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Volume II

Wesclen Vilar Nogueira
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Wesclen Vilar Nogueira
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Apresentação

Neste segundo volume do E-book *Realidades e Perspectivas em Ciência dos Alimentos* as áreas de abrangência das pesquisas foram expandidas, contribuindo para o acesso ao conhecimento numa linguagem contextualizada e de fácil compreensão.

As pesquisas e reflexões abordadas nos capítulos foram realizadas por pesquisadores de diversas unidades da Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) e Instituições de Ensino Superior (IES) públicas (Universidade Federal do Amazonas, Universidade Federal do Ceará, Universidade Federal do Rio de Janeiro, Universidade Federal do Espírito Santo, Universidade Federal de Rondônia, Universidade Federal do Oeste do Pará, Universidade Federal do Rio Grande, Universidade do Estado do Amazonas, Universidade Estadual do Ceará, Universidade Tecnológica Federal do Paraná) e privadas (Centro Universitário IDEAU).

O conteúdo abordado demonstra a multidisciplinaridade da área de Ciência dos Alimentos sobre diferentes aspectos e realidades. As pesquisas abordam desde o emprego de compostos bioativos na produção de alimentos, desenvolvimento de novos produtos, avaliação da composição química e microbiológica de *commodities*, até alternativas para reutilização de resíduos agroindustriais na produção de alimentos.

Que o E-book *Realidades e Perspectivas em Ciência dos Alimentos* seja de grande proveito e, ofereça subsídios teórico-metodológicos para profissionais da área de Ciência dos Alimentos e áreas afins.

Wesclen Vilar Nogueira

SUMÁRIO

Chapter VII

Peppers the genera Capsicum as bioactive compounds sources: a review

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INTRODUCTION

Peppers the genera *Capscicum* pertain to family Solanaceae, botanic specie with particular characteristics, because produces fruits of spicy taste, that vary ranging from low or hight pungency (Ribeiro et al., 2008). Are cultivated in warm climate regions worldwide, especially in Asia, northern America, Europe, and tropical and subtropical Africa (Thampi, 2004).

This Genus present great morphological variation, fruits with diverse sizes, shapes, colors, and varying degree of pungency. The pungency is an exclusive feature to this genus because to capsaicinoids, especiallly the capsaicin and dihydrocapsaicin. The ripe fruit is generally red but varies from milky yellow to purple. The format varies according to the species, can be fruits elongated, round, triangular, conical, and square fruits (Costa, Henz, 2007). Peppers also present great variation of vitamins composition, antioxidant compounds, and other phytochemicals (Chuah et al., 2008).

This sensory attributes of color, taste and pungency makes peppers so popular and provide their use in different research fields, food industry, pharmaceutical, cosmetics and even chemical agent production.

Capsicum peppers are of social and economic importance for agribusiness. Total pepper production increased by 25 % in a decade (2006 – 2016). In 2018, the global production of *Capsicum* peppers was 40.9 million tons in a cultivated area of 3.8 million hectares. The main producers were: China (45.3%), Mexico

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(8.4%), Turkey (6.3%), Indonesia (6.2%), India (4.6%), Spain (3.1 %), Nigeria (2.0%), Egypt (1.9%), United States (1.7%), Algeria (1.6%) and Tunisia (1.1%) (FAOSTAT, 2019).

Due to its tropical climate, Brazil has a wide variety of fruits of the genus *Capsicum*, are found domesticated, semidomesticated and wild species throughout the country. The domesticated species *C. annuum L.*, *C. Baccatum*, *C. Chinense* and *C. frutescens L.* are widely cultivated, especially in the Southeast and Midwest (Ribeiro et al., 2008). Current official data on the production of these crops in Brazil were not found, however Pinheiro et al. (2012) describe that the production in 2012 comprised about 75 thousand tons cultivated in 5 thousand hectares. Pinto et al. (2016) state in your research that the cultivation of peppers increases considerably each year in Brazil.

In addition to the wide consumption of fresh pepper, several products derived from peppers are widely used in the industry, such as oleoresin, enriched extracts, paprika, which is widely used as a spice in meat products, soups, sauces and snacks, contributing to pungency, color attributes and flavor (Buckenhüskes, 2003; Baenas et al., 2018).

Recently, researches have considered the use of pepper and or its sub-products as antimicrobial or antioxidant agents, due to the variety of bioactive substances contained in your composition (Baenas et al., 2018).

Research carried out in the area of foods seeks ever more informations in relation to the benefits of foods healthy, advances in analytical methodologies and chemical and functional properties of bioactive compounds in natural matrices such in peppers and their by-products (Sanatombi; Rajkumari, 2019; Srinivasan 2015; Buckenhüskes, 2003) .

The interest in bioactive compounds in peppers has increased due their benefits on human health established in several studies, especially about the antioxidant, anti-inflammatory and analgesic properties of capsaicin, which is a bioactive exclusive to *Capsicum* genus peppers (Srinivasan, 2005; Sanatombi; Rajkumari, 2019). The pharmacological biological effects of Capsaicin are widely proved, such as: hypolipidemic and hypoglycemic potential, restoration of resistance and vigor, reduction of obesity and diabetes, thermogenic action, influence and beneficial effects on the gastrointestinal and respiratory systems, fatigue decrease, cancer prevention, among others (Chaiyasit et al., 2009; Srinivasan, 2005; Sanatombi; Rajkumari, 2019, Srinivasan, 2015).

Literature data show significant differences on the content of bioactive compounds in peppers. Distinct techniques used in both extraction and quantification, pepper variety, climatic and atmospheric conditions at the place of cultivation, can influence the content of these compounds and, consequently, the antioxidant properties (Boyukbayram et al., 2006).

For better understanding of genus *Capsicum* peppers and their properties, this chapter compiles data the different types of fruits, characteristics and biological activities already researched.

PEPPERS THE GENUS *CAPSICUM*

The most bibliographies describe that pepper of the genus *Capsicum* are native of the American continent, but the exact origin is controversial, some researchers believe be original is the Amazon basin, while others believe it have arisen could be Central America or Mexico (Bontempo, 2007; Carvalho et al., 2006; Ribeiro et al. 2008).

Capsicum species are divided into three categories: domesticated plants, which have undergone certain genetic changes and do not survive under natural conditions, need human cultivation, semidomesticated plants with a lower degree of dependence on human cultivation and wild ones, present in natural environments, reproduce in abundance, especially in the Amazon region, do not require any human cultivation (SanatombiRajkumari, 2019; Ribeiro et al., 2008).

Genus *Capsicum* has around 27 known species only five domesticated and largely cultivated: *C.annuum var. annuum*, *C. baccatum var. pendulum*, *C. chinense*, *C. frutescens*, and *C. pubescens*; among those, only *C. pubescens* is not cultivated in Brazil (Figure 1). There is three the semi-domesticated species: *Capsicum annuum var. glabriusculum*, *Capsicum baccatum var. praetermissum* and *Capsicum baccatum var. baccatum*, the others are wild species (Ribeiro et al., 2008; Costa; Henz, 2007).

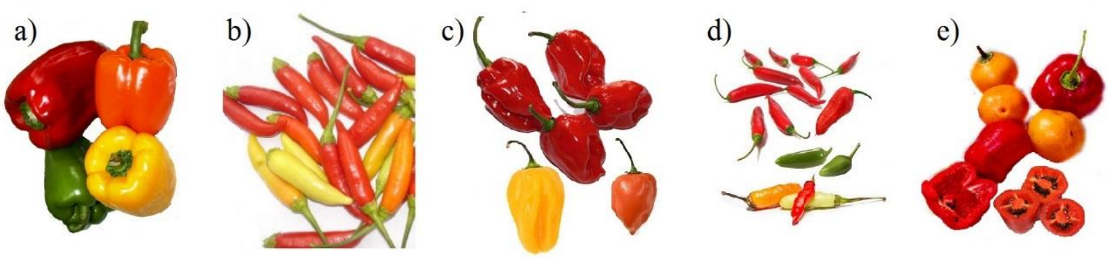

Figure 1. Peppers domesticated of the genus *Capsicum*: *annuum var. annuum* (a); *baccatum var. pendulum* (b); *chinensense* (c); *frutescens* (d); *pubescens* (e). Source: the authors 2020.

It is possible to differentiate species and varieties according to their morphological characteristics, observed mainly in the flowers (Ribeiro et al., 2008; Carvalho et al., 2006). In botanical terms, the fruit is fleshy and involves the seeds in a hollow structure. Their multiple shapes, sizes, colors, and pungencies are responsible for the variation among the different species (Carvalho et al., 2006; Pinto et al., 2016).

Capsicum pepper is cultived of out worldwide. Of the five domesticated species, the cultivation of the species *C. annuum var. annuum* is widely developed in Mexico and Central America; *C. frutescens* in the southeastern Brazil as far as Central America; the variety *C. baccatum var. pendulum* has its largest production in Bolivia and southeastern Brazil; *C. chinense*, is the most Brazilian of the domesticated species, originaty

Amazon Basin and *C. pubescens* is grown in Peru, Bolivia, Chile, Argentina and Ecuador (Carvalho et al., 2006; DeWitt; Bosland, 2009, Thampi 2004).

In Brazil, *Capsicum* peppers are produced in almost all states, the main producers are: Minas Gerais, São Paulo, Goiás, Ceará and Rio Grande do Sul. Part of this production is exported from different forms, such as paprika, paste, dehydrated and ornamental preserves (Ribeiro et al., 2008).

Pungency is an important attribute of the fruits of the genus *Capsicum*, it comes from alkaloids, present only in this genus (Jarret et al., 2007). Capsaicin and dihydrocapsaicin are the main capsaicinoids responsible for up to 90% of the total pungency of the peppers fruits, they differ only by a double bond in the aliphatic chain of capsaicin making it less lipophilic than dihydrocapsaicin (Garcés-Claver et al., 2006; DeWitt; Bosland, 2009). Besides them, at least nine smaller capsaicinoids have already been found, they are: norcapsaicin, nordihydrocapsaicin, homocapsaicin I, homodihydrocapsaicin I, homocapcaicin II, homodihydrocapsaicin II, n-vanillyl nonanamide, n-vanillyl octanamide and n-vanillyl. Figure 2 shows the chemical structures of the most important (Garcés-Claver et al., 2006).

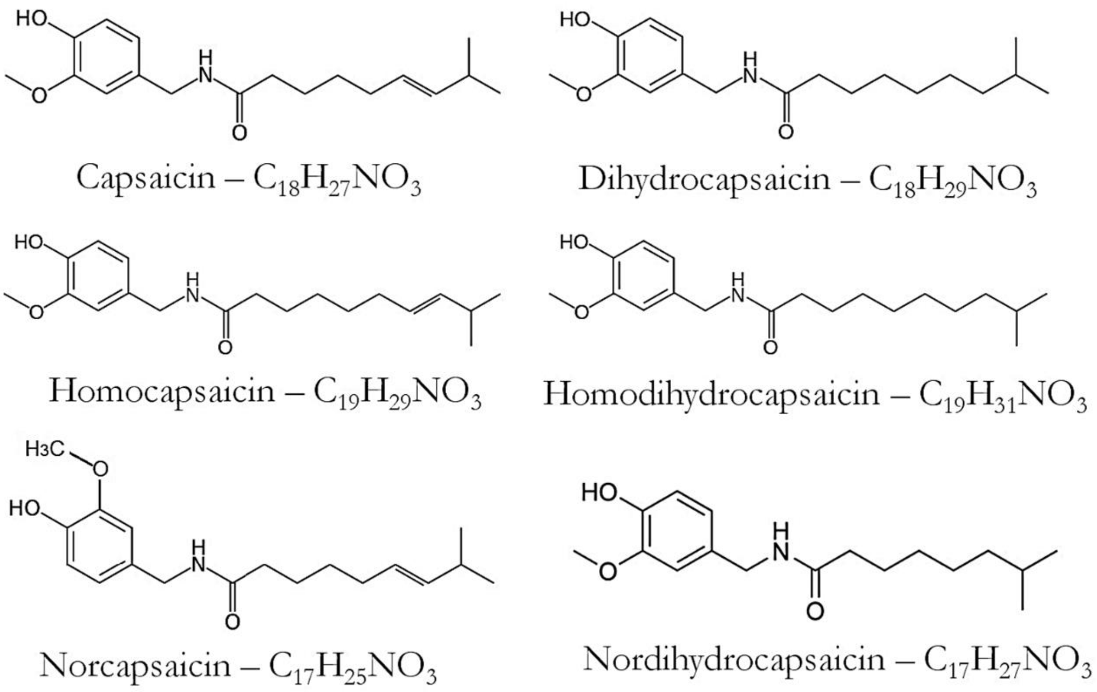

Figure 2. Molecular structure of the main capsaicinoids in *Capsicum* peppers. Source: the authors 2018.

The pungency can be measured in using specific devices such as LC (high efficiency liquid chromatography), by Scoville Heat Units in scale ('Scoville Heat Units-SHU') or for by Scoville organoleptic test (dilution and proof procedure). The SHU value can vary from zero to 15,000.00 (pure capsaicin) of Scoville units, the higher the value of Scoville heat units, the greater the concentration of capsaicin and consequently the more pungent it´s the pepper (Carvalho et al., 2006; Barnett, 2006).

Five levels of pepper pungency can be considered in relation to the Scoville scale, measured in heat units (SHU): 0-700 (not pungent), 700-3,000 (slightly pungent), 3,000-25,000 (moderately spicy), 25,000-70,000 (highly pungent), and greater than 80,000 (extremely spicy) (Scoville, 1912; Gonzalez-Zamora et al., 2013). Table 1 shows the qualifications of the five domesticated species of *Capsicum* peppers.

Table 1. Different varieties of *Capsicum* peppers and their specifications. Source: the authors 2010.

Variety	Types of peppers	Origin and production	Characteristics	Use	Pungency (SHU)	References
Annuum var. *annuum.*	Bell pepper, caiena, jalapeno, cherry Pepper, chili, cone Pepper, creen capsicum, red Pepper.	Native of North America and the north of South America. Produced worldwide.	Species encompass different types, colors, and sizes of peppers, the pungencies variety from litle to very peppery.	Smaller peppers are more pungent, used mainly as condiment; non pungent bell peppers are used in salads and food preparation.	Variation of around zero for bell pepper (low pungency) to 40,000 for caiena (high pungency)	DeWitt; Bosland, 2009; Lim, 2013; Reifschneider et al., 2009; Barnett, 2006.
Baccatum var. *pendulum*	Aji pepper (aji rojo), cambuci, peri-peri pepper, peperoncini, chifre de veado.	Native of South America, especially Peru and Bolivia. Largely cultivated in Argentina, Colombia, Equator, and Brazil.	Mildly pungent, the fruits are generally small, oval or round; The fruits are red when ripe.	When fresh are used in sauces and cannings, when dehydrated (in flakes) is used as seasoning.	Varies from 5,000 to 50,00 Scoville units to ají amarillo chili and aji.	Reifschneider et al., 2009; Costa; Henz, 2007; Barnett, 2006; Lim, 2013.
Chinensense	Habanero, malagueta, murici, bode, murupi, cumari do Pará (yellow chili).	Typical to the Amazon Basin. Largely cultivated in Brazil and Mexico.	Great variability of forms (round and oval), colors (from yellow to red), and pungency.	Highly used in the preparation of pepper sauces.	Soft to strong, reaching up to 500 thousand Scoville units for habanero, one of the most peppery in the world.	Costa; Henz, 2007; Carvalho et al. 2006; Barnett, 2006; Lim, 2013
Frutescens	Tabasco, cayenne pepper, piri piri, jindungo pepper, african chillies.	Native of tropical regions of America. Produced majorly in Brazil, Portugal, Africa, and South of Asia.	Low morphological variation, generally small, red, tapered and conical fruits.	Largely used in the production of canning and pepper sauces.	High pungency content, with variation from around 50,000 to 100,000 Scoville units.	Costa; Henz, 2007; Carvalho et al., 2006; Barnett, 2006; Lim, 2013.
Pubescens	Rocoto or locoto pepper, manzano, caballo, peron pepper.	Native of Bolivia, found mainly in South America and Central America. Develops well in subtropical climates and is resistant to low temperatures.	It is the most distinguished of the *Capsicum* species. Has Black seeds and present hairiness on the pulp.	Used as condiment for specific dishes, such as ceviche.	Extremely pungent (30,000 to 50,000 Scoville units).	(DeWitt; Bosland, 2009; Barnett, 2006; Yamamoto et al., 2013).

ASSESSMENT THE NUTRITIONAL AND FUNCTIONAL CHARACTERISTICS OF PEPPERS

Several vegetables are considered as potential sources of bioactive compounds with antioxidant activity. The antioxidants favor human health and food preservation. The action of antioxidants in our body or in the food itself is to reduce the oxidation of active substances, such as free radicals, some antioxidants block chain reactions by reacting with free radicals, there are also antioxidants that empower effects of other compounds present in the product (Baenas et al., 2018; Pieroni et al. 2004). Free radicals are unstable molecules that have an unpaired chemically reactive electron that tends to quickly associate with other positively charge molecules with which it can react or oxidize, antioxidants act by donating an electron and the free radical is neutralized (Fiedor; Burda, 2014; Baenas et al., 2018, Haminiuk et al., 2012).

Our cells produce free radicals during the process of burning the oxygen, this process occurs to convert nutrients absorbed from food into energy. Our organism has enzymes capable of controlling the action of free radicals and keeping the body stable. However, some external factors such as incorrect diet, intake fatty foods or with many chemical additives and alcohol consumption, contribute to the excess of free radicals. Peppers are excellent sources of various health-related compounds, such as vitamins A, E and C, fatty acids, folic acid, zinc, carotenoids, polyphenols, flavonoids, specific alkaloids such as capsaicinoids, that providing to fruits antioxidant properties considerables (Bontempo, 2007; Marti et al., 2011).

Bioactive compounds, as well as capsaicionoids, are derived from the phenylpropanoid pathway (Arora et al., 2011). Carotenoids, phenolic acids, capsaicinoids and flavonoids are the main phytochemicals investigated and found in peppers. They are synthesized by plants as a result of adaptation to abiotic and biotic stress (Shetty, 2004). Phenolic acids, such as caffeic acid, cinnamic acid and ferulic acid, flavonoids, such as myricetin and quercetin, are the very abundant in peppers (Asnin; Park, 2015). The capsaicinoids of peppers are amides, which, in part, are derived from a phenolic portion, Sora et al. (2015) state that there are correlations between the levels of capsaicin and dihydrocapsaicin with the content of phenolic compounds and antioxidant activity obtained in different types of peppers of the genus *Capsicum*. Therefore, greater the pungency, the greater the concentration of capasaicinoids in the fruit.

These compounds have a high antioxidant power in addition to several physiological and pharmacological properties (Guil-Guerrero et al. 2006; Baby; Ranganathan 2016; Baenas et al., 2018). Figure 3 illustrates the oxireduction effect of the main alkaloids of peppers of the genus *Capsicum*.

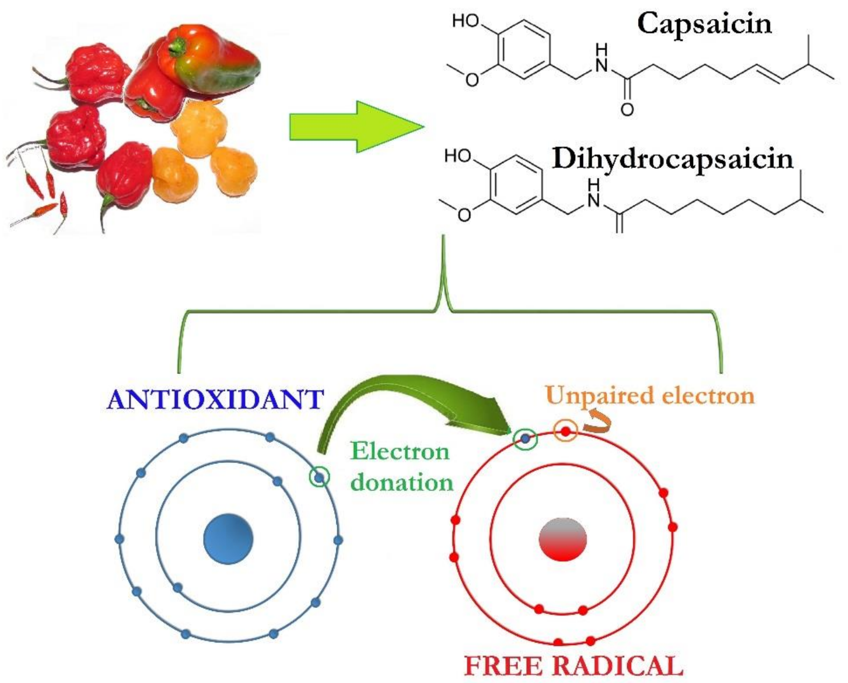

Figure 3. Antioxidant action of capsaicinoids in reducing free radicals. Source: the authors 2020.

Kobata et al. (2013) describe in their study capsinoids and their dihydro-capsiato and nordihydrocapsiate derivatives, which are non-spicy compounds found only in some varieties of sweet or non-pungency peppers (*Capsicum annuum var. Annuum*), similar to capsaicinoids, your main structure contains essential a fatty acid ester with vanyl alcohol. A promising alternative for those who abstain from foods containing capsaicin due to pungency (Ludy et al., 2012). Their mechanisms of action are still poorly investigated, however, they demonstrated considerable antimicrobial activity (Bacon et al., 2017).

The choice of the bioactive compounds extraction method is not always a process that the authors fully explain, the decision possibly being defined according to the convenience or availability of the equipment. However, it is essential to evaluate the extraction method used, since it can have a direct influence on the analysis result (Asnin; Park, 2015).

Table 2. Comparison of modern methods for extracting bioactive in peppers from the genus *Capsicum*. Source: the authors 2020.

Method	Description	Advantages and disadvantages	References
Ultrasound assisted extraction (UAE)	Occurs by action to mechanical waves in low frequency, which are responsible for generation and collapse of microbubbles causing punctual areas high pressure and temperature in the solution, breaking up vegetables cells favoring extraction.	Reduced analysis time, simple, inexpensive and efficient, but it is difficult to control the system temperature, as the bath water heats up with use and also the energy supplied for extraction is not easily quantifiable.	De Aguiar et al., 2016; Barbero et al., 2008.
Extraction with supercritical fluid (SFE)	A fluid is used, usually carbon dioxide (CO2) in supercritical conditions, which has intermediate properties between gas and liquid, allowing the greater solvency power of the fluid used in the extraction.	Extract free of organic solvent residues; ability to separate different fractions (capsaicinoids and carotenoids) at the same time; possibility of extracting thermolabile compounds; limited capacity to extract polar compounds.	Saini; Keum, 2018; De Aguiar et al., 2016.
Microwave assisted extraction (MAE)	Energy obtained by electromagnetic radiation is used, which is converted into heat, promoting an increase in temperature and pressure, breaking membranes to extract the compounds of interest.	It is a fast and economical method, but depending on the frequency used it can cause thermal degradation and cis-trans isomerization of carotenoids.	Bendjersi et al., 2016; Barbero et al., 2006.
Enzymatic assisted extraction (EAE)	Suitable enzymes are used to catalyze reactions with specificity and regioselectivity under mild processing conditions and in aqueous media.	Organic solvents are not used, which results in higher extract quality, low energy consumption, but expensive multi-enzyme preparations are required and is only possible extraction in small volumes due to enzymatic availability in completely hydrolyzing cell walls.	Baiano 2014; Baenas et al., 2018.
Pressurized liquid extraction (PLE)	Use of solvents at elevated temperatures, using pressure to keep the solvent in a liquid state above the boiling point. The pressure facilitates the transport of the solvent into the matrix improving the solubility of the target compound.	Smaller quantity solvent and extraction time, but the use of high temperatures can compromise the thermolabile compounds.	Mustafa, Tuner, 2011; Barbero et al., 2006.

Several researches point the importance of optimizing extraction parameters for each matrix evaluated, especially the sample quantity, solvent volume, temperature and time (Barbero et al., 2006; Sora et al. 2015; Haminiuk et al. 2012). The choice of the appropriate solvent is one of the crucial steps for efficient extraction, one must consider the polarity of the compounds of interest must be considered, the concentration, stabilitythe compounds and chemical composition of the analyzed vegetable (Saini, Keum, 2018).

The literature offers a wide variety of methods for the extraction and characterization of bioactive compounds in peppers (fresh, dried and crushed) (Yamamoto et al., 2013; Baenas et al., 2018). The most cited classic methods are Soxhlet extraction, maceration and magnetic stirring, and modern methods such as ultrasound assisted extraction (UAE), extraction with supercritical fluid (SFE), microwave assisted extraction (MAE), enzymatic assisted extraction (EAE) and pressurized liquid extraction (PLE) (Saini; Keum, 2018; Barbero et al., 2008).

Baenas et al. (2018) describe that classic methods provide efficient extraction of bioactive substances, but require a long extraction time and a large amount of solvents, factors that limit their application due to their high cost, due to environmental and safety issues. Modern methods, on the other hand, require little extraction time with minimal use of organic solvent, with less impact on the environment. In addition to obtaining extracts of greater purity, with the highest total yield when compared to traditional methods (Saini, Keum, 2018). Table 2 describes some modern extraction methods.

There many bioactive compounds present in peppers, among the main ones studied are the phenolic compounds (flavonoids, phenolic acids, tannins, among others), ascorbic acid and carotenoids. All of these compounds are widely evaluated by colorimetric methods using spectrophotometry that employ reactives specific as chromophores, reagents and organic salts that react with the compounds to be determined (Ignat et al., 2011; Naczk; Shahidi, 2004).

These analytical methods are widely used for purposes of quantification and general detection of phenolic content, among others, as they are relatively simple and low cost methodologies and if used correctly they are considered effective, with good reproducibility of the results (Haminiuk et al., 2012; Ignat et al., 2011).

Among the most used methodologies for the analysis of phenolic compounds, many authors use the Folin-Ciocalteau reagent assay, the chemical composition of this reagent includes phosphomolybdic acid and phosphotungstic acid, which are reduced from the tested extracts. An alkaline pH medium allows reducing substances (phenolic) to dissociate a proton, leading to the formation of the phenolate anion. This anion is capable of reducing the Folin-Ciocalteu reagent to form molybdenum oxide and tungsten oxide. These oxides have a blue tint that is detectable in the spectrum band at approximately 760 nm, enabling the quantification of these substances through spectrophotometry (Huang; Prior 2005; Singleton; Rossi, 1965; Haminiuk et al., 2012).

Table 3. Phenolic compounds quantified in different peppers por methodologies distinct by the spectrophotometric. Source: the authors.

Sample and Reference	**Condition**	**Solvent**	**Total phenolic**
Ripe Caiena (*C. Annuum*) (Bae et al., 2012).	Lyophilized	Acetone	65.9[a]
Ripe Caiena (*C. Annuum*) (Bae et al., 2012).	Lyophilized	Methanol and water (80 and 20)	35.7[a]
Ripe Jalapenho (*C. Annuum*) (Bae et al., 2012).	Lyophilized	Ethyl acetate	51.6[a]
Ripe Red pepper (*C. annuum,* L. var. *Hungarian*) (Vega-Gálvez et al., 2009).	Fresh	Water	1.359[b]
Ripe Jalapenho pepper (C.*annuum*) (Sandoval-Castro et al., 2017).	Lyophilized	Ethanol and water (80: 20)	1,312[b]
Ripe Cayenne pepper (*Capsicum frutescens* L) (Santos et al., 2015).	Fresh	Super citric fluid	0.858[b]
Ripe C. frutescens var. baccatum (Olatunji; Afolayan, 2019)	Fresh	Water	90.86[b]
Ripe C. frutescens var. baccatum (Olatunji; Afolayan, 2019)	Fresh	Ethanol	221.21[b]
Ripe Habanero (*C. Chinese*) (Oboh et al., 2007).	Fresh	Water	103.2[d]
Green Habanero (*C. Chinese*) (Oboh et al., 2007).	Fresh	Water	73.7[d]
Ripe Habanero (*C. Chinese*) (García-López et al., 2019)	Fresh	Acetone	1,286[b]
Ripe bell pepper (*C.annuum*) (Lin; Tang, 2007).	Lyophilized	Water	180.3[d]
Green bell pepper (*C.annuum*) (Lin; Tang, 2007).	Lyophilized	Water	206.0[d]
Ripe chili spur bell pepper *(C.annuum*) (Wangcharoen; Morasuk, 2007).	Fresh	Ethanol and water (95 and 5)	1.85[b]

a = mg equivalent of catequin per g of extract. b = mg equivalent of Gallic acid per g of fresh sample.
c = µmol equivalent of catequin per g of fresh sample. d = mg equivalent of Gallic acid per 100 g of fresh sample.

Table 3 indicates some quantifications of the phenolic compounds found in different types of pepper, processes, and methodologies of extraction assessed through the Folin Ciocalteau method.

The analysis of the data presented in Table 3 reveals considerable variation in the phenolic quantification of peppers by the same identification method, which is attributed to a series of factors, such as the type of sample, extraction method, fruit condition (fresh, lyophilized, ripe, green), solvent used, time of extraction, fruit variety. Even considering so many variations reinforce the importance of if optimizing the extraction of bioactive compounds to obtain optimal conditions of planning, since the colorimetric methodology only quantifies of bioactive extracted.

In 1990, the first article to use liquid chromatography to analyze peppers was published (Wehmeyer et al., 1990). The authors evaluated the pungent principle of *Capsicum* pepper to develop a new class of analgesics, with tests in vitro and later in mice, this ex vivo research was carried out to determine the main route of metabolism of these new compounds (intestinal and/or hepatic).

Instrumental methodologies like: such as high performance liquid chromatography (LC), LC coupled with mass spectrophotometer, gas chromatography, high efficiency liquid chromatography in reverse phase, mass spectrometry, capillary electrophoresis, among others, can be are used in the individual determination of bioactives in peppers for analytical separation, quantification and identification of capsaicinoids and other bioactive substances (Mendes et al., 2019; Baenas et al., 2018; Sora, et al. 2015; Slatnar et al., 2014).

Advanced analytical techniques, such as capillary liquid chromatography and micellar electrokinetic chromatography, can be useful for analyzing compounds in peppers (Tolstikov et al., 2003; Elder et al., 2010). There is still no definition of an ideal technique for this type of study, it is essential to conduct more research on the pepper extract matrix and to develop validation of new analytical protocols to offer advantages over these methods (Asnin; Park, 2015).

Instrumental analyzes, especially chromatographic, demand high value both in the acquisition of equipment and in the maintenance and preparation of samples, a factor that may be a limitation for some laboratories, however, they are able to perform the separation and individual identification of each bioactive compound in the sample, while colorimetric methods only detect and quantify them in general (Mendes et al., 2019; Asnin; Park, 2015; Ignat et al., 2011).

The antioxidant capacity of peppers are evaluated especially by employing chemical tests in vitro, such as reduction of the antioxidant capacity using copper (CUPRAC), absorption capacity of oxygenated radicals (ORAC), Ferrous Ion Reduction Method (FRAP), organic radical removal capacity (ABTS), peroxidation of 2.2 - diphenyl - 1 - picrylhydrazyl (DPPH), and inhibition of oxidation of β - carotene in the presence of linoleic acid (system β - carotene linoleic acid) (Haminiuk et al., 2012). A comparison of the tests in vitro applied to assess the antioxidant activity in several studies that evaluated peppers reveals that systems β-carotene and DPPH are more commonly used.

In order to verify the antioxidant capacity of peppers, the analyses encompass the assessment of the quality of phenolic compounds identified in the samples as well as the guarantee that such compounds correspond to the bioactive substances that provide the fruits with antioxidant capacity.

Due to the diversity of bioactive compounds available in peppers, with different mechanisms of action, it is very important to choose on a method what verify the antioxidant action of the existing compounds. It is recommended to employ more than one method for a safe, accurate representation of the actual antioxidant activity of the samples (Haminiuk et al., 2012; Sucupira et al., 2012). However, many authors studying peppers found high correlations among the methods employed (Medina-Juarez et al., 2012; Sora et al., 2015), which is an indication of redundancy when using three methods to assess antioxidant activity.

Sora et al. (2015) researched different *Capsicum* peppers regarding their antioxidant capacity by applying the DPPH, ABTS, and FRAP methods and found that the FRAP system is the most adequate for this type of assessment.

The assessment of the antioxidant activity using biological tests (ex vivo), with both mice and cells, although not common, is important to understand the actual benefits to health. Assessing the antioxidant activity with cell tests includes the development of a model of cell culture to quantify the antioxidant activity in a more biologically representative way than the conventional methodologies for the chemical capacity of antioxidants (in vitro). In animal tests, the pepper or its extract will be inserted into your diet for further evaluation of the effects (Yaffe, et al., 2013; Maistro et al. 2011).

Table 4. Research data that evaluated the antioxidant activity of peppers ex vivo. Source: the authors.

Sample	**Application**	**Culture (*ex vivo*)**	**Results**
Ethiopian pepper, seeds of *Ashanti* and *guineense* peppers (Adefegha; Oboh, 2012).	Methanol extract and HCl (1:1)	Diet for mice	Diet supplementation with etíope pepper proved more promising due to higher phenolic quantification. Values ex vivo were higher than values *in vitro*.
Bell pepper (*C. baccatum*) (Matsuo; Hitomi, 2007).	Soup	Diet for mice	The antioxidant activity ex vivo was higher than ex vivo.
Red bell pepper, green bell pepper, and chili pepper (Wei et al., 2010).	Acetone extract and water (1:2)	Cell cultures – HepG2	Red bell pepper presented higher antioxidant activity and phenolic composition.
Red pepper (Seori; Kyung-Hyun, 2011).	Aqueous extract	Diet for fish	The extracts presented good antioxidant activities both ex vivo and in vitro; a cholesterol reduction occurred.
Capel Hot (*Capsicum annuum* L., cv.) (Materská et al., 2015).	Ethanol solution (80:20)	Cell culture of human blood	The compounds presented weak antioxidant activities, but high radioprotector potential.
Black pepper (Hwang et al., 2011).	Different aqueous solutions	Human cells of fibrosarcoma HT - 1080.	Piperine indicated potential antioxidant against cancer.
Capsicum annuum L. (Jang et al., 2011).	Solution with ovalbumin	Diet induced with oral probe in mice	The results suggest that the extract can be an efficient oral treatment for allergy inflammation of the respiratory tract because of its antioxidant activity.

Pure capsaicin (Kim; Moon, 2004).	Not reported in the study	Tratamento em células MCF10A	Capasaicin was suggested to have strong chemoprevention characteristic for breast cancer.
Piper longum L (Zou et al., 2016)	Piperlongumine (PL).	In human gastric cancer cells and mices.	PL treatment markedly reduces tumor cell burden. Overexpressed in gastric cancer cell lines and human gastric cancer tissues.
Piper longum L (Wang et al., 2019).	Piperlongumine (PL).	colorectal cancer and tumor-bearing mice	The tests improved the tumor response to both single and fractionated radiation, resulting in a significant increase of survival rate of tumor-bearing mice.

There is research that has studied the behavior of these antioxidants in cell culture, mice, fish, and some more recent ones have tested in humans, all of which have shown positive results regarding the preservation of oxidation. Table 4 lists some descriptions for each publication. The quantification and validation of antioxidant activity, especially ex vivo, are necessary to evaluate the phenolics and alkaloids that determine the pungency of each pepper, since the spicy characteristic of the fruits is an important attribute of each variety and is related to its antioxidant activity (Jarret et al., 2007). It is suggested carry out research comparing peppers with varying pungencies in ex vivo systems.

BENEFITS OF PEPPERS

After described the antioxidant properties in relation to the bioactive contained in *Capsicum* peppers, especially capsaicinoids it is confirmed that these fruits have chemopreventive potential evidenced in research and numerous health.

Benefits the bioavailability of the components of the peppers is extremely important because this determines the amount and speed in which the active ingredient will be absorbed, getting available for its performance as an antioxidant. Hervert-Hernández et al. (2010) investigated the in vitro intestinal bioavailability of carotenoids and polyphenols from red peppers and related that the amount of antioxidants relesed by the action of digestive enzymes was about this 75% for total polyphenols and up to 49% for β-carotene among others, so 50 to 80% of these compounds are bioavailable to reach the colon and be fermented potentially. Regarding capsaicin and dihydrocapsaicin, its absorption rate in ex vivo evaluations was 80% in the jejunum, 70% in the ileum and 50% in the stomach (Rollyson et al., 2014).

Ludy and Mattes (2011), Westerterp-Plantenga et al. (2005) and Yoshioka et al. (1998), claim that a daily consumption between 1-3.5 g of peppers is already sufficient to guarantee the beneficial effects of the fruit. These studies demonstrate benefits especially in relation to weight control, reinforcing thermogenesis and increasing fat oxidation. In addition, a small oral dose of capsaicin demonstred therapeutic action and prevent oxidative damage in rat livers (Giri, et al., 2017).

Peppers stimulate appetite and benefit digestion with is alkaloids that cause burning, inflammation and desensitization, stimulating sensory nerves. Intake of peppers increases salivation and stimulates gastric secretion and gastrointestinal motility, in addition to releasing endorphins that promote a feeling of well-being after eating (Sim; Sil, 2008).

The fat-soluble compounds contained in peppers act as natural antioxidants due to the reducing force of the hydroxyl group and capture superoxide anions or free radical reducing agents, drifting in less reactive radicals, consequently stimulating the immune system by delaying the aging process, among other activities biological (Chuah et al., 2008; Podsedek, 2007; Deepa et al., 2007).

Capsaicionoides and other bioactive substances present in peppers, especially capsaicin, had their anti-inflammatory activities proven in in vitro and ex vivo tests, proinflammatory substances were reduced and necrosis was observed in tumors, collaborated with immune responses related to infections from cancer, inflammatory dieses and autoimmune diseases (Allemand et al., 2016).

The carotenoids contained in red peppers, among other compounds, were tested in rats with adjuvant-induced inflammation and it was observed that they played an important role in regulating blood pressure in addition to reducing the fraction of mucoproteins present occur in different inflammatory processes (Boiko, et al., 2017). Therefore, the intake of products containing capsaicinoids helps to prevent inflammation and oxidative stress in the human body, preventing the development of chronic and neurodegenerative diseases.

CONCLUSION

Peppers are spices consumed all over the world, they are considered nutritious and functional foods because they contain alkaloids and phenolic compounds as main components. In this chapter several methodologies for extraction and quantification of bioactives in peppers of the genus *Capsicum* have been reported.

Most studies with peppers evaluate their antioxidant properties by in vitro methods of quantification of total phenolics, little data is found on the identification of active molecules of these matrices. There is demand for research with more precise on the main alkaloids of the peppers most produced and consumed and real proof of antioxidant activity either in ex vivo experiments or in food products.

The application of its main alkaloids in foods, drugs, cosmetics should be evaluated for expanding the resources of these compounds in the market.

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ÍNDICE REMISSIVO

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V

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