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Published online: 26 Mar 2020.

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REVIEW

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# Factors affecting the capsaicinoid profile of hot peppers and biological activity of their non-pungent analogs (Capsinoids) present in sweet peppers

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## ABSTRACT

Capsaicinoids are acid amides of $C_9$–$C_{11}$ branched-chain fatty acids and vanillylamine and constitute important chemical compounds of *Capsicum annuum* together with their non-pungent analogs (capsinoids) which have an impressive list of health benefit properties (i.e., analgesia, anti-obesity, thermogenic, cardiovascular, gastrointestinal, antioxidant, anti-bacterial, anti-virulence, anti-inflamatory, anti-diabetic, inhibits angiogenesis, and improves glucose metabolism) . In this review, the state of art on how capsaicinoids are affected by different pre- and postharvest factors is discussed together with their biological activity. For instance, high light intensity and heat treatments may reduce capsaicinoid content in fruits probably due to the loss of activity of capsaicin synthase (CS) and phenylalanine ammonia lyase (PAL). The pungency in peppers varies also with environment, genotype or cultivar, node position, fruiting and maturity stages, nitrogen and potassium contents. As the fruit mature, capsaicinoid levels increase. Fruits from the second node tend to have higher accumulation of pungency than those of other positions and the pungency decreases linearly as the node position increase. Sodium hydroxide treatment reduces the pungency of pepper fruit as it hydrolyzes and modifies one of the features (vanillyl group, the acid-amide linkage and alkyl side chain) of capsaicin molecule. Salt and water stress increase PAL and capsaicin synthase activity and increase the capsaicinoid accumulation in fruit, by negatively regulating peroxidase activity at appropriate levels. Future research must be directed in better understanding the changes of capsinoids during pre and post-harvest management, the causal drivers of the loss of activity of the aminotransferase gene (*pAMT*) and if possible, studies with genetically modified sweet peppers with functional *pAMT*. Available data provided in this review can be used in different agricultural programs related to developing new cultivars with specific pungency levels. The contents of capsaicinoids and capsinoids in both fresh fruits and marketed products are also of remarkable importance considering the preferences of certain niches in market where higher added-value products might be commercialized.

## Introduction

### Origin of capsicum annuum

*Capsicum annuum* (L.) belongs to the nightshade (Solanaceae) botanical family (Rudrappa 2016) and is one of the five domesticated pepper species, in which include *Capsicum baccatum, Capsicum chinense, Capsicum frutescens and Capsicum pubescens* (Kraft et al. 2014). They have been cultivated for more than 9000 years, with the earliest cultivation having taken place in South and Central America (George Mateljan Foundation (GMF) 2018). Mexico was probably the center of origin and domestication of *Capsicum annuum* in the narrow sense, based on archeological, paleoclimatic, mid-Holocene, linguistic, and genetic data (Kraft et al. 2014), while *Capsicum chinense* originated

in the Amazonian region and *Capsicum frutescens* in the coastal region at the Southern part of South America (Grubben and Denton 2004; Kraft et al. 2014; Zonneveld et al. 2015). Peru and Bolivia constitute the primary center of diversity of cultivated capsicum, center of origin of *Capsicum pubescens* and one of America's wild pepper hotspots (Zonneveld et al. 2015). Shortly, after the discovery of America by Christopher Columbus, the Spanish and Portuguese took capsicum pepper (hot and sweet ones) to Europe, from where especially the hot pepper was dispersed to all tropical and subtropical areas worldwide. By the end of 17th century, it was grown as popular vegetable and spice everywhere in the tropics and several distinct types and landraces have been developed (Grubben and Denton 2004). Species less frequently under cultivation include *Capsicum*

*baccatum* and *Capsicum pubescens* (World of Chillies 2016). Capsicum presents a wide biodiversity and the chemical composition within the genus presents also a great variability (Antonio, Wiedemann, and Veiga Junior 2018).

## Importance and nutritional value of peppers

Capsicum fruits are consumed fresh, dried or after processing. Non-pungent fruit (pungency ranging from zero to very minimal hotness - Rudrappa 2016), usually called sweet peppers are eaten raw in salads, but more commonly cooked, fried or processed together with other foods. They are consumed in such quantity per serving that they constitute a real table vegetable contributing to the nutritional value of the meal. The most pungent types including chilies, bird pepper, and aromatic hot peppers are consumed in small quantities, being considered as condiment or spice for seasoning and stimulating appetite. As many intermediate forms exist, there is no strict borderline between the use of capsicum as spice and vegetable (Grubben and Denton 2004; Kang and Kole 2013).

Peppers (hot or sweet) contains an impressive list of nutrients on which disease preventing and health promoting properties have been claimed (Lang et al. 2009; Wahyuni et al. 2011). Among others, the hot peppers contain small amounts of the health promoting alkaloid capsaicin ($C_{18}H_{27}NO_3$, 8-methyl-*N*-vanillyl-6-nonenamide), which confers a pharmacodynamic as well as organoleptic properties and the sweet peppers contain capsinoids, a non-pungent analog of capsaicin with valuable biological activity (Lang et al. 2009; Nilius and Appendino 2013).

Fresh sweet peppers, red or green, are rich sources of vitamin C. This vitamin is especially concentrated in red peppers at the highest levels. It also contains good amounts of vitamin A. In addition, it contains antioxidant flavonoids such as luteolin, quercetin, hesperidin; carotenoids ($\alpha$- and $\beta$-carotene, lycopene, lutein, zeaxanthin, and cryptoxanthin) and hydroxycinnamic acids (ferulic and cinnamic acids). Sweet pepper has adequate contents of essential minerals as, for instance, iron, copper, zinc, potassium, manganese, magnesium, and selenium. Further, sweet peppers are also a good source of B-complex group of vitamins, such as niacin, pyridoxine, riboflavin, pantothenic acid, biotin, folate, cobalamin and thiamin (George Mateljan Foundation (GMF)), 2018; Rudrappa 2016; Wahyuni et al. 2011; Guzman et al. 2010; Camara et al. 1980a, 1980b – see Table 1).

According to the USDA National Nutrient Database for Standard Reference (Release April, 2018), the nutritional composition of green sweet pepper in raw state is (per 100 g of edible portion) 93.89 g water, 0.86 g protein, 0.17 g total lipids (fat), and 0.43 g ash. In minerals, 10 mg calcium, 175 mg potassium, 20 mg phosphorous, 0.34 mg iron, 10 mg magnesium, 3 mg sodium, and vitamins 7.4 µg vitamin K, 0.37 mg vitamin E (alpha-tocopherol), 0.48 mg niacin, and 10 µg folate. Interestingly, sweet pepper is also rich in carbohydrates, i.e., 6.64 g, with 2.40 g total sugars and 1.70 g total dietary fiber, corresponding to an energy source of 20 kcal. Regarding the vitamins, the total amount of ascorbic acid (vitamin C) is 80.4 mg and vitamin A is 370 IU (18 µg RAE) per 100 g of edible portion. However, like other vegetables, they are quite perishable, resulting in high losses in post-harvest periods due to storage problems, marketing, and inappropriate processing technologies (Faustino, Barroca, and Guiné 2007; USDA, 2016). There is a huge intra-specific (natural hybridization due to evolution processes and human intervention) and inter-specific (endogenous and exogenous factors) chemical variability within each species (Antonio, Wiedemann, and Veiga Junior 2018).
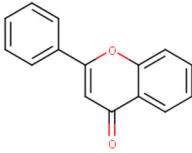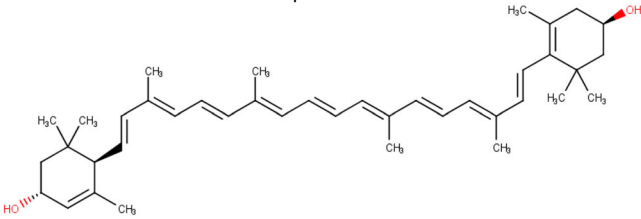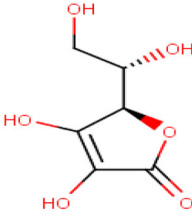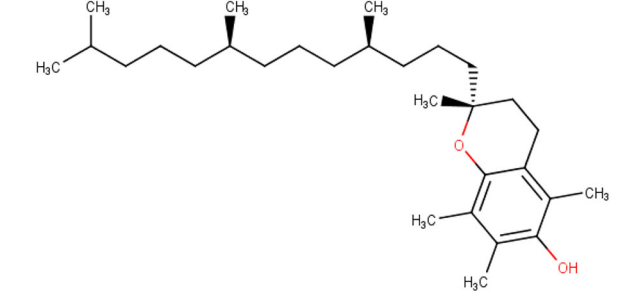
## Capsaicinoids and capsinoids: Diversity, biosynthesis and application

### Capsaicinoids diversity

Capsaicin itself and dihydrocapsaicin are the dominat capsaicinoids present in all the hot peppers studied (80–90%; Barbero et al. 2016; Roth 2014; Usman et al. 2014). Antonious and Jarret (2006) evaluated ninety accessions of peppers including *chinense, frutescens, baccatum* and *annuum* and found that the concentration varies greatly. Such findings were also reported by Zewdie and Bosland (2001) while researching 200 accessions and Reilly, Crouch, and Yost (2001). These two compounds also produce the greatest sensory impact and their contents also determine the overall pungency of a pepper. Nevertheless, minor components also make a contribution to the overall pleasure conferred owing to their diverse sensory characteristics (Garcés-Claver et al. 2006; Kosuge and Furuta 1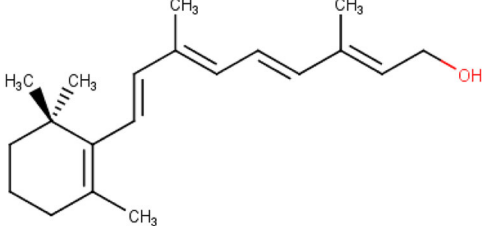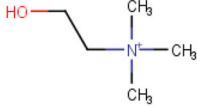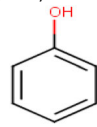970; Othman et al. 2011; Peña-Alvarez, Ramírez-Maya, and Alvarado-Suárez 2009; Roth 2014). The ratio of capsaicin to dihydrocapsaicin is species specific: 1: 0.8 in *C. annuum*, 2: 1 to 1: *1.5 in* C. baccatum*, 2.5: 1 in* C. chinense/frutescens, and 1: 1.5 in* C. pubescens *(Roth 2014). Extensive reports exists with regard to the percentage of capsaicinoid components with emphasis on capsaicin, dihydrocapsaicin, nordihydrocapsaicin and homodihydrocapsaicin in *Capsicum annuum, Capsicum frutescens,* C. *baccatum* and C. *pubescens*. According to Hui et al. (2010) the ratio is 1:1 for *Capsicum annuum*, 2:1 for C. *frutescences* and *baccatum* and 0.7:1 for C. *pubescens*, which shows that is there a variation within the genus. As it can be observed in Table 2, the amount of other capsaicinoids (norhydrocapsaicin and homodihydrocapsaic) is lower than 20% of total capsaicinoids present in peppers (Table 2). The pungent oral sensation (measured in Scoville scale and reported in Scoville Heat Units - SHU, as a function of capsaicin concentration) of capsaicin and dihydrocapsaicin increases rapidly in the middle and rear portions of the tongue and gums, and persists for a long time, whereas for homodihydrocapsaicin the effect develops more slowly, and only in the rear of the mouth. Nordihydrocapsaicin is perceived as milder in the frontal region of the tongue, and its effect diminishes more rapidly (Table 2). According to Yazawa et al. (1989), most pungent lines of peppers also produce the non-pungent analogs of capsaicin (capsinoids) but in trace amounts.

Studies with isotopically labeled metabolites have shown that the amino acid phenylalanine is the starting point for

**Table 1.** In depth nutritional profile of sweet peppers.

| Sweet peppers | Chemical group of nutrients |
|---|---|
| | Flavonoids<br>B-complex vitamins<br>Carotenoids<br>Vitamin C<br>Omega 3 and 6<br>Vitamin E<br>Vitamin K<br>Vitamin A<br>Calories<br>Saturated and Polyunsaturated (PUFAs) fatty acids<br>Choline<br>Proteins, Aminoacids, Ash, Minerals and Carbohydrates<br>Phenolic compounds |

(*continued*)

**Table 1.** Continued.

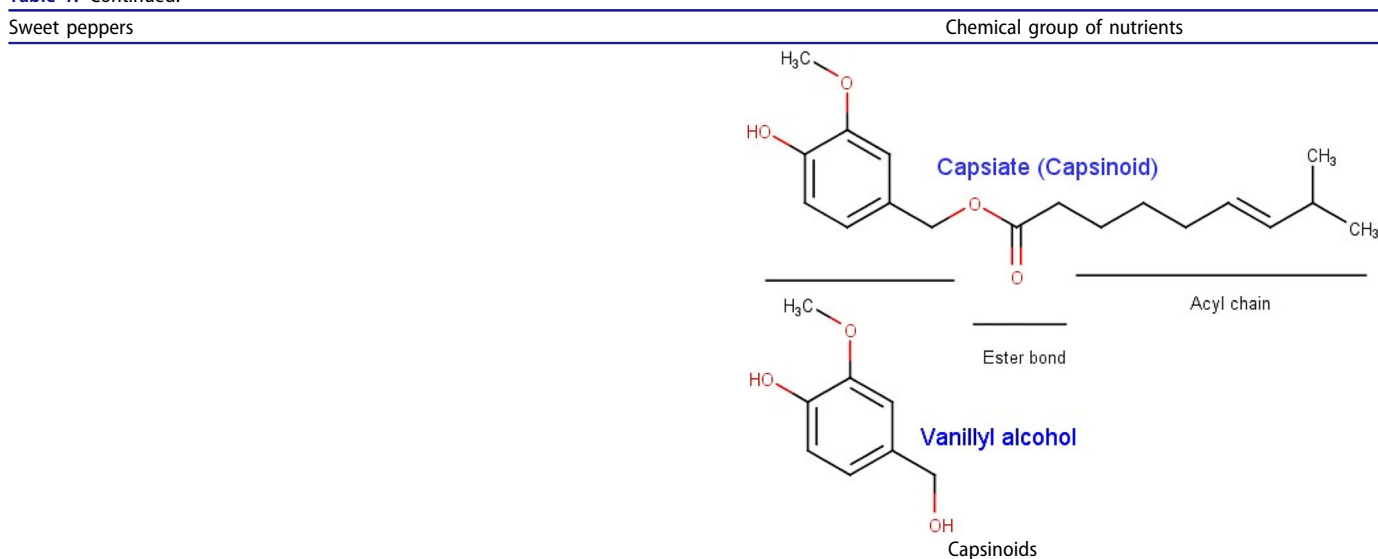| Sweet peppers | Chemical group of nutrients |
|---|---|



Capsinoids

Figure adapted by the author using George Mateljan Foundation (GMF)) 2018 available information and MarvinSketch 19.9 software.

**Table 2.** Percentage of the occurrence of Capsaicinoids in the tree species of peppers among the total compounds found (Roth 2014).

| | Capsaicin | Dihydrocapsaicin | Nordihydrocapsaicin | Homodihydrocapsaicin |
|---|---|---|---|---|
| C. annum | 44–51% | 31–37% | 8–15% | <1% |
| C.chinense & frutecens | 62–77% | 17–29% | 0.6–6% | <3% |
| C. baccatum | 38–40% | 50–51% | <1.1% | <2% |

the aromatic portion of all these molecules, with the various carboxylic acids segments coming from the amino acids valine, leucine, or isoleucine, depending upon the chain length and branching pattern (Aza-González, Núñez-Palenius, and Ochoa-Alejo 2011; Zhang et al. 2016). The enzyme capsaicin synthase (CS) joins the two fragments through an amide linkage in the final synthetic step. Capsaicin synthase was reportedly isolated from the placenta of Capsicum and found to be a protein with a molecular weight of 38 kDa. Its activity showed to be well correlated with the concentration of capsaicin in the tissue (Garcés-Claver et al. 2006; Kosuge and Furuta 1970; Othman et al. 2011; Peña-Alvarez, Ramírez-Maya, and Alvarado-Suárez 2009; Roth 2014). Contrary to commonly held belief, the seeds are not a source of capsaicin. Instead, capsaicin-producing glands are to be found in the upper layer of the placenta (Figure 1). At very high capsaicin concentrations, however, the compound can diffuse into neighboring tissue. A similar migration also frequently occurs in the course of processing and drying (Antonio, Wiedemann, and Veiga Junior 2018; Lang et al. 2009; Roth 2014).

## Capsaicinoid biosynthesis

Peppers are the major source of capsaicinoids (Luo, Peng, and Li 2011), a group of well-known pungent phenolic alkaloid compounds found in the fruit of chili pepper plant, whereas capsinoids are the non-pungent analogs of capsaicinoids that occur in sweet peppers. The structural characteristics of capsaicinoids that determine the pungency have been associated to the presence of an amide bond
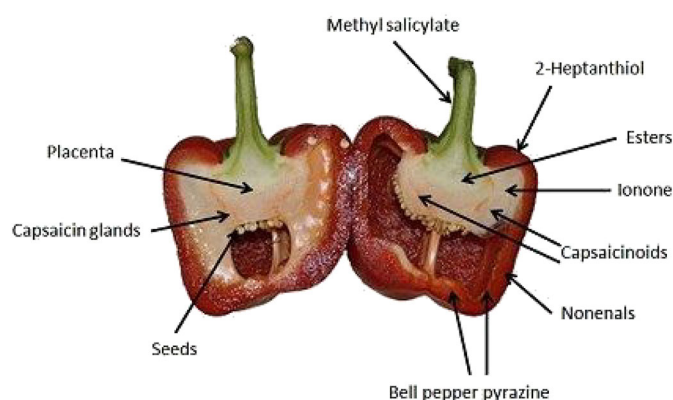


**Figure 1.** Anatomical details of the pepper fruit showing the sites of biosynthesis of the hotness compounds responsible by the pungent spiciness (Roth 2014).

connecting a vanillyl ring and an acyl chain (Castillo et al. 2007), as illustrated in the Figure 2. The carbon chain be (i) unsaturated and ramified (i.e., capsaicin), (ii) saturated and ramified (i.e., dihydrocapsaicin) and (iii) saturated and linear (i.e., nonivamide – Antonio, Wiedemann, and Veiga Junior 2018). Stewart et al. (2007) reported that the pungency is controlled via Pun1 locus which encondes a putative acyltransferase. The biosynthesis of capsaicinoids is a genetically controlled trait and depends on the cultivar or genotype. Such characteristic is also regulated during fruit development and can be influenced by the environment (Aza-González, Núñez-Palenius, and Ochoa-Alejo 2011). Literature regarding the genes involved in the biosynthesis of capsaicinoids has also advanced (Liu et al. 2013; Zhang et al. 2016). Han et al. (2013) also reported that capsinoid
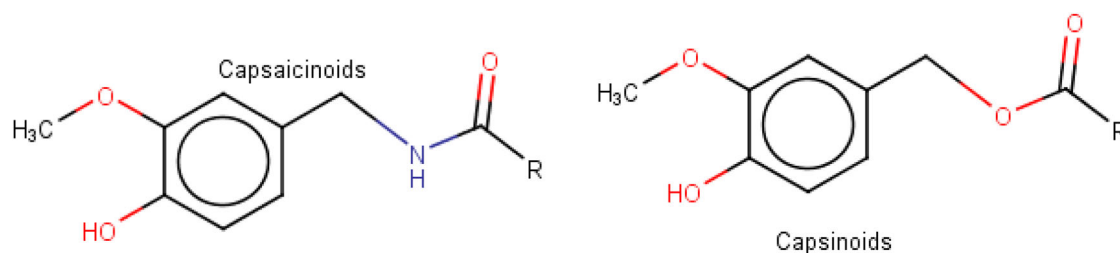
**Figure 2.** Structural characteristics of capsaicin which determines its pungency, adapted by the author using MarvinSketch 19.9 software.

**Table 3.** Chemical structure of capsaicinoids and capsinoids.



Chemical structures were produced by the author using MarvinSketch 19.9 software.

biosynthesis is controlled by *Pun1* gene. Previous reports have shown capsaicin synthase (CS) gene and the putative aminotransferase (*pAMT*). In a research of Jang et al. (2015), they showed also that a substitution of a *pAMT* allele results in High levels of capsinoids. Strong evidence of the involvement of a MYB transcription factor in the regulation

of capsaicinoid biosyntehsis was also presented by Arce-Rodríguez and Ochoa-Alejo (2017).

Capsaicinoids consists of mainly two isomers, namely, capsaicin and dihydrocapsaicin, whereas capsinoids also have two major isomers, capsiate and dihydrocapsiate (see Table 3 for details of the structure). Other naturally

occurring capsaicinoids include nordihydrocapsacin, homodihydrocapsaicin, homocapsaicin, norcapsaicin, and nornorcapsaicin, differing in the fatty acid chain length (Curry et al. 1999). Capsaicinoids are fatty acid amides linked with vanillylamine, whereas capsinoids are fatty acid esters linked with vanillyl alcohol (Huang et al. 2014). The molecular structure of capsaicinoids is resistant to ionizing radiation and readily decomposes when exposed to temperatures over 80 °C due to cleavage of carbon-nitrogen bond. The vanillyl group acts as proton donor and provides the ability to stabilize radical species and interact with cellular membranes, enzymes and nervous receptors (Antonio, Wiedemann, and Veiga Junior 2018). The phenolic functional group within the vanillyl enables capsaicinoids to suffer a phase transfer when exposed to basic conditions in which can form phenolate ion, which may form a salt in the presence of metals, increasing their solubility in aqueous solutions (Antonio, Wiedemann, and Veiga Junior 2018).

The pathway leading to capsaicin formation has two distinct arms (Figure 3), one that contributes the fatty acid moiety, usually formed via CoA derivatives of an amino acid like valine (valine conversion to 8-methyl-6-nonenyl CoAa acid – pathway II) and the other is an aromatic component that is derived from the phenylpropanoid biosynthesis (phenylalanine conversion to vanillylamine - pathway I, Sukrasno and Yeoman 1993). The two pathways are connected in a capsaicinoid synthase-catalyzed condensation reaction between vanillylamine and 8-methyl-6-nonenyl-CoA (Weber et al. 2014). The initial steps of phenylpropanoid biosynthesis are similar to ones in other plants that give rise to a number of metabolic intermediates, but the steps from ferulate to vanillylamine production is unique to Capsicum (Sukrasno and Yeoman 1993). According to Zhang et al. (2016), capsaicinoids can be synthesized via both the phenylpropanoid and the branched-fatty-acid pathways. Numerous enzymes are involved in capsaicin biosynthesis. Phenylalanine ammonia-lyase (PAL) is the first enzyme in the phenylpropanoid pathway, in which cinnamic acid is formed from phenylalanine 4, followed by cinnamac 4-hydroxylase, 4-Coumarate: Coenzyme A Ligase, hydroxycinnamoyl transferase, coumarate3-hydroxylase, caffeoyl-CoA 3-Omethyltransferase, and a putative aminotransferase (pAMT or VAMT) to form vanillylamine. In the branched-fatty-acid synthesis pathway, the precursor 8-methyl-6nonenoyl-CoA is produced via the fatty-acid synthesis cycle by a series of several enzymatic reactions. In the end, vanillyamine and 8-methyl-6-nonenoyl-CoA, which are produced as two branched chains, are converted to capsaicin by acyl-transferase (Zhang et al. 2016). A key step in the biosynthetic route to capsaicin is represented in the Figure 4.

## Capsinoid biosynthesis

Sweet peppers are the source of nature capsinoids, which share similar structure with capsaicinoids. Comparing with capsaicinoids, capsinoids are less pungent and easily broken down in the normal aqueous conditions. The fundamental structure of capsinoids is an ester of an aliphatic hydroxyl group in vanillyl alcohol with a fatty acid (Kobata, Kawaguchi, and Watanabe 2002). Capsiate and dihydrocapsiate have same acyl residue as that in the corresponding capsaicinoids capsaicin and dihydrocapsaicin (see Table 3) except for replacement of a peptide (NH) by an ester bond (O). The replacement of peptide with ester causes non-pungency of capsinoid. Non-pungency capsiate makes it more palatable and less toxic than capsaicin (Jang 2014; Jang et al. 2015). Biosynthesis of capsinoids is caused by mutations in the pAMT gene resulting in pAMT suppression of the formation vanillylamine from vanillin. Dysfunction of pAMT shunts synthesis vanillylamine into vanillyl alcohol. According to Arce-Rodríguez and Ochoa-Alejo (2019) it was a non-sense mutation of pAMT caused by T nucleotide insertion followed by GA nucleotides, producing a stop codon. This mutation affects the translation of pAMT and its enzymatic activity to produce vanillylamine. Pepper can produce both capsaiciniods and capsinoid when have functional Pun1. Quatitative control of capsinoid synthesis may be affected by other factors besides pAMT and CS. The Same genetic factors controlling capsaicinoid accumulation may be involved in capsinoid accumulation (Jang 2014; Jang et al. 2015). Chemically, they are synthesized by the condensation of vanillyl alcohol and fatty acid chloride. The enzymatic synthesis (Figure 5) catalyzed by the lipase of these capsaicin analogs has been extensively studied (Castillo et al. 2007). Such enzymatic synthesis can be either by transacylation with natural oils or fatty acid derivatives in n-hexane (Kobata et al. 1999); using natural oils as an acyl donor (Kobata et al. 1998a) or by amidation of vanillylamine with fatty acid derivatives (Kobata et al. 1998b).

Capsinoids were discovered in sweet pepper cultivar (CH-19 sweet). The production is associated to specificic mutations in the putative aminotransferase (p-AMT) responsible for the reductive amination of vanillin, the key step in the biosynthesis of capsaicin in pepper fruits. In the absence of a functioning aminotransferase, vanillic alcohol, and not vanillamine is produced and this alcohol is eventually acylated to capsinoids (Nilius and Appendino 2013; Sutoh et al. 2006; Tanaka et al. 2010a, 2010b). Most pungent lines also produce trace amounts of capsinoids (Yazawa et al. 1989). Capsinoids have valuable pharmaceutical properties in which will be discussed below. Sutoh et al. (2006) using ^{14}C radioactivity confirmed that capsinoids are formed from phenylalanine and valine and are also controlled by Pun1 locus (Han et al. 2013). Recently, Tsurumaki and Sasanuma (2019) reported that there are more pAMT mutants unconsciously in other sweet bell peppers that cause the loss of pungency.

## Application of capsaicinoids

Capsaicinoids are extraordinarily versatile agents, as testified by almost 1000 patents covering the use of the natural product, their synthetic analogs, and capsicum oleoresin in fields ranging from pharmacology and nutrition to chemical weapons and shark repellence (Appendino 2008). Capsaicinoids
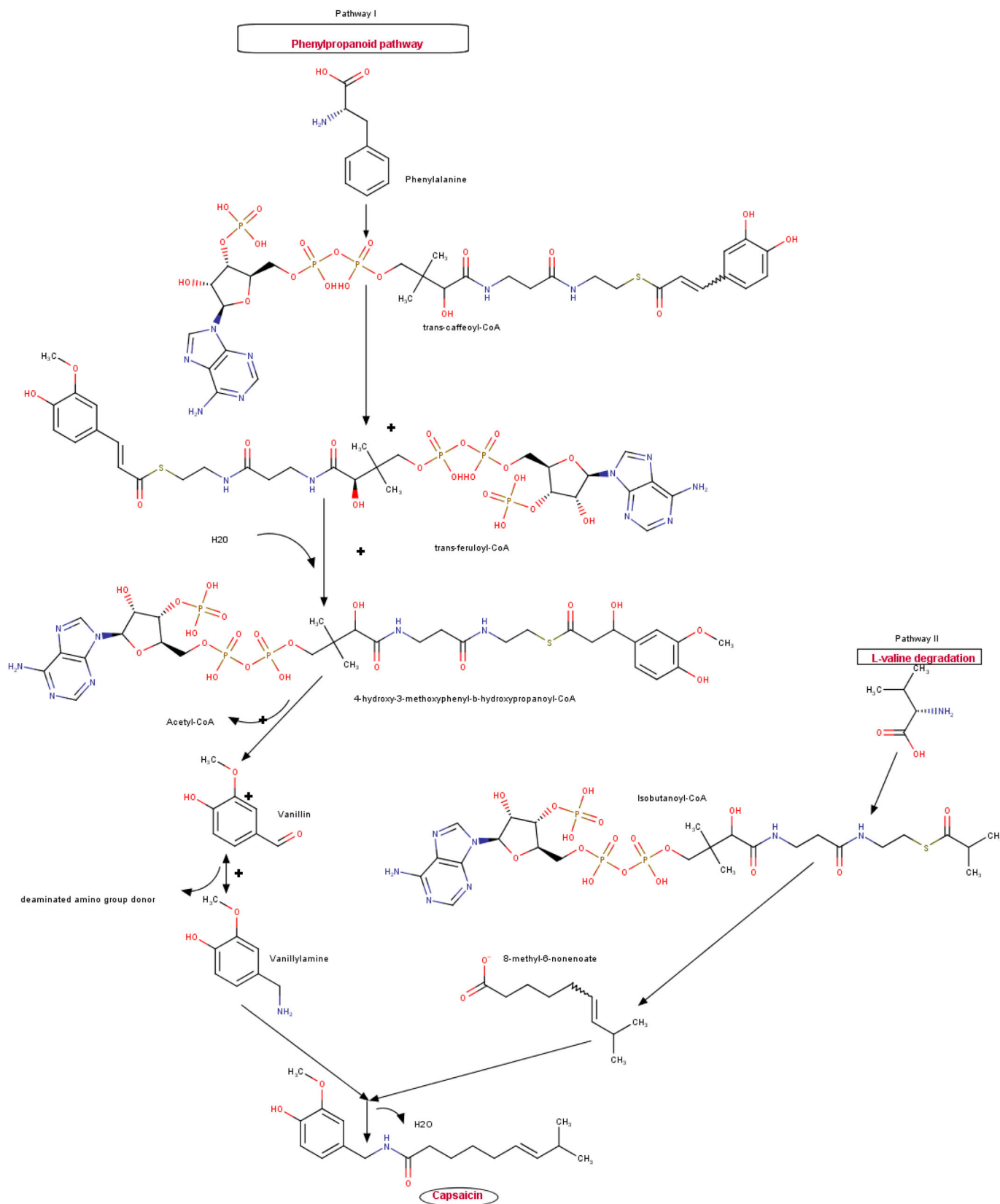
**Figure 3.** Simplified biosynthetic pathways of capsaicinoids via phenylalanine and L-valine degradation. Chemical structures were produced by the author using MarvinSketch 19.9 software.

are found to exert the activities of analgesia and anti-obesity (they accentuate the impact of caloric restriction on body weight loss – Kang et al. 2007). Capsaicin, the major pungent capsaicinoid stimulates the sympathoadrenal system

that mediates the thermogenic and anorexigenic effects of capsaicinoids. Capsaicin supplementation attenuates or even prevents the increase in hunger and decrease in fullness, as well as the decrease in energy expenditure and fat oxidation,

which normally result from energy restriction. These effects may postpone the occurrence of resistance to lose fat during a weight loss program and facilitate the maintenance of body weight in a post-obese state. Evidence also highlights the plausibility of an indirect effect of capsaicin on energy balance via its analgesic effects, which may improve sleep and ultimately facilitate the regulation of energy balance (Baranidharan and Bhaskar 2015; Uller, 2016; Tremblay, Arguin, and Panahi 2016). Capsaicinoid consumption has also been associated with enhanced metabolism (Laird 2009). Therefore, those secondary metabolites may have a potential value in clinics for pain relief, cancer prevention, and weight loss. In addition, capsaicinoids also display the benefits on cardiovascular and gastrointestinal system. It has been shown that capsaicinoids are potential agonists of capsaicin receptor or transient receptor potential vanilloid subfamily member 1 (TRPV1). They could exert the effects not only through the receptor-dependent pathway, but also through the receptor-independent one (Luo, Peng, and Li 2011). Capsaicin was also reported to have anti-bacterial

and antivirulence properties (Careaga et al. 2003; Cichewicz and Thorpe 1996; Dorantes et al. 2000; Marini et al. 2015; Molina-Orres, García-Chávez, and Ramírez-Chávez 1999; Nascimento et al. 2014; Omolo et al. 2014). Anti-carcinogenic (Clark and Lee 2016), analgesic (Brederson, Kym, and Szallasi 2013; Simone, Baumann, and LaMotte 1989), anti-inflammatory (Kim et al. 2003; Zimmer et al. 2012), chemo-preventive and chemotherapeutic (Jun et al. 2007; Surh 2002; Yang et al. 2009), and anti-diabetic properties (Narang, Jiraungkoorskul, and Jamrus 2017) were also reported in the literature. It has also shown to reduce triglycerides and LDL cholesterol levels in obese individuals (Sahin et al. 2018; Urbina et al. 2017) and activate brown adipose tissue (Saito and Yoneshiro 2013; Snitker et al. 2009).

## Biological activity of non-pungent capsaicin analogs (capsinoids)

So far, it has been found that capsinoids possess the biological properties of being antitumoral, antioxidant, and anti-obesity compounds. Since capsinoids are less toxic than capsaicinoids, they may be advantageous over capsaicinoids in clinical applications such as cancer prevention and weight of loss (Luo, Peng, and Li 2011)by activating sympathetic nervous system. Some studies have also shown that capsinoids increase energy expenditure (Tremblay, Arguin, and Panahi 2016, Luo, Peng, and Li 2011). Capsinoids promote metabolism and suppress accumulation of fatty (Ohnuki et al. 2001). Two week administration of capsiate increased mRNA level of uncoupling proteins and thyroid hormones which play important role in energy expenditure and thermoregulation and as a result, increased metabolic rate and promoted fat oxidation (Masuda et al. 2003). They have also the anti-cancer activity by blocking vascular endothelial growth factor (VEGF)-induced proliferation without irritating response, and induce nociceptive response by binding and activating the vanilloid type 1 receptor -TRPV1 (Macho et al. 2003). As summarized by Luo, Peng, and Li (2011) and reported also by Antonio, Wiedemann, and Veiga Junior (2018), capsinoids presents benefits in pain relief and prevents cardiovascular and gastrointestinal
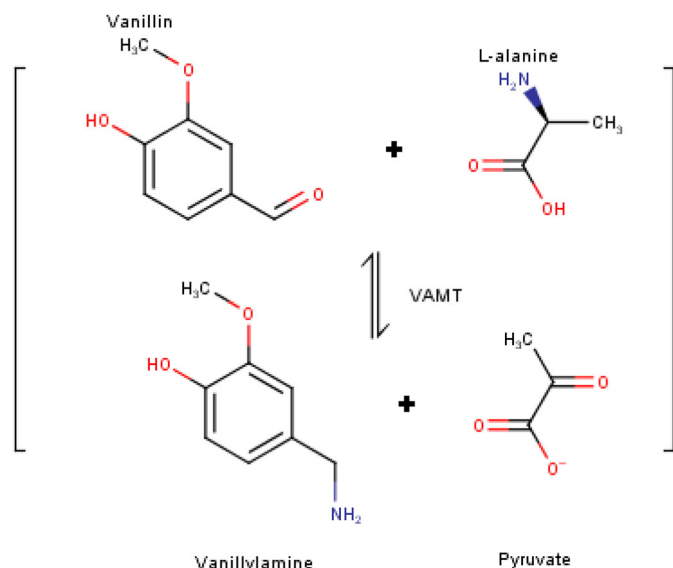
**Figure 4.** A key step in the biosynthetic route to capsaicin – conversion of vanillin to vanillylamine via transamination of the aldehyde moiety into the corresponding amine. VAMT-vanillin aminotransferase. Chemical structures were produced by the author using MarvinSketch 19.9 software.
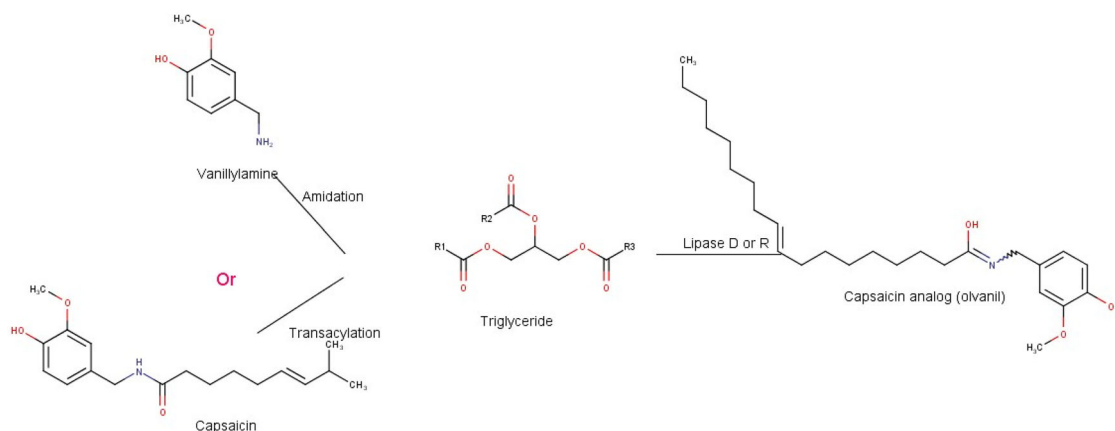
**Figure 5.** Lipase-catalyzed capsinoid biosynthesis via amidation of vanillylamine and transacylation of capsaicin with fatty acids as acyl donors ( adapted from Kobata et al. 1999; 1998a; 1998b ). Chemical structures were produced by the author using MarvinSketch 19.9 software.

diseases. In vitro experiments of Rosa et al. (2003) showed that capsinoids protect linoleic acid against free radical attach. Kwon et al. (2013) showed that capsinoids improve glucose metabolism by improving insulin sensitivity in diabetic rats, reduce body weight gain, visceral fat accumulation, serum leptin levels and improved glucose tolerance.

Capsiate inhibits angiogenesis and vascular permeability via inhibition of kinase activity in vitro. It inhibited vascular endothelial growth factor, cell proliferation, chemotatic motility and capillary-like tube formation of primary cultured human endothelial cells (Pyun et al. 2008). The immunosuppressive activity by inhibiting the activation of the transcription factor (NF-kB) was reported in an experiment performed by Sancho et al. (2002). Capsiate and dihydrocapsiate were found to inhibit UV-B induced skin inflammation by inhibiting Src family kinases and epidermal growth factor receptor in vivo (Lee et al. 2010) and downregulates uncoupling protein-3, enhances muscle oxidation capacity and decrease abdominal fat content in vivo (Faraut et al. 2009). Extensive literature regarding the biological activity of capsinoids exists and here are some of such claimed applications (Baboota et al. 2018; Josse et al. 2010; Naves et al. 2019; Rogers et al. 2018).

## The impact of pre- and postharvest factors on the capsaicinoid profile of *Capsicum annuum*

The level of particular phytochemical varies according to the cultivar, climatic conditions, growing locations, agronomic factors, and harvest factors (including maturity stage). Besides these pre-harvest factors, various post-harvest stages and food processing operations have a major influence on the stability of phytochemicals (Tiwari and Cummins 2013). Capsaicinoid regulation should be affected by genotype, transcriptional, translational, functional and external stimuli such as light, atmospheric carbon dioxide, altitude, wounding, water and temperature (Arce-Rodríguez and Ochoa-Alejo 2019).

### Pre-harvest factors

### Effect of light on the Capsaicinoid contents of hot peppers
It is well known that light is an ecological factor which can affect metabolite production. As an important environmental factor, light is not only the major source of energy for plant photosynthesis and carbon assimilation, but also a key signal, regulating plant growth and development. Light intensity is an important factor in capsaicinoid formation and accumulation (Iwai, Suzuki, and Fujiwake 1979). Optimum light intensity for peppers is $1400\,\mu mol/m^2$ s. Reducing the light intensity may result in either a positive or a negative effect on the capsaicinoid accumulation, depending on the species, the degree of shading, and other agricultural practices (Jeeatid et al. 2017). High light intensity levels have been reported to reduce capsaicinoid accumulation (Gurung et al. 2011). Because peppers belong to the nightshade family, shading could improve capsaicinoid

accumulation. Similarly higher levels of capsaicinoid accumulation were found at 50% and 70% shade of full light intensity in comparison with control - no-shading (Jeeatid et al. 2017). In a study of Gangadhar et al. (2012) using light emitting diodes (LEDs) and HPLC analysis of chili fruit, it was revealed enhanced capsaicinoid contents in blue LEDs when compared with fluorescent light. In a study from Valiente-Banuet and Gutiérrez-Ochoa (2016), the pungency (i.e., capsacinoid level) of hot peppers were not affected by shading levels. According to Arce-Rodríguez and Ochoa-Alejo (2019), light positively influence accumulation of capsaicinoids.

### Effect of genotype, node position, fruiting, and maturity stage on capsaicinoids
Variation in pungency can be attributed to genotypic traits, plant growth stage, fruiting, maturity stage, and environmental differences. Significant differences in total capsaicinoid amount among individuals of a single homozygous genotype have been reported (Harvell and Bosland 1997). The effect of genotype, environment, and genotype-by-environment interaction on the total capsaicinoids and on individual capsaicinoids were also studied by Zewdie and Bosland (2000a). Significant differences were observed among the genotypes and among genotype-environment interactions. Among the genotypes in a certain environment, the within-genotype variances were also reported to be significantly different (Zewdie and Bosland, 2000a). Capsaicinoid contents depend on the genotype and also change during fruit development. Moreover, environmental and nutritional conditions occurring during the cultivation of peppers can affect the capsaicinoid content (Garcés-Claver et al. 2007). In their research, capsaicin and dihydrocapsaicin contents varied largely among families, as families did not respond similarly in producing these capsaicinoids when their fruits were grown in spring and summer, with some families showing no increase, whereas in others, the increase was more than 2-fold. Heterosis for the pungency trait, assessed by the capsaicin and dihydrocapsaicin contents in fruits, was found, indicating the existence of epistasis, over-dominance, or dominance complementation. Non-pungent parent alleles contributed to the capsaicin and dihydrocapsaicin contents, since transgressive segregation did occur (Garcés-Claver et al. 2007).

The effect of genotype and ripening was also studied by Gnayfeed et al. (2001), who observed differences in capsaicin, dihydrocapsaicin, and nordihydrocapsain between cultivars. All studied cultivars presented lower levels of capsaicinoids at mature green stage, and the onset of climacteric ripening caused their content to augment. Capsaicinoids reached their maximum at the color break or red stage and then declined (Gnayfeed et al. 2001). Smith et al. (2006) also reported the influence of cultivars/genotypes in pepper fruit quality attributes as evidenced by year to year variation in fruit quality attributes. The content of bioactive compounds may vary according to genetic and environmental factors. Bae et al. (2014) studying the impact of cultivar, fruit maturity stage (mature versus immature), and growing season

observed that mature peppers generally showed the highest content of capsaicinoids compared to immature ones. The amount of capsaicinoids was lower in the second season, demonstrating the genotype/environment interaction effect in the production.

The evolution of total and individual capsaicinoids (nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homo-capsaicin, and homodihydrocapsaicin) during fruit ripening in peppers were also reported by Barbero et al. (2014). Capsaicinoids begin to accumulate gradually in the peppers from the beginning of its development up to a maximum concentration. From this point, there is initially a sharp decrease in the total capsaicinoid content, followed by a gradual decrease until day 80 of ripening.

The fruit node position was also reported to affect the capsaicinoid level in peppers. Zewdie and Bosland (2000b) studied the pungency of pepper fruits from five node positions (second, third, fourth, fifth, and sixth from the base) using a double haploid line in a greenhouse and in the field during two years. Fruits harvested from the second node had higher mean pungency than did those from other node positions. The pungency decreased in a predominantly linear fashion as node position increased. Except in the first field experiment, fruit set at the sixth node had the lowest pungency level. The higher pungency at the lower nodes was related to the number of fruits developed. At early developmental stages, there are fewer fruits per plant and competition among fruits for a substrate may be minimal. However, at later growth stages, there are more fruits per plant and competition for substrates may be high, resulting in a low amount of capsaicinoid production per fruit (Zewdie and Bosland, 2000b). Contrarily, Barrera et al. (2008) studying the physiological behavior of fruit growth stages (from cell division and transient peak of respiration to plateau – full maturity) and ripening observed increases in capsaicinoids (capsaicin and dihydrocapsaicin) in pungent peppers was unaltered. The variability in bioactive compounds during maturity stage has also been reported previously by Conforti, Statti, and Menichini (2007). In their research with peppers in 3 maturity stages (small green, green, and red) reduction in phenolics and increase in carotenoids during maturity have been detected. The accumulation of capsaicinoids and their analogs (capsinoids) during growth stages after flowering was also studied by Iwai, Suzuki, and Fujiwake (1979). Capsaicinoids were firstly detected 20 days after flowering, and reached maximal contents around 40 days after flowering, gradually decreasing later. The capsaicinoid composition did not show any appreciable change throughout the stages after flowering (Iwai, Suzuki, and Fujiwake 1979). Capsaicinoids were observed to increase with fruit development or maturation process (Estrada et al. 2000). Estrada et al. (2002) also studied the amount of capsaicinoids in vegetative organs in relation to fruiting and detected a spatial gradient in the content of capsaicinoids along the stem. These compounds were reported to be consistently more abundant in apical fruits than in fruits belonging to middle and basal segments. Analysis of the two principal capsaicinoids in fruits showed that the proportion of capsaicin was always higher than that of dihydrocapsaicin. Capsaicinoids were also found to be present in vegetative organs, such as stem and leaves. In this case, the proportion of individual capsaicinoids was different in respect to fruits, and dihydrocapsaicin was found to be more abundant. To find out whether the capsaicinoids in vegetative organs came from the fruits, they removed the floral buds and fruit formation was prevented. Curiously, capsaicinoids were not detected in the stem and leaves of floral bud-deprived plants, suggesting that they did originate from the fruit (Estrada et al. 2002).

## Effect of thermal stress and atmospheric carbon dioxide on the capsaicinoids

Fruits of tropical and subtropical plant species developed a series of biochemical mechanisms in response to changes in temperatures leading to an increase of leakage of electrolytes, increase of respiration, ethylene synthesis, accumulation of toxins, and finally cell collapse (Finger and Pereira 2016). High temperatures may influence the activity of capsaicin synthase enzyme that may help to accumulate augmented amounts of capsaicin. Rahman, Inden, and Hossain (2012) studied capsaicin content in green and ripe fruit in six cultivars of *Capsicum* by applying high temperature (29.9 °C) and low temperature (24.1 °C). The capsaicin content in fruit increased in high temperature treatment over low temperature treatment. Besides, higher contents of those secondary metabolites were detected in ripe fruits than in green ones grown under high temperature (Arce-Rodríguez and Ochoa-Alejo 2019, Rahman, Inden, and Hossain 2012; Rahman and Inden 2012). Capsaicinoids were also observed to increase with increase of carbon dioxide levels (Arce-Rodríguez and Ochoa-Alejo 2019).

## Effect of mineral nutrition on the capsaicinoids

The capsaicinoid content is genetically controlled, but also subject to environmental variables such as fertilization levels. Estrada et al. (1998) investigated the effect of mineral supplementation (N-P-K) during vegetative growth and flowering and found that capsaicinoids increased favorably with mineral supplementation. They argued that the fertilization regime may alter the balance of the competition between capsaicinoid biosynthesis and the accumulation of lignin-like substances in the cell wall (Estrada et al. 1998). Johnson and Decoteau (1996) studied the effect of $N_2$ (1 to 30 mM) and K (1 to 12 mM) fertilization on the pungency of peppers and found that $N_2$ at 1 mM negatively affected capsaicinoid contents, as K did not affect the pungency of peppers. Similarly, the effect of $N_2$ (applied as 0, 1, 7.5, 15, 22, and 30 mM urea) or K fertilization (0, 1, 3, 6, 9, and 12 mM of $K_2SO_4$) on pepper fruit pungency was also studied, revealing higher capsaicin contents in plants under N fertilization at 15 mM (Medina-Lara et al. (2008). In this turn, potassium did not appear to play a role in capsaicinoid metabolism in pepper (Medina-Lara et al. 2008). Monforte-González et al. (2010) also reported that capsaicin accumulation is related to nitrate contents in the placenta and potassium may also

affect pepper pungency due to its positive effect on fruit development. In an experiment with hydroponically cultured peppers, lower levels of nitrate application reduced capsaicin content in fruit placenta and variations in potassium levels did not result in capsaicinoid accumulation (Monforte-González et al. 2010). Kosmidou, Kefalas, and Gerasopoulos (2013) studied the effect of sodium hydroxide on the capsaicinoids during 60 days of preservation and observed that capsaicin content was reduced by 25% and 50%, following 0.5 or 1% NaOH treatment, respectively. Besides, pepper organoleptic pungency was also decreased following 1% NaOH treatment. Kosmidou, Kefalas, and Gerasopoulos (2013) claimed that pungency is reduced due to alkaline treatment (NaOH) hydrolysis which modify one of the features (i.e., vanillyl group, the acid-amide linkage and the alkyl side chain) of capsaicin molecule. Das et al. (2016) in an experiment in two different locations observed also that nutrient management has influence in pungency level. According to their research NPK + vermicompost and producing in alluvial soil type elevated the pungency of peppers.

### Effect of salt stress on the capsaicinoids

Salt tolerance is an important topic of study due to the loss of productive agricultural lands in several regions worldwide. Cropland salinization is a growing concern because salinity negatively affects almost 10 million hectares of agricultural world land annually (Arrowsmith et al. 2012). Plants may react to salt stress in a variety of ways (Uarrota et al. 2018). Salt can affect nutrient uptake in plants and, in particular, nitrogen uptake can be reduced by high salinity, in turn reducing plant growth and affect capsaicinoids. Previous research in peppers has shown that salinity increases oxidative stress and antioxidant defense system (Lee 2011) and reduced the photosynthetic rates (Urrea-López, de la Garza, and Valiente-Banuet 2014). Phenylalanine activity (the key enzyme in capsaicinoid biosynthesis) was reported to increase at high levels of salt stress (El-Kaaby, Ai-Anny, and Almaliky 2017) and fruits of peppers have been considered to be more sensitive to salinity (Azuma et al. 2010).

In order to explore the relationship between abiotic stress and fruit capsaicin content, Arrowsmith et al. (2012) treated pepper plants with 0.0, 0.5, 1.0, and 1.5% NaCl solution. Plants exposed to the two highest salinity treatments showed higher amounts of capsaicin compared to control and lowest salinity group. The impact of salt stress (25 mM, 50 mM, 100 mM, 150 mM, and 200 mM) on the capsaicin accumulation was also evaluated in three capsicum cultivars by Maurya et al. (2014). Accumulation of that secondary metabolite was increased in fruits of plants exposed to low salt concentration as compared to control ones, while it was found to be decreasing in fruits following exposure of plants to high salt concentrations, a contradictory result from those of Arrowsmith et al. (2012).

### Effect of water deficit on the capsaicinoids

Water is the most limiting factor for crop production, and consequently crops have to deal with it frequently. Drought-induced loss in crop yield probably exceeds losses from all other causes, since both the severity and duration of the stress are critical (Reddy et al. 2016; Uarrota et al. 2018). Water stress in peppers has been argued to be a multidimensional problem causing different physiological and morphological disturbances, such as changing free amino acid composition, decreasing photosynthetic pigments, and reducing enzyme activities (Krishnamurthy et al. 2016). According to Rao, Laxman, and Shivashankara (2016), a primary response of plants subjected to drought stress is the growth arrest. Shoot growth inhibition under drought reduces metabolic demands of the plant and mobilizes metabolites for the synthesis of protective compounds required for osmotic adjustment.

Regarding the pungency in peppers, capsaicinoid content was reported to increase under water deficit (Phimchan et al. 2012). Capsaicinoid levels increased for all cultivars studied when subjected to drought stress. Previously, Estrada et al. (1999) had observed higher amounts of capsaicinoids (capsaicin and dihydrocapsaicin) in pepper fruits of water-stressed plants than that in control plants. The authors suggested that water stress has a strong effect upon the accumulation of capsaicinoids which is the result of competition between biosynthesis of capsaicinoids and other phenylpropanoid metabolites. Ruiz-Lau et al. (2011) also observed increases in capsaicin and dihydrocapsaicin contents in fruits of peppers under water stress and a paradoxical decrease in capsaicin synthase activity, probably due to the lower activity of peroxidases and augumented synthesis of capsaicin in respect to its degradation. In a research of Sung, Chang, and Ting (2005) with water stressed peppers, the activities of phenylalanine ammonia lyase (PAL) and capsaicin synthase (CS) were observed to increase and the activity of peroxidases (PODs) were observed to decrease with stress. PODs have important role in oxidizing vanillin, vanillylamine and capsaicinoids. The lower level of PODs with water stress may have effect on capsaicinoid catabolism (Díaz et al. 2004; Martinez-Romero et al. 2004). The role of PODs on the capsaicinoid degradation under water deficit was also studied by Zamudio-Moreno et al. (2014) by withholding irrigation by 7 or 9 days after anthesis, while control plants were watered daily. Withholding irrigation by 9 days induced capsaicinoid accumulation in placental tissue through lowered degradation, or low activity of PODs. Capsaicin synthase activity was not found after 60 days post-anthesis. The correlation between PODs and capsaicinoids was totally dependent of the fruit maturity stage, which suggests that PODs may not be the sole pathway in the degradation process of capsaicinoids in peppers (Zamudio-Moreno et al. 2014). The effect of drought stress (mild and moderate stress) on pepper contents of capsaicin and dihydrocapsaicin was also studied by Qiong et al. (2015). The results showed that the contents of capsaicin and dihydrocapsaicin were increased by 63.36% and 63.03%, respectively, then the control under mild drought stress, 60 days after flowering. Jeeatid et al. (2018) investigated the influence of water stress (daily irrigation -control, every 2 d, every 3 d and every 4 d) on capsaicinoid accumulation of pepper cultivars with different pungency levels after anthesis. The highest capsaicinoid

yield was found at every 2 days of water regime, and the increase of capsaicinoid yield was attributed to increasing the absolute capsaicinoid content and reducing the dry fruit yield as compared to the control. The results suggest that appropriate water stress can increase capsaicinoid yield in some cultivars, but not in all pepper ones. In a study from Valiente-Banuet and Gutiérrez-Ochoa (2016), the pungency (i.e., capsacinoid level) of hot peppers were not affected by irrigation frequencies.

## Post-harvest factors

### Effect of heat treatment on the capsaicinoids

Cooking and processing methods can alter their sensory attributes and contents of functional compounds. For example, ascorbic acid content (AA) − 27–70%, $\beta$-carotene 16–60% and lycopene were reduced in sweet peppers, by several processing methods including grilling, canning, and pickling (Greco et al. 2007). Reduction in 15–87% of AA and 1–45% of $\beta$-carotene by heat treatments were also found in sweet peppers by Ornelas-Paz et al. (2013). Contrarily, mild heat treatments applied to fresh-cut peppers seemed to preserve some of their bioactive compounds during storage (Sgroppo and Pereyra 2009).

Ornelas-Paz et al. (2010) studied the effect of cooking (boiling − 96 °C and grilling – 210 °C) on capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) of pungent peppers. The authors claimed moderate losses (1.1–28.1%) in capsaicinoids induced by boiling, while grilling caused a significant increase by 2.6– to 92.49%) in the content of those compounds. Proportion of individual capsaicinoids was similar in raw and cooked peppers. Heat processing by boiling for 10 min, 20 min and pressure cooking for 10 min at 15 pounds per square inch of area (psi, in SI units $1\,\text{psi} \approx 6896\,\text{N/m}^2$) studied by Suresh, Manjunatha, and Srinivasan (2007) revealed capsaicin losses from red pepper ranging from 18% to 36%, with maximum loss observed in pressure cooking. Victoria-Campos et al. (2015) found similar results. The bioaccessibility of capsaicinoids from green peppers decreased as the intensity of heat treatment (boiling at 94 °C/12.5 min and grilling at 210 °C/13.2 min) increased. Additionally, the impact of boiling on the capsaicinoid concentration was dependent on the capsaicinoid type and ripening stage of the fruit. Capsaicinoid content in hot peppers and its activity were not affected by the postharvest processes (Blanco-Rios, Medina-Juarez, and Gamez-Meza 2018).

### Effect of packaging and storage conditions

Available research information regarding the effect of storage temperature and packaging materials on capsaicinoids concentrations is scarce in the literature. Previous research with hot peppers revealed that the level of capsaicinoids decreases under different packaging (polyethylene and jute bags) and storage temperatures (20 °C, 25 °C, and 30 °C). Overall, the decrease in capsaicinoids concentrations in polyethylene was found to be lower when compared to those stored in jute bags. In both reported conditions, the decrease was accentuated while increasing the temperature and time of storage (Iqbal et al. 2015). Contrarily, when green peppers were frozen using liquid nitrogen, packed in polyethylene bags and stored at −20 °C in darkness during 6 months, a typical trend was not found. In another study (Giuffrida et al. 2014) who tested the storage of peppers at room temperature (20–24 °C) and at low temperature (−18 °C), a progressive decrease in capsaicinoid amount at room temperature following linear kinetics has been found. Capsaicinoids decreased by 75% when compared to their initial value. Contrarily, at low temperatures, capsaicinoids were almost unaltered.

## Concluding remarks and future directions

Capsaicinoid accumulation is greatly influenced by pre- and postharvest factors. High light intensity and heat treatments reduce capsaicinoid content probably due to the loss of activity of capsaicin synthase and phenylalanine activity. Storage in jute bags combined with high temperatures and longtime of storage may result in loss of capsaicin levels. The pungency in peppers varies according to environmental factors, genotype or cultivar, node position, fruiting and maturity stage. As the fruit matures, capsaicinoid levels increase. Fruits from the second node show higher accumulation of pungency than those in other positions and the pungency decreases linearly as a node position increase. There must have another local of biosynthesis of capsaicinoids besides of plancetal tissue, as observed by the presence of these compounds in vegetative organs (stem and leaves). Temperatures during fruit growth stages affect the enzymatic activity and activate the capsaicinoid production. Nitrogen fertilization has also a positive effect on the capsaicinoid biosynthesis and potassium has no direct effect on the accumulation of these compounds, but over the fruit development stages. Sodium hydroxide treatment reduces the pungency of fruits by modifying capsaicin molecule in vanillyl group, the acid-amide linkage or the alkyl side chain. Salt and water stress increase the PAL activity, capsaicin synthetase activity, and increases the capsaicinoids accumulation in fruits by negatively regulating the activity of peroxidases, but appropriate levels of water stress and salinity must be observed. Extensive literature has shown that capsaicinoids have valuable biological activities such as analgesia, anti-obesity, thermogenic, cardiovascular, gastrointestinal, antioxidant, anti-bacterial, antivirulence, antiinflamatory and anti-diabetic while capsinoids share similar properties of those previous referred for their analogs and confers also the property of inhibiting angiogenesis, and improving glucose metabolism. Currently, there is lack of studies in the literature aiming to undestanding the changes of capsinoids during pre- and post-harvest management. Future directions must be to better understanding the causal drivers of loss of activity of the aminotransferase gene (pAMT) and if possible, studies with genetically modified sweet peppers with functional pAMT.

## Acknowledgements

## Disclosure statement

## Funding

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