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Antioxidant Assays in Phytonutrient Research: Translating Laboratory Innovations into Practical Applications

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ABSTRACT: There is great promise for improving nutrition and health thanks to phytonutrients' antioxidant qualities and health advantages. Their capacity to combat oxidative stress and associated illnesses emphasizes the importance of precisely evaluating their antioxidant characteristics. This study concludes by providing a comprehensive and critical critique of the current approaches to measure the antioxidant activity of phytonutrients. It dives into the fundamentals, benefits, drawbacks, and most recent developments of commonly used antioxidant assays, giving the reader a comprehensive grasp of the topic. This recapitulation of the review's goal in the end reinforces the reader's primary takeaway. Research on several antioxidant tests, such as FRAP, ORAC, DPPH, and ABTS, is consolidated in this review. It looks at each assay's performance traits, technological advances, and techniques. The review also assesses the incorporation of many assays to thoroughly examine phytonutrient potency and its uses in the food industry and nutritional science. The review shows how antioxidant tests have advanced significantly, improving sensitivity, accuracy, and physiological relevance. It demonstrates how these tests can be used practically to guarantee food quality, create supplements, and offer nutritional advice. The paper also lists the difficulties today, including the intricacy of antioxidant mechanisms, test variability, and the requirement for assay standardization. The practical value of the research is emphasized by highlighting the significance of antioxidant tests for quality assurance, adulteration detection, and shelf-life extension in the food business.

Keywords: Pharmaceutical Research; Food Quality Control; Oxidative Stress; Phytochemical Evaluation; Nutritional Science; Antioxidant Potency

1. Introduction

Antioxidant molecules are essential for scavenging free radicals and averting cell harm because they contribute an electron or a hydrogen atom. Unpaired electrons make free radicals extremely erratic and reactive. Antioxidants donate electrons to free radicals, neutralizing them and reducing their reactivity. Examples of typical antioxidant structures include the lengthy chains of conjugated double bonds seen in carotenoids, vitamins C and E, and the hydroxyl groups attached to aromatic benzene rings found in phenolic compounds. These antioxidants neutralize free radicals and stop additional harm by giving them electrons or hydrogen atoms. Vitamin E, a fat-soluble

antioxidant, prevents lipids in cell membranes from oxidizing, and vitamin C, a water-soluble antioxidant, provides electrons to neutralize free radicals. Antioxidants protect cells from DNA damage caused by oxidative stress, which may help reduce cancer risk. The hallmark of cancer is unregulated cell division, in which free radicals can promote and induce mutations. By scavenging free radicals, antioxidants preserve DNA integrity and prevent the start and spread of cancer. Moreover, phenolic chemicals and carotenoids are required for scavenging free radicals. Essential functional groups, like the hydroxyl in phenolic substances and the carboxyl in vitamin C, stimulate antioxidant action. Antioxidants mostly eliminate free radicals through the electron and hydrogen atom transfer process. A few examples of antioxidants are the fat-soluble antioxidant vitamin E, which has a chromanol ring with a lengthy phytyl tail, beta-carotene, a carotenoid with conjugated double bonds, and vitamin C, a water-soluble antioxidant with numerous hydroxyl groups. Several fruits and vegetables contain flavonoids, which are antioxidants, such as selenium, a trace mineral found in nuts and seeds. Antioxidants are vital for maintaining cellular health and preventing diseases associated with oxidative stress because they fight free radicals. Oxidative stress-related disorders can arise from cellular and tissue damage caused by an imbalance between antioxidants and free radicals. The severity of these conditions can range from minor skin issues to more severe diseases like cancer and heart disease. Our understanding of their chemical structures and modes of action highlights their significance in biological systems and empowers us to take control of our health. Antioxidants can give them electrons to change free radicals into more stable molecules. This procedure can be shown as:

Antioxidant– H + Free Radical → Oxidized Antioxidant + Stable Molecule – H

(Rao, 2016; Santos-Sánchez et al., 2019; Sisein, 2014).

Antioxidants, whether enzymatic or non-enzymatic, protect against reactive oxygen species (ROS). Non-enzymatic antioxidants like vitamins A, C, E, and K, minerals like zinc and selenium, and polyphenols (flavonoids) indirectly reduce ROS. On the other hand, enzymatic antioxidants like catalase and superoxide dismutase (SOD) directly destroy ROS. While α-tocopherol and other hydrophobic antioxidants protect cell membranes, ascorbic acid and other hydrophilic antioxidants interact with blood plasma and cell cytoplasm. The body produces primary and secondary endogenous antioxidants. Catalase and SOD are examples of primary antioxidants, also known as preventive antioxidants, which neutralize potentially accessible radical sources before they initiate oxidative chain reactions and hence stop the production of new free radicals. One example of an enzyme that catalyzes the transformation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) or regular molecular oxygen (O_2) is superoxide dismutase (SOD). By transforming superoxide radicals into less reactive species, SOD prevents the highly reactive chemicals known as superoxide radicals from damaging cells.

$$2O_2 + 2H+ \rightarrow O_2 + H_2O_2$$

One of the best examples of an antioxidant is the enzyme catalase, which breaks down hydrogen peroxide, a potentially dangerous consequence of numerous metabolic processes, into oxygen and water. This process dramatically lessens the possibility that hydrogen peroxide may transform into hydroxyl radicals, which are incredibly harmful to cells. Catalase's capacity to change hydrogen peroxide into innocuous molecules demonstrates the protective function of antioxidants in cellular health. As a result of this approach, we feel reassured about our health.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Secondary, chain-breaking antioxidants are critical components of our body's defence system. They prevent the spread of free radical chain reactions, preventing any potential harm. A chain reaction is often initiated by a free radical reacting with a molecule to produce another free radical. Secondary antioxidants effectively halt the chain reaction and stabilize the reactive species by giving free radicals electrons or hydrogen atoms, safeguarding the integrity and well-being of our cells. Secondary antioxidants include Vitamin E or tocopherol: This fat-soluble vitamin protects cell membranes from oxidative damage by its potent antioxidant capabilities that break down chains. Lipid radicals are stabilized, and vitamin E stops lipid peroxidation by giving them a hydrogen atom.

Lipid Radical + Vitamin E → Stable Lipid + Oxidized Vitamin E

Ascorbic acid, or vitamin C, is a water-soluble antioxidant that can give electrons to free radicals in watery settings to neutralize them. Additionally, it aids in the regeneration of oxidized vitamin E, regaining its antioxidant power. Vitamin C effectively stops the chain

reaction that creates free radicals because it can donate electrons.

Free Radical + Vitamin C→ Stable Molecule + Oxidized Vitamin C

Antioxidants that are created internally by the body are Endogenous. They consist of non-enzymatic antioxidants, including glutathione, uric acid, and coenzyme Q10, as well as enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase. The body controls these antioxidants' synthesis and activity to preserve cellular redox balance. In this state, the production of free radicals is balanced by the body's ability to neutralize them with antioxidants. These include glutathione, uric acid, and coenzyme Q10. On the other hand, antioxidants derived from the food we consume are known as exogenous antioxidants. They consist of carotenoids (like lycopene and beta-carotene), polyphenols (like resveratrol and flavonoids), and vitamins (like C and E). Exogenous antioxidants are essential for counteracting free radicals that the body comes into contact with from outside sources, including pollution, radiation, and food consumption. They support the body's endogenous antioxidant defence mechanism. Exogenous antioxidants found in foods, herbs, and dietary components are essential for clinical research and the treatment of disease because they can suppress lipid peroxidation and donate electrons to oxidative radicals. Resveratrol in red wine, quercetin in apples, lycopene in tomatoes and flavonoids in green tea are the examples. These antioxidants can be easily incorporated into a healthy diet, providing a natural and effective way to boost our body's antioxidant levels and protect against oxidative stress. This empowers us to make informed and beneficial choices for our health.

Synthetic antioxidants, developed to assess and compare natural antioxidants' activity in food, have proven safe and effective. They are used in processed meals to keep them fresher longer and prevent oxidation, especially in the case of fatty acids. The European Food Safety Authority (EFSA) has determined that BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), two common synthetic antioxidants, are safe up to predetermined intake limits. The flavour, colour, and freshness of animal food products can be preserved with TBHQ (tert-butylhydroquinone), and octyl gallate is safe because it decomposes into innocuous chemicals when consumed. Despite its effectiveness, rodent renal cystic disease has been associated with NDGA (nordihydroguaiaretic acid). Still, with proper use and regulation, synthetic antioxidants can be a safe and reliable tool in food preservation (Atta et al., 2017; Shalini Kapoor and Sivakumar Joghi Thatha, 2015).

2. Antioxidants' Significance in Health and Illness

2.1. Reducing Inflammation: Antioxidants are essential in reducing inflammation because they neutralize free radicals and reactive oxygen species (ROS), which can initiate inflammatory pathways. There is a connection between chronic inflammation and conditions like arthritis, heart disease, and some types of cancer. Antioxidants assist in reducing inflammatory reactions and the chance of developing certain chronic illnesses by reducing oxidative stress. Giving free radicals electrons or hydrogen atoms suppresses pro-inflammatory signalling pathways like NF-kappa B, which lowers the synthesis of inflammatory cytokines and chemokines and avoids oxidative damage. Furthermore, antioxidants prevent tissue damage caused by oxidative stress, regulate immunological responses by controlling T lymphocytes and macrophages, and facilitate cellular repair processes by assisting in the elimination of damaged molecules and the regeneration of healthy cells (Mittal et al., 2013).

2.2. Cardiovascular Fitness: Antioxidants are essential for cardiovascular health because they protect against the oxidative modification of low-density lipoprotein (LDL) cholesterol, significantly contributing to atherosclerosis development. Heart attacks and strokes are more likely to occur when oxidized low-density lipoprotein (LDL) deposits plaque in arteries. Antioxidants like vitamin E and polyphenols reduce the risk of cardiovascular disease by stopping LDL oxidation. Additionally, they enhance endothelial function by increasing the synthesis of nitric oxide, decreasing chronic inflammation associated with the development of arterial plaque, and improving blood vessel flexibility to avert hypertension. Consuming a diet high in foods high in antioxidants, such as fruits, vegetables, nuts, and seeds, helps to prevent atherosclerosis, preserve blood vessel integrity, and improve heart health in general. Additionally, they reduce chronic inflammation associated with the development of arterial plaque, promote nitric oxide synthesis in endothelial cells to improve endothelial function, and widen blood vessels to prevent hypertension. Eating a diet high in foods high in

antioxidants, such as fruits, vegetables, nuts, and seeds, protects blood vessel integrity, lowers the risk of atherosclerosis, and improves heart health in general (Mangge et al., 2014).

2.3. Neuroprotection: Since the brain uses oxygen quickly and has a significant lipid content, oxidative stress poses a severe risk to neuroprotection. This is where the role of antioxidants is relevant. Antioxidants that defend brain cells from oxidative damage linked to neurodegenerative illnesses like Parkinson's and Alzheimer's include polyphenols, vitamins C and E, and antioxidant-rich diets. Through their ability to scavenge free radicals, boost cellular defences, and reduce neuroinflammation, they facilitate oxidative stress and play a critical role in maintaining cognitive function. Additionally, antioxidants may be able to delay the course of neurological illnesses. Antioxidant-rich diets are potent for supporting cognitive function, brain health, and defence against age-related neurodegeneration and cognitive decline. With this knowledge, we may make good decisions for our health and well-being. (Lee et al., 2020).

2.4. Cancer Prevention: Antioxidants protect cells from DNA damage caused by oxidative stress, which may help reduce cancer risk. The hallmark of cancer is unregulated cell division, in which free radicals can promote and induce mutations. By scavenging free radicals, antioxidants preserve DNA integrity and prevent the start and spread of cancer. Certain antioxidants improve the immune system's capacity to identify and eliminate cancerous cells. They promote immune cell activity, lessen inflammation, guard against carcinogenic agents, and decrease the formation of cancer cells by causing apoptosis and stopping proliferation. Consuming a diet high in antioxidant-rich fruits, vegetables, nuts, and seeds can help lessen the chance of developing many types of cancer while also improving general health(Bennett et al., 2012).

2.5. Oxidative Stress-Related Diseases:

Antioxidants play a pivotal role in preventing and managing various acute and chronic illnesses that are exacerbated by oxidative stress. Long-term oxidative stress can damage the beta cells in the pancreas, neurons in Alzheimer's disease, and joints in arthritis. By reducing oxidative stress, antioxidants provide a crucial defense against environmental stressors such as radiation, pollution, and toxic chemicals. These stressors rapidly generate free radicals, which can cause significant cellular damage. Antioxidants counteract these free radicals, aiding the body's detoxification processes and preserving cellular integrity. They also enhance immune system function, promote healthy aging, and help prevent chronic diseases, including diabetes, neurological disorders, and cardiovascular disease. Including antioxidant-rich foods and supplements in the diet can significantly improve overall health and reduce the risk of diseases caused by oxidative stress. (Guo et al., 2022; Reddy, 2023; Sharifi-Rad et al., 2020).

3. Phytonutrients

Phytonutrients, sometimes called phytochemicals, naturally exist in plants. Although these substances are not considered vital nutrients like vitamins and minerals, their potential to improve health has been acknowledged. Because of phytonutrients, fruits, vegetables, herbs, and spices have rich colors, flavors, and aromas. They assist in the growth and reproduction of plants and protect them from pests, illnesses, and UV radiation. Phytonutrients are thought to have anti-inflammatory, antioxidant, and other bioactive qualities that benefit human nutrition. They may have preventive benefits against long-term illnesses like heart disease, several types of cancer, and neurological conditions. Phytonutrients have various health-promoting properties, including flavonoids, carotenoids, phenolic acids, and glucosinolates. Including a wide variety of vibrant fruits and vegetables in the diet is a valuable strategy to reap the benefits of different phytonutrients and promote general health. Because of their anti-inflammatory and antioxidant qualities, phytonutrients such as phenolic acids, flavonoids, and anthocyanins have been shown to have significant cognitive advantages. After 12 weeks of consuming 250 mg/day of V. vinifera extract, older persons with depression, anxiety, and cognitive impairments showed improvements due to anthocyanins, which are prevalent in colourful fruits. 143 mg of blueberry anthocyanins improved children's reaction times. While elderly participants consuming 201 mg of anthocyanins preserved cognitive function in those with mild impairment, those taking 387 mg of anthocyanidins showed more excellent brain activity and memory. At 993 mg/day, flavonoids dramatically enhanced cognitive performance in older people. Phenolic acids improve focus and memory, especially chlorogenic acid (300 mg/day) (Ditu et al., 2018; Marcus, 2013; Monjotin et al., 2022). Phytonutrients, also called phytochemicals, are a broad class of plant

substances with unique chemical and biological properties. These chemicals fall under several categories according to their functional characteristics and chemical makeup:

1. Phenolic Substances:

a. Flavonoids: The largest class of phytonutrients, flavonoids, is known for their anti-inflammatory and antioxidant qualities. Anthocyanins (found in berries and red cabbage) quercetin (found in apples and onions) and catechins (found in green tea) are the examples.

b. Phenolic acids: These substances, which are present in a variety of fruits, vegetables, and whole grains, have antioxidant properties. Ferulic acid (found in oats and rice bran) and chlorogenic acid (found in coffee and apples) are two examples.

2. **Carotenoids:** Carotenoids are pigments that give many fruits and vegetables their yellow, orange, and red hues. They are precursors to vitamin A and have antioxidant qualities. Examples are lutein (found in spinach and kale), lycopene (found in tomatoes and melons), and beta-carotene (found in carrots and sweet potatoes).

3. **Glucosinolates:** Presumably anticancer, glucosinolates are primarily found in cruciferous vegetables, including cabbage, broccoli, and Brussels sprouts. Bioactive chemicals like isothiocyanates are produced when these molecules are broken down during chewing and digesting.

4. **Terpenes:** Aromatic terpenes are present in plant essential oils. They possess a range of biological actions, such as antibacterial and antioxidant qualities. Examples are linalool (in lavender) and limonene (in citrus fruits).

5. **Plant sterols:** These substances can help decrease blood cholesterol levels since they share structural similarities with cholesterol. Nuts, seeds, plant oils, and whole grains all contain them.

6. **Sulfur-Containing Compounds**: These substances give allium foods like garlic, onions, and shallots unique flavors and health advantages. Examples of substances with antioxidant and anticancer properties are organosulfur compounds and allyl sulfides (Ayse Tulin and Ebru, 2017; Wahlqvist and Wattanapenpaiboon, 2020). Table-1 shows the antioxidant phytonutrients with their sources and benefits in human illnesses.

Table-1. Sources of Phytonutrients as Antioxidants

Category	Phytonutrient	Sources	Benefits	References
Fruits	Vitamin C (Ascorbic Acid)	Citrus fruits, strawberries, kiwi, guava, papaya, mango, pineapple	↑ Immunity; ↑collagen synthesis	(Mandl et al., 2009)
	Anthocyanins	Blueberries, strawberries, raspberries, blackberries, cherries, grapes (especially red/purple)	Anti-inflammatory; ↓cardiovascular disease; ↓ neurodegeneration	(Khoo et al., 2017; Prior and Wu, 2006)
	Flavonoids	Citrus fruits (hesperidin), apples (quercetin), grapes (catechins), berries, cherries, plums	Anti-inflammatory, anticancer	(Spencer, 2008; Yao et al., 2004)
	Vitamin E (Tocopherols and Tocotrienols)	Avocado, kiwi, mango, nuts (almonds, hazelnuts, peanuts), seeds (sunflower seeds), spinach, Swiss chard	↓Cardiovascular disease; ↑skin health	(Sen et al., 2006; Traber and Atkinson, 2007)
	Carotenoids	Beta-carotene (carrots, sweet potatoes, pumpkins), lycopene (tomatoes, watermelon), lutein and zeaxanthin (spinach, kale, collard greens)	↑ Eye health, anticancer, ↑ Immunity	(Krinsky et al., 2003; Rao and Rao, 2007)
	Quercetin	Apples, onions, citrus fruits, berries, tomatoes, leafy greens, broccoli	Anti-inflammatory; ↓cardiovascular disease	(Boots et al., 2008)

Vegetables	Glucosinolates	Cruciferous vegetables (broccoli, Brussels sprouts, cauliflower, cabbage, kale)	Anti-cancer	(Dinkova-Kostova and Kostov, 2012; Traka and Mithen, 2009)
	Phenolic Acids	Spinach, kale, artichokes, peppers, tomatoes, potatoes	Anti-inflammatory; ↓cardiovascular disease	(Balasundram et al., 2006; Manach et al., 2004)
	Selenium	Mushrooms (shiitake, button), spinach, broccoli, asparagus	↑ Immunity	(Fairweather-Tait et al., 2011; Rayman, 2000)
	Resveratrol	Grapes (especially red), blueberries, cranberries, peanuts	Anti-inflammatory; ↓cardiovascular disease; ↓ neurodegeneration	(Baur and Sinclair, 2006; Vang et al., 2011)
	Chlorophyll	Leafy green vegetables (spinach, kale, Swiss chard), green beans, peas	Support detoxification, promote cellular health.	(Egner et al., 2003; Ferruzzi and Blakeslee, 2007)
	Vitamin K	Leafy green vegetables, Brussels sprouts, parsley	Essential for blood clotting and bone health	(Shearer and Newman, 2008; Walther et al., 2013)
Herbs	Rosmarinic Acid	Rosemary, oregano, thyme, sage	Anti-inflammatory; supports ↑ digestive, ↑ cognitive, and ↑ immune health.	(De Oliveira et al., 2019; Petersen and Simmonds, 2003)
	Flavonoids	Parsley, basil, cilantro	Anti-inflammatory, anticancer; ↓cardiovascular disease; and ↑ immunity	(Knekt et al., 2002; Panche et al., 2016)
	Limonene	Citrus herbs like lemon balm, lemon	Anti-inflammatory; antimicrobial	(Sun, 2007)
	Carnosic Acid	Rosemary	↓cardiovascular disease; ↓ neurodegeneration	(Anwar and Qadir, 2021; de Oliveira, 2018)
	Apigenin	Parsley, chamomile, celery seeds	Anti-inflammatory; anticancer, ↑ Immunity, ↑ digestion; ↓ neurodegeneration	(Patel et al., 2007; Shukla and Gupta, 2010)
Spices	Curcumin	Turmeric	Anti-inflammatory; ↑ joint health; ↑ brain function	(Aggarwal et al., 2007; Gupta et al., 2013)

	Gingerol	Ginger	Anti-inflammatory; anticancer; ↑ digestion, ↑ immunity	(Pan et al., 2008; Shukla and Singh, 2007)
	Cinnamaldehyde	Cinnamon	Anti-inflammatory; antimicrobial; ↑ cardiac health	(Gruenwald et al., 2010; Kirkham et al., 2009)
	Capsaicin	Chili peppers like cayenne, jalapeño	Anti-inflammatory; anticancer	(Baboota et al., 2014; Srinivasan, 2016)
	Quercetin	Onions (ground onion powder)	Anti-inflammatory; ↑cardiovascular health	(Boots et al., 2008)
	Allicin	Garlic (and garlic powder)	Antimicrobial; anticancer	(Borek, 2001; Iciek et al., 2009)
	Piperine	Black pepper	Anti-inflammatory; ↑ digestion	(Srinivasan, 2007)
	Tannins	Cloves	Anti-inflammatory	(Sharma et al., 2021)
	Eugenol	Cloves, cinnamon	Antimicrobial	(Pramod et al., 2010)
Whole Grains	Tocopherols (Vitamin E)	Whole wheat, oats, barley, brown rice, quinoa	↓Cardiovascular disease ; ↑skin health	(Traber, 2007; Traber and Stevens, 2011)
	Selenium	Whole wheat, brown rice, oats, barley, quinoa	↑ immunity; supports thyroid health.	(Fairweather-Tait et al., 2011; Rayman, 2000)
	Phenolic Acids	Whole wheat, oats, barley, brown rice	Anti-inflammatory; ↑cardiovascular health; ↑ digestion; anticancer	(Balasundram et al., 2006; Scalbert and Williamson, 2000)
	Lignans	Flaxseed, oats, barley, rye	↑cardiovascular health; anticancer	(Adlercreutz, 2007; Peterson et al., 2010)
	Beta-Glucan	Oats, barley	Anticancer, ↓ cholesterol; ↑cardiovascular health;	(Chan et al., 2009; Murphy et al., 2020; Musco, 2013)
	Ferulic Acid	Whole wheat, oats, brown rice	Anti-inflammatory; ↓Cardiovascular disease ; ↑skin health; neuroprotective	(Zhao et al., 2004)
	Quercetin	Buckwheat, whole wheat	↑cardiovascular health; ↑ immunity; anti-allergic	(Boots et al., 2008)

	Polyphenols	Oats, barley, brown rice, quinoa	↑Cardiovascular health	(Pandey and Rizvi, 2009)
	Tannins	Sorghum, barley	Anti-inflammatory; ↑cardiovascular health; ↑ digestion	(Chung et al., 1998)
	Phytic Acid	Oats, brown rice, barley	↑ mineral absorption, ↑ digestive health, anticancer	(Kumar et al., 2010; Reddy, 2001)
Nuts	Vitamin E (Tocopherols)	Almonds, hazelnuts, sunflower seeds	↓Cardiovascular disease; ↑skin health	(Traber and Stevens, 2011)
	Selenium	Brazil nuts, sunflower seeds	↑ immunity; supports thyroid health.	(Fairweather-Tait et al., 2011)
	Polyphenols	Walnuts, pecans	Anti-inflammatory; ↑cardiovascular health	(Duthie and Crozier, 2000)
	Flavonoids	Pistachios	Anti-inflammatory, anticancer; ↓cardiovascular disease; and ↑ immunity	(Kim and Je, 2017)
	Resveratrol	Peanuts	Anti-inflammatory; ↑cardiovascular health; anti-aging	(Baur and Sinclair, 2006)
	Lignans	Flaxseeds	Hormonal balance; anticancer	(Adlercreutz, 2007)
	Phytic Acid	Almonds, walnuts	↑ mineral absorption; ↑ digestive health; anticancer	(Kumar et al., 2010)
Seeds	Alpha-Linolenic Acid (ALA)	Flaxseeds, chia seeds	Anti-inflammatory; ↑cardiovascular health; ↑ brain function	(Gillingham et al., 2011; Riediger et al., 2009)
	Lutein and Zeaxanthin	Pumpkin seeds	↑ eye health and protect against age-related macular degeneration	(Ma and Lin, 2010; Moeller et al., 2000)
	Catechins	Green tea seeds (when consumed whole)	↑Cardiovascular health; anticancer	(Cabrera et al., 2006; McKay and Blumberg, 2002)
	Phenolic Acids	Sesame seeds	Anti-inflammatory; ↑cardiovascular health; ↑ digestion; anticancer	(Taylor and Duodu, 2015)
	Tocopherols (Vitamin E)	Sunflower seeds	↓Cardiovascular disease ; ↑skin health	(Traber and Stevens, 2011)

4. Antioxidant Assays

DPPH Assay: Despite its shortcomings in solvent compatibility, potential interference, and non-specificity, it is still widely used in many industries and research fields to screen and compare the antioxidant capacity of various substances. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, based on decreasing the DPPH radical—a persistent free radical with a deep violet color—is widely used for assessing

antioxidant capability. The transformation of DPPH into a colorless or pale-yellow molecule by antioxidants giving electrons or hydrogen atoms is a sign of antioxidant activity. This concept serves as the foundation for the assay's procedure. Making a DPPH solution is the first step, which involves dissolving DPPH in methanol until the concentration reaches 0.1 mM. The antioxidant sample is then diluted using methanol or another appropriate solvent. The mixture is then allowed to rest at room temperature in the dark for about thirty minutes after a fixed volume of the DPPH solution and a fixed volume of the antioxidant sample are combined. Using a spectrophotometer, the absorbance is determined at 517 nm following incubation. A drop in absorbance signifies a decrease in DPPH, a gauge of the sample's antioxidant activity. In this process, control and blank samples are also employed. The solvent-free DPPH is blank, and the DPPH solution with the antioxidant is the control. The DPPH Scavenging Activity (%) formula can determine the fraction of DPPH radical scavenging activity which measures antioxidant activity.

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula provides a consistent method for assessing the antioxidant activity of different samples. It is a crucial component of the DPPH test because it allows for the measurement and comparison of antioxidant activity, which aids in determining the relative effectiveness of various compounds as antioxidants. The antioxidant concentration needed to lower the starting DPPH concentration by 50% is known as the IC50, and this can also be used to express the results. Lower IC50 values show greater excellent antioxidant activity.

Thanks to its versatility and broad spectrum of antioxidant susceptibility, the DPPH test is a valuable tool across numerous fields. Its wide spectrum of antioxidant sensitivity allows it to be used in many different applications, and its ease of use and speed make it suitable for high-throughput screening. The DPPH test, despite its limitations, is versatile enough to be applied to various industries. The DPPH assay's numerous practical uses across multiple industries prove its importance and adaptability. In the food and beverage sector, the DPPH assay plays a crucial role in evaluating the antioxidant content of food items, drinks, and dietary supplements. This is significant because it guarantees the purity and nutritional value of the products, instilling confidence in their quality. The assay also enables the identification of products with high concentrations of antioxidants, which are commonly associated with health benefits. Despite its limitations in solvent compatibility, possible interference, and non-specificity, the DPPH assay is still widely used to screen and compare the antioxidant capacity of different compounds in many industries and research sectors. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a popular technique for evaluating antioxidant capacity, is based on the reduction of the DPPH radical—a persistent free radical with a deep violet color. Antioxidant activity is demonstrated by the change of DPPH into a colorless or pale-yellow molecule when antioxidants donate electrons or hydrogen atoms. The DPPH assay plays a significant role in agricultural research, where it looks at the antioxidant properties of different plant species and varieties, helping to create nutrient-dense and hardy crops. This aspect of the DPPH assay gives hope for the future of agriculture. The audience is informed and aware of the adaptability of the DPPH assay by these real-world applications.(Kedare and Singh, 2011; Yamauchi et al., 2024).

ABTS Assay: The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) assay is frequently used to assess a substance's antioxidant capacity. The assay's foundation is the creation of the blue-green ABTS radical cation (ABTS•+). The antioxidant content in the sample reduces ABTS•+ to a colorless form; the degree of this reduction can be observed by measuring a decrease in absorbance at a specific wavelength, often 734 nm. A sequence of steps administers the ABTS test. ABTS must first be dissolved in water to a concentration of 7 mM in order to create an ABTS solution. This process requires meticulous attention to detail. Next, the ABTS solution is mixed with potassium persulfate to a final concentration of 2.45 mM, which yields the ABTS radical cation (ABTS•+). This combination is left to react at room temperature for 12 to 16 hours in the dark. After a few days, the resultant ABTS•+ solution is stable and can be kept for further use. The next step is to put together a practical fix. Either water or ethanol is added to the ABTS•+ solution until the absorbance at 734 nm is 0.70 ± 0.02. The antioxidant sample, produced at different concentrations by dilution in ethanol,

water, or another suitable solvent, is mixed with this working solution. The combination is incubated at room temperature for a predetermined period, often six minutes. Using a spectrophotometer, the absorbance at 734 nm is determined following incubation. The antioxidant's capacity to scavenge additional ABTS•+ is revealed when absorbance decreases. Accurate measurement requires blanks and controls. To compensate for any inevitable drop in absorbance, a control sample with the ABTS•+ solution and no antioxidants is added. By utilizing a blank sample containing the solvent but not ABTS•+, background absorbance is considered.

$$\text{ABTS•+ Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

This formula is a crucial component of the ABTS test since it makes it possible to measure and compare antioxidant activity, which helps ascertain the relative effectiveness of various compounds as antioxidants. An alternative way to express the data is using Trolox equivalent antioxidant capacity (TEAC). TEAC measures the sample's antioxidant activity against that of Trolox, a water-soluble vitamin E homolog. This comparison makes it easier to compare the results of different studies by providing a standard measurement of the sample's antioxidant activity. The ABTS test has several benefits. Its versatility is explained by its ability to assess lipophilic and hydrophilic antioxidants. The test's speed and repeatability can be advantageous for high-throughput screening. However, there are certain drawbacks to this tactic. The ABTS•+ solution requires twelve to sixteen hours of preparation time. Furthermore, the choice of solvent and other colored chemicals in the sample may alter the absorbance measurements and require careful calibration. Despite these drawbacks, the benefits of the ABTS assay are significant and should be appreciated by the audience.(Cano et al., 2023; Dawidowicz and Olszowy, 2011).

FRAP Assay: The ferric-reducing antioxidant power, or FRAP assay, is a well-known technique for determining a substance's antioxidant potential. It works because antioxidants can change ferric ions (Fe3+) into ferrous ions (Fe2+). In this mechanism, 2,4,6-tripyridyl-s-triazine (TPTZ) forms a blue-colored complex that may be seen by measuring absorbance at 593 nm. The sample's antioxidant strength is correlated with the intensity of the color shift.

The FRAP reagent, made up of acetate buffer, TPTZ solution, and ferric chloride solution combined in a specific ratio, is prepared first. The FRAP reagent is mixed with prepared test samples at different concentrations. A spectrophotometer measures absorbance following an incubation period at 37°C to promote the reduction reaction. Precision is maintained by considering background absorbance and using control samples devoid of antioxidants and blanks. Ferrous equivalents (Fe2+), calculated using a standard curve based on known ferrous sulfate concentrations, are commonly used to quantify antioxidant activity. Results can also be represented as Trolox equivalent antioxidant capacity (TEAC), a comparison between the sample and the vitamin E mimic Trolox. Its benefits are the FRAP assay's simplicity, repeatability, and specificity for determining direct reducing power. It is used in many industries, including agriculture research, cosmetics, medicines, and food and beverage industries. It is more appropriate for hydrophilic antioxidants, though, as it does not directly measure the radical scavenging activity. Outcomes may also be impacted by interference from additional reducing agents in the samples.(Benzie and Strain, 1996; Pulido et al., 2000).

ORAC Assay: The Oxygen Radical Absorbance Capacity, or ORAC, is a commonly used technique to assess a substance's antioxidant capacity. This test measures how well antioxidants scavenge oxygen radicals, especially peroxyl radicals, preventing the oxidation of a fluorescent probe, often fluorescein. Antioxidants slow down the oxidative destruction of the fluorescent probe, which eventually causes a decrease in fluorescence. This

process is triggered by free radical generators like AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride). The ORAC assay's basic premise is that antioxidants prevent fluorescein from being oxidatively degraded. Fluorescein is oxidized when a free radical generator is present, which causes a drop in fluorescence. This drop in fluorescence is delayed by the antioxidants in the sample, which neutralize the peroxyl radicals. There are multiple steps in the process. To get a working concentration of 70–80 nM, fluorescein is first dissolved in a phosphate buffer (75 mM, pH 7.4) to create a fluorescein solution. Subsequently, the antioxidant sample is diluted in the phosphate buffer or another suitable solvent to prepare it at different concentrations. A fixed volume of the antioxidant sample and a fixed volume of the fluorescein solution are mixed in a microplate well. The combination is then given a predetermined amount of the radical generator AAPH, with a final concentration of usually about 12 mM. The incubation process starts immediately, and using a fluorescence microplate reader, fluorescence is monitored at regular intervals (e.g., every minute) for a set amount of time (e.g., 60–90 minutes). Typically, 485 nm and 520 nm are chosen as the excitation and emission wavelengths, respectively. Controls and blanks are given to adjust for background fluorescence and fluorescein auto-oxidation. A blank sample is the fluorescein solution without AAPH, while a control sample has the fluorescein solution with AAPH but not the antioxidant. The ORAC test has several benefits. It is essential for biological systems since it quantifies antioxidant activity utilizing peroxyl radicals in a biologically relevant setting. Whether using lipophilic or hydrophilic antioxidants, it is adaptable and efficient. Because of its extreme sensitivity, it can also detect very little antioxidant activity. There are, however, several restrictions with the ORAC test. Its complexity is increased by the need for specialized tools, like a fluorescent microplate reader, and cautious reagent management. The process could take some time because it requires constant monitoring. Moreover, the accuracy of the results may be impacted by the sample including other fluorescent chemicals that interfere with the measurements. The ORAC test has several uses across multiple sectors. In the food and beverage business, it is used to evaluate the antioxidant content of meals, drinks, and nutritional supplements. It assesses the antioxidant capacity of synthetic substances, drugs, and natural extracts in pharmaceutical research. The cosmetics industry uses the ORAC test to determine an ingredient's potential for antioxidants in skincare and beauty products. The test in agricultural research examines the antioxidant properties of different plant species and cultivars (Cao et al., 1993; Huang et al., 2005).

5. Comparative Analysis of Antioxidant Assays

The DPPH assay is a sensitive method for assessing an antioxidant's ability to scavenge the stable DPPH radical. After the antioxidant sample has been mixed with a DPPH solution, the absorbance drop at 517 nm is calculated. Because of its high sensitivity, the test is a reliable and essential tool that can differentiate between various antioxidants at different doses, giving users confidence in its correctness. It functions well with other antioxidants because it is simple to administer and provides immediate benefits. It does not, however, provide information on particular antioxidant types or their actions and is solvent-dependent. The DPPH test's appropriateness, speed, and simplicity make it worthwhile for high-throughput screening. It can analyse lipophilic and hydrophilic antioxidants, among many others. Quantitative data are obtained by measuring the absorbance reduction at 517 nm. Its lack of specificity stems from its inability to test antioxidant methods or types exactly, merely their ability to scavenge the DPPH radical. If an alternative solvent is used, comparability can be compromised. However, the DPPH radical, while not a perfect reflection of physiological conditions, is a widely accepted model for studying antioxidant activity *in vitro*.

The ABTS assay, a versatile method, assesses antioxidants' capacity to squelch the enzymatically generated ABTS•+ radical cation, indicated by a drop in absorbance at 734 nm. Like the DPPH assay, it is susceptible, widely applicable, and versatile—it functions well with both lipophilic and hydrophilic antioxidants. The assay's flexibility allows it to be adjusted to work with different antioxidants, making it an adaptable tool in your research arsenal.

However, it takes time to prepare the ABTS•+ solution, which colored compounds can hamper. The ABTS test is flexible in timing because it uses the stable ABTS•+ radical cation and may be used with both lipophilic and hydrophilic antioxidants. It offers quantifiable outcomes that are comparable to antioxidants. Measuring interference may occur from colored compounds or molecules with similar absorbance qualities, and the production of the ABTS•+ radical cation can be time-consuming. Like the DPPH assay, it doesn't offer comprehensive details on antioxidant mechanisms other than scavenging free radicals.

Using a ferric-tripyridyltriazine complex, the FRAP test converts ferric ion (Fe3+) to ferrous ion (Fe2+), detected at 593 nm. This allows antioxidants to be evaluated for their capacity to reduce. It pinpoints the decrease in ferric ions, emphasizing the ability of antioxidants to donate electrons. The test is straightforward and scalable, making it easy to use and measure reducing capacity directly. However, it only applies to hydrophilic antioxidants and does not evaluate radical scavenging directly. The FRAP assay directly measures the reducing capacity of antioxidants, which also sheds light on how well they donate electrons. It has predictable, quantifiable results based on color change, providing a sense of reassurance, and has repeatable, standardizable results across investigations. Other reducing agents can influence the assay in the sample, assess reducing power broadly without distinguishing between particular antioxidant processes, and can only evaluate hydrophilic antioxidants.

The ORAC assay, which measures antioxidants' ability to prevent peroxyl radicals from oxidatively degrading a fluorescent probe, is particularly biologically relevant. Its high sensitivity accurately reflects antioxidant activity in physiological situations. The technique calculates the fluorescence decay period using peroxyl radicals produced by AAPH. This involves measuring the decrease in fluorescence over time as the peroxyl radicals degrade the fluorescent probe. The ORAC test is advantageous for quantifying radical scavenging; nevertheless, it requires a complicated setup, takes a long time, and can be influenced by other fluorescent chemicals. The ORAC assay evaluates the radical scavenging capacity against peroxyl radicals to quantify antioxidant activity in a physiologically relevant setting. It can identify low antioxidant activity and works well with various antioxidants and complicated materials, such as biological fluids and natural extracts. However, its high sensitivity requires a complex setup with specialized equipment and careful reagent management. Moreover, other luminous chemicals in the sample may interfere with the process, making it time-consuming.(Chaves et al., 2020; Dudonné et al., 2009; Floegel et al., 2011; Thaipong et al., 2006).

6. Antioxidant Assays and Phytonutrients

6.1. Relevance to Nutritional Science

In nutritional research, antioxidant assays are vital because they offer critical information about the antioxidant capacity and content of different foods and dietary supplements. By detecting and measuring antioxidant molecules, these tests aid in nutrient profile and allow dietitians to suggest foods high in antioxidants for a balanced diet. They make it possible to analyze nutrients in detail, including macronutrients, bioactive substances, and vital vitamins and minerals. Antioxidant assays support the relationship between individual antioxidants and health benefits, the comparison analysis of different food types, and the suggestion of foods for particular medical conditions. The advancement of nutritional science research and innovation impacts public health recommendations and dietary standards. Moreover, incorporating antioxidant information into nutrient profiles enhances food labelling and guides consumers toward healthier selections. In conclusion, antioxidant testing helps create dietary guidelines and consumer education, enhance nutritional profiling, and promote better dietary choices for long-term health and well-being (Munteanu and Apetrei, 2021).

6.2. Assessment of Phytonutrient Potency

Antioxidant tests are required to assess the effectiveness of phytonutrients, naturally occurring compounds present in plants and linked to several health advantages. These

studies determine which phytonutrients are the strongest by comparing them, assessing their ability to scavenge free radicals, and gauging how well they function against oxidative stress. They also evaluate the bioavailability of phytonutrients, investigate how they work, and provide funding for research on disease prevention. Antioxidant tests assist individualized nutrition by identifying an individual's specific antioxidant level and recommending certain phytonutrients. They also look into the synergistic effects of mixing phytonutrients for more significant health benefits. Antioxidant assays provide essential information for research, product development, and dietary recommendations. This helps to maximize the health benefits of phytonutrients and fosters trust in the validity of evidence-based recommendations and formulations (Mendonça et al., 2022).

6.3. Guiding Dietary Recommendations

Antioxidant assays play a critical role in shaping dietary recommendations by providing empirical data that aid in developing guidelines to promote public health via nutrition. These tests evaluate a food's capacity to contain antioxidants, helping to find foods that have significant health benefits. By quantifying the quantity of antioxidants in various meals, antioxidant assays enable us to suggest antioxidant-rich diets that include whole grains, berries, nuts, and veggies. These assays produce evidence-based recommendations by allowing the inclusion of foods with high antioxidant content and promoting a balanced diet with various antioxidant sources. Antioxidant data also guides public health programs, enhances nutritional education, and encourages the development of functional foods and supplements. In addition to providing individualized nutrition programs and assessing an individual's antioxidant status, they offer dietary suggestions for skin and cognitive health to promote healthy ageing. Furthermore, assays encourage the use of natural foods instead of processed ones and morally sound, environmentally friendly, and health-conscious food choices (Bibi Sadeer et al., 2020).

6.4. Role in Food Industry

Antioxidant assays are also capable of identifying synthetic compounds used to imitate natural antioxidant levels and verifying the authenticity of drugs. In addition to enhancing regulatory compliance and quality control, antioxidant assays monitor process integrity and enhance customer safety and product integrity. Assay technologies are continuously evolving, strengthening their capacity for detection and guarding against fresh methods of adulteration. Antioxidant assays also aid in regulatory compliance, direct the creation of new goods, and enhance the nutritional value of food items. Antioxidant assays assist in developing health-promoting goods and upholding strict standards in the food sector by giving vital data(Sadowska-Bartosz and Bartosz, 2022)

6.5. Detection of Adulteration

Antioxidant assays offer analytical techniques to identify deviations from expected antioxidant profiles, essential for identifying food product contamination or adulteration. They establish baseline antioxidant levels to compare accurate goods to samples that raise concerns. Assays that detect reduced levels of antioxidants can reveal potential adulteration by diluting or substituting inferior compounds. They can also identify synthetic compounds used to imitate natural antioxidant levels and verify the authenticity of drugs. In addition to enhancing regulatory compliance and quality control, antioxidant assays monitor process integrity and improve customer safety and product integrity. Assay technologies are continuously evolving, strengthening their capacity for detection and guarding against fresh methods of adulteration (Loi and Paciolla, 2021).

6.6. Application in Pharmacology

Antioxidant tests are used in pharmacology to evaluate the potential antioxidant activity of therapeutic candidates. These assays are also employed in drug discovery to identify compounds that can lower oxidative stress. Whether conducted on synthetic or natural compounds, these studies help us understand how drugs prevent oxidative damage by identifying prospective therapeutic options and illuminating antioxidant activity mechanisms. Remarkably, antioxidant tests also contribute significantly to medical research by helping to

discover therapeutic targets and supporting disease biology. They provide direction for the creation of antioxidant-based treatments for oxidative stress-related illnesses. In preclinical research, they are essential because they assess the efficacy of antioxidants in animal models and offer recommendations for human safety and efficacy trials. They also aid in optimising and developing new drugs, guaranteeing the stability and effectiveness of antioxidants in the finished product (Egbujor et al., 2021; Zehiroglu and Ozturk Sarikaya, 2019).

6.7. Understanding Health Benefits

Antioxidant assays are essential for determining the health benefits of meals, supplements, and other goods. They assess how well these products reduce oxidative stress and neutralize free radicals, aiding in evaluating their shielding properties against long-term illnesses. By identifying foods high in antioxidants, these tests help to influence dietary guidelines. More importantly, they support public health campaigns, connecting individuals to a more significant cause of promoting health and well-being. They also verify health claims by providing empirical evidence supporting the advantages of goods advertised with antioxidant qualities. Lastly, they ensure that functional foods have sufficient antioxidants for health and well-being(Wahlqvist, 2013).

6.8. Pharmaceutical and Nutraceutical Development

Antioxidant tests are widely employed in developing pharmaceuticals and nutraceuticals to evaluate the potential health benefits and therapeutic uses of antioxidants produced from natural sources. By checking natural substances and plant extracts for antioxidant activity, they aid in identifying bioactive components— these assays direct formulation optimisation by identifying the ideal concentration and combination of antioxidant-rich components for maximal performance. Preclinical research assesses the antioxidant capacity of possible medication candidates, offering initial information on how well they can reduce inflammation and oxidative stress. Antioxidant assays also evaluate pharmacokinetics and bioavailability, which affects dosing guidelines and administration systems. They support toxicity and safety evaluations, guaranteeing beneficial antioxidant levels free from side effects. Antioxidative tests monitor the state and efficacy of antioxidants in human subjects undergoing clinical trials, evaluating their effects on biomarkers associated with oxidative stress, inflammation, and the onset of illness. They are integrated into quality control processes to ensure product potency and uniformity, support submissions, adhere to health and safety requirements, and offer vital scientific proof for regulatory compliance(Ziarati et al., 2023).

6.9. Shelf-Life Extension and Reduction of Food Waste

Antioxidant tests are essential for extending the shelf life of food goods because they monitor and optimize antioxidant levels to prevent oxidative deterioration, which can lead to rancidity, bad flavors, color changes, and nutritional loss. These tests let companies compare synthetic and natural antioxidants, determine the optimal amounts to protect food without compromising taste or safety and select packaging that preserves antioxidant activity. Additionally, they aid in creating the best storage practices, ensure consistent antioxidant levels during production, and enhance product formulations by utilizing synergistic antioxidants, which are combinations of antioxidants that work together to provide greater protection than the sum of their individual effects. These tests also stabilize fortified meals, and design processing techniques. Antioxidant assays also demonstrate that products remain safe and of the highest quality for the duration of their shelf lives, which is essential information for regulatory compliance. Because antioxidant tests prolong product shelf life and preserve quality, they are essential for minimizing food waste in the food business. They monitor antioxidant levels to stop oxidative deterioration, improve stability in formulations, and advance preservation methods. Finding efficient antioxidants reduces food waste across the supply chain, stops spoiling, and enhances distribution and storage procedures. All of these factors support sustainability. By preserving the nutritional content and freshness of food goods, these assays also help with effective inventory management, guarantee regulatory compliance, and increase

consumer confidence by promoting the consumption of nutrient-dense meals. (Lu and Pham-Mondala, 2018; Zahid et al., 2024).

7. Challenges and Limitations

Antioxidant assays pose several difficulties and constraints, but they are crucial in industry and research for determining antioxidant capacity and directing applications. Assays with different techniques, like DPPH, ABTS, FRAP, and ORAC, produce variable results and make comparisons challenging. Because of variables related to bioavailability and metabolism, in vitro experiments could not precisely represent in vivo activity. Interpretations can become more difficult due to the influence of the meal matrix and component interactions on test results. Since most assays only evaluate individual antioxidants, synergistic effects may be noticed. The sensitivity, dynamic range, and lack of standardized units in assays make precise measurement and comparison even more difficult. Temporal stability problems and interference from non-antioxidant substances can distort the results. High prices and technological limitations restrict accessibility, and discoveries must be validated by complementary approaches, such as clinical trials, due to the complexity of biological systems. Antioxidant tests' dependability and usefulness can be increased by addressing these issues through methodological advancements and standardization(Apak, 2019; Hermans et al., 2007).

8. Future prospects

Antioxidant assays are critical in promoting innovation in various sectors by offering essential information on antioxidants' mechanisms, effectiveness, and applications. These tests help in the identification of novel antioxidants from synthetic and natural sources, the comprehension of their mechanisms of action, and the creation of improved antioxidant formulations. To increase the efficiency of antioxidants in the human body, they guide the optimization of extraction and processing methods and aid in assessing bioavailability. Furthermore, assays promote developing novel food and beverage products, pharmaceutical uses, and unique delivery technologies. Assays are used in the cosmetics industry to verify the antioxidant qualities of substances used in anti-ageing products. Additionally, they support individualized treatment and nutrition and advocate for sustainable sourcing. Antioxidant assays impact environmental applications to decrease food waste and improve animal health by providing antioxidant-rich feed. They also influence public health regulations. They also work as teaching resources, teaching upcoming scientists how to assess antioxidant activity and its effects on health. In general, antioxidant assays propel industry, science, and health improvements by serving as a basis for many innovations. Antioxidant assays pose several difficulties and constraints, but they are crucial in industry and research for determining antioxidant capacity and directing applications. Assays with different techniques, like DPPH, ABTS, FRAP, and ORAC, produce variable results and make comparisons challenging. Because of variables related to bioavailability and metabolism, in vitro experiments could not precisely represent in vivo activity. Interpretations can become more difficult due to the influence of the meal matrix and component interactions on test results. Since most assays only evaluate individual antioxidants, synergistic effects may be noticed. The sensitivity, dynamic range, and lack of standardized units in assays make precise measurement and comparison even more difficult. Temporal stability problems and interference from non-antioxidant substances can distort the results.

The future of phytonutrient and antioxidant studies holds great promise for various fields. New technologies and multidisciplinary methods will revolutionize our understanding of these substances. With advancements enabling personalized antioxidant strategies based on individual genetic profiles and health conditions, customized medicine is set to play a significant role. Future cardiovascular health, neuroprotection, and cancer prevention studies will explore new antioxidant molecules and their synergistic effects with other treatments. Technological advancements in agriculture could lead to crops with enhanced phytonutrient profiles, and new extraction and synthesis techniques will maximize their application in

functional foods and supplements. The accuracy and applicability of these tests will be further enhanced with the development of high-throughput, real-time antioxidant assays and better standardization. The food sector will increasingly rely on these tests to validate health claims, ensure quality, and extend shelf life. Antioxidant assays, with their potential to support the development of pharmaceuticals and nutraceuticals, will play a crucial role in the healthcare industry. They will also aid in detecting food adulteration. Overcoming challenges such as assay uniformity and individual variability will require ongoing creativity and collaboration, leading to more potent health interventions and reduced food waste(Andrei Florin, 2021; Sen et al., 2010).

9. Conclusion

In conclusion, the antioxidant properties of phytonutrients hold immense potential for advancing nutrition and health. They offer a promising avenue for combating oxidative stress and related disorders, inspiring us with their present possibilities. This review critically examined the methodologies used to evaluate antioxidant activity, highlighting their fundamentals, advancements, and practical applications in food science and nutritional research. While significant progress has been made in improving assay sensitivity, accuracy, and physiological relevance, challenges such as variability and standardization remain. Addressing these challenges will further enhance the reliability and applicability of antioxidant assays, paving the way for innovative solutions in health promotion and food quality assurance.

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References

Adlercreutz, H., 2007. Lignans and human health. Critical reviews in clinical laboratory sciences 44(5-6), 483-525.

Aggarwal, B.B., Sundaram, C., Malani, N., Ichikawa, H., 2007. Curcumin: the Indian solid gold. The molecular targets and therapeutic uses of curcumin in health and disease, 1-75.

Andrei Florin, D., 2021. Recent Advances in Antioxidant Capacity Assays, in: Viduranga, W. (Ed.) Antioxidants. IntechOpen, Rijeka, p. Ch. 29.

Anwar, F., Qadir, R., 2021. Carnosic acid and carnosol, A centum of valuable plant bioactives. Elsevier, pp. 261-274.

Apak, R., 2019. Current Issues in Antioxidant Measurement. Journal of agricultural and food chemistry 67(33), 9187-9202.

Atta, E.M., Mohamed, N.H., Abdelgawad, A.A., 2017. Antioxidants: An overview on the natural and synthetic types. Eur. Chem. Bull 6(8), 365-375.

Ayse Tulin, O., Ebru, K., 2017. Phytochemicals in Fruits and Vegetables, in: Viduranga, W., Naofumi, S. (Eds.), Superfood and Functional Food. IntechOpen, Rijeka, p. Ch. 8.

Baboota, R.K., Murtaza, N., Jagtap, S., Singh, D.P., Karmase, A., Kaur, J., Bhutani, K.K., Boparai, R.K., Premkumar, L.S., Kondepudi, K.K., 2014. Capsaicin-induced transcriptional changes in hypothalamus and alterations in gut microbial count in high fat diet fed mice. The journal of nutritional biochemistry 25(9), 893-902.

Balasundram, N., Sundram, K., Samman, S., 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food chemistry 99(1), 191-203.

Baur, J.A., Sinclair, D.A., 2006. Therapeutic potential of resveratrol: the in vivo evidence. Nature reviews Drug discovery 5(6), 493-506.

Bennett, L.L., Rojas, S., Seefeldt, T., 2012. Role of Antioxidants in the Prevention of Cancer. Journal of Experimental & Clinical Medicine 4(4), 215-222.

Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry 239(1), 70-76.

Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G., Mahomoodally, M.F., 2020. The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations. Antioxidants 9(8), 709.

Boots, A.W., Haenen, G.R., Bast, A., 2008. Health effects of quercetin: from antioxidant to nutraceutical. European journal of pharmacology 585(2-3), 325-337.

Borek, C., 2001. Antioxidant health effects of aged garlic extract. The Journal of nutrition 131(3), 1010S-1015S.

Cabrera, C., Artacho, R., Giménez, R., 2006. Beneficial effects of green tea—a review. Journal of the American College of Nutrition 25(2), 79-99.

Cano, A., Maestre, A.B., Hernández-Ruiz, J., Arnao, M.B., 2023. ABTS/TAC Methodology: Main Milestones and Recent Applications, Processes.

Cao, G., Alessio, H.M., Cutler, R.G., 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free radical biology and medicine 14(3), 303-311.

Chan, G.C.-F., Chan, W.K., Sze, D.M.-Y., 2009. The effects of β-glucan on human immune and cancer cells. Journal of hematology & oncology 2, 1-11.

Chaves, N., Santiago, A., Alías, J.C., 2020. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. Antioxidants 9(1), 76.

Chung, K.-T., Wong, T.Y., Wei, C.-I., Huang, Y.-W., Lin, Y., 1998. Tannins and human health: a review. Critical reviews in food science and nutrition 38(6), 421-464.

Dawidowicz, A.L., Olszowy, M., 2011. Antioxidant properties of BHT estimated by ABTS assay in systems differing in pH or metal ion or water concentration. European Food Research and Technology 232(5), 837-842.

De Oliveira, J.R., Camargo, S.E.A., De Oliveira, L.D., 2019. Rosmarinus officinalis L.(rosemary) as therapeutic and prophylactic agent. Journal of biomedical science 26(1), 5.

de Oliveira, M.R., 2018. Carnosic acid as a promising agent in protecting mitochondria of brain cells. Molecular neurobiology 55(8), 6687-6699.

Dinkova-Kostova, A.T., Kostov, R.V., 2012. Glucosinolates and isothiocyanates in health and disease. Trends in molecular medicine 18(6), 337-347.

Ditu, L.-M., Grigore, M.E., Camen-Comanescu, P., Holban, A.M., 2018. Chapter 1 - Introduction in Nutraceutical and Medicinal Foods, in: Grumezescu, A.M., Holban, A.M. (Eds.), Therapeutic, Probiotic, and Unconventional Foods. Academic Press, pp. 1-12.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., Mérillon, J.-M., 2009. Comparative Study of Antioxidant Properties and Total Phenolic Content of 30 Plant Extracts of Industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC Assays. Journal of agricultural and food chemistry 57(5), 1768-1774.

Duthie, G., Crozier, A., 2000. Plant-derived phenolic antioxidants. Current opinion in lipidology 11(1), 43-47.

Egbujor, M.C., Egu, S.A., Okonkwo, V.I., Jacob, A.D., Egwuatu, P.I., Amasiatu, I.S., 2021. Antioxidant drug design: historical and recent developments. J. Pharm. Res. Int 32(41), 36-56.

Egner, P.A., Muñoz, A., Kensler, T.W., 2003. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 523, 209-216.

Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E., Hurst, R., 2011. Selenium in human health and disease. Antioxidants & Redox Signaling 14(7), 1337-1383.

Ferruzzi, M.G., Blakeslee, J., 2007. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. Nutrition research 27(1), 1-12.

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S.I., Chun, O.K., 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Journal of food composition and analysis 24(7), 1043-1048.

Gillingham, L.G., Harris-Janz, S., Jones, P.J., 2011. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 46, 209-228.

Gruenwald, J., Freder, J., Armbruester, N., 2010. Cinnamon and health. Critical reviews in food science and nutrition 50(9), 822-834.

Guo, J., Huang, X., Dou, L., Yan, M., Shen, T., Tang, W., Li, J., 2022. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal Transduction and Targeted Therapy 7(1), 391.

Gupta, S.C., Patchva, S., Aggarwal, B.B., 2013. Therapeutic roles of curcumin: lessons learned from clinical trials. The AAPS journal 15, 195-218.

Hermans, N., Cos, P., Maes, L., De Bruyne, T., Vanden Berghe, D., A, J.V., Pieters, L., 2007. Challenges and Pitfalls in Antioxidant Research. Current Medicinal Chemistry 14(4), 417-430.

Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays.

Journal of agricultural and food chemistry 53(6), 1841-1856.
Iciek, M., Kwiecień, I., Włodek, L., 2009. Biological properties of garlic and garlic-derived organosulfur compounds. Environmental and molecular mutagenesis 50(3), 247-265.
Kedare, S.B., Singh, R.P., 2011. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology 48(4), 412-422.
Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M., 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & nutrition research.
Kim, Y., Je, Y., 2017. Flavonoid intake and mortality from cardiovascular disease and all causes: A meta-analysis of prospective cohort studies. Clinical nutrition ESPEN 20, 68-77.
Kirkham, S., Akilen, R., Sharma, S., Tsiami, A., 2009. The potential of cinnamon to reduce blood glucose levels in patients with type 2 diabetes and insulin resistance. Diabetes, obesity and metabolism 11(12), 1100-1113.
Knekt, P., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., Hakulinen, T., Aromaa, A., 2002. Flavonoid intake and risk of chronic diseases. The American journal of clinical nutrition 76(3), 560-568.
Krinsky, N.I., Landrum, J.T., Bone, R.A., 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annual review of nutrition 23(1), 171-201.
Kumar, V., Sinha, A.K., Makkar, H.P., Becker, K., 2010. Dietary roles of phytate and phytase in human nutrition: A review. Food chemistry 120(4), 945-959.
Lee, K.H., Cha, M., Lee, B.H., 2020. Neuroprotective Effect of Antioxidants in the Brain. International Journal of Molecular Sciences 21(19), 7152.
Loi, M., Paciolla, C., 2021. Plant Antioxidants for Food Safety and Quality: Exploring New Trends of Research. Antioxidants 10(6), 972.
Lu, F., Pham-Mondala, A., 2018. Trends in synthetic and natural antioxidants for shelf life extension of meat and meat products. https://www.aocs.org/stay-informed/inform-magazine/featured-articles/trends-in-synthetic-and-natural-antioxidants-for-shelf-life-extension-of-meat-and-meat-products-november/december-2018?SSO=True. (Accessed 19th July 2024).
Ma, L., Lin, X.M., 2010. Effects of lutein and zeaxanthin on aspects of eye health. Journal of the Science of Food and Agriculture 90(1), 2-12.
Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., 2004. Polyphenols: food sources and bioavailability. The American journal of clinical nutrition 79(5), 727-747.
Mandl, J., Szarka, A., Banhegyi, G., 2009. Vitamin C: update on physiology and pharmacology. British journal of pharmacology 157(7), 1097-1110.
Mangge, H., Becker, K., Fuchs, D., Gostner, J.M., 2014. Antioxidants, inflammation and cardiovascular disease. World journal of cardiology 6(6), 462.
Marcus, J.B., 2013. Chapter 7 - Vitamin and Mineral Basics: The ABCs of Healthy Foods and Beverages, Including Phytonutrients and Functional Foods: Healthy Vitamin and Mineral Choices, Roles and Applications in Nutrition, Food Science and the Culinary Arts, in: Marcus, J.B. (Ed.) Culinary Nutrition. Academic Press, San Diego, pp. 279-331.
McKay, D.L., Blumberg, J.B., 2002. The role of tea in human health: an update. Journal of the American College of Nutrition 21(1), 1-13.
Mendonça, J.d.S., Guimarães, R.d.C.A., Zorgetto-Pinheiro, V.A., Fernandes, C.D.P., Marcelino, G., Bogo, D., Freitas, K.d.C., Hiane, P.A., de Pádua Melo, E.S., Vilela, M.L.B., Nascimento, V.A.d., 2022. Natural Antioxidant Evaluation: A Review of Detection Methods. Molecules 27(11), 3563.
Mittal, M., Siddiqui, M.R., Tran, K., Reddy, S.P., Malik, A.B., 2013. Reactive Oxygen Species in Inflammation and Tissue Injury. Antioxidants & Redox Signaling 20(7), 1126-1167.
Moeller, S.M., Jacques, P.F., Blumberg, J.B., 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. Journal of the American College of Nutrition 19(sup5), 522S-527S.
Monjotin, N., Amiot, M.J., Fleurentin, J., Morel, J.M., Raynal, S., 2022. Clinical Evidence of the Benefits of Phytonutrients in Human Healthcare. Nutrients 14(9), 1712.
Munteanu, I.G., Apetrei, C., 2021. Analytical Methods Used in Determining Antioxidant Activity: A Review. International Journal of Molecular Sciences 22(7), 3380.
Murphy, E.J., Rezoagli, E., Major, I., Rowan, N.J., Laffey, J.G., 2020. β-glucan metabolic and immunomodulatory properties and potential for clinical application. Journal of Fungi 6(4), 356.

Musco, N., 2013. Role of soluble and insoluble polysaccharides in omnivore and carnivores nutrition: a review. Journal of Nutritional Ecology and Food Research 1(4), 247-261.
Pan, M.H., Hsieh, M.C., Kuo, J.M., Lai, C.S., Wu, H., Sang, S., Ho, C.T., 2008. 6-Shogaol induces apoptosis in human colorectal carcinoma cells via ROS production, caspase activation, and GADD 153 expression. Molecular nutrition & food research 52(5), 527-537.
Panche, A.N., Diwan, A.D., Chandra, S.R., 2016. Flavonoids: an overview. Journal of nutritional science 5, e47.
Pandey, K.B., Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity 2(5), 270-278.
Patel, D., Shukla, S., Gupta, S., 2007. Apigenin and cancer chemoprevention: progress, potential and promise. International journal of oncology 30(1), 233-245.
Petersen, M., Simmonds, M.S., 2003. Rosmarinic acid. Phytochemistry 62(2), 121-125.
Peterson, J., Dwyer, J., Adlercreutz, H., Scalbert, A., Jacques, P., McCullough, M.L., 2010. Dietary lignans: physiology and potential for cardiovascular disease risk reduction. Nutrition reviews 68(10), 571-603.
Pramod, K., Ansari, S.H., Ali, J., 2010. Eugenol: a natural compound with versatile pharmacological actions. Natural product communications 5(12), 1934578X1000501236.
Prior, R.L., Wu, X., 2006. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. Free radical research 40(10), 1014-1028.
Pulido, R., Bravo, L., Saura-Calixto, F., 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of agricultural and food chemistry 48(8), 3396-3402.
Rao, A.V., Rao, L.G., 2007. Carotenoids and human health. Pharmacological research 55(3), 207-216.
Rao, V.R., 2016. Chapter 7 - Antioxidant Agents, in: Penta, S. (Ed.) Advances in Structure and Activity Relationship of Coumarin Derivatives. Academic Press, Boston, pp. 137-150.
Rayman, M.P., 2000. The importance of selenium to human health. The lancet 356(9225), 233-241.
Reddy, N.R., 2001. Occurrence, distribution, content, and dietary intake of phytate, Food phytates. CRC Press, pp. 41-68.
Reddy, V.P., 2023. Oxidative Stress in Health and Disease. Biomedicines 11(11), 2925.
Riediger, N.D., Othman, R.A., Suh, M., Moghadasian, M.H., 2009. A systemic review of the roles of n-3 fatty acids in health and disease. Journal of the American Dietetic Association 109(4), 668-679.
Sadowska-Bartosz, I., Bartosz, G., 2022. Evaluation of The Antioxidant Capacity of Food Products: Methods, Applications and Limitations. Processes 10(10), 2031.
Santos-Sánchez, N.F., Salas-Coronado, R., Villanueva-Cañongo, C., Hernández-Carlos, B., 2019. Antioxidant compounds and their antioxidant mechanism. Antioxidants 10, 1-29.
Scalbert, A., Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. The Journal of nutrition 130(8), 2073S-2085S.
Sen, C.K., Khanna, S., Roy, S., 2006. Tocotrienols: Vitamin E beyond tocopherols. Life sciences 78(18), 2088-2098.
Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y., De, B., 2010. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res 3(1), 91-100.
Shalini Kapoor, M., Sivakumar Joghi Thatha, G., 2015. Members of Antioxidant Machinery and Their Functions, in: Sivakumar Joghi Thatha, G. (Ed.) Basic Principles and Clinical Significance of Oxidative Stress. IntechOpen, Rijeka, p. Ch. 4.
Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P.V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M.E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A.O., Setzer, W.N., Calina, D., Cho, W.C., Sharifi-Rad, J., 2020. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Frontiers in Physiology 11.
Sharma, K., Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Sharma, R., Gat, Y., Kumar, A., 2021. Health effects, sources, utilization and safety of tannins: a critical review. Toxin Reviews 40(4), 432-444.
Shearer, M.J., Newman, P., 2008. Metabolism and cell biology of vitamin K. Thrombosis and haemostasis 100(10), 530-547.
Shukla, S., Gupta, S., 2010. Apigenin: a promising molecule for cancer prevention. Pharmaceutical research 27, 962-978.
Shukla, Y., Singh, M., 2007. Cancer preventive properties of ginger: a brief review. Food and chemical toxicology 45(5), 683-690.

Sisein, E.A., 2014. Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences 2(2), 110-118.
Spencer, J.P., 2008. Flavonoids: modulators of brain function? British journal of nutrition 99(E-S1), ES60-ES77.
Srinivasan, K., 2007. Black pepper and its pungent principle-piperine: a review of diverse physiological effects. Critical reviews in food science and nutrition 47(8), 735-748.
Srinivasan, K., 2016. Biological activities of red pepper (Capsicum annuum) and its pungent principle capsaicin: a review. Critical reviews in food science and nutrition 56(9), 1488-1500.
Sun, J., 2007. D-Limonene: safety and clinical applications. Alternative medicine review 12(3), 259.
Taylor, J.R., Duodu, K.G., 2015. Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. Journal of the Science of Food and Agriculture 95(2), 225-237.
Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of food composition and analysis 19(6-7), 669-675.
Traber, M.G., 2007. Vitamin E regulatory mechanisms. Annu. Rev. Nutr. 27(1), 347-362.
Traber, M.G., Atkinson, J., 2007. Vitamin E, antioxidant and nothing more. Free radical biology and medicine 43(1), 4-15.
Traber, M.G., Stevens, J.F., 2011. Vitamins C and E: beneficial effects from a mechanistic perspective. Free radical biology and medicine 51(5), 1000-1013.
Traka, M., Mithen, R., 2009. Glucosinolates, isothiocyanates and human health. Phytochemistry reviews 8, 269-282.
Vang, O., Ahmad, N., Baile, C.A., Baur, J.A., Brown, K., Csiszar, A., Das, D.K., Delmas, D., Gottfried, C., Lin, H.-Y., 2011. What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. PloS one 6(6), e19881.
Wahlqvist, M.L., 2013. Antioxidant relevance to human health. Asia Pacific Journal of Clinical Nutrition 22(2), 171-176.
Wahlqvist, M.L., Wattanapenpaiboon, N., 2020. Phytonutrients as Bioactive Food Components, Food and Nutrition. Routledge, pp. 311-321.
Walther, B., Karl, J.P., Booth, S.L., Boyaval, P., 2013. Menaquinones, bacteria, and the food supply: the relevance of dairy and fermented food products to vitamin K requirements. Advances in nutrition 4(4), 463-473.
Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J., Gotoh, H., 2024. DPPH Measurements and Structure—Activity Relationship Studies on the Antioxidant Capacity of Phenols. Antioxidants 13(3), 309.
Yao, L.H., Jiang, Y.-M., Shi, J., Tomas-Barberan, F., Datta, N., Singanusong, R., Chen, S., 2004. Flavonoids in food and their health benefits. Plant foods for human nutrition 59, 113-122.
Zahid, M., Khalid, S., Raana, S., Amin, S., Javaid, H., Arshad, R., Jahangeer, A., Ahmad, S., Hassan, S.A., 2024. Unveiling the anti-oxidative potential of fruits and vegetables waste in prolonging the shelf stability of vegetable oils. Future Foods 10, 100328.
Zehiroglu, C., Ozturk Sarikaya, S.B., 2019. The importance of antioxidants and place in today's scientific and technological studies. Journal of Food Science and Technology 56(11), 4757-4774.
Zhao, Z., Egashira, Y., Sanada, H., 2004. Ferulic acid is quickly absorbed from rat stomach as the free form and then conjugated mainly in liver. The Journal of nutrition 134(11), 3083-3088.
Ziarati, P., Tajik, S., Cruz-Rodriguez, L., 2023. A Nutraceuticals with high antioxidant properties based on fruits and medicinal plants. Biological Environment and Pollution 3(1), 15-24.