotenoids (Adadi et al., 2018). Pumpkin flesh and byproducts, including peels and seeds, are particularly rich in carotenoids, making them valuable for developing functional foods and nutraceuticals (Lima et al., 2019). For instance, one cup of canned pumpkin delivers 1,906 mg of retinol activity equivalents (RAE), fulfilling over 200% of the daily value for vitamin A (USDA, 2021).

Pumpkins are also a source of other bioactive compounds, such as polyphenols and tocopherols (Pereira et al., 2020). Pumpkins are rich in essential minerals, potassium, and magnesium, which are critical for heart health, immunity, and muscle function as well as in fibers which support digestive health and help in maintaining stable blood sugar levels. Low calories and high fiber content make pumpkin a satiating food that supports weight control (Pinna et al., 2023).

Pumpkin is an excellent raw material for food applications due to its adaptability to various growing conditions, nutritional richness, and versatility in processing. Thriving in warmer climates with high yields and minimal maintenance, it is a sustainable, cost-effective choice for large-scale production, particularly in resource-limited regions (Kulczyński & Gramza-Michałowska, 2019). Pumpkins can be processed into purees, powders, or flours, extending shelf life (Sharma et al., 2020) and enhancing usability in diverse food products such as baked goods and baby food processing (Kim, Kim, Kim, Choi & Lee, 2016). While comparable to carrots and sweet potatoes in terms of β-carotene content (Table 1), pumpkins offer additional carotenoids like lutein and zeaxanthin (de Carvalho, Ortiz, de Carvalho, Smirdele & de Souza Neves, 2017), contributing to their value as functional ingredients with antioxidant properties.

Additionally, pumpkins offer a longer shelf life than leafy vegetables, preserving their nutrient integrity during storage and processing (El-Ramady, Domokos-Szabolcsy, Abdalla, Taha & Fári, 2015). These attributes make them a culturally significant, low-cost, and versatile crop ideal for addressing food security and develo-

Table 1.
Carotenoid content in pumpkins *vs.* other carotenoid-rich raw materials

Raw material	Predominant carotenoids	Average total carotenoid content (mg/100g)	Additional attributes	References
Pumpkins	β-carotene (80%), lutein, zeaxanthin, α-carotene	1.0–10.0 (varies by cultivar)	High β-carotene bioavailability; easy to process and store	Provesi et al., 2011; Rodriguez-Amaya (2019)
Carrots	β-carotene (70–90%), α-carotene	6.0–9.0	Excellent source of provitamin A; widely consumed	Arscott & Tanumihardjo (2010)
Sweet potatoes	β-carotene (80%)	8.5–15.0	High β-carotene content, particularly in orange-fleshed varieties	Bengtsson, Namutebi, Alminger & Svanberg (2008)
Tomatoes	Lycopene (90%), β-carotene, lutein	3.0–10.0	Lycopene-rich, but less provitamin A activity	Martínez-Valverde, Periago, Provan & Chesson (2002)
Green leafy vegetables	Lutein, zeaxanthin, β-carotene	2.0–15.0	High antioxidant capacity; prone to rapid nutrient degradation	Granado, Olmedilla & Blanco (2003)

ping value-added products (Hosen et al., 2021).

Carotenoid content in pumpkins and influencing factors

Pumpkins are rich in carotenoids, primarily β-carotene, which is responsible for their bright orange color, as well as other carotenoids such as lutein, zeaxanthin, and α-carotene. As already mentioned, β-carotene is the predominant carotenoid in pumpkins, often comprising up to 80% of the total carotenoid content (Provesi et al., 2011). The concentrations of carotenoids in pumpkins can vary depending on the cultivar, growth conditions, and ripeness. Lutein is present in smaller quantities compared to β-carotene but still plays a significant role in the nutritional value of pumpkins. The concentration typically ranges from 0.1 to 1 mg per 100g of pumpkin flesh, depending on the variety. Zeaxanthin is often found in similar quantities to lutein in pumpkins, contributing to the overall antioxidant capacity of the fruit.

Alpha-carotene is another carotenoid present in pumpkins. It is a less potent provitamin A carotenoid compared to β-carotene but still contributes to vitamin A intake (Rodriguez-Amaya, 2019).

For example, while analyzing the species *C. pepo*, *C. moschata*, and *C. maxima*, β-cryptoxanthin was detected only in the pulp of *C. maxima*, while β-carotene was found in the peel, seeds, and pulp of all three species (Kim et al., 2016). HPLC analysis was used to determine α- and β-carotene and their isomers in the pulp of *C. moschata*. The results showed that β-carotene was the most abundant, while α-carotene was present at approximately three times less concentration.

Additionally, Kurz, Carle, & Schieber (2008) reported relatively high levels of lutein in eight pumpkin varieties belonging to *C. maxima*, *C. pepo*, and *C. moschata*. Provesi et al. (2011) also investigated the concentration of major carotenoids by HPLC in two pumpkin cultivars, *C. moschata* and *C. maxima*, and found that the most abundant carotenoids in *C. moschata* were β-carotene and α-carotene, with smaller amounts of violaxanthin and lutein. In the samples of *C. maxima*, β-carotene, violaxanthin, and lutein were the most dominant. In many varieties of *C. maxima*, lutein rather than β-carotene is the predominant carotenoid, with this species showing the greatest variation in carotenoid composition across its cultivars (Azevedo-Meleiro & Rodriguez-Amaya, 2007).

The levels of carotenoids in pumpkins are significantly influenced by cultivation practices, storage conditions, and processing methods. Understanding these factors is crucial for optimizing their nutritional value and health benefits. Carotenoids, due to the presence of double bonds in their structure, are highly reactive and prone to degradation during food processing and storage. Factors such as light, heat, acidity, and oxygen can lead to oxidative and cis–trans isomerization reactions, resulting in the loss of color and diminished biological activity. The stability of these compounds is influenced by numerous variables, including the type and physical form of the carotenoid, oxygen availability, presence of metal ions, light exposure, intensity of heat treatment, and the nature of the food matrix (Rodriguez-Amaya, 2001).

The availability of essential nutrients like nitrogen, potassium, and magnesium in the soil affects carotenoid biosynthesis. Fertilization practices that balance these nutrients can enhance carotenoid production in pumpkins (Biesiada, Nawirska, Kucharska & Sokól-Letowska, 2009). Carotenoid synthesis in pumpkins is stimulated by light. Higher exposure to sunlight during the growing period enhances the accumulation of β-carotene and other carotenoids by activating key enzymes in the carotenoid biosynthesis pathway (Pérez-Gálvez, Viera & Roca, 2020). Shaded cultivation or inadequate sunlight can lead to reduced carotenoid content. Moderate water stress has been shown to increase the concentration of secondary metabolites, including carotenoids, as a stress response in plants. However, excessive or insufficient irrigation can impair plant growth and carotenoid biosynthesis (Uarrota, Stefen, Leolato, Gindri & Nerling, 2018). Likewise, storage conditions greatly influence carotenoid content in pumpkins. Storing pumpkins at cooler temperatures in dark conditions helps preserve carotenoids, as exposure to heat, oxygen, and light during storage leads to carotenoid oxidation and degradation, reducing their nutritional value (Rodriguez-Amaya, 2019). For example, storing pumpkin pieces resulted in a reduction of total carotenoid content, with a decrease of approximately 40% when kept in the refrigerator and around 20% when stored in the freezer for a period of 80 days (Dutta, Chaudhuri & Chakraborty, 2009). Controlled atmospheric storage, with reduced oxygen levels and increased carbon dioxide, has been found to effectively retain carotenoid levels for longer periods (Jaswir et al., 2014). Provesi et al. (2011) reported no significant changes in carotene concentrations during the 180-day storage of pumpkin purees. This stability was attributed to factors such as enzyme inactivation during processing, low levels of dissolved oxygen in the product, storage temperatures below 30 °C, and protection from light exposure. Harvesting pumpkins at full maturity maximizes carotenoid content. Immature harvesting results in underdeveloped carotenoid levels, while overripe pumpkins may begin to degrade carotenoids during storage (Dutta et al., 2009).

Additionally, processing conditions are essential for preserving and enhancing the carotenoid levels in pumpkins, thereby maximizing their nutritional and functional potential. Thermal processing, such as steaming or boiling, can increase carotenoid bioavailability by breaking down cell walls and releasing carotenoids bound to proteins or cell structures. For example, steaming pumpkins or pureeing them has been shown to enhance β-carotene bioavailability while minimizing its degradation (Provesi & Amante, 2015). The total carotenoid content even increased in thermally treated (blanched) pumpkin pieces, with a more pronounced rise observed at 55 °C compared to 95 °C (Dutta et al., 2009). Mechanical processing, such as blending, can increase carotenoid bioavailability by breaking down physical barriers, making carotenoids more accessible for absorption in the human digestive system (Rodriguez-Amaya, 2019). However, prolonged exposure to high temperatures during cooking or canning can lead to significant carotenoid losses due to oxidation and isomerization. On the other hand, freezing helps retain carotenoid content by inhibiting enzymatic activity and oxidative processes. Similarly, freeze-drying preserves carotenoids better than traditional drying methods like sun or oven drying, which can cause substantial losses due to heat and light exposure (Kļava, Kampuse, Tomsone, Kince & Ozola, 2018). In a study examining the drying of 12 different *C. maxima* and *C. pepo* varieties, the highest carotenoid retention was achieved through freeze-drying, followed by vacuum-microwave drying, vacuum drying, and, lastly, conventional convective drying (Nawirska, Figiel, Kucharska, Sokół-Łętowska & Biesiada, 2009).

CONCLUSIONS

The antioxidant system represents a multi-layered system that combines enzymatic pathways, small-molecule antioxidants, and dietary contributions to protect against oxidative stress. Enhancing these protections through a diet rich in antioxidants, alongside supporting endogenous systems, is critical for reducing the risk of oxidative stress-related chronic diseases and maintaining overall health. Carotenoids play a crucial antioxidant role by neutralizing reactive oxygen species (ROS). Among carotenoid-rich alternative crops, pumpkins are highlighted for their high β-carotene content and high bioavailability, and additional carotenoids, including lutein and zeaxanthin, which contribute significantly to their antioxidant properties. These attributes make pumpkins an excellent dietary source of carotenoids, suitable for functional foods and nutraceutical applications. Given their wide availability, affordability, and versatility in both fresh and processed forms, pumpkins have considerable potential to contribute to dietary carotenoid intake, particularly in regions where vitamin A deficiency remains a public health concern. Including pumpkin-based foods in regular diets could help improve carotenoid status in vulnerable populations. Therefore, future research should focus on breeding pumpkin varieties with enhanced carotenoid content, emphasizing biofortification as a sustainable strategy to combat global nutritional deficiencies.

AUTHOR CONTRIBUTIONS

Conceptualization, M.D.B.J. and B.M.K..; Writing-original draft preparation, M.Đ.M..; Writing-review and editing, M.D.B.J., A.M.S., M.M.K. and B.M.K..; Supervision, M.D.B.J.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ULOGA KAROTENOIDA U SPREČAVANJU OKSIDATIVNOG STRESA: ISTRAŽIVANJE ANTIOKSIDATIVNOG POTENCIJALA BUNDEVE KAO FUNKCIONALNE KULTURE

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Sažetak: Ovaj pregledni rad ima za cilj proučavanje antioksidativnih svojstava karotenoida i njihove uloge u ublažavanju oksidativnog stresa izazvanog reaktivnim kiseoničkim vrstama (ROS). Povišeni nivoi ROS-a povezani su sa oštećenjem ćelija, što doprinosi hroničnim bolestima poput karcinoma, kardiovaskularnih poremećaja i neurodegenerativnih stanja. Karotenoidi, prirodni pigmenti prisutni u raznim vrstama voća i povrća, pokazuju značajnu antioksidativnu aktivnost neutralisanjem ROS-a i zaštitom ćelijskih komponenti. Ovaj pregledni rad ističe mehanizme kojima karotenoidi ublažavaju oksidativni stres, sa posebnim naglaskom na njihovu sposobnost da hvataju slobodne radikale i sprečavaju dalja oštećenja lipida, proteina i DNK. Bundeva (*Cucurbita* spp.) se gaji na malim površinama, ali se ističe kao vredna, alternativna biljna vrsta, zahvaljujući visokom sadržaju karotenoida, potencijalu za gajenje u održivim uslovima i raznovrsnoj primeni u razvoju funkcionalne hrane i nutraceutika. Među izvorima bogatim karotenoidima, bundeve se izdvajaju visokim sadržajem β-karotena i dodatnim karotenoidima poput luteina i zeaksantina. Ova jedinjenja ne samo da jačaju antioksidativnu odbranu već i obezbeđuju provitamin A, doprinoseći ukupnom zdravlju. Razmatrani su faktori koji utiču na sadržaj karotenoida u bundevama, uključujući proizvodne prakse i uslove skladištenja nakon žetve. Takođe analiziran je uticaj metoda prerade na biodostupnost karotenoida, sa naglaskom na tehnike, poput kuvanja na pari i liofilizacije, koje optimizuju očuvanje nutrijenata. Ključni nalazi naglašavaju značaj bundeve kao održivog i ekonomičnog izvora karotenoida, pogodnog za razvoj funkcionalne hrane. Zaključak istraživanja ukazuje na potrebu za oplemenjivanjem sorti bundeve s većim sadržajem karotenoida i optimizacijom metoda prerade, a sve u cilju zdravstvenih benefita. Povećanje broja proizvoda na bazi bundeve koji će biti deo redovne ishrane predlaže se kao praktična strategija za ublažavanje efekata oksidativnog stresa i kao podrška javnom zdravlju.

Ključne reči: *antioksidanti, karotenoidi, bundeva, oksidativni stres, reaktivne kiseonične vrste (ROS)*

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