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A CRITICAL REVIEW OF SCREENING METHODS TO DETERMINE THE ANTIOXIDANT CAPACITY IN LEGUME

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Abstract

Legumes are a rich source of bioactive compounds such as phenolic or polyphenolic compounds, particularly tocopherols. Legumes antioxidants are widespread for their radical scavenging proprieties as active biologic compounds belonging to various chemical classes. Polyphenols are the most studied molecules of both nutritional and pharmaceutical interest. Furthermore, an overview concerning the antioxidant capacity and determination is mandatory for the precise and accurate method selection, involving cost-effectiveness and time-saving, towards gathering networks between research and development fields. The current review aims to summarize the presence of the natural antioxidant, their multiple biological effects, and the various approaches to methods determinations.

***Key words**: antioxidant activity, legumes, phenolic compound, vitamin.*

INTRODUCTION

Legumes are a rich source of bioactive compounds such as phenolic acids, flavanols, flavones, flavanols, flavanones, isoflavones, anthocyanins, and tannins, (Nicolás-García et al., 2021).

Phenolic compounds are present in all anatomical parts of plants (Amarowicz et al., 2017) and exhibited mostly in the seed tegument, represented by anthocyanins, condensed tannins, kaempferol glucoside, and quercetin. Whereas phenolic acids such as ferulic, synaptic, chlorogenic, and other hydroxycinnamic acids are found primarily in the cotyledon (Nurzyńska-Wierdak et al., 2019). Several authors have emphasized the importance of phenolic compounds such as natural biocontrol agents (Dresch et al., 2014) implicated in the resistance of some grapevine cultivars to fungi, oomycetes, bacteria, phytoplasma, and viruses; one of the most well-known properties of these compounds is their antioxidative activity (Aouey et al., 2016), which allows them to scavenge free radicals and might have positively health outcomes (Waffo-Teguo et al., 2008). The antioxidant activity of phenolic compounds extracted from legume seeds has been previously studied using several in vitro chemical assays. Antioxidants can neutralize free radicals and reduce the risk of damage (Hayat et al., 2009). By competing with free radicals, antioxidants function as chain-breaking inhibitors, interrupting the chain process, with major role in apoptosis and cell-signalling mechanism (Halliwell, 1996).

The current review aims to summarize the presence of the natural antioxidant, their multiple biological effects, and the various approaches to methods determinations.

MATERIALS AND METHODS

The summarised and synthetised information of the current review were literature-based assessments (Figure 1).

The systematic review methodology was employed by using scientific database available, such as Google Scholar, Science direct, Web of Science, Scopus, Springer link, and MDPI. The identification protocol was based on keyword, year, and article type filtration (Figure 1).

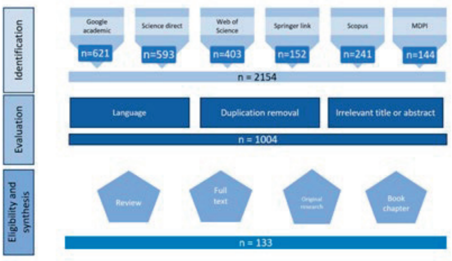

Figure 1. Identification of relevant information

1. Endogenous antioxidant

1.1. Enzymatic antioxidant

This chapter provides an overview of two protein antioxidants (with enzymatic activity), which are the first line of defence against oxidative stress on the body: superoxide dismutase (SOD) and catalase (CAT).

Superoxide dismutase (SODs) is a class of enzymes that operate as the first line of antioxidant defence by dismutation extremely reactive superoxide radicals into hydrogen peroxide and molecular oxygen. There are four isozymes of superoxide dismutase (Table 1).

Table 1. Identification of four isozymes of Superoxyde dismutase (adapted by Fink & Scandalios, 2002)

Isozymes of SOD	Metal cofactors	Localizations	References
SOD1	Cu / Zn	Cytosol Chloroplast	(Mccord et Fridovich, 1969); Laukkanen, 2016 ; Fink et Scandalios, 2002)
SOD2	Mn / Fe	Mitochondria	(Laukkanen, 2016 ; Weisiger et Fridovich, 1973)
SOD3	Cu / Zn	Extracellular	(Laukkanen, 2016; Marklund, 1982)
SOD4	Ni	Aerobic soil bacteria	(Wuerges et al., 2004 ; Anju et al., 2013)

Catalase is a tetrameric porphyrin-containing enzyme that is mainly found in peroxisomes. It catalyzes the conversion of H_2O_2 to water and molecular oxygen in two steps (Aslani et Ghobadi, 2016):

Catalase-Fe (III) + H2O2→compound A
Compound A + H2O2→catalase-Fe (III) + 2H2O + O2

CAT is one of the most active catalysts produced by nature, it plays a crucial role in systems that have evolved to allow organisms to live in aerobic environments. Because of its evolutionary conservation, wide distribution, and capacity to rapidly degrade hydrogen peroxide, CAT provides the cell with a highly efficient mechanism for removing hydrogen peroxide. Then when cells are challenged for energy and rapidly produce H_2O_2 via "emergency" catabolic processes, H_2O_2 is destroyed in an energy-efficient manner by CAT. So, there is a net gain in decreasing equivalents and, as a result, cellular energy (Scandalios, 2005).

2. Exogenous antioxidants

Over the decades, people's health prevention and diet were a preoccupation of worldwide scientists (Xu et al., 2017; Dominguez-Perles et al., 2020; Loizzo et al., 2021). Antioxidants were previously studied for their effects through anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer (Manach et al., 2004; Xu et al., 2017; Menga et al., 2023). Moreover, exogenous antioxidants are found especially in food such as vegetables, fruits, medicinal plants, or cereals (Xu et al., 2017). The representative components of exogenous antioxidants found in plant sources are often divided into phenols, carotenoids, and vitamins (Baiano et al., 2015).

2.1. Phenolic structure

The chemical structure of the phenolic compounds includes one or many aromatic rings which have annexed one or numerous AOH (hydroxyl) groups in different, free, combined, or bound statuses (Wang et al., 2015; Telles et al., 2017). Phenolic compounds have many health benefits, they act as antioxidants, anticarcinogenic antimutagenic, antifungal, and anti-inflammatory (Alshikh et al., 2015). Considering that, legumes are a rich source of bioactive phenolic compounds, some of these phenolic compounds are concentrated in the seed coat of legumes (Gan et al., 2016;) and include phenols, flavonoids, and phenolic acids (Hossain et al., 2021). Legume seeds are essential in the human diet because they are excellent sources of protein, fiber, minerals, vitamins, and bioactive compounds (Magalhães et al., 2017). Essentially sources of legumes are categorized into mature legumes, including dry beans, dry peas, lentils, and fava beans; and immature legumes which are considered green beans and peas, respectively (FAO, 2018). Regarding Kalili et al., 2022, the fava beans concentrations ranged from 49.5 to 594.4 mg

GAE/ g for the total phenols, a range of 0.7 mg to 3.4 mg QE/g for flavonoids, and for tannins was on average from 4.9 mg to 73.91 mg TAE/g dry weight.

2.1.1. Phenolic acids

Phenolic acids (Table 2) are similar to phenol carboxylic acids, being an organic compound, which belongs to the aromatic carboxylic acids class with a C6-C1 type skeleton (Heleno et al., 2015). However, phenolic acids are the main source of bioactive chemical substances, from the class of phenolic components, which are discovered in various natural foods or even beverages intended for human consumption (Zhang et al. 2020). Furthermore, this metabolite occurs in second place, after the flavonoids, which concurs with the distinctive organoleptic characteristics of food (Zhang et al., 2016).

The phenolic acids are a different class which includes hydroxybenzoic acid derivatives (p-hydroxybenzoic, protocatechuic, gallic, vanillic, and syringic acids) and hydroxycinnamic acid derivatives (caffeic, chlorogenic acid, ferulic, p-coumaric) (Singh et al., 2017). PCA (protocatechuic acid) is a water-soluble benzoic acid derivative that reduces metabolic disorders associated with obesity (Ormazabal et al., 2021) and has anti-atherosclerotic, anti-inflammatory, antineoplastic, analgesic, antibacterial, hepatoprotective and antiviral effects (Kakkar et al., 2014). Gallic acid has antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, gastroprotective, and neuroprotective effects (Choubey et al., 2015). Chlorogenic acid is a good antioxidant, but it is also involved in several biological activities, antifungal activities, and antimutagenic activities, provides protection against cardiovascular diseases (Mirali et al., 2014) and reduces the process of carcinogenesis (Shin et al., 2015; Telles et al., 2017). The chlorogenic acid present in cereals, as well as other legumes, has been shown that inhibits the activity of digestive amylase, and prevents the degradation of food and its contamination with microorganisms (Heidtmann-Bemvenuti et al., 2011; Pagnussatt et al., 2013; Telles et al., 2017). Ferulic acid, like chlorogenic acid, is known for its antioxidant activity, anticancer properties and helps in the prevention and therapy of diabetes. (Kumar & Prothi, 2014; Telles et al., 2017).

Common as well as fava beans (*Vicia faba* L.), like food in human nutrition, gathered its study in countless research projects, both for the component rich in phenolic compounds (Sigh et al., 2017), especially for their oxidative activity, as well as for the role of preventing countless diseases (Beninger et al., 2003; Menga et al., 2023). In addition, a total of 10 phenolic compounds were identified in the fava bean extracts, comprising four hydroxybenzoic acids (protocatechuic, p-hydroxybenzoic, vanillic, and syringic), three hydroxycinnamic acids (chlorogenic, p-coumaric and trans-ferulic) and three flavonoid-related compounds (Johnson et al., 2021).

For instance, lentils (*Lens culinaris* L.) are well known for their antioxidant effect (through polyphenols concentrations) within the human metabolism and protective outcome from diseases such as cardiovascular degeneration or different types of cancer. Additionally, depending on the varieties of the colors, lentils range from black, brown, red, and green, and this characteristic is given a different antioxidant report (Tienda-Vasquarez et al., 2023). In lentils extracts, p-hydroxybenzoic acid and gallic aldehyde were reported as major hydroxybenzoic acids, and trans-ferulic, trans-p-coumaric, and sinapic acids were reported as major hydroxycinnamic acids (Amarowicz et al., 2009; Mustafa et al., 2022).

Moreover, phenolic acids identified from water-based extracts from green peas (*Pisum sativum* L.) are quinic acid, 5-caffeoylquinic acid, gallic acid and p-coumaric acid, ferulic acid, protocatechuic acid were not identified (Castaldo et al., 2022). In soybean (*Glycine max*), 8 phenolic acids were identified, among them three in a larger quantity such as chlorogenic acid having values from 78.35 to 221.04 μg Gallic acid equivalents (GAE)/g DW, p-hydroxybenzoic acid 38.92 to 196, 40 μg GAE/g DW and caffeic acid 137.43 to 240.28 μg GAE/g DW for black soybean (Zhu et al., 2018).The total phenolic content (TPC) in selected legume seeds is reported in Table 2. The highest values of TPC were recorded in beans, with a range that varies from 92.85 to 151.04 mg GAE/g (Chaeib et al., 2011), and the legumes with the lowest content in phenolic acids are green peas and yellow peas with values between 0.65 to 1.14 mg GAE/g (Han and Baik, 2008; Xu et al., 2007). Moreover, TPC for soybean is lower than other relevant studies that showed total phenolic acid content for soybean ranged from 13.35 to 21.49 mg GAE/g DW (Zhu et al., 2018). Other studies showed that total phenolic content in chickpeas, lentil, and soybean was recorded as 3.12 ± 0.07, 9 ± 0.02, 10.22 ± 0.01 mg GAE g^{-1} (Naz et al., 2023).

2.1.2. Flavonoids

Flavonoids (Table 2) as a secondary metabolite (De Luna et al., 2020) which contribute to health benefits (Juca et al., 2020) especially in humans' bioavailability and biological activity (Maleki et al., 2019). Besides, flavonoids were intensely studied for their nutritional benefits with antioxidant activity (Shen et al., 2022). Moreover, the well-known role of flavonoids in plants is the implication in giving coloration to fruits and flowers (Williamson et al., 2018). However, the general classification of the flavonoids is structured in flavonols, flavones, isoflavones, anthocyanidins, flavanones, flavanols, and chalcones and each of them is distributed in different food sources (Shen et al., 2022).

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content (CTC) were reported in various legume beans

Legumes	TPC	TFC	CTC	References
Faba beans	92,85 to 151,04 mg GAE/g	11,87 to 43,86 mg RE/g	309,28 to 958,77 mg CE/g	Chaieb et al. (2011); Baginsky et al. (2013)
Soybeans	0,81 to 5,89 mg GAE/g	1.06 to 4.04 mg CE/g	0,37 to 1,96 mg CE/g	Kumar V. et al. (2010) ; Xu et al. (2007)
Lentils	4,86 to 9,60 mg GAE/g	3,04 to 4,54 mg CE/g	3,73 to 10,20 mg CE/g	Xu et al. (2007)
Green peas	0, 65 to 0,99 mg GAE/g	0,05 to 0,15 mg CE/g	0,23 to 0,61 mg CE/g	Han and Baik (2008) Xu et al. (2007)
Yellow peas	0,85 to 1,14 mg GAE/g	0.09 to 0,17 mg CE/g	0,22 to 0,59 mg CE/g	Singh et al. (2017) ; Xu. et al. (2007)
Chickpeas	0,98 to 2,2 mg GAE/g	0,72 mg CE/g	0,52 mg/CE g	Han and Baik (2008); Xu. et al. (2007)

Furthermore, legumes such as Fava beans have been considered one of the oldest plants in the world, being nutritionally efficiently used fresh or dry (Kalili et al., 2022), for being rich in flavonoids with up to 16 in total (El Feky et al., 2018). On the other hand, studies on legumes such as common bean (*Phaseolus vulgaris* L.) reported flavonoid concentrations up to 252 mg CE/100 g DW (dry weight) (Yang et al., 2020). Legume seeds including lentils, soybeans, common beans, or peas are nutritionally rich in flavonoids and also contribute to reducing the risk of human affection, respectively type 2 diabetes, and obesity (Zhang et al., 2015). In addition, the potential nutraceutical properties of peas (*Vicia faba* L.) could be considered a healthy option used in human nutrition for the antioxidant compounds (Loizzo et al., 2021). Yellow or green peas are cultivated and scientifically studied worldwide for their pivotal nutrients (Kumari & Deka, 2021) and are extremely useful in curing diabetes, cardiological affections, cancers, and numerous degenerative diseases for their antioxidant properties (Oh et al., 2019; Roy et al., 2020; Kumari & Deka, 2021). Regardless of the pea's color classification, isoflavones commonly accumulate in these legumes. On the other hand, soybeans are rich in isoflavones as well (Shen et al., 2022).

2.1.3. Tannins

Tannins (Table 2) are plant compounds produced by the condensation of simple phenolic compounds and have a complex chemical structure. They are generally divided into hydrolyzable and condensed/non-hydrolyzable tannins (Delavan-3-ol polymers) (Xu et al., 2007). Condensed tannins (proanthocyanin) are phenolic compounds that are found in large amounts in legume seeds such as lentils, yellow or green peas, soybeans, and common beans (Xu et al., 2021).

The condensed tannin content (CTC) in selected legume seeds is reported in Table 2. Beans and lentils are rich in tannins. Beans have the highest content of condensed tannins among the six legumes with content of 309.28 to 958.77 mg CE/g (Chaieb et al., 2011), and the lowest content was observed in peas, 0, 23 to 0.61 mg CE/g for green pea (Xu et al., 2007) and 0.22 to 0.59 mg CE/g for yellow pea (Singh et al., 2017). Other studies have shown that the content values of condensed tannins in Fava beans can vary from 1.9 mg/g (Karatas et al., 2017) to 2586 mg/100 g catechin equivalents (Weihua et al., 2015), these variations can be explained by environmental conditions, geographical area, genetic variability, and quantification methods (Martineau-Côte et al., 2022).

Tannins have been shown to reduce the digestibility and bioavailability of bean proteins because they bind proteins and form insoluble complexes (Karatas et al., 2017; Martineau-Côté et al., 2022).

2.2. Carotenoids

Carotenoids are natural bioactive compounds found in legumes and are known to be important antioxidants with pigmentation properties, these functions establish their role as valuable nutritional additives associated with numerous health benefits (Myrtsi et al., 2023; Altuner et al., 2022). Carotenes typically are hydrocarbons (Qudah, 2009) that contain only carbon and hydrogen. Carotenes are divided into two main groups: carotenes or hydrocarbon carotenoids composed only of carbon and hydrogen atoms and xanthophylls are oxygenated hydrocarbon derivatives that contain at least one oxygen function (Grigore et al., 2023). Beta-carotene and lutein are known as carotenoids and lutein and zeaxanthin as xanthophylls (Qudah, 2009; Kumar et al., 2015). In the human diet, the most important carotenoids are β-carotene (Figure 2), α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene (Rao & Rao, 2007).

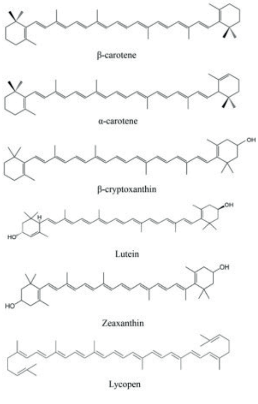

Figure 2. Chemical structures of the carotenoids found most often in the legumes

Legumes are included in the diet for their important sources of carotenoids (including provitamin A) and tocopherols (vitamin E), which play pivotal roles in the prevention of inflammatory processes, as well as coronary, and neuromuscular disorders and maintaining eye health (Turco et al., 2016). The most important carotenoids identified in legumes were beta-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin (Qudah, 2009). Another study has identified 8 lutein and zeaxanthin isomers from legumes where the highest content of carotenoids identified were in red beans (8.29-20.95 μg/g) and lentils (4.53-21.34 μg/g), followed by black soybeans (4.41-6.09 μg/g) and cowpea (6.62-9.46 μg/g) (Kan et al., 2018). Fava beans contain three important carotenoids beta-carotene, lutein and zeaxanthin. In bean seeds, the highest concentration of carotenoids is represented by lutein (Qudah, 2009). Concerning the content of the total carotenoids in Fava beans, this was 1.97 μg (Qudah, 2009), in another article, varying from 5.7 mg/g at 8.4 μg FW (fresh weight) (De Cillis et al., 2019). Soybeans are mostly considered an important source of carotenoids compared to other legumes such as corn and peas. Furthermore, lutein was detected in all varieties studied, while only 37% and 65% of the varieties contained β-carotene and zeaxanthin, respectively. Regarding the carotenoid content of lentils in the study of Zang et al. 2014, lutein and zeaxanthin were identified in almost 20 varieties of lentils. The total carotenoid content (TCC) varied between 5.32 and 28.1 lg/g DW and the total carotenoid index (TCI) varied from 4.64 to 19.6 lg/g DW (Zang et al., 2014). The total content of lutein and zeaxanthin (total of all trans and cis isomers) ranged between 4.32-17.3 lg/g DW and 0.32-2.73 lg/g DW and were significantly different ($p < 0.05$) among the 20 lentil cultivars studied. The highest content of carotenoids in lentils is lutein (all-trans-lutein 64%-78%) followed by zeaxanthin (all-trans-zeaxanthin 5%-13%) (Zang et al., 2014). In pea seed and cotyledons violaxanthin, lutein, and β -carotene were identified and in chickpeas, lutein was reported as the main carotenoid, followed by zeaxanthin and β -cryptoxanthin. Another study has identified, violaxanthin, lutein, zeaxanthin, and b-carotene were identified in the mature pea and chickpea seeds și a low concentration of b-cryptoxanthin was detected in chickpeas (Arevalo et al., 2020).

2.3. Vitamins

Vitamins represent natural substances essential in small quantities to normal metabolism. Oxidative stress occurs due to the inability of the body's antioxidant defense mechanism; therefore, it is recommended to administer exogenous antioxidant supplements (Julia et al., 2019) such as vitamin A, vitamin C (Mohamed et al., 2020) and vitamin E (Kan et al., 2018). Moreover, in vegetables can be included the well-known and numerous benefits of fava beans for human health, after the content of carbohydrates, proteins, and antioxidants, to be taken into account are the vitamins, mostly B complex, ascorbic acid (Alghamdi, 2009; Mohamed et al., 2020), retinol (Didier et al., 2023) and tocopherol (Kan et al., 2018).

2.3.1. Retinol (vitamin A)

Taking into account the general function of retinol, this is a vitamin A which is essential for human metabolism and development, being involved in the immune system, reproduction function, skin defense and numerous other functions about memory or vision. The group named Vitamin A, include retinol derivatives which are known as retinoids used on the whole medical and beauty domain (Ferreira et al., 2020). Furthermore, bioactive forms of retinol which are metabolized at the human cell level, are represented by retinal and retinoic acid, retinyl being intended for depository (Ferreira et al., 2020).

On the other hand, retinol is a nutrient component found in soybean sprouts, with a concentration of up to 34 RE/100 g (Plaza et al., 2003). Additionally, legumes with content of vitamin A are soybeans with 22UI/100g (USDA, 2019), lentils has 8 UI/100 g (USDAa, 2019), fava beans being the most rich legume in vitamin A, with 333 UI/100 g (USDAb, 2019).

2.3.2. Ascorbic acid (vitamin C)

The oxidative role of ascorbic acid, frequently known as vitamin C, makes this subject important for numerous research (Njus at al., 2020). Studies in the field show that vitamin C is a multifunctional metabolite (Bilska et al., 2019) that has many properties, however, three biological functions are proper to be mentioned. Among these is counted the function of being a reducing agent for enzymatic reactions, a scavenger of free radicals, and for the action as antioxidant which eliminates reactive oxygen species. In addition, antioxidants could be taken from natural exogenous sources such as legumes, fruits, seeds, and many others (Hossain et al., 2021). For decades, people use food processing in different ways, microwaves, boiling, heat, and autoclaving; however, cooking processes make physicochemical changes of nutrient composition and vitamins too. Moreover, vitamin C can easily degrade under usual cooking treatments (Uherova et al., 1993). Nevertheless, published research results regarding the nutritional composition of fava beans, that taking ascorbic acid with a high antioxidant potential is a good option for the production of healthy foods (Hossain et al., 2021). Additionally, legumes such as pigeon peas (*Cajanus cajan* (L.) Huth), contains 39.00 g/100 g of vitamin C for immature seeds (Hossain et al., 2021).

2.3.3. Tocopherol (vitamin E)

Vitamins represent a small amount of the total nutrients in vegetables compared to phenols or flavonoids. Tocopherol, also known as vitamin E, as an exogenous compound, has an important antioxidant effect on human metabolism (Baiano et al., 2015; Menga et al., 2023). In addition, scientists in the field discovered that vitamin E, includes several isoforms such as: α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. Apart fromthe antioxidant effect of α- and β-tocopherol, in human health a big contribution has γ-tocopherol, especially for its anti-inflammatory and antitumor impact; and δ-tocopherol for preventing many cancerous types (pulmonary, breast, colon etc.) and lipid accumulation (Azzi, 2018; Azzi, 2019).

Vitamin E, is more abundant in beans, peas and lentils, being found particularly as γ-tocopherol (Amarowicz et al., 2009; Martín-Cabrejas et al., 2019). Moreover, regarding to a study of Kan et al., 2018, where they studied almost 29 legumes, including soybean, pea and lentil, the total tocopherol composition was in different ranges: 120.96±2.48a; 58.40±0.7g and 41.28±0.24i, respectively. Furthermore, the antioxidant effect of fava bean, could be taken into account as legumes for its 0.08 mg of alpha tocopherol which were found in a study conducted by Jahreis et al. (2016). Gamma tocopherol being not determined (Jahreis et al., 2016). Additionally, legumes such as pigeon pea contain 0.39 g/100 g of vitamin E in immature seed, regarding Kuraz Abebe 2022.

3. Conducted assays for determining the antioxidant activity and antioxidant capacity

The antioxidant capacity of legumes differs according to biological variation (Table 3) and is reported throughout a wide range (Ketnawa et al., 2022). The highest natural endogenous antioxidant levels can be influenced by technological processing (Garrido-Galand et al., 2021) including seed germination (coatings) (Gu et al., 2022) and are important. The high number of phenolic antioxidants in seed hulls is an essential concern and it is important to evaluate the methods and instruments that can be specific and cost-effective for establishing the antioxidant potential. Analyzing the antioxidant content of legumes typically involves laboratory methods that measure the levels of specific antioxidant compounds or antioxidative capacity. The antioxidant capacity of legumes refers to their ability to neutralize or scavenge free radicals and prevent oxidative damage (Pisoschi et al., 2021; Mihai et al., 2022). Antioxidants in legumes primarily include phenolic compounds, flavonoids, vitamins (such as vitamin C and vitamin E), carotenoids, and other bioactive components. These antioxidants work synergistically to protect cells and tissues from oxidative stress and contribute to overall health and well-being (Crupi et al., 2023). Various methods have been developed to measure the antioxidant content in legumes. These methods include spectrophotometric assays (Diniyah et al., 2020), such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, and oxygen radical absorbance capacity (ORAC) assay, among others. These assays provide quantitative measurements of the antioxidant capacity of legumes by assessing their ability to scavenge free radicals or donate electrons. In addition to spectrophotometric methods, chromatographic techniques (Adebo et al., 2021; More et al., 2022; Zhu et al., 2020).

4. Benefits of antioxidants for human and livestock

Antioxidants in legumes help neutralize harmful free radicals and reduce oxidative stress in the body (Zhang et al., 2016). Oxidative stress (Surai, 2020) has been linked to various chronic diseases, and a diet rich in antioxidants can help protect against their development. They can help reduce inflammation, lower blood pressure, improve blood lipid profiles, and enhance blood vessel function, thereby reducing the risk of heart disease and stroke. Some antioxidants found in legumes, including phytochemicals like flavonoids and polyphenols, have been shown to have anti-cancer properties, and provide protection against certain types of cancer, such as colon, breast, and prostate cancer (Almatroudi et al., 2023). The antioxidants in legumes can help reduce inflammation by neutralizing free radicals and modulating inflammatory pathways, contributing to a lower risk of chronic inflammatory conditions, including arthritis and inflammatory bowel diseases (Zhang et al., 2016). Legumes, with their high antioxidant content and low glycemic index, are beneficial for individuals with diabetes or those at risk of developing diabetes. Legumes are rich in dietary fiber, which promotes healthy digestion and prevents constipation. The antioxidants in legumes may also have prebiotic properties, nourishing beneficial gut bacteria and supporting a healthy gut microbiome, which is essential for digestive health (Kalili, 2022). Legumes can serve as a cost-effective feed ingredient for livestock production. Using legumes as a feed ingredient can help reduce feed costs, especially in regions where legumes are locally grown and readily available (Formato et al., 2022; Corino et al., 2021). Providing a diverse and nutritious diet is essential for maintaining optimal animal health and welfare. Legumes have a relatively lower environmental impact compared to some other feed ingredients. Their nitrogen-fixing ability reduces the need for synthetic nitrogen fertilizers, which can help minimize nitrogen runoff and its associated environmental consequences (Formato et al., 2022; Wang et al., 2022). Including legumes in livestock feed may contribute to improved meat quality, increased omega-3 fatty acid content, enhanced flavor profiles, and other desirable characteristics in animal products, thereby meeting consumer demands for high-quality and nutritious food (Corino et al., 2021).

Table 3. Conducted assays for determining the antioxidant activity and antioxidant capacity

Spectrophotometric methods			
Assays	Wavelength	Mechanisms of detection	References
2,2-diphenyl-1-picrylhydrazyl (DPPH)	510-520 nm	Measures the ability of antioxidants to scavenge the stable free radical DPPH.	(Diniyah et al., 2020)
Total antioxidant capacity (TAC)	450-490 nm	Measures the overall antioxidant capacity of a sample. These assays assess the ability of antioxidants to neutralize free radicals or inhibit oxidation in a chemical reaction	(Y. Zhang et al., 2020)
Folin-Ciocalteu (FC)	760-765 nm	Determines the total phenolic content in legumes. The reaction between phenolic compounds and the Folin-Ciocalteu reagent produces a color that can be measured.	(AU - Apea-Bah et al., 2022)
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)	645-650 nm	Method to assess the antioxidant activity of samples. ABTS is oxidized by the action of antioxidants, resulting in a color change that can be measured spectrophotometrically. This assay provides information about the ability of antioxidants to neutralize free radicals.	(González-Osuna et al., 2023)
Ferric Reducing Antioxidant Power (FRAP)	593 nm	Measures the reducing capacity of antioxidants in a sample. It involves the reduction of a ferric-tripyridyl triazine complex to a ferrous form by antioxidants, leading to a color change that can be quantified using a spectrophotometer. The FRAP assay provides information on the electron-donating capacity and overall antioxidant power of a sample.	(X. Zhang, Zhang, et al., 2021)
Trolox Equivalent Antioxidant Capacity (TEAC)	450-490 nm	Measures the overall antioxidant capacity of a sample. These assays assess the ability of antioxidants to neutralize free radicals or inhibit oxidation in a chemical reaction.	(Arevalo et al., 2020)
Oxygen Radical Absorbance Capacity (ORAC)	485-535 nm	Measures the antioxidant reaction with peroxil radicals.	
Nitric Oxide (NO) Scavenging Assay	540 nm	Measures the ability of antioxidants to scavenge nitric oxide radicals. The reaction between antioxidants and nitric oxide leads to the formation of a stable-colored product, which can be quantified spectrophotometrically.	(Xu et al., 2021)
Lipid Peroxidation Assay (TBARS)	535 nm	This assay involve the reaction between lipid peroxidation products and a chromogenic reagent, resulting in the formation of a colored complex that can be measured.	(Kasaiyan et al., 2023)
Hydroxyl Radical Scavenging Assay	460 nm	Evaluates the ability of antioxidants to scavenge hydroxyl radicals. Hydroxyl radicals are highly reactive and can cause oxidative damage.	(Verni et al., 2019)
Superoxide Radical Scavenging Assay	560-562 nm	Measures the ability of antioxidants to scavenge superoxide radicals. Superoxide radicals are highly reactive and can contribute to oxidative stress.	(Haile & Kang, 2019)
Cupric Reducing Antioxidant Capacity (CUPRAC)	455-490 nm	Measures the reducing capacity of a sample by its ability to reduce cupric ions (Cu2+) to cuprous ions (Cu+).	(Mitic et al., 2023)
Total Flavonoid Content (TFC) Assays		Measures of the concentration of flavonoids in legume samples, which contribute to their antioxidant activity.	(Liu et al., 2022)
Total Carotenoid Content	450-460 nm	Mseasues the concentration of carotenoids in legume samples. Spectrophotometric methods are commonly employed for this purpose.	(Vieira et al., 2016)
Prussian blue	725 nm	Detection of ferric (Fe^{3+}) ions. It involves the formation of a blue-colored complex called Prussian blue when ferric ions react with potassium ferrocyanide.	(Ahmad, 2021)

Aluminum chloride flavonoid	415-425 nm	Determine the total flavonoid content in samples	(Sharma & Giri, 2022)
Sodium borohydride reduction	500-800 nm	The method for determining the total reducing power or antioxidant capacity of a sample	(Singh et al., 2021)
Electrochemical methods			
Cyclic Voltammetry		The electrochemical technique that measures the current as a function of applied potential-redox behavior of antioxidants in legumes and electrochemical activity.	(Sakinah & Fikri, 2023)
Potentiometric Titration		Measures changes in electrical potential as a titrant is added to a sample to determine the antioxidant content by titrating against a known oxidative reagent or measuring the potential changes associated with the oxidation or reduction of antioxidants.	(Rico et al., 2021)
Electrochemical Impedance Spectroscopy		Measures the impedance of an electrochemical system as a function of frequency. It can provide information about the charge transfer processes and surface properties of antioxidants in legumes, offering insights into their antioxidant capacity.	(Tolun & Altintas, 2023)
Square Wave Voltammetry		Measures the resulting current. It can be used to determine the concentration of specific antioxidants or assess their redox behavior.	(Diniyah et al., 2020)
Chemiluminescence Detection		It is used in conjunction with electrochemical methods to detect the antioxidant capacity of legumes - inhibiting or reducing the chemiluminescence reaction, providing an indirect measure of their antioxidant activity.	(Minguillón et al., 2022)
Electrochemical Sensor Arrays		By using different electrodes, each selectively sensitive to a specific antioxidant or antioxidant group, sensor arrays can provide simultaneous detection of multiple antioxidants in a sample.	(Kumar Mehata et al., 2022)
Conductometric Measurements		Assess changes in the electrical conductivity of a sample due to redox reactions or interactions with antioxidants. This method can be used to determine the antioxidant capacity or monitor the antioxidant content in legumes.	(Szparaga et al., 2021)
Chromatographic Methods			
High-performance liquid chromatography (HPLC) analysis		Used to separate and quantify individual antioxidants in legumes. These methods provide high specificity and sensitivity for the identification and quantification of antioxidant compounds. MS is often coupled with chromatographic techniques (such as HPLC-MS or GC-MS) to identify and quantify specific antioxidants in legumes. MS provides highly accurate molecular weight determination, allowing for the identification of antioxidant compounds based on their mass spectra.	(Adebo et al., 2021; AU - Apea-Bah et al., 2022; More et al., 2022; Zhu et al., 2020)
Gas chromatography (GC) Mass Spectrometry (MS)			(Farag, Sharaf El-Din, Aboul-Fotouh Selim, et al., 2021)

CONCLUSIONS

The consumption of beans can meet the challenges of balanced diets, both individually and globally. With the aim of changing eating habits, we need to offer consumers a range of products that are less outdated and more practical to cook.

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