

Aspects Relating to Mechanism of the Capsaicinoids Biosynthesis

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Editorial

Capsaicin, the main component of the hot pepper, is the most common natural product, being represented by over 16000 records in SciFinder data base and also mentioned in Webster English dictionary. Capsaicin is a drug, confirmed by the almost 1000 patents covering the use of the original product, synthetic analogues and the oleoresin from pepper. Comments proving aspects of capsaicin's biomedical relevance are shown in scientific literature. Suzuki and Iwai's information of Chemistry, Biochemistry, distribution and ecological aspects of capsaicin remained unknown, still, for two more decades. The taxonomy of *Capsicum* spp. (Solanaceae) type is also controversial.

There are twenty two wild species and five common species known together with the over 2000 cultural derived from the former ones. Wild species have been less investigated and the most known data concerning the distribution of vanillyl cognate in pepper refer to growing. *Capsicum* type is apparently endemic in the highlands of Bolivia and Peru, but peppers are now grown worldwide in warm and temperate regions. It was estimated that agricultural territories for pepper cultivation correspond to the size of a country like Switzerland [1]. Capsaicinoids appear as complex mixtures of the analogues, whose profile is under epigenetic and genetic control. Even within the same species, analogues mixture changes depending on the investigated organ, because the transport from the place of synthesis is apparently more efficient for some structural types than for the others.

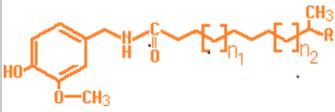
Taxonomic value of capsaicinoids is difficult to assess, so the way to distribute these compounds is inconsistent, in one species. It is possible to identify clusters characterized by only one common signature of capsaicinoids in different species cultivars [2]. Capsaicinoids with the linear chain C9–C12 are constituents found only in traces of oleoresin, which contains mainly branches of capsaicinoids.

Acyl part of these compounds is produced by way of branched chain fatty acids [3]. Depending on the amino acid that acts as the starting precursor for acylation, various capsaicinoids are formed [4].

Capsaicinoids iso-type, such as isomer of dihydrocapsaicin (ISO) and homocapsaicin I are derived from valine and leucine the path iso butiril-CoA and izovaleril-coenzima A, while the anteiso series, such as homocapsaicin II, result from isoleucine through 2-methylbutyryl-CoA. Norcapsaicin polimetilen branch has one carbon less than capsaicin [5] (Table 1).

The biogenetic origin of this group has not been investigated yet and neither has the one of capsaicinoids with odd straight chains which is probably derived from propionyl-CoA or precursors. A cetoacyl synthesis, expressed during fruit ripening, is apparently responsible for the formation of capsaicinoids acyl chain from the acyl reserve corresponding the beginning with acyl-CoA [6].

After various cycles of elongation with malonil CoA (2 for bishomocapsaicin, 3 for capsaicin (CPS, C₁₈H₂₇NO₃) and 4 for bishomocapsaicin) capsaicinoid acids might result [7].

Compound	n ₁	α	n ₂	R	
1a	1	+	0	-CH ₃	capsaicin
1b	1	-	0	-CH ₃	dihydrocapsaicin
1c	3	+	0	-CH ₃	bis-homocapsaicin (*)
1d	4	+	0	-CH ₃	tris-homocapsaicin (*)
1e	0	+	0	-CH ₃	norcapsaicin
1f	0	-	0	-CH ₃	nor-dihydrocapsaicin
1g	1	+	1	-CH ₃	homocapsaicin I
1h	1	-	1	-CH ₃	homodihydrocapsaicin I (*)
1i	1	+	0	-C ₂ H ₅	homocapsaicin II
1j	1	-	0	-C ₂ H ₅	homodihydrocapsaicin II (*)
1k	0	-	0	-C ₂ H ₅	homo nor-dihydrocapsaicin (*)
1l	1	-	0	-H	nonivamid
1m	2	-	0	-H	decivamid (*)
1n	3	-	0	-H	undecivamid (*)
1o	4	-	0	-H	dodecivamid (*)
1p	1	+	0	-CH ₂ OH	α-hydrocapsaicin
1q	2	+	0	-CH ₃	homocapsaicin (**)

(*) detected but non-isolated; (**)Erroneously reported as a constituent of *Capsicum*, due to confusion with homocapsaicins I and II

Table 1: Natural capsanoids.

According with the biogenetic generation, treatment of placental tissues of *Capsicum* species with cerulenine, a known inhibitor of fatty acid synthase, suppresses CAP's production and leads to accumulation vanillylamine. The variation of capsaicinoids acids derives from the path followed by the carbonyl group of the acyl starter which is processed after the first stage of elongation. Following the reduction of the ketone and alcohol dehydration process, the double bond can be maintained (unsaturated compounds series) or lowered (saturated compounds series) [8]. Vanilamin, a unique compound of hot pepper, is produced

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from vanilla, through the reaction of the aminotransferase depending on pyrophosphate, sensitive to inhibition with aminoxyacetate (AOA). In accordance with the hypothesis that MAN (mannose) and not the vanilamin is the limiting factor for capsaicin synthase, the degree of inhibition of aminotransferase reaction is poorly correlated with suppression of capsaicin.

Thus, an inhibition of 80% of the aminotransferase reaction only causes a reduction of 20% of the production of capsaicin, confirming once again that MAN and not the vanilamin is a limiting factor for the final acylation [9]. Vanillin biosynthesis in hot pepper is probably similar to that which occurs in the vanilla pod and arising from phenylalanine via cinnamic acid, coumaric acid and caffeic acid to ferulic acid, following oxidation to vanillin [10]. There seems to be an adjustment of vanilamin and precisely at the Coffee-O-methylation level [11], because the concentration of cinnamic and coumaric is dependent on that of capsaicin, while there is a clear correlation between the strength of the hot pepper and ferulic acid accumulation [12]. Capsaicinoids are metabolized by the P_{450} enzyme, found in the entire body, especially in liver, kidneys and lungs. Metabolism's speed is significantly lower in kidneys' microsomes than liver's microsomes, suggesting that respiratory tissues are poorly equipped to metabolize capsaicin and are therefore particularly susceptible to its toxic effects [13].

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), an alkaloid found in the fruit of *Capsicum* spp., is responsible for the pungency of *C. annuum* fruits and is unique to the genus [14]. Capsaicin's metabolism is oxidative, given the presence of lipophilic character and vulnerable vanillyl fragments (Figure 1).

There are three positions of oxidation, namely the phenyl nucleus, susceptible to O-demethylation and ortho-hydroxylation, benzyl position and terminal isopropyl group. In addition to α -hydroxylation compounds (1p) and α -1-hydroxylation was also found dehydrogenation at 8,9-dehydrocapsaicin and cyclization N, α -1-macrocylic.

Availability of sophisticated analytical methods for separation and

quantification of capsaicinoids allowed studying their distribution and kinetics of formation [15].

Capsaicinoids are unevenly distributed in plants and fruits. Placenta is their place of biosynthesis, from where they are then translocated to other plant parts (pericarp, seeds and leaves). This is a striking contrast to that observed with other Solanaceae plants, where alkaloids are produced in the roots and then translocated to aerial parts. Simple leaks can be responsible for the detection of capsaicin in the seeds, because of strict anatomical connection with the placenta [16].

Capsaicinoid are synthesized in epidermal cells of the placenta and then they are gathered just below the cuticles on the surface of the placenta as drops which can then be easily separated by pressing, releasing their content on the seeds and inner parts of the fruit. Almost 85% of capsaicinoids contained in hot peppers is located in the placenta, while only 6% is found in the pericarp and 8% in the seeds [17]. The enzymes on the pathway are: ACL, acyl carrier protein; BCAT, branched-chain amino acid transferase; BKDH, 3-methyl-2-oxobutanoate dehydrogenase; CA_3H , coumaric acid 3-hydroxylase; CA_4H cinnamic acid 4-hydroxylase; COMT, caffeic acid O-methyltransferase; CS capsaicinoid synthase; FAT, acyl-ACP thioesterase; KAS, β -ketoacyl ACP synthase; PAL, phenylalanine ammonia lyase [18]. Several studies have shown a well-defined concentration gradient in fruit, the highest concentration being found in the basal and apical parts [19].

Distinctions in composition between different parts of the plants were found, suggesting a subtle but obscure physiological significance, capsaicinoid signature of any organ. It has been observed that, while the capsaicin has been the major compound of capsaicinoids in the fruit of some species of *C. annuum*, dihydrocapsaicin has been more abundant in the vegetable organs, like roots or stems [20].

Only in the fruit the content and profile of capsaicinoids depend mainly on the stage of maturation.

Capsaicinoids can be detected after only one week after flowering, but there are differences in the kinetics of production, the highest concentration being reached after two and four weeks, depending on cultivation. At 50 days after flowering capsaicinoids concentration starts to decrease by oxidative metabolism [21]. Basically, the maximum concentration of capsaicinoids is achieved when green fruit begin to change color. Interestingly, in one plant, the fruit collected at the same time after flowering have the same type of capsaicinoids but the content of capsaicinoids is different.

Whose concentrations present a gradient along the stem with the highest concentration in the top of the fruit compared with the middle or fruit [22]. Capsaicinoids accumulation is dependent on environmental variables such as temperature, soil moisture, light, fertilization level. In general, drought and high temperature during the night causes capsaicinoids synthesis, explaining the major strength of the peppers in tropical areas compared to those grown in the temperate or humid climate.

"GRAS" is an abbreviation for Generally Recognized As Safe, which is an American Food and Drug Administration (FDA) meaning that a chemical or compounds added to food is considered safe by experts, and is exempted from the usual Federal Food, Drug, or Cosmetic Act (FFDCA) food additive tolerance requirements. High membrane permeability is a significant in the mechanism of antimicrobial action, where compounds may damage the membranes and cause a loss of

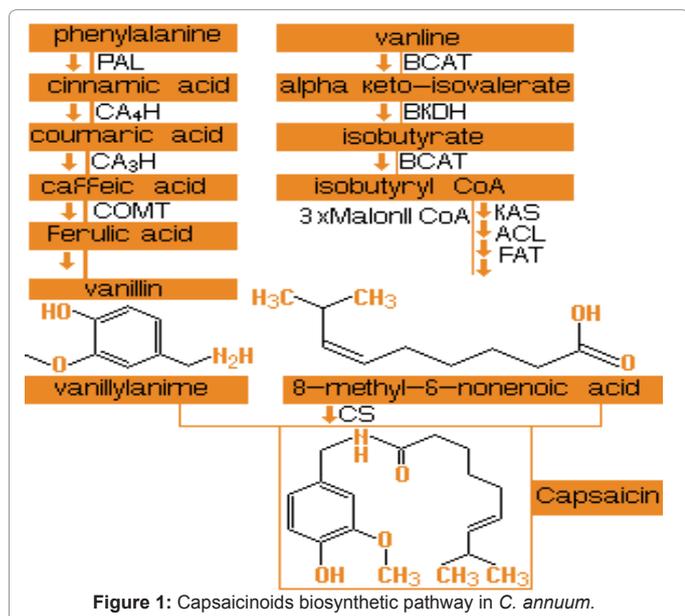


Figure 1: Capsaicinoids biosynthetic pathway in *C. annuum*.

cellular integrity and possibility cell death [23]. Capsaicinoids are used in food, that because of their consequence on bacteria [19]. The main key on the mode of impact of capsaicinoids on bacteria is that non-dissociated (non-ionized) capsaicinoids can infiltrate the bacteria cell wall and split the usual physiology of certain types of bacteria. This mode is called pH-sensitive, meaning that they cannot approve a wide internal and external pH gradient. At passive diffusion of capsaicinoids into the bacteria, where the pH is near or above neutrality, the capsaicinoids will dissociate and lower the bacteria internal pH, leading to circumstance that will impair or stop the growth of bacteria [8,20].

In another aspect, the anionic part of the capsaicinoids that cannot escape the bacteria in its dissociated form will accumulate within the bacteria and disrupt many metabolic functions, leading to osmotic pressure increase, incompatible with the survival of the bacteria [21,22].

Starting from the idea that food is used, spiced with capsaicin, for which logistical and financial investment have been made (over \$ 500 million / year in the U.S.) and from ascertained fact that the consumption of *C. annuum* (foods containing *C. annuum*) [24] is increasing, there has been considered that the biosynthesis of the alkaloids in hot pepper (based on bibliographical studies) their separation and identification could be a study of interest (an alternative) for food use.

References

1. Bosland PW, Baral JB (2007) 'Bhut Jolokia': the world's hottest known chile pepper is a putative naturally occurring interspecific hybrid. HortScience 42: 222-224.
2. Ramalho do Rêgo E, Monteiro do Rêgo M, Cruz CD, Finger FL, Dias Casali VW (2011) Phenotypic diversity, correlation and importance of variables for fruit quality and yield traits in Brazilian peppers (*Capsicum baccatum*). Genet Resour Crop Ev 58: 909-918.
3. Wu F, Eannetta NT, Xu Y, Durrett R, Mazourek M, et al. (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *Capsicum*. Theor Appl Genet 118: 1279-1293.
4. Kroumova AB, Wagner GJ (2003) Different elongation pathways in the biosynthesis of acyl groups of trichome exudate sugar esters from various solanaceous plants. Planta 216: 1013-1021.
5. Sutoh K, Kobata K, Yazawa S, Watanabe T (2006) Capsinoid is biosynthesized from phenylalanine and valine in a non-pungent pepper. *Capsicum annuum* L. cv. CH-19 sweet. Biosci Biotechnol Biochem 70: 1513-1516.
6. Blum E, Mazourek M, O'Connell M, Curry J, Thorup T, et al. (2003) Molecular mapping of capsaicinoid biosynthesis genes and quantitative trait loci analysis for capsaicinoid content in *Capsicum*. Theor Appl Genet 108: 79-86.
7. Park M, Park J, Kim S, Kwon J-K, Park HM, et al. (2012) Evolution of the large genome in *Capsicum annuum* occurred through accumulation of single-type long terminal repeat retrotransposons and their derivatives. Plant J 69: 1018-1029.
8. Markai S, Patrice MA, Mabon F, Baguet E, Billault I, et al. (2002) Natural deuterium distribution in branched-chain medium-length fatty acids is nonstatistical: a site-specific study by quantitative ²H NMR spectroscopy of the fatty acids of capsaicinoids. ChemBiochem 3: 212-218.
9. Pandhair V, Gosal SS (2009) Capsaicin production in cell suspension cultures derived from placenta of *Capsicum annuum* L. fruit. Indian J Agric Chem 22: 78-82.
10. Nuñez-Palenius H, Ochoa-Alejo N (2005) Effect of phenylalanine and phenylpropanoids on the accumulation of capsaicinoids and lignin in cell cultures of chili pepper (*Capsicum annuum* L.). In Vitro Cell Dev-PL 41: 801-805.
11. Gutiérrez-Carbajal M, Monforte-González M, Miranda-Ham M, Godoy-Hernández, G, Vázquez-Flota F (2010) Induction of capsaicinoid synthesis in *Capsicum chinense* cell cultures by salicylic acid or methyl jasmonate. Biol Plantarum 54: 430-434.
12. Walton NJ, Mayer MJ, Narbad A (2003) Vanillin. Phytochemistry 63: 505-515.
13. Reilly CA, Yost GS (2006) Metabolism of capsaicinoids by P450 enzymes: a review of recent findings on reaction mechanisms, bio-activation, and detoxification processes. Drug Metab Rev 38: 685-706.
14. Seung-Joon A, Badenes-Pérez FR, Heckel DG (2011) A host-plant specialist, *Helicoverpa assulta*, is more tolerant to capsaicin from *Capsicum annuum* than other noctuid species. J Insect Physiol 57: 1212-1219.
15. Monforte-González M, Guzmán-Antonio A, Uuh-Chim F, Vázquez-Flota F (2010) Capsaicin accumulation is related to nitrate content in placentas of habanero peppers (*Capsicum chinense* Jacq.). J Sci Food Agric 90: 764-768.
16. Díaz J, Pomar F, Bernal A, Merino F (2004) Peroxidases and the metabolism of capsaicin in *Capsicum annuum* L. Phytochem Rev 3: 141-157.
17. Mazourek M, Pujar A, Borovsky Y, Paran I, Mueller L, et al. (2009) A Dynamic Interface for Capsaicinoid Systems Biology. Plant Physiol 150: 1806-1821.
18. Higashiguchi F, Nakamura H, Hayashi H, Kometani T (2006) Purification and structure determination of glucosides of capsaicin and dihydrocapsaicin from various *Capsicum* fruits. J Agric Food Chem 54: 5948-5953.
19. Wahyuni Y, Ballester A-R, Sudarmonowati E, Bino RJ, et al. (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. Phytochemistry 72: 1358-1370.
20. Hoffmann L, Besseau S, Geoffroy P, Ritzenthaler C, Meyer D, et al. (2004) Silencing of hydroxycinnamoyl-coenzyme A shikimate/quininate hydroxycinnamoyltransferase affects phenylpropanoid biosynthesis. Plant Cell 16: 1446-1465.
21. Patrick MR (2005) Manual of Clinical Microbiology. (6th edn), ASM Press, Washington.
22. Nachay K (2011) FDA issues food safety rules. Food Technology 65(6): 14-14.
23. Butnariu M, Caunii A, Putnoky S (2012) Reverse phase chromatographic behaviour of major components in *Capsicum Annuum* extract. Chem Cent J 6: 1-6.
24. McIlvain S, Chen W, Mamiya PH, Burch R, Carter RB, et al. (2004) Preparation and Purification of Synthetic Capsaicin. 10745.