proteinspotlight
> ONE MONTH, ONE PROTEIN <

Issue 263, December 2023

www.proteinspotlight.org

hot

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The further south and east you go, the spicier food tends to get. A spice many of us store in the kitchen cupboard are chili peppers. Fresh or dried, crushed or finely sliced and thrown into a sauce, chili peppers can set your palate on fire to varying degrees. It has to do with the amount you fling in, but also the kind of pepper you choose to chop. The substance that sparks off the well-known mixture of burn and sometimes pain is known as capsaicin – and the sensation of pain is exactly what we are supposed to feel. Chili peppers do not produce capsaicin for human pleasure and cuisine; they make it to ward off predators. No other mammal would dream of adding hot pepper to its meal! Not all peppers are pungent, however. Take bell peppers, for example. Do they produce capsaicin? No. Instead, they produce what is known as capsiate, which is not hot. Both capsaicin and capsiate are derived from the same pathways but, at the very last steps, a vanillin substrate is modified in either one of two ways. The resulting product is capsiate or capsaicin – both of which, surprisingly, are synthesized by the same enzyme, a synthase known as PUN1.

kettle and flames, woodcut

by Eric Ravilious (1903-1942)

Pungent chili peppers and their non-pungent counterpart, bell peppers also known as paprikas, belong to the plant genus *Capsicum.* These peppers first grew in Central America, possibly in the region of today's Bolivia, and are believed to be one of the earliest domesticated plants, with archaeological evidence dating back 7000 years. Like many other much-appreciated South American plants, Christopher Columbus brought several pungent peppers back to Spain, from where they made their way across Europe to the rest of the world. Over the centuries, human fondness for the kick chili pepper gives to food has never ceased to grow, so much so that it has become one of the planet's major spice crops, with about 40 million tons of chili pepper fruits produced globally and on a yearly basis – although part of this production is used for medicinal and industrial purposes.

Why are peppers sometimes pungent? Pungency keeps predators away – especially mammals that like fruit. When you slice a fresh chili pepper in two, you will notice a line of small whitish seeds that are attached to a pithy formation in the fruit's centre, full of placental cells. It is in this pithy formation that capsaicin – like capsiate for that matter – is synthesized. When an animal crunches a chili pepper fruit, capsaicin is released and heads straight for the animal's nociceptors, triggering off the sensation of pain. This is exactly what happens in our mouths, and on our skin for that matter. Once burnt by a chili pepper, there is a fair chance that a frugivorous mammal will not try to eat one again. Pungency is the plant's very effective way of protecting its seeds, and therefore its progeny.

Surprisingly, birds can eat hot peppers without suffering from any kind of pain. This is because birds do not have nociceptors for capsaicin. What is more, the seeds pass straight through a bird's digestive tract untouched so that the pepper's seeds are still able to germinate once ejected – whereas with mammals, the seeds are ground between molars or broken-down during digestion, thus losing all capacity for germination. Why would birds be insensitive to capsaicin? Birds are wonderful dispersers of plant seeds, such as chili pepper seeds of course. In fact, birds have hugely expanded the current habitat of *Capsicum* while also contributing to its genetic diversity. So, it could only have been a good move for Nature to select birds that are oblivious to chili pepper pungency while keeping the seeds intact as they pass through the avian digestive tract.

Pungency is thus caused by capsaicin, itself located in the pithy part of the inside of fresh chili pepper fruits. The compound was isolated in the early 1900s. Its chemical composition was determined at the turn of the 20th century, yet it took many more years to pin down the biosynthetic pathways leading to it. Today we know that capsaicin is synthesized by way of two independent biosynthetic pathways: one which begins with the amino acid phenylalanine, and the other with valine. A series of events turns phenylalanine into vanillylamine on the one hand, and valine into the branched fatty acid 8-methyl-6-nonenoyl-CoA on the other. Vanillylamine and 8-methyl-6-nonenoyl-CoA are then condensed by a capsaicin synthase that catalyses an amide bond formation to produce capsaicin: 8-methyl-*N*-vanillyl-6-nonenamide. Capsaicin synthase, or PUN1, does the job on its own, as a monomer, with no additional help from cofactors or associated proteins.

What about peppers like paprika? Does their lack of spice have anything to do with PUN1? Surprisingly: yes. Remember, capsaicin is produced by the condensation of vanillylamine and 8-methyl-6-nonenoyl-CoA. Just one step upstream, vanillin is converted to vanillylamine by an aminotransferase called pAMT. However, it turns out that vanillin can also be reduced to vanillyl alcohol by an alcohol dehydrogenase known as CAD1, or cinnamyl alcohol dehydrogenase. In the absence of vanillylamine, PUN1 simply turns to vanillyl alcohol to which it adds the same fatty acid 8-methyl-6-nonenoyl-CoA thus catalysing the formation of the non-pungent capsiate. So pungency depends on whether vanillin is converted to vanillyl alcohol or to vanillylamine. But what decides on whether a pepper will be hot or not? CAD1 happens to be involved in lignin synthesis, and has therefore been around for a long time in peppers, as it has in all plants. pAMT was probably acquired later in time, selected by certain varieties of *Capsicum* as a means to fight off predators with capsaicin. It remains to be proved, but pAMT can be seen as something that is optional in peppers. The spice of a pepper would then depend on the level of pAMT expression.

There is a chance that evolution coaxed non-pungent peppers to become pungent peppers. Certainly, ever since capsaicin was discovered, it has not only been widely used as a spice but also as an analgesic to… relieve pain. It is used to treat human disorders such as obesity, diabetes, cancer and cardiovascular diseases, although evidence that it actually does help remains scant. Capsaicin is equally used to fight off pests such as voles, squirrels, insects and dogs, and in the form of pepper spray in riot controls or even, in some countries, it is carried in the bottom of bags to be pulled out when needed as a means of self-defence. Certainly, since Columbus and his travels, capsaicin has become an undeniable part of our lives. It is unexpected, too, that humans have chosen to season their food with something that has been modelled to cause pain. Although recent findings do suggest that capsaicin triggers off positive effects in the human brain. An explanation perhaps.

Cross-references to UniProt

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Cinnamyl alcohol dehydrogenase 1, *Capsicum annuum* (Capsicum pepper): B5LAT8
Vanillin aminotransferase, *Capsicum annuum* (Capsicum pepper): E1AQY3

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