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REVIEW

Methods for determination of antioxidant capacity of traditional and emergent crops of interest in Mexico: An overview

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Abstract

Reactive oxygen species are produced by aerobic organisms, including humans, because of metabolism. They can oxidise biomolecules and cause degenerative and cardiovascular diseases (diabetes, atherosclerosis, and neurological damage, among others). However, the consumption of different plant products is related to the prevention of reactive oxygen species–mediated damage because they contain various antioxidants that inhibit the oxidation of biomolecules. Some important natural antioxidants are carotenoids, flavonoids, polyphenols, and vitamins. These molecules are found in various crops produced in Mexico, some of which have been cultivated for a long time, while others have emerged in recent years. The study of the antioxidant capacity of these crops has increased over time. Different methods are used to determine this capacity, depending on the type of antioxidants. In this review, we analyse the antioxidant quantification methods of various crops of interest in Mexico (traditional and emergent), as well as their relationship to prevent the oxidation of biomolecules.

Keywords: reactive oxygen species; natural antioxidants; extraction methods; quantification methods: Mexican crops; antioxidant capacity.

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1. Introduction

For many years, many studies have been carried out related to the determination of the antioxidant capacity of different agricultural products around the world, and sometimes, the identification of the molecules that confer such antioxidant activity, for which different determination methods have been used, including DPPH, ABTS and Folin–Ciocalteu. However, many papers reported the use of various methods, regardless of the nature of the antioxidant molecules, which leads to misinterpretation of the data obtained. Likewise, many studies have been published in which the antioxidant capacity of agricultural products grown in Mexico was determined, mainly tomatoes, coffee and grapes, although in recent years, the interest in other products has increased. The aim of this review is to analyse the methods of determination of antioxidant capacity of various traditional and emergent crops of interest in Mexico, as well as their relationship to prevent the oxidation of biomolecules.

2. Redox State

Metabolism is a set of oxidation-reduction reactions that are part of fundamental processes in all living beings, including humans, providing bioenergy and maintaining vital functions. Those reactions define the redox state both intracellularly and extracellularly. A constant equilibrium is not established because, in different cellular compartments or organs, such reactions cause variations in the redox state – that is, there is a thermodynamic imbalance (Kemp et al., 2008; Sies et al., 2017).

The thermodynamic imbalance that prevails in cellular metabolism produces different molecules that are highly reactive, called reactive species, which may contain molecules of different chemical natures, such as oxygen, chlorine, bromine, nitrogen, sulphur, selenium, and carbonyls, among others. Of these, some are free radicals and others are non-radicals (Del Rio, 2015; DeLeon et al., 2016; Labunskyy et al., 2014; Poole, 2015; Sies et al., 2017). Likewise, it is important to mention that, of all the

reactive species, the most studied are oxygen species due to their role in metabolic regulation and cell cycles (Sies et al., 2017; Sies, 2020).

2.1 Reactive Oxygen Species

Aerobic organisms produce reactive oxygen species (ROS) from the molecular oxygen used in respiration processes. These molecules are produced mainly by mitochondria, NADPH oxidases (NOX), and autoxidation of xenobiotics (Aguirre & Lambeth, 2010; Cakmak & Gulcin, 2019; Gulcin, 2020; Starkov, 2008; Woolley et al., 2021). ROS – such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), the hydroxyl radical ($OH\cdot$), the superoxide anion (O_2^-), nitric oxide ($NO\cdot$), peroxynitrite ($ONOO^-$), and the peroxy radical ($ROO\cdot$) – are highly unstable and can damage important biomolecules (proteins, carbohydrates, lipids, and nucleic acids (Figure 1) (Gulcin, 2020; Huyut et al., 2017; Yang & Lian, 2020). On the other hand, ROS production is involved in the maintenance of cellular homeostasis, cytodifferentiation, and signalling mechanisms in response to adverse environmental conditions (Gulcin, 2020; Miranda-Hernández et al., 2016; Pérez-Guzmán et al., 2016; Yang & Lian, 2020). Likewise, ROS are the main factors in the development of different pathologies and senescence in all types of cells (Starkov, 2008), and their physical and chemical properties allow them to interact with many molecules, which causes great cellular damage (Woolley et al., 2021). Additionally, ROS have an especially significant role in organisms that have an immune system: they can be directly related to the energy requirement, and it has been shown that some immune cells produce ROS to eliminate pathogens (Yang & Lian, 2020).

Cells possess both enzymatic and non-enzymatic mechanisms to prevent ROS-mediated oxidation of biomolecules (Aguirre et al., 2005; Garza-López et al., 2015). Among the enzymatic mechanisms are superoxide dismutases (SOD), catalase (Cat), and peroxidases (POX) (Aguirre et al., 2005). The non-enzymatic antioxidants include molecules that sequester ROS, such as carotenoids, polyphenols, vitamins, flavonoids, and polyols (Gulcin, 2020; Hallsworth & Magan, 1996; Sies, 2020). This sequestration prevents ROS from reacting with and damaging biomolecules.

2.2 Oxidative Stress

The interaction between reactive species and biomolecules can cause oxidative stress, which is defined as the imbalance between oxidant and antioxidant molecules in favour of oxidants (Aguirre & Lambeth 2010; Sies 2020). Oxidative stress leads to a redox imbalance, cell damage, and death. However, as mentioned previously, moderate oxidative stress is part of the signalling and cytodifferentiation mechanisms in many organisms (Aguirre et al., 2005; Fridovich, 1998; Pérez-Guzmán et al., 2016; Tlecutil-Bersitain et al., 2010).

3. Antioxidants

Antioxidants are molecules capable of inhibiting the oxidation of other molecules. There are various definitions depending on the context of the knowledge area. In the case of oxidative stress, an antioxidant is defined as any molecule that 'scavenges' ROS or regulates antioxidant defence or inhibits ROS production (Figure 1) (Gulcin, 2020; Huyut et al., 2017; Sies, 1997). On the other hand, in terms of food, an antioxidant is a molecule that, when consumed, slows, prevents, or inhibits damage to macromolecules (Gulcin, 2020; Halliwell, 1997; Sies, 1997).

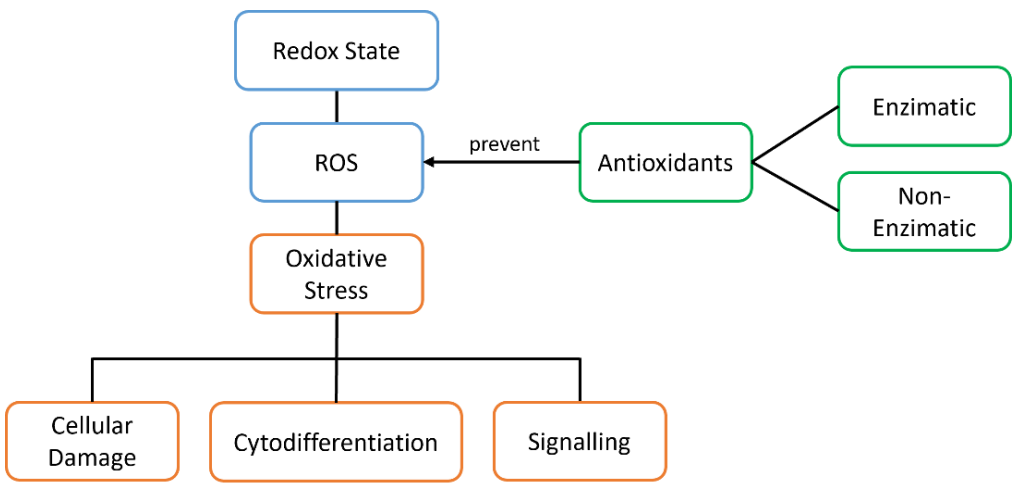

Figure 1. Effect of cellular redox state and interaction with antioxidants.

In recent years, there has been greater attention placed on antioxidants in the food industry and in human health because they are capable of reducing ROS-mediated damage to biomolecules that could cause various harmful effects in humans, such as atherosclerosis, diabetes, cardiovascular and neurological diseases, skin lesions, and rheumatoid arthritis, among others (Cakmakci et al., 2015; Gulcin, 2020; Huyut, et al., 2017; Pan et al., 2020; Rahal et al., 2014). Numerous studies have shown that antioxidants retard lipid peroxidation to maintain both physical and sensory characteristics of food during storage (Bursal et al., 2013; Oztazkin et al., 2015). Additionally, antioxidants protect proteins from oxidation and subsequent interaction with other molecules that could cause protein malfunction (Gulcin, 2020; Sindhi et al., 2013). The consumption of different fruits and vegetables and products derived from them has long been associated with the prevention of many chronic diseases and the reduction of the effects of ageing, mainly due to the antioxidant activity of molecules contained in these products (polyphenols, flavonoids, carotenoids, etc.) (Cömert & Gökmen, 2018; Ganesan et al., 2011; Huang et al., 2005; Moure et al., 2001; Oztazkin et al., 2015; Pan et al., 2020).

3.1 Natural Antioxidants

Worldwide, there is a trend to consume natural products, such as plants, that contain considerable levels of antioxidants, which protect against the adverse effects of ROS. In addition, they do not represent risks to human health (Gulcin, 2020; Hou et al., 2003; Shahidi & Ambigaipalan, 2015). Plants produce a large amount of secondary metabolites that they use as protection against possible damage to biomolecules (Gulcin, 2020). In a similar view, researchers have suggested included compounds with antioxidant activity in food consumed by humans to contribute to the protection against ROS-mediated damage (Gulcin, 2020; Rice-Evans et al., 1996). Natural antioxidants have great diversity regarding their structure and chemical composition. The main groups of antioxidants are carotenoids, flavonoids, lignans, phenolic acids, stilbenes, tannins, and vitamins (Figure 2) (Balasundram et al., 2006; Cömert & Gökmen, 2018; Davey et al., 2002; Fiedor & Burda, 2014; Gulcin, 2020; Nimse & Pal, 2015; Rice-Evans et al., 1996).

3.1.1 Carotenoids: Including α-carotene, β-carotene, lycopene, lutein, and cryptoxanthin – are fat-soluble pigments with antioxidant activity that are found mainly in plants (Figure 3a), as well as in some microorganisms. The basic structure consists of a chain of at least 40 carbons with several double bonds. The consumption of carotenoids in the human diet is associated with a reduction in ROS-mediated damage and prevention of the development of cancer and some cardiovascular diseases (Cömert & Gökmen, 2018; Gulcin, 2020; Monego et al., 2017).

3.1.2 Flavonoids: Are polyphenolic antioxidants produced as secondary metabolites in plants. Their aromatic rings stabilise unpaired electrons from free radicals (Figure 3b).

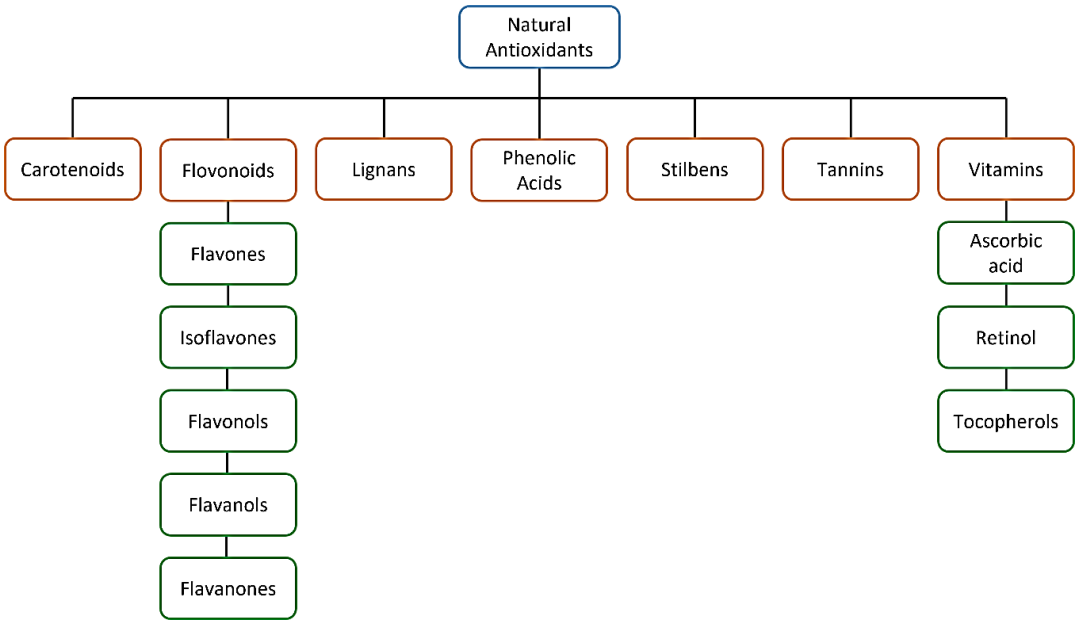

Figure 2. Main groups of natural antioxidants contained in plants.

Flavonoids can be divided into flavones, isoflavones, flavonols, flavanols, and flavanones. The representative members of this antioxidant class are genistein, quercetin, luteolin, catechin, and herperetin (Balasundram et al., 2006; Cömert & Gökmen, 2018; Gulcin, 2020; Harborne et al., 1999; Samsonowicz & Regulska, 2017).

3.1.3 Lignans: Are structurally diverse polyphenolic antioxidants (Figure 3c), derived from shikimic acid, that are widely present in plants – indeed, they can be found in all parts of plants. These molecules can prevent different types of cancer in humans (Cui et al., 2020; Gulcin, 2020, Markurin et al., 2019).

3.1.4 Phenolic Acids: Are phenolic compounds that consist mainly of benzoic or propenoic acid with carboxylic groups (Figure 3d). They are found in plants and their derivatives and have a significant contribution to human nutrition. They provide protection against diseases caused by oxidative damage such as cancer, heart disease and glaucoma. The most important molecules belonging to this group are p-hydroxybenzoic acid, vanillic acid, coumaric acid, caffeic acid, and syringic acid, among others (Clifford & Scalbert, 2000; Coban et al., 2007; Gulcin, 2020; Rice-Evans et al., 1996).

3.1.5 Stilbenes: Are also phenolic compounds with antioxidant activity at very low concentrations (Figure 3e). These molecules are involved in the prevention of cancer and cardiovascular diseases caused by ROS. Resveratrol is the most studied stilbene, mainly due to its presence in wine (Cömert & Gökmen, 2018; Krawczyk, 2019).

3.1.6 Tannins: Are phenolic antioxidants (Figure 3f), produced by many plants that are important in cancer prevention. The most important molecule is tannic acid (Gulcin, 2020; Shahidi & Ambigaipalan, 2015).

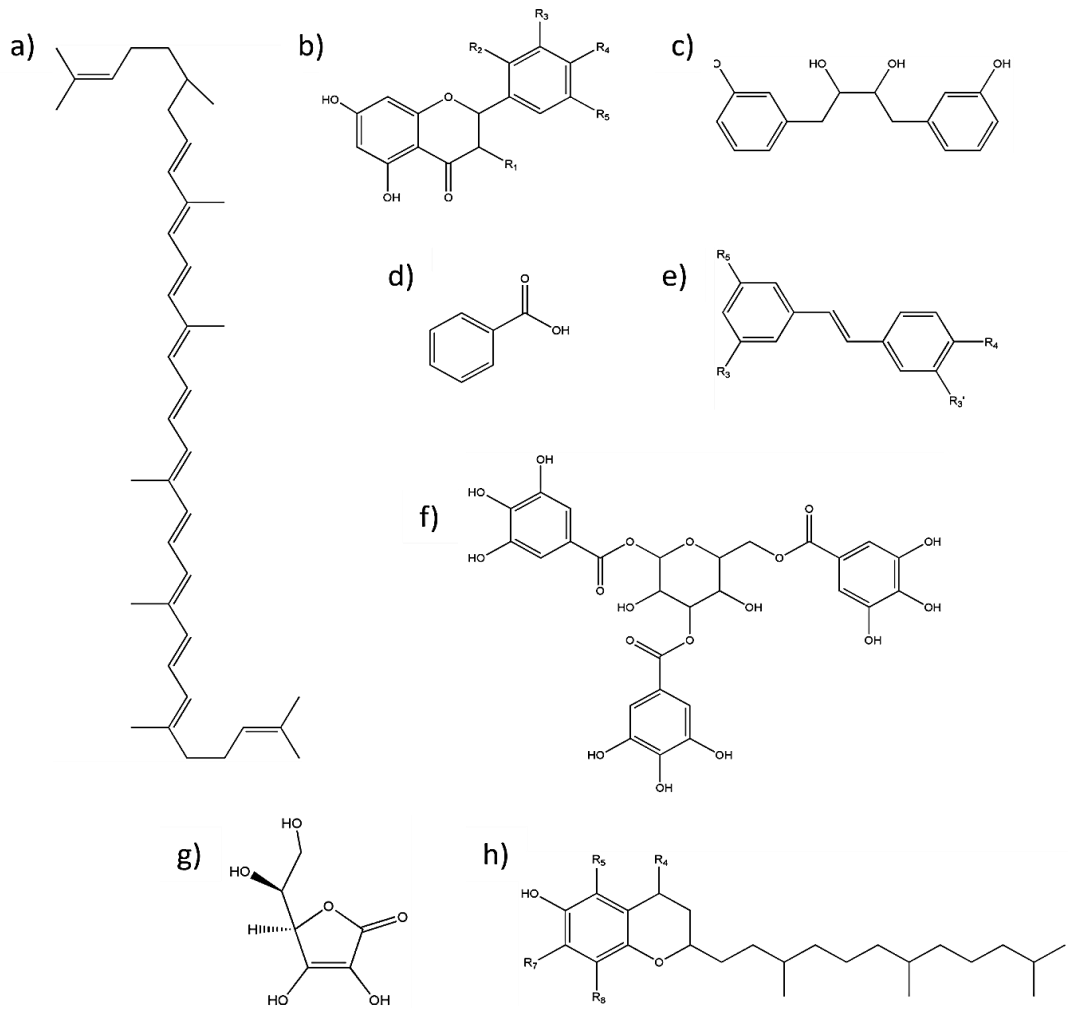

Figure 3. Common structures of natural antioxidants. a) Carotenoids, b) Flavonoids, c) Lignans, d) Phenolic acids, e) Stilbenes, f) Tannins, g) Ascorbic acid (Vitamin C), h) Tocopherols (Vitamin A). The structures were made using ChemOffice® software v. 2024 (Cambridge University).

3.1.7 Vitamins: Are a heterogeneous group of antioxidants including ascorbic acid (vitamin C) (**Figure 3g**), tocopherols (vitamin E) (**Figure 3h**), and retinol (vitamin A). Tocopherols – which are widely present in plants – were among the first groups of antioxidants to be identified. They are fat-soluble molecules that prevent lipid peroxidation (**Cetinkaya et al., 2012**; **Gulcin, 2020**; **Nimse & Pal, 2015**). On the other hand, ascorbic acid is a water-soluble antioxidant present in many fruits and plants. Given its water solubility, it can be absorbed in the gastrointestinal tract and prevent ROS-mediated damage. It is also involved in the tocopherol regeneration process (**Barros et al., 2011**; **Cömert & Gökmen, 2018**; **Davey et al., 2000**). Retinol is a carotenoid that prevents lipid peroxidation and exerts beneficial effects on the skin, eyes, and internal organs. Additionally, it has a protective function against photooxidant processes in plants (**Carocho & Ferreira, 2013**; **Gulcin, 2020**).

4. Antioxidant capacity determination methods

When measuring the antioxidant capacity, it is necessary to consider the type of matrix, the extraction material, and the physical state. Of course, the reaction mechanism involved in the determination method must also be considered: it is necessary to know what will be measured to interpret and compare the results correctly (**Figure 4**). It is important to note that the antioxidant capacity can be altered by how long the material has been stored and the steps required for the extraction. Moreover, each method has inherent factors that influence its use: the ease or complexity of the measurement, the stability and availability of the reagents used, its reproducibility, the amount of sample required, and the cost of equipment and reagents. Before choosing a determination method, it is advisable to carry out a critical analysis of the available options to ensure reliable results (**Gulcin, 2020**).

4.1 Extraction Methods of Antioxidant Compounds

4.1.1 Solvents

The compounds found in plants and fungi present a wide range of chemical structures, which makes it difficult to choose a single solvent to extract compounds with antioxidant potential. In addition, the use of some methods such as maceration, shaking, and Soxhlet extraction is recommended to increase solid-liquid extraction through diffusion (**Jaimez-Ordaz et al., 2021**). When various solvents are used, the temperature must be considered because it can lead to losses due to volatilisation. The extraction time must also be optimised: upon reaching equilibrium by diffusion, the solvent is saturated, and the extraction is finished. At this point, the antioxidant compounds should be quantified; otherwise, they could be oxidised by light, denatured, or polymerised before being quantified, which would lead to errors in the results. Due to the polarity of the compounds with antioxidant potential, the most used solvents are water, ethanol, methanol, and mixtures of these in different proportions. Other reasons for the use of these solvents are the cost, the ease of their handling, their accessibility, and the innocuousness (safety) of the extracts (**Table 1**).

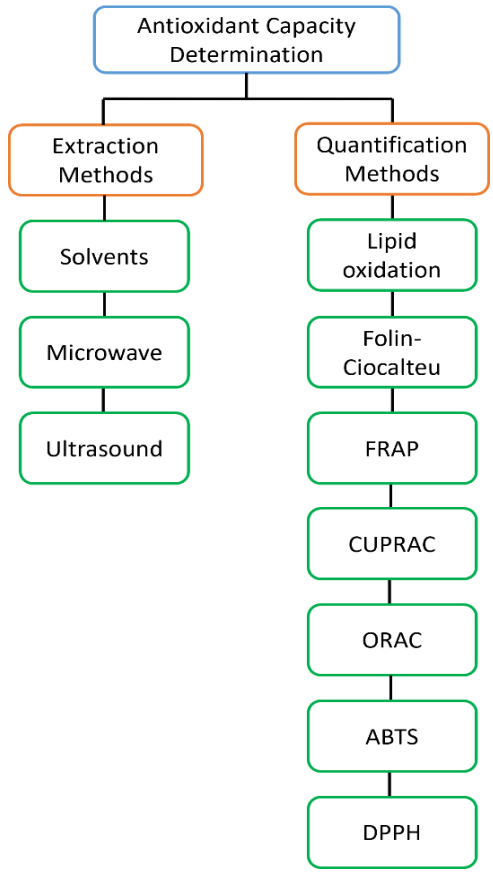

Figure 4. Methods of extraction and quantification used for determination of antioxidant capacity.

4.1.2 Microwave extraction

The extraction of compounds with antioxidant capacity from plant matrices, including flowers and agri-food products, has been carried out with microwaves for several decades. The optimal microwave exposure to extract antioxidants is 0.5–1 min (**Jaimez-Ordaz et al., 2021**). The published studies indicate that microwave-assisted extraction has advantages over conventional or even ultrasonic extraction: the heating is more efficient because of the vibration of the molecules in the medium with the solvent, which makes the extraction more efficient and faster. Microwave extraction also has various advantages over conventional extraction, such as shorter extraction

times, less solvent consumption, and specificity of the extracted molecules (Singh et al., 2011). It is important to know the polarity and size of the compounds of interest to extract them efficiently. Singh et al. (2011) recommended carrying out a response surface methodology: evaluate the effect of the concentration of the proposed solvents, the extraction time, and the microwave power level to optimise the process and obtain the best yields (Table 1).

4.1.3 Ultrasonic extraction

Temperature is an important factor for the extraction of phenolic compounds; however, it usually makes it difficult to standardise and reproduce the same method at different times. For example, many authors mention extraction at 'room temperature', although sometimes the exact temperature is not included.

Table 1
Characteristics of extraction methos of antioxidant compounds

Extraction method	Fundament	Characteristics	Reference
Solvents	Chemical extraction	Expensive, ease of handling	Jaimez-Ordaz et al., 2021
Microwave extraction	Heating	More efficient, faster extraction	Singh et al., 2011
Ultrasonic extraction	Acoustic cavitation	Greater release of compounds	Mendez-Flores et al.,2018

Table 2
Quantification methods of antioxidant capacity

Method	Advantages / Disadvantages	Type of quantification / Expression of the result	Examples of use in agriculture and byproducts
Lipid oxidation inhibition	The assay evaluates the ability of polyphenolic compounds to prevent the oxidation of biomolecules, such as lipids	Indirect	Oregano (Arcila-Lozano et al., 2004)
	The determined compounds have the potential to prevent human body diseases through cellular and molecular oxidation processes, not the total antioxidant capacity of the sample	% antioxidant activity	
Folin–Ciocalteu method	Most frequently used to determine the total phenolic content. It was developed for protein analysis and uses tyrosine as the phenolic amino acid	Direct	Barley and after it had been malted and roasted (Gallegos-Infante et al., 2010)
	That measuring the total phenolic content does not specifically indicate the antioxidant capacity	IC50	
Ferric reducing antioxidant power (FRAP)	The mechanism of this reaction involves an electron transfer, while other methods scavenge free radicals.	Indirect	Pigmented maize (Mihn, 2021). Honey (Mendoza-Bacilio et al., 2022)
	Should be performed along with other methods to obtain a more complete picture of the antioxidant capacity of the samples.	µM equivalents of Trolox/g of sample µM Fe(II)/100 g of sample	
Cupric reducing antioxidant capacity assay (CUPRAC)	It has faster kinetics, greater specificity, and sugars and citric acid do not interfere.	Direct	Commercial tea (Cárdenas et al., 2014)
	This method is applicable to relatively insoluble food matrices	Equivalents of Trolox / mol dm^{-3}	
Oxygen radical absorbance capacity assay (ORAC)	Method used to measure the peroxyl radical scavenging capacity	Indirect	Cactus, berry fruits (Montiel-Sánchez et al., 2021)
	This assay determines the total antioxidant capacity, instead of specific compounds and it is commonly used in food industry	mM equivalents of Trolox	
2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay (ABTS)	The assay is representative of the reactions that occur within cells, and it has the advantages of being stable and fast	Indirect	Berries (Virgen-Carrillo et al., 2022).
	This assay has been criticised for being non-specific for phenolic compounds that are reduced by other non-phenolic compounds	Equivalents of Trolox	
α,α-Diphenyl-β-picrylhydrazyl radical scavenging assay ((DPPH)	Used to quantify the antioxidant capacity by evaluating free radicals because of its scavenging activity of non-enzymatic antioxidants	Direct	Wine (Espitia-López et al., 2014)
	The antioxidant capacity reported is lower compared to other assays. It does not react with compounds such as flavonoids lacking a hydroxyl group in the B ring, nor does it react with aromatic acids that contain only one hydroxyl group	TEAC, gallic acid equivalents, or the IC50 or IC25	

Mendez-Flores et al. (2018) reported ultrasonic extraction, as an alternative to isolate phenolic compounds with antioxidant capacity from the Mexican rambutan. An advantage of this method is that the temperature does not need to be controlled. This method employs acoustic cavitation of a sample to extract compounds with adequate yields. Moreover, it improves the solvent–sample relationship, leading to greater release of compounds of interest. For the extraction of phenolic compounds, the mass-to-volume ratio (g/mL) is evaluated, as well as the ultrasonic extraction time (min) and the ratio of ethanol to water (extraction solvent). Once the optimal extraction conditions have been established, the extract is separated by column chromatography to recover the polyphenolic fraction and, finally, the solvent is evaporated to recover the polyphenols as a fine powder (Table 1).

4.2 Antioxidant capacity quantification methods

4.2.1 Lipid oxidation inhibition

Lipid oxidation inhibition is used as an indirect way to measure the antioxidant capacity. This method involves evaluating the ability of polyphenolic compounds to prevent the oxidation of biomolecules, such as lipids. The compounds determined from the measurement can be considered to have the potential to prevent diseases of the human body caused by cellular and molecular oxidation processes (Mendez-Flores et al., 2018). For this assay, linoleic acid is used as the lipid source. The sample is prepared in a mixture of Tween 20, 0.02 M acetate buffer (pH 4.0), and ethanol. Subsequently, the sample is homogenised, $FeCl_2$-EDTA is added, and it is incubated at 37°C to induce oxidation. After incubation, it is mixed with 0.1 M NaOH in ethanol and the absorbance is measured at 232 nm, with 10% ethanol as the blank. The result is expressed as a percentage of antioxidant activity. The antioxidant effect of Mexican oregano (*Lippia graveolens*) has been measured using this method. Arcila-Lozano et al. (2004) reported the highest antioxidant capacity when the oregano was dried in the shade and in the sun. This antioxidant capacity from aromatic plants is a consequence of the hydroxyl groups in the phenolic compounds, which protect biomolecules from free radical damage. The authors also mentioned that the antioxidant capacity depends on the type of solvent used in the extraction. The extracts obtained by using lipophilic solvents contain the compounds with the highest antioxidant capacity due to their polarity (Table 2).

4.2.2 Folin–Ciocalteu method

The Folin–Ciocalteu method is used most frequently to determine the total phenolic content. Initially, it was developed for protein analysis and uses tyrosine as the phenolic amino acid (Folin & Ciocalteu, 1927). It is important to mention that measuring the total phenolic content does not specifically indicate the antioxidant capacity. Nevertheless, some of these phenolic compounds could be directly related to the antioxidant capacity of the sample. Hence, there is a relationship between the total phenolic content and the antioxidant capacity. The results of this method are expressed as gallic acid, caffeic acid, catechin, chlorogenic acid, or ferulic acid equivalents (Gulcin, 2020) (Table 2).

One of the most cited articles using the Folin–Ciocalteu methodology was published by Singleton et al. (1999): it has more than 12500 citations as of 2024. This method is described as easy to use and measures oxidisable compounds, such as proteins, and not only polyphenols. It is highly recommended to carry out other assays such as high-performance liquid chromatography (HPLC) that identify the different compounds and their concentration to know the specificity of the antioxidant compounds and thus to deduce their contribution to the total phenolic content. To carry out the methodology, the sample and the blank must be analysed by spectrophotometry at 760 nm, at which the blue colour shows the maximum absorbance. There are some modifications such as the one used by González-Mendoza et al. (2022), who measured the absorbance at 765 nm to analyse the total phenolic content in different Mexican craft beers. The authors added 1 mL of the Folin-Ciocalteu reagent, similarly to Singleton et al. (1999), then added 20% (w/v) sodium carbonate to the samples and incubated them in the dark at 25°C for 60 min. In both studies, the researchers used gallic acid as a standard and the results are expressed as gallic acid equivalents. Singleton et al. (1999) mentioned that the assay includes monophenols and gives predictable reactions (but varies based on the reactive groups per molecule) with the types of phenols found. Because various phenols react different, it is common to express the results as gallic acid equivalents. Gallegos-Infante et al. (2010) used the Folin–Ciocalteu method to evaluate the antioxidant capacity of fresh Mexican barley and after it had been malted and roasted. The phenolic extract after malting and roasting had a higher antioxidant capacity (IC50). The authors mentioned that this increase is associated with the release of phenolic compounds linked to the decomposition of the cellular constituents because of the heat treatment.

4.2.3 Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay is a spectrophotometric method that indirectly quantifies the antioxidant capacity, based on the reduction of

Fe^{3+} (iron complex, yellow) to Fe^{2+} (iron complex, blue), which has less antioxidant power (Benzie & Strain, 1996). The higher the antioxidant capacity of the analysed sample, the higher the Fe^{2+} concentration, and the more intense the absorbance at 630 nm. The mechanism of this reaction involves an electron transfer, while other methods scavenge free radicals. Cömert & Gökmen (2018) mentioned that the FRAP assay underestimates the antioxidant capacity that some compounds, such as thiols and proteins, may have because it does not measure free radicals. Hence, the FRAP assay should be performed along with other methods to obtain a more complete picture of the antioxidant capacity of the samples. New methods have been proposed to overcome the disadvantages of the FRAP assay, such as the cupric antioxidant capacity reduction (CUPRAC) assay (section 4.2.4), which does not present the obstacles shown by FRAP because the redox reaction and the kinetics are faster based on the potential of the copper ion compared with the iron ion. In addition, this method allows quantifying all types of antioxidants, including thiols and proteins (Borahan et al., 2022; Cömert & Gökmen, 2018) (Table 2).

The FRAP assay has been used to determine the levels of phenolic compounds in Mexican pigmented maize samples (Mihn, 2021). These authors found that the colour presented by the different samples does not determine their antioxidant capacity. They confirmed these results by performing the α, α-diphenyl-β-picrylhydrazyl (DPPH) assay (section 4.2.6). Besides, the FRAP assay was the best method to differentiate the samples for the antioxidant capacity. In another study, Mendoza-Bacilio et al. (2022) evaluated the influence of colour on phenolic compounds and the bioactive properties of honey from Guerrero, Mexico. They reported that the differences in the antioxidant properties between the samples could be a consequence of the diversity of the phytochemical compounds of the plants and their geographical origins. There was a correlation between the FRAP assay results and the colour of the honey samples.

4.2.4 Cupric reducing antioxidant capacity assay

The cupric reducing antioxidant capacity (CUPRAC) assay was introduced by Apak et al. (2004). It is based on the potential of the copper ion due to the action of neocuproine, a chromogenic oxidising agent – Cu (II) is oxidised to Cu(I). The reaction results in a colour change from colourless to yellow, and the absorbance is measured at 450 nm. This assay has advantages over other methods such as the FRAP assay: it has faster kinetics, greater specificity, and sugars and citric acid do not interfere (Table 2). Cárdenas et al. (2014) quantified the antioxidant capacity of various samples of commercial tea with the CUPRAC assay using an electrochemical approach to compare it with a traditional spectrophotometric approach. The authors emphasised some advantages such as a reduction in the interference due to turbidity and an extended linear analytical range. They recommend using this method to measure antioxidants in oils, fats, and cosmetics.

4.2.5 Oxygen radical absorbance capacity assay

The oxygen radical absorbance capacity assay (ORAC) is a method to measure the peroxyl radical scavenging capacity. The ORAC assay is based on the use of generators of these radicals, which oxidize fluorescent probes, and it is measured by fluorescence decay. Antioxidants scavenge peroxyl radicals, which causes a lower rate of florescence decay (Skendi, 2021; Zhong & Shahidi, 2015) (Table 2). This assay determines the total antioxidant capacity, instead of specific compounds and it is commonly used in food industry (Osorio-Arias et al., 2020). The most used peroxyl radical generators are AIBN (α, α-azobisiso-butyronitrile), ABAP (2,2-azobis (2-amidinopropane) dihydrochloride), AMVN: 2,2′-azobis(2,4-dimethyl-valeronitrile) and AAPH (2, 2′-azobis(2-amidino-propane) dihydrochloride) (Zhong & Shahidi, 2015). The disadvantage of the ORAC assay is the use of specific equipment and reagents to measure fluorescence, which in some cases are not accessible.

The ORAC assay was used to measure the antioxidant capacity of betalains and phenolic compounds of cactus berry fruits in Mexico (Montiel-Sánchez et al., 2021). The authors found that the antioxidant capacity was directly proportional to the amount of ascorbic acid present in the sample. Another product in which the antioxidant capacity was evaluated using the ORAC assay is coffee. Liao et al. (2022) determined the antioxidant capacity of coffee infusions from Mexico, Brazil, Ethiopia and Tanzania.

4.2.6 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay is a spectro-photometric method. The radical is generated by oxidation with potassium persulphate. The samples are incubated with the reagent for 30 min in the dark at 25 °C and the decrease in absorbance at 734 nm is measured due the decolourisation of the ABTS radical cation (due to its reduction by antioxidants). This assay has been criticised for being non-specific for phenolic compounds that are reduced by other non-phenolic compounds (Palomino et al., 2009). However, the ABTS radical scavenging assay, like the FRAP assay, is useful when the potential is less than 0.7 V. Hence, the

ABTS radical scavenging assay is representative of the reactions that occur within cells, and it has the advantages of being stable and fast (Rojas-Barquera & Narváez-Cuenca, 2009). The ABTS radical cation reacts with different compounds that contribute to the antioxidant capacity but are not necessarily polyphenols. It is common to express the results from this assay as the Trolox equivalent antioxidant capacity (TEAC) (Table 2). The ABTS assay was used to determine the antioxidant potential of eight varieties of berries from southern Jalisco, Mexico (Virgen-Carrillo, et al., 2022). The authors freeze-dried the samples for conservation before the assay. Among the compounds characterised by Electrospray Ionization Quadropole Time-of-Flight Mass Spectrometry (ESI-Q-TOF-MS) that contribute to the antioxidant capacity, the authors identified anthocyanins, phenolic acids, flavanols, and flavonols. The results obtained indicate that blackberries have biological potential to be used in the preparation of functional foods.

4.2.7 α,α-Diphenyl-β-picrylhydrazyl radical scavenging assay

The α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay has been used since the 1950s. This assay involves a notable colour change from intense violet to yellow when DPPH is reduced by donating a hydrogen atom. This colour change is detected by measuring the absorbance at 517 nm. This method is widely used because it is easy, stable, fast, efficient, and inexpensive, and the radical has a long life due to its inability to dimerise. This assay is used to quantify the antioxidant capacity by evaluating free radicals because of its scavenging activity of non-enzymatic antioxidants. The antioxidant capacity reported by the DPPH radical scavenging assay is lower than that reported by other assays. For example, the ABTS radical scavenging assay, which reports the TEAC, generally indicates a higher antioxidant capacity because the ABTS radical cation has low selectivity and reacts with any hydroxylated aromatic compounds (Gulcin, 2020). DPPH is more selective: it does not react with compounds such as flavonoids lacking a hydroxyl group in the B ring, nor does it react with aromatic acids that contain only one hydroxyl group. Hence, the DPPH radical scavenging assay results are more reflective of the actual antioxidant capacity of the samples (Palomino et al., 2009). The results of the DPPH radical scavenging assay are expressed as the TEAC, gallic acid equivalents, or the IC50 or IC25. Hence, the results can be compared with the results obtained by other methods. However, the comparison is not recommended because, as mentioned above, the molecules quantified by other less selective methods do not represent the molecules with a real antioxidant potential (Table 2).

The DPPH radical scavenging assay has been used to quantify the antioxidant capacity of a Mexican ruby cabernet red wine from the state of Querétaro. The Mexican market demands sweet drinks, so researchers produced a wine with overripe grapes to ensure there would be residual sugars after fermentation. The result was a sweet red wine that the researchers subjected to two ageing temperatures in the bottle (4 and 18°C). The ageing occurred in the vineyard's artificial cellar. Subsequently, the researchers determined the antioxidant capacity of both wines and found a significant difference after 6 months of ageing. This difference was a consequence of the final composition of the phenolic compounds because the polymerisation and oxidation reaction rates had been modified by temperature (Espitia-López et al., 2014).

5. Antioxidant capacity of traditional and emergent crops of interest in Mexico

According to data published by the Panorama Agroalimentario 2024 of the Agrifood and Fisheries Information System (SIAP), Mexico ranked 11th worldwide in the production of agricultural crops in 2023 (SIAP, 2024). Additionally, 21.6 million hectares were cultivated, from which approximately 299 million tons of different products were obtained. Moreover, Mexico ranked seventh regarding exports: there were approximately 51 billion dollars in sales to different countries, including the United States, Japan, Canada, China, and Guatemala, among others (SIAP, 2024).

5.1 Amaranth

Amaranth (*Amaranthus sp.*) is an important grain in Mexico. Indeed, in 2023 about 6,200 tons were produced and per capita consumption was 46 g (SIAP, 2024). It is considered a new source of nutrients and contains many molecules with antioxidant potential (López-Mejía et al., 2014). The concentrations of phenolic compounds, such as tocopherols and polyphenols, and the antioxidant capacity have been determined mainly with the Folin–Ciocalteu, DPPH, and ABTS methods (Sarker et al., 2018; Venskutonis & Kraujalis, 2013). Muñiz-Márquez et al. (2014) extracted compounds from the whole plant with ethanol as a solvent. They performed HPLC and reported different concentrations of phenolic compounds and different antioxidant activity with the DPPH and ABTS radical scavenging assays. López-Mejía et al. (2014) compared extraction methods and solvents using amaranth leaves and seeds and the relationship with the antioxidant capacity (DPPH and ABTS radical scavenging assays) and the total phenolic content (Folin–Ciocalteu method). In addition, the total

antioxidant capacity (Folin–Ciocalteu, DPPH, and ABTS) of the leaves of different varieties of amaranth has been determined (Sarker et al., 2020, Skwaryło-Bednarz et al., 2024). Bucky et al. (2024) evaluated the effect of light on phenolic content and antioxidant capacity (Folin–Ciocalteu and FRAP, respectively) on red amaranth. The authors demonstrated that blue light caused an increase of antioxidant capacity.

Flours based on amaranth have been produced and their antioxidant capacity has been established by the ORAC method and the total phenolic content by the Folin–Ciocalteu method (Milán-Carrillo et al., 2012). The antioxidant activity of amaranth seeds has also been determined with the DPPH and ORAC assays when applying different methods of conservation and processing in the medium and long term (Pazinatto et al., 2013). In addition, alternatives have been studied to improve the nutritional properties of amaranth, including its antioxidant capacity, through drought stress. The authors found an increase in antioxidant capacity, using the DPPH, ABTS, and HPLC methods (Sarker & Oba, 2018). Sandoval-Sicairos et al. (2020) compared the antioxidant capacity of flours obtained from germinated and non-germinated amaranth seeds using the ORAC assay. Additionally, Cárdenas-Hernandez et al. (2016) added flour and dry leaves of amaranth in wheat paste and to evaluate its impact on the antioxidant capacity of the final product, which they determined with the Folin-Ciocalteu, ABTS, DPPH, and FRAP methods.

5.2 Avocado

Avocado (*Persea americana*) is a crop of great importance to Mexico. In 2023, Mexico was the largest global avocado producer, with 2.4 million tons produced (SIAP, 2024). Avocado contains carotenoids and phenolic compounds, which is why the antioxidant capacity of this fruit has been extensively studied (Ameer, 2016; Ochoa-Zarzosa et al., 2021). The ABTS, DPPH, and ORAC methods have been used most frequently to determine the antioxidant capacity. Wang et al. (2010) observed the highest antioxidant activity in avocado seeds based on the DPPH and ORAC assays. Dabas et al. (2019) determined the antioxidant capacity of crushed Hass avocado seeds by using the ORAC assay. Likewise, Miramontes-Corona et al. (2024) determined total phenolic content and antioxidant capacity of seeds of Hass and Criollo varieties, using Folin–Ciocalteu and FRAP methods. The authors identified the antioxidant components and determined their antimicrobial activity.

Castro-Lopez et al. (2019) measured the antioxidant capacity of leaf extracts from different avocado varieties using the DPPH and ABTS radical scavenging assays. The authors found that the Plátano-Delgado and Criollo 6 varieties had the highest antioxidant capacity.

In another study, the authors found the highest antioxidant capacity, based on the DPPH radical scavenging assay, of 11 avocado varieties in the peel (Corrales-García et al., 2019). Moreover, the antioxidant capacity, determined by the ORAC assay, of avocado oil is independent of the number of phenolic compounds (Espinosa-Alonso et al., 2017). García-Solís et al. (2009) determined the antioxidant capacity (using the FRAP and DPPH assays), the total phenolic content by the Folin–Ciocalteu method, and the β-carotene concentrations by HPLC. They compared the levels with other widely consumed agricultural products in Mexico. Based on the DPPH and ORAC assays, Villa-Rodríguez et al. (2011) observed that the antioxidant capacity and the total phenolic content increase as the fruit matures. Another study showed that the avocado pressing process, necessary to produce guacamole, did not affect the carotenoid levels (Jacobo-Velázquez & Hernández-Brenes, 2012). Cenobio-Galindo et al. (2019) evaluated the effect of a nanoemulsion that covered the avocado fruit. This approach prevented the antioxidant capacity (based on DPPH radical scavenging) and the total phenolic content (based on the Folin–Ciocalteu method) from decreasing during 60 days of storage. Chuacharoen et al. (2024) encapsulated avocado seed extracts, subsequently the authors determined the antioxidant capacity (using DPPH and FRAP assays) and evaluated the effect on leukemia cell lines.

5.3 Berries

Berries are a group of round-shaped fruits – of various bright colours and with a sour or sweet flavour – that are generated from the ovary of a single flower. Strawberries, blackberries, raspberries, and blueberries are included in this category and together they represented 2.25% of the national agricultural gross domestic product (GDP) of Mexico (SIAP, 2016). Mexico is the sixth largest producer of blueberries (*Vaccinium* spp.) in the world, with 80,133 tons produced in 2023. Regarding raspberries (*Rubus idaeus*), 190,412 tons were produced in 2023, which placed Mexico as the second largest producer in the world. Regarding strawberries (*Fragaria ananassa*), 641,552 tons were produced in Mexico in 2023, making it the fifth largest producer in the world. The production of blackberries (*Rubus ulmifolius*) was 238,122 tons in 2023, making Mexico the leading producer (SIAP, 2024). These berries contain secondary metabolites with aromatic rings and hydroxyl groups that contribute to the antioxidant capacity. It is important to mention that the total phenolic content and the antioxidant activity differs among berries (Jiang et al., 2022).

Chen et al. (2013) determined the total polyphenolic content with the Folin–Ciocalteu method and the antioxidant activity with FRAP assay in 12 different berries; they associated this activity with the anthocyanin content. Guevara-Terán et al. (2022) studied the influence of altitude on antioxidant capacity of strawberry cultivation by using the FRAP, DPPH, and ORAC assays. Salazar-Montoya et al. (2022) analysed the antioxidant activity (based on ABTS and DPPH radical scavenging) in blackberry pulp pastes. Researchers have also studied the relationship between the tannin concentration and the antioxidant capacity, which they determined with the ORAC method (Sánchez-Velázquez et al., 2019). Virgen-Carrillo et al. (2022) compared the antioxidant capacity and total phenolic content, determined by the DPPH and Folin–Ciocalteu methods, respectively, of seven varieties of blackberries, blueberries, and raspberries produced in the state of Jalisco, located in western Mexico. Caicedo-Narváez and Hernández-Carrión (2022) compared the antioxidant capacity and total phenolic content (using the DPPH and Folin–Ciocalteu methods, respectively) of fresh and dried Biloxi variety blueberries. They found no significant differences in these response variables. López-Corona et al. (2022) mentioned that raspberry extracts can be used to prevent degenerative diseases. They attributed this benefit to the antioxidant capacity, which is commonly measured with the DPPH, ABTS, FRAP, and ORAC assays. Petrov-Ivanković et al. (2024) determined the antioxidant activity (using FRAP, ABTS, DPPH and CUPRAC assays) and phenolic content (by Folin–Ciocalteu method) of raspberry and strawberry pomaces, as well as their *in-vitro* prebiotic capacity.

5.4 Cocoa

In 2023, Mexico produced 29,047 tons of cocoa (*Theobroma cacao* L.), ranking 14th worldwide. Belgium was the largest buyer of this fruit (SIAP, 2024). This agricultural product has a 10% phenolic content – these compounds are responsible for its antioxidant and anti-inflammatory activity and cardiovascular protection effects (Hernández-Hernández et al., 2019). Hernández-Hernández et al. (2022) determined the antioxidant capacity with the DPPH radical scavenging assay in intact cocoa beans and only the cotyledons. There were no differences in the activity. Quiroz-Reyes et al. (2013) compared the antioxidant activity, using the DPPH and FRAP assays, of polyphenol extracts obtained from cocoa beans with ultrasonic and maceration extraction methods. Ruesgas-Ramón et al. (2020) determined the total phenolic content of the cocoa husk with the Folin–Ciocalteu and HPLC methods; the former method quantified a higher total phenolic content. Hernández-Hernández et al. (2018) reported different bioactive compounds in various genotypes of Mexican cocoa using different extraction methods. They related the total phenolic content (determined with the HPLC and Folin–Ciocalteu methods) to the antioxidant capacity, which they determined with the DPPH radical scavenging assay. In a similar study, Avendaño-Arrazate et al. (2021) determined the antioxidant capacity, using the DPPH and ABTS radical scavenging assays, of 26 Mexican cocoa phenotypes. Cortez et al. (2024) evaluated the total phenolic content and antioxidant capacity (Folin–Ciocalteu and DPPH assays, respectively) of Peruvian cocoa during different postharvest processes.

There have been various studies in which the authors evaluated products derived or processed from cocoa. Tovar-Perez et al. (2017) evaluated the antioxidant capacity, using the ABTS and DPPH radical scavenging assays, in hydrolysates and peptide fractions of cocoa glutelin. In another study, Quiroz-Reyes et al. (2014) obtained cocoa-based gelatine nanocapsules for use in the food industry. They then determined the antioxidant activity with the DPPH radical scavenging assay. Similarly, Calva-Estrada et al. (2019) developed a cocoa liquor nanoemulsion and determined the antioxidant activity with the DPPH and ABTS radical scavenging assays. Moreover, Orbe-Chamorro et al. (2024) evaluated different fermentation parameters on the phenolic content (using Folin–Ciocalteu method) and the antioxidant capacity (by ABTS assay) of different Ecuadorian cocoa varieties, to produce liquor and butter. The authors found that the phenolic compounds decreased through fermentation time, which is desirable on those processed cocoa products.

5.5 Coffee

Cherry coffee (*Coffea arabica*) production in Mexico has increased in the last 5 years. In 2023, 12.2 million tons were harvested, which represents 1.7% of the country's agro-industrial production (SIAP, 2024). This crop is an important source of antioxidants with significant health benefits, and an inverse correlation has been reported between coffee consumption and the effects of diseases such as diabetes mellitus, cancer, and Parkinson's disease (Liao et al., 2022). Given these health benefits, Liao et al. (2022) determined the total phenolic content and the antioxidant capacity with the Folin–Ciocalteu and DPPH methods, respectively, of coffee samples from different countries and with different degrees of roasting. Shen et al. (2022) compared the antioxidant capacity of flowers, leaves, and coffee beans using the ABTS, DPPH, and FRAP assays. Casas-Junco et al. (2021) determined the sensory, physicochemical, and antioxidant capacity (based on ABTS radical scavenging assay) properties of roasted coffee treated with cold plasma, to maintain

the main characteristics of the coffee. Additionally, Munguía-Ameca et al. (2018) determined the antioxidant capacity with the FRAP assay of fresh, silage, and sun-dried coffee pulp. Changes in the antioxidant capacity of coffee pulp have also been evaluated, using the FRAP assay, during the silage process; it did not change compared to non-silage samples (Salinas-Ríos et al., 2014). López et al. (2013) extracted bioactive compounds from coffee pulp by lactic fermentation and determined the antioxidant capacity based on the DPPH and ABTS radical scavenging assays. It was higher compared with non-fermented pulp.

Martínez-Ruiz et al. (2018) studied the evolution of antioxidant activity in Mexican coffee beverages, using the DPPH and FRAP assays, by keeping it in a filter coffee machine for 8 h. They observed a decrease in the antioxidant capacity after 2 h. Hervert-Hernández and Goñi (2011) assessed the contribution of the consumption of coffee beverages to the health of women with obesity in Mexico. They measured the total phenolic content and the antioxidant capacity with the Folin–Ciocalteu and DPPH methods, respectively. Cañas et al. (2022) studied the stability of phenolic compounds obtained from coffee beans in simulated digestion systems. Recently, more importance has been given to the reuse of coffee residues, such as ground grain, husk, and silver skin, among others, for use as a food additives or sources of compounds with antioxidant activity. In these studies, the authors used the ABTS and DPPH radical scavenging assays to determine the antioxidant capacity (Macías-Garbett et al., 2022; Osorio-Arias et al., 2020; Vargas-Sánchez et al., 2023).

5.6 Corn

Maize (*Zea mays*) is the most important cereal in Mexico. National production in 2023 was 27.6 million tons, which represents 87.8% of grain production (SIAP, 2024). Mexico has a wide variety of coloured corn (purple, blue, red, cherry, white, black, and yellow), which contain phenolic compounds such as anthocyanins. These compounds give maize a greater antioxidant capacity than other cereals (Corona-Terán et al., 2017). Therefore, various studies have been carried out, including characterisation of different corn varieties native to Mexico. Corona-Terán et al. (2017) observed that the black and purple varieties had the highest antioxidant capacity, determined by the DPPH radical scavenging assay. These varieties also had the highest anthocyanin concentrations. Paucar-Menacho et al. (2017) optimised the germination time and temperature for purple corn, in relation to the total phenolic content and the antioxidant capacity determined with the Folin–Ciocalteu and ORAC methods, respectively. Mihn (2021) evaluated the influence of temperature on the concentrations of anthocyanins and phenolic compounds and the antioxidant capacity of pigmented corn (without specifying colour). The author found that at temperatures above 100 °C, the antioxidant capacity (based on DPPH radical scavenging) and the total phenolic content (based on the Folin–Ciocalteu assay) decreased drastically.

Montero-Vargas et al. (2020) predicted the antioxidant capacity of corn hybrids based on mass fingerprinting and data mining. Harakotr et al. (2014) compared the antioxidant capacity of coloured corn varieties at different maturation stages based the DPPH radical scavenging assay. Capocchi et al. (2016) studied the differences in the antioxidant capacity of four Italian corn varieties, using the FRAP and DPPH assays, as well as the relationship with the flavonoid content. Nemzer et al. (2019) compared the antioxidant capacity with the ORAC assay of non-germinated grains and sprouts of purple corn. The grains showed the highest antioxidant capacity.

Considering that the consumption of corn masa (nixtamal) and tortillas is extremely important in Mexico, the antioxidant capacity, anthocyanin concentration, and total phenolic content of grains, masa, and tortillas of purple, white, red, and blue corn varieties have been determined (López-Martínez et al., 2011; Magaña-Cerino et al., 2020). The authors observed that the grains had the highest phenolic content and antioxidant activity (based on the DPPH and ABTS radical scavenging assays). In contrast, there were no differences between the processed masa (nixtamal) and the tortillas. It is important to mention that studies have been carried out on other foods derived from corn, such as traditional pinole and popcorn. The authors determined the antioxidant capacity with the DPPH and FRAP assays and the total phenolic content with the Folin–Ciocalteu method (Coco & Vinson, 2019; Sánchez-Herrera et al., 2014).

Likewise, it has been made bread with different amounts of purple corn (Wang et al., 2024). The authors demonstrated that anthocyanins contained in the corn could inhibit aging of bread, increased antioxidant capacity (using DPPH and ABTS assays) and even, improves digestion 'in-vitro'.

Öztürk & Uzun (2024) characterized some yellow and purple corn lines from Thailand, China, Argentina and Peru, and the results showed that the purple corn samples had the greater antioxidant capacity (determined by DPPH method).

The above shows that there has been an increase in interest in using corn varieties with greater antioxidant capacity.

5.7 Grape

Grape (*Vitis vinifera*) is a crop of great interest due to its antioxidant properties, with the seeds and peels containing the greatest number of compounds that contribute to this characteristic. Likewise, the by-products derived from this fruit are also relevant in the human diet to prevent the harmful effects of free radicals (Espitia-López et al., 2014; Molina-Quijada et al., 2010). In 2023, Mexico produced 385,260 tons of fruit grapes but only 77,000 tons of the grape varieties destined for the industrial production of juice and wine (SIAP, 2024).

Molina-Quijada et al. (2010) determined the antioxidant capacity of the Mexican table grape peel, using the ABTS and DPPH radical scavenging assays, and the total phenolic content using the Folin–Ciocalteu method. Other researchers have analysed the complete fruit of different cultivars from different parts of the world using the ABTS, DPPH, FRAP, and ORAC assays (Kisaca & Gazioglu-Sensoy, 2023; Liang et al., 2014; Zeghad et al., 2019). Of note, most of the studies related to grapes have focused on wine. The leaves of grape plants are also an important source of phenolic compounds and antioxidant activity (determined by DPPH radical scavenging; Fernandes et al., 2013). Additionally, grape juice extracted from immature fruit also contains phenolic compounds and antioxidant activity (measured with the Folin–Ciocalteu and DPPH methods, respectively) (Bayram & Elgin-Karabacak, 2022). Montagner et al. (2022) determined the antioxidant capacity of grape seeds of the Merlot variety, from winemaking waste, with the DPPH, ABTS, and FRAP assays.

There are too many studies emphasising the great antioxidant capacity of wine. This characteristic depends on the grape variety and post-vinification processing (Xu et al., 2014). In addition, the maturation method also contributes to differences in the antioxidant capacity of wines produced with different grape varieties from different wine regions (Espitia-López et al., 2014; Mendez-Trujillo & González-Mendoza, 2021; Muñoz-Bernal et al., 2021). Likewise, Lingua et al. (2016) compared the antioxidant capacity and phenolic composition of different grape varieties, in the different steps of wine production, from immature fruit to the final product, using the FRAP, ABTS, DPPH, and Folin–Ciocalteu methods. They reported differences in these properties among the grape varieties and developmental stages.

Finally, due to the economic importance of grapes, the impact of some diseases on the antioxidant capacity, determined with the FRAP assay, of some grape varieties has been studied (García et al., 2022).

5.8 Jamaica (Roselle)

Jamaica, also known as roselle (*Hibiscus sabdariffa*), is a plant used to prepare infusions and teas in Central America and Asia (Coria-Ávalos et al., 2022). In 2019, Mexico produced 7,889 tons of this plant (SADER, 2021). The calyces are the most used parts of the jamaica plant; they contain alkaloids, ascorbic acid, flavonoids, polyphenols, and anthocyanins, all of which contribute to the antioxidant and anti-inflammatory activity. They are also an important source of dietary fibre (Coria-Ávalos et al., 2022; Sáyago-Ayerdi & Goñi, 2010). Therefore, much attention has been paid to the characterisation and composition of calyces. The antioxidant capacity of this flower has been measured, using the ABTS, DPPH, and FRAP assays, and compared with other agricultural by-products (Ságayo-Ayerdi & Goñi, 2010). Additionally, aqueous and ethanolic extracts of jamaica calyxes have been obtained. Their antioxidant properties have been determined with the ABTS, DPPH, and FRAP assays, and the total phenolic content has been determined with the Folin–Ciocalteu method. The results differed depending on the solvents and their combinations (Fernández-Arroyo et al., 2011; Salazar-González et al., 2012). Sindi et al. (2014) characterised extracts of dried flowers grown in Sudan, using water, methanol, ethyl acetate, and hexane as solvents, and determined the antioxidant capacity (based on the ABTS and DPPH radical scavenging assays) as well as the total phenolic content (using the Folin–Ciocalteu method).

Preciado-Saldaña et al. (2019) formulated and characterised functional drinks based on jamaica and green tea containing different concentrations of the extracts, also modifying the extraction temperatures. Subsequently, the authors determined the total phenolic content with the Folin–Ciocalteu method and the antioxidant capacity with the DPPH and FRAP assays. Jabeur et al. (2017) measured the antioxidant activity with the DPPH radical scavenging assay as well as the antibacterial and antifungal activity of organic extracts of jamaica flowers and inflorescences. Navidad-Murrieta et al. (2020) developed and characterised microcapsules of extracts of this plant. They reported variable antioxidant activity based on the DPPH, ABTS, and FRAP assays, depending on the extract concentration. In recent years, the antibacterial activity of jamaica extracts has been assessed to increase the shelf life of agricultural products and by-products, including tomatoes, strawberries, and carrots (Gómez-Aldapa et al., 2017; Gutiérrez-Alcántara et al., 2015, 2016). Moreover, Ali et al. (2023) evaluated the effect of some native Australian fruits and spices, including roselle, on the activity of α-glucosidase, which is related to type 2 diabetes.

The authors quantified total phenolic content (by Folin–Ciocalteu method) and antioxidant activity (using DPPH and ABTS assays), as well as the potential of phenolic compounds to inhibit in silico α-glucosidase activity was determined.

5.9 Pitahaya

Pitahaya (*Hylocereus* spp.), also known as dragon fruit, is an exotic crop that has recently gained economic importance due to its high antioxidant capacity and is also a source of natural colourants (Verona-Ruiz et al., 2020). In 2021, Mexico produced 10,867 tons of this crop. However, the production of this fruit is more important in Central and South America (Constantino et al., 2021; Zitha et al., 2022). Researchers have analysed the nutraceutical potential and antioxidant capacity of red pitahaya by using the FRAP and DPPH assays (Tenore et al., 2012). Furthermore, Hua et al. (2018) compared three different cultivars of pitahaya, focusing, among other things, on the antioxidant capacity (determined with the ABTS and FRAP assays). Barkociová et al. (2021) compared the antioxidant capacity of four pitahaya species using the DPPH, ABTS, and FRAP assays. They found that antioxidant activity differed depending on the species and the method used. Researchers have also paid special attention to the phenotypic differences of the pitahaya species. Attar et al. (2022) related these differences to the total phenolic content (measured by the Folin–Ciocalteu method) and the antioxidant capacity (determined by the DPPH radical scavenging assay). The bioactive and antioxidant characteristics of pulps of different species of this fruit, grown organically (Angonese et al., 2021), as well as in different stages of plant development (Zitha et al., 2022), have also been studied. On the other hand, Reyes-García et al. (2024) determined the composition and physicochemical, technical and antioxidant properties of flours obtained from peel and flesh of Central American pitahaya (*Hylocereus ocamponis*). The study showed that peel flour had the higher antioxidant activity (using DPPH and ABTS assays). In other study, some natural preharvest elicitors were used in yellow pitahaya (*Selenicereus megalanthus*) to improve antioxidant capacity and phenolic content (determined by ABTS and Folin–Ciocalteu methods, respectively) (Erazo-Lara et al., 2024).

5.10 Tomato

Tomato (*Solanum lycopersicum*) is a widely consumed fruit throughout the world. Indeed, it is a fundamental part of the diet of many countries, including Mexico. In 2023, Mexico produced 3.64 million tons of tomatoes (SIAP, 2024). Tomatoes are a source of carotenoids and flavonoids that counteract the effects of ROS (Vega-López et al., 2022). The antioxidant activity has been widely determined in tomatoes with different techniques and subjecting the plants to different conditions to improve this activity. Figueroa-Cares et al. (2018) measured the total phenolic content and the antioxidant capacity with the Folin–Ciocalteu and DPPH methods, respectively, in different varieties and genotypes native to Mexico. Delgado-Vargas et al. (2018) determined the phenolic and antioxidant profiles of cherry tomatoes from Sinaloa, Mexico, by using the HPLC, Folin–Ciocalteu, and ABTS methods. The authors found that caffeoylquinic acids and rutin were the compounds most closely associated with the antioxidant activity of this tomato variety.

Kim et al. (2014) observed that blue light stimulates the production of phenolic compounds, antioxidants, and flavonoids in tomato seedlings. In this sense, Baenas et al. (2021) compared the production of carotenoids and the antioxidant capacity (based on the FRAP assay) in tomatoes grown in the dark and with LED light. Moreover, Vitale et al. (2022) determined the antioxidant capacity, with the FRAP assay, of Micro-Tom tomatoes subjected to different wavelengths of light. The authors observed that RB light increased the antioxidant activity.

Pinela et al. (2019) determined the phenolic composition, by HPLC, and the antioxidant capacity, with the DPPH radical scavenging assay, of different samples of preserved tomato germplasm by hydroalcoholic extraction. Martínez et al. (2020) evaluated the effect of osmotic stress, induced with salt, on the antioxidant capacity, using the FRAP and DPPH assays, of wild and domesticated tomatoes. Finally, researchers have assessed the relationship between different production methods and postharvest treatments and the antioxidant capacity (based on the ORAC and FRAP assays) and the total phenolic content (based on the Folin–Ciocalteu method) (Batziakas et al., 2022; Vega-López et al., 2022). Cruz-Chamorro et al. (2024) observed that the riper the tomatoes, the greater phenolic content (using Folin–Ciocalteu method) and antioxidant capacity (measured by FRAP, ABTS, ORAC, and DPPH assays) of three organically-grown tomato cultivars. Due to the great importance of tomatoes, the relationship between the response to stimuli in tomato cultivation, transcription factors, and antioxidant capacity has even been studied (Ma et al., 2024).

5. Current challenges and perspectives

The antioxidant capacity of agricultural products has been determined worldwide (Gulcin, 2020). In addition, interest in emerging crops has increased due to their potential antioxidant capacity and greater availability to consumers. However, many authors used

different methods of extraction of antioxidant compounds interchangeably. Likewise, several quantification methods were used at the same time and subsequently compared with each other, sometimes regardless of the chemical nature of the antioxidant or the limitations of the methods. On the other hand, most of the published articles were limited to quantifying the antioxidant capacity; few of them identified the molecules, and, to a lesser extent, the direct impact on human health was evaluated.

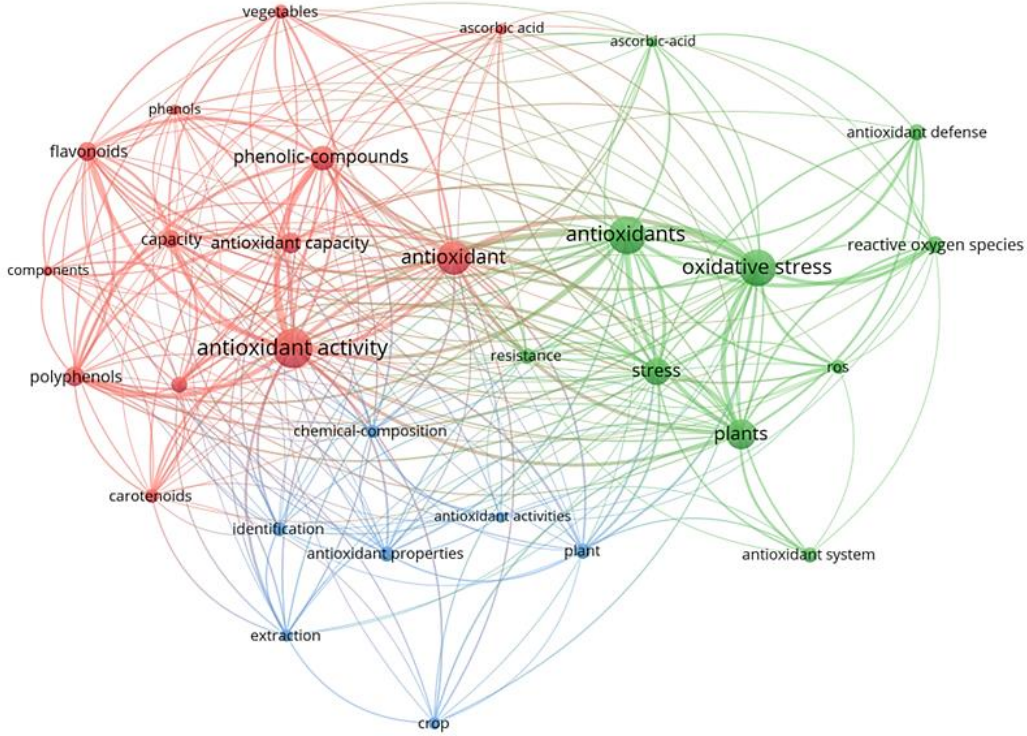

Figure 5. Keyword co-occurrence analysis of articles published in the period 2020-2024. The data was obtained from Web of Science (Clarivate®) (search criteria: KEYWORDS: "Antioxidant AND crop") using VOSviewer® software (https://www.vosviewer.com/).

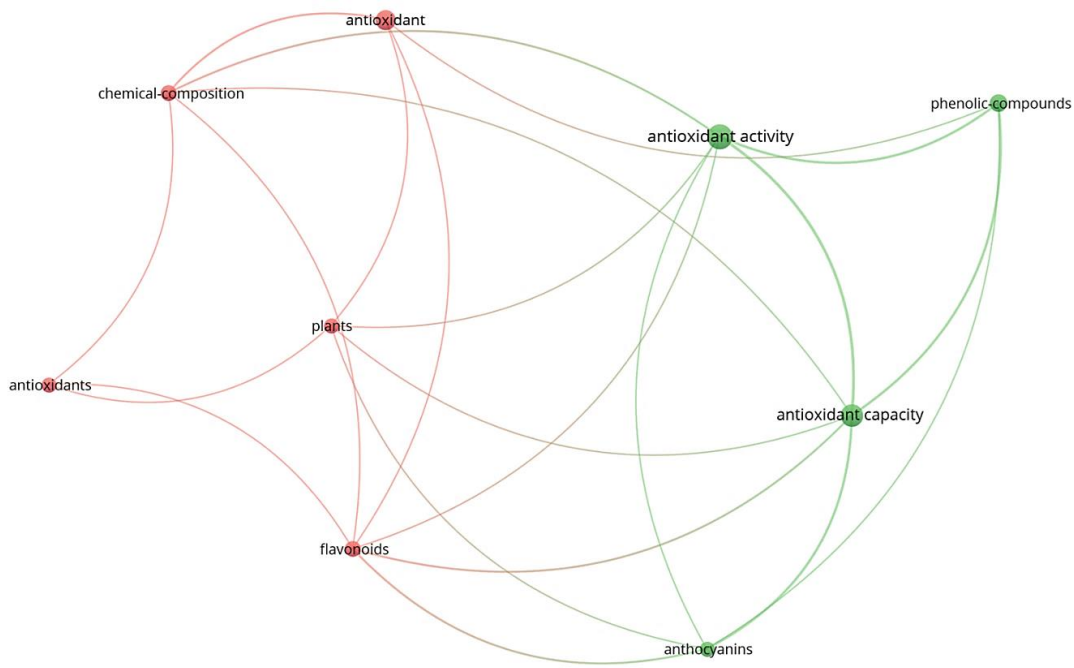

Figure 6. Keyword co-occurrence analysis of articles published in the period 2020-2024. The data was obtained from Web of Science (Clarivate®) (search criteria: KEYWORDS: "Antioxidant AND crop AND mexico") using VOSviewer® software (https://www.vosviewer.com/).

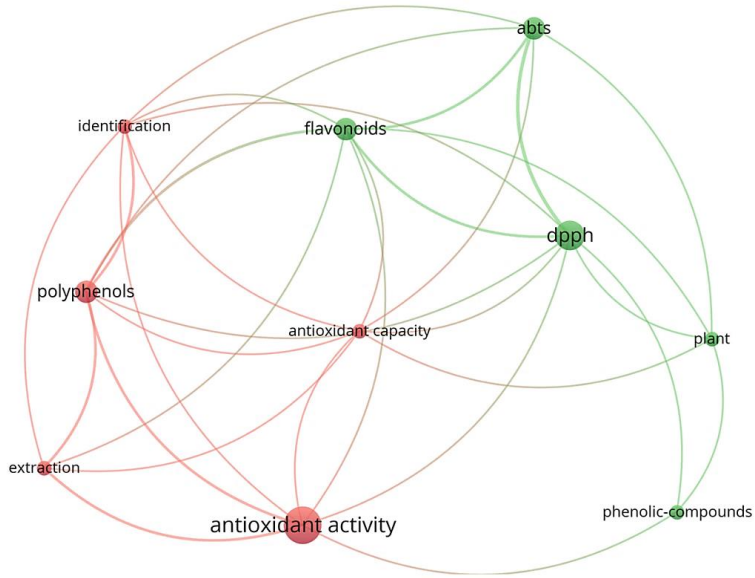

Figure 7. Keyword co-occurrence analysis of articles published in the period 2020-2024. The data was obtained from Web of Science (Clarivate®) (search criteria: KEYWORDS: "Antioxidant AND dpph AND abts AND folin AND crop") using VOSviewer® software (https://www.vosviewer.com/).

On the other hand, a bibliometric analysis of the papers published in the period 2020-2024 was carried out in Web of Science (Clarivate®), first with the keyword's "antioxidant" and "crop", which obtained a total of 9434 papers. Subsequently, a co-occurrence analysis was performed using the VOSviewer® software (Figure 5). Three nodes can be observed, in which the relationship between the keywords is varied due to the way of expressing some concepts such as antioxidant activity, antioxidant capacity, phenols, phenolic compounds, etc. Some keywords are even repeated, with the difference that some contain hyphens. Despite this, the three nodes have the concept of antioxidants in common.

Additionally, another bibliometric analysis was carried out with the keywords "antioxidant", "crop", and "mexico" (Web of Science, Clarivate®), in the same period (2020-2024) to analyze the publications related to crops produced in Mexico, obtaining 55 results. The co-occurrence analysis of the data obtained (VOSviewer®) is shown in Figure 6, where two nodes are observed, and the concepts of antioxidant activity, antioxidant capacity, and plants stand out.

Finally, a third bibliometric analysis was carried out, using the keywords "antioxidant" and "dpph", "abts", "folin" and "crop" (Web of Science, Clarivate®), delimiting the period 2020-2024, with which only 11 results were obtained, which indicates that the determination of antioxidant capacity in crops, using the most common methods, has decreased considerably. Figure 7 shows the co-occurrence analysis of the data obtained (VOSviewer®), with only 2 nodes, particularly the red node in which the concept of identification stands out, related to polyphenols and extraction, while in the green node, dpph and abts stand out. It is important to highlight that in all co-occurrence analyses carried out, no word related to the effect on consumers appeared. This underscores the need for future publications to focus not only on the adequate quantification of antioxidant capacity and antioxidant activity, but also on the direct impact on consumers, thereby increasing consumer awareness in this area.

6. Conclusions

The increase in the consumption of agricultural products that contain high levels of antioxidants has promoted research on various crops, as well as their production. Researchers have used numerous assays to assess the antioxidant capacity – and sometimes they have compared the results from different assays. These findings have inspired an ongoing dialogue about which method provides the best estimate of the antioxidant capacity. In recent years, there has been increased attention to identify the compounds that confer antioxidant activity in agricultural products and by-products. However, few articles have evaluated the actual effects of these antioxidants when consumed by humans. In Mexico, there has been increased interest in the antioxidant capacity of agricultural products. This phenomenon has had an impact on the annual agricultural production reported by the

Mexican government. Moreover, new crops with a high content of molecules with antioxidant activity – such as berries, pitahaya and amaranth – have been introduced, and the production of staple crops for the Mexican diet, such as corn, tomato, coffee, avocado, and cocoa, has increased. Additional studies are necessary to characterise antioxidants in the different crops of interest and to relate the antioxidant capacity to these compounds, using the correct methods, and to assess their effects on human health.

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CRediT authorship contribution statement
B. Flores-Chávez: investigation and supervision. **S. Hernández-León:** investigation and supervision. **A. L. Guzmán-Elizalde:** investigation. **J. Espitia-López**: investigation and writing – review and editing. **P. M. Garza-López:** conceptualisation, investigation, supervision, and writing – review and editing.

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Data availability
The data will be made available on request.

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