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> ONE MONTH, ONE PROTEIN <

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the intimacy of flavour

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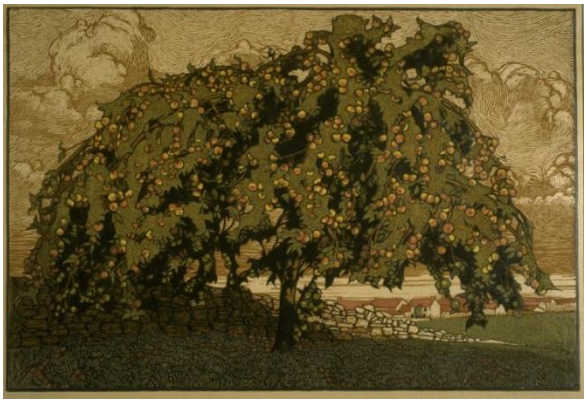
Apples. Toffee apples. Baked apples. Dried apples. Apple tart, apple pie, apple crumble, apple cake, apple strudle, apple pudding, apple purée, apple cider, apple wine, apple juice. Who has not tasted one of these ? Who has not been greeted by the house-warming smell of apple tart cooking in the oven? Does a week go by without biting into the flesh of an apple or helping ourselves to a bit of apple-something? How many of us grab an apple as we leave home to have a light and sweet snack at hand? And when you were a child, how longingly did you look at the toffee apples displayed on stalls at circuses or village fairs? Much like dogs, apples have been our companions for a very long time now; they are one of the most popular fruits eaten by humans – and on a global scale. Native of central Asia, over the millennia and thanks to its pleasing and refreshing taste, the apple travelled to the West where, in one form or another, it became almost as common as eating a slice of bread. Besides a very practical shape and size, a lot has to do with its flavour, which cultivators have tinkered with extensively through cross-breeding. What is it, though, that actually produces the taste of an apple? The answer is a blend of volatile esters that are synthesized by enzymes, one of which is alcohol acyl transferase 1, or AAT1.



François-Louis Schmied (1873-1941)

Apple trees are said to have originated in Central Asia, more precisely in the Tien Shan Mountains of Kazakhstan where they were domesticated between 4,000 and 10,000 years ago. Before domestication, apple trees – which will have resembled what we now call crab apple trees – probably depended on large animals for their dissemination because of the size of their fruit. In fact, archaeological records show that the original apple trees seem to have spread extensively before the Ice Age kicked in, during which time they gradually lost their original seed spreaders, i.e. large animals, whose numbers dropped drastically. After this glacial period, apple trees, and indeed different varieties of apples, began to spread again thanks to domestication but also because the Tien Shan Mountains happened to be at the heart of a very important trading route: the Silk Road. From Central Asia, apples gradually travelled westwards reaching Europe, and were finally taken to the American continent by European colonists. Today, the original Asian wild apple has given rise to over 7,500 different varieties of apple, and almost 90 million tonnes are produced worldwide each year.

It is hardly surprising that a fruit which has become so popular on our plates or, in liquid form, in our glasses, is also an integral part of various mythologies and religions. In Norse mythology, for instance, the apple grants eternal youthfulness or fertility and was placed besides the deceased in their early graves. In Greek mythology, apples are a symbol of love. Throwing an apple to someone was a way of declaring one's love, for example, while catching it meant that you accepted their love. In Christian tradition, however, the apple lost its virtues of youthfulness, fertility and love to become a symbol for both good and evil – it all depended what you did with it. This change in symbolism, or indeed this confusion, has been tentatively explained by the origin of the apple's name in Latin, *malus*, which can mean both 'apple' and 'evil'. The 'forbidden fruit' of Eden, though depicted as an apple by artists, is in fact not identified in the Bible.

If apples became so widespread and popular among humans, it is because they taste good. The aroma and flavour of an apple is a complex blend of hundreds of volatile compounds including alcohols, aldehydes, ketones, sesquiterpenes and esters. The flavour of one apple, for instance, can be the result of a combination of 30 or more esters. It is the volatile esters that are responsible for the fruity taste of apples – the taste we go for. In fact, ripe apples are simply fruit out to flirt, when their flavour, flesh and colour have matured and their sole aim in their short-lived life is to seduce seed dispersers. Esters are abundantly produced in the cortex and skin of apples that are ripening and are the end product of a metabolic pathway which begins with an aldehyde to finish off with a volatile ester. The very last step is performed by alcohol acyl transferase 1 (AAT1).

AAT1 belongs to the BAHD superfamily of transferases, where B, A, H and D are the first letters of the first four enzymes of this kind to be discovered. BAHD enzymes are not specific to apples but found throughout the plant kingdom, and have been studied in other popular fruits such as strawberries, melons, bananas and papaya. AAT1 is situated at the very end of a fatty acid pathway which ultimately provides alcohols and cofactors (coenzyme A, or CoA) to AAT1 – with which it will create esters. How? First, let us describe the protein's structure. AAT1 is a globular protein, modelled a little like a doughnut through which runs a channel. Similar to all enzymes from the BAHD superfamily, AAT1 has two conserved domains that are essential for its function. One of the conserved domains – the active site motif – is located in the channel. On one side of the active site is a binding site for CoA which rapidly binds an acetyl to form acetyl-CoA; on the other side, there is a site for an alcohol. Once both entities are bound to AAT1, AAT1 leaps into a two-step action, first by forming a tetrahedral intermediate which it then breaks down into a volatile ester and free CoA.

Alcohol acyl transferases (AATs) are able to bind a wide range of substrates. Likewise, AAT1 also binds more than one substrate, with preference going towards alcohols which give rise to hexyl esters. Consequently, the esters produced – and hence the apple's flavour – all depend on the nature as well as the number of substrates (alcohols and CoAs) present in an apple. The combination of different esters will lend a given variety of apple its distinctive flavour, much in the way perfume is created by combining a variety of scents. It is apparently simple combinations such as these which inspired cultivators to cross-breed different varieties of apples, thus, unknowingly perhaps, juggling with their metabolic pathways and shuffling their substrates to create new flavours.

An apple's flavour, however, cannot be stripped down to the sole presence of substrates and AAT1. It also depends on the presence of other AAT enzymes, as it does their number, their regulation and their kinetic properties with regards to substrate concentrations. As an example, the second step in ester synthesis proceeds far more rapidly for some alcohols than it does for others. Furthermore, when we talk about the flavour of an apple, we are really talking about its taste at a certain stage of development, i.e. when it is ripe and ready to disseminate its seeds. Just remember the taste of an unripe apple... Though apples are part of our daily diet, we do not spread apple trees in the way large animals did before the Ice Age made an appearance, but we certainly have managed to spread the fruit in other most imaginative ways: not only across the planet but into recipes, fairy tales and myths. An apple a day…

Cross-references to UniProt

Alcohol acyl transferase 1 allele RGa, *Malus domestica* (Apple): Q64FJ6
Alcohol acyl transferase 1 allele GSa, *Malus domestica* (Apple): A0A498KFL4

References

1. Souleyre E.J.F., Chagne D., Chen X., et al.
   The AAT1 locus is critical for the biosynthesis of esters contributing to 'ripe apple' flavour in 'Royal Gala' and 'Granny Smith' apples
   The Plant Journal 78:903-915(2014)
   PMID: 24661745

2. Souleyre E.J.F., Greenwood D.R., Friel E.N., et al.
   An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple flavor
   The FEBS Journal 272:3132-3144(2005)
   PMID: 15955071

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