Would you eat a brown apple?

1 December 2010

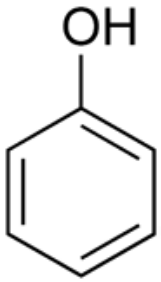
Have you ever cut up an apple to take for lunch, or prepared apples for a fresh fruit tray only to have them turn an unappealing shade of brown? You're not alone. There's nothing wrong with brown apple slices, but they certainly don't look nice, which discourages some people from eating as many apples as they should. Apples are a healthy snack and anything that gets people to eat more fruit could be considered beneficial.

Sliced apples that don't brown are in demand. Many children and some adults have hard time biting into whole apples. In addition, there is much convenience in being able to eat one slice at a time, no matter where you are. Some companies are producing bagged sliced apples that have been treated with a chemical solution to keep them from browning, and you can find them in some schools and in places like McDonald's and Subway restaurants, but that has its own complications, including what some say is an off-taste and additional plastic waste.

A Canadian company has developed apples that won't turn brown, which has the potential to solve this problem and get more people eating an apple a day. In this post, I'll discuss the chemistry behind browning and the science behind non-browning fruits and vegetables.

The enzymatic reaction that turns apples brown within minutes is a major problem for home cooks and professional chefs alike. Just Google how to stop an apple from turning brown and you'll get 2,770,000 results, including a pretty cool at-home apple browning experiment guide (pdf) by the Australian Institute of Food Science and Technology.

Why do apple, potatoes, avocados, peaches, and many other fruits and vegetables turn brown when cut or bumped?



Phenol via Wikipedia

Phenols are a whole category of compounds found naturally in a lot of foods. Most plant phenols are fine for humans to consume and some even seem to have anti-cancer properties and slow aging, but some are toxins, some may cause cancer, and many do things like reduce the absorption of iron from food. The enzyme polyphenol oxidase catalyzes a variety of reactions among phenols. Polyphenol oxidase oxidizes phenolic compounds into quinones and then links the quinones into pigments that make the surface of light colored produce look brown. There is

actually a whole family of polyphenol oxidases that each work on slightly different molecules, and each plant, animal, or bacterium may have many different genes for different types of polyphenol oxidases.

Dr. Anne Marie Helmenstine describes the chemistry behind the prevention of apple browning on About.com:

> The reaction can be slowed or prevented by inactivating the enzyme with heat (cooking), reducing the pH on the surface of the fruit (by adding lemon juice or another acid), reducing the amount of available oxygen (by putting cut fruit under water or vacuum packing it), or by adding certain preservative chemicals (like sulfur dioxide). On the other hand, using cutlery that has some corrosion (as is seen with lower quality steel knives) can increase the rate and amount of the browning by making more iron salts available for the reaction.

All of the methods to deter browning have some effect on taste or texture, which is sometimes ok, sometimes not, depending on what you plan to do with the apples. If you're baking a pie, or putting apples in a salad, a little lemon or salt probably doesn't matter, but if you're preparing apples for a fruit tray for guests to savor with cheese and wine, any apple contaminants are unacceptable.

Frank N. Foode and a large Pink Lady apple. The apple was beautiful and delicious, but would have browned terribly if it had been prepared in advance for a fruit tray, salad, or similar fresh use.

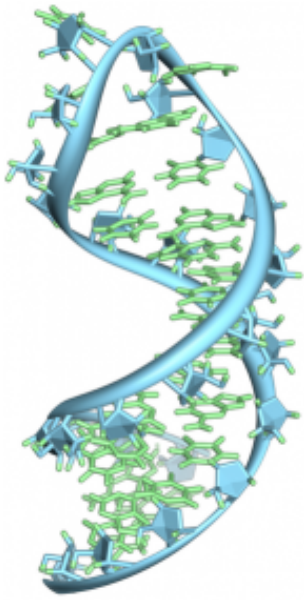
Okanagan Specialty Fruits, a Canadian fruit breeding company in Summerland, British Columbia, has developed a way to keep apples from browning without the need for special heat or chemical treatments. How did they do it? The short story is that they silenced the gene that makes the polyphenol oxidase enzyme so that the enzyme is no longer produced. No enzyme, no browning.

As for the details, we don't have many. If you've read any of the articles about these apples, you know that most of the stories are short on science and short on facts. The company isn't telling much on their website*, and hasn't published any peer-reviewed papers on their process (probably because they don't want anyone to steal their ideas), so we'll have to wait until the APHIS risk assessment for petition 10-161-01p is made public. Until then, an AP article by Shannon Dininny gives an important clue. USDA asked to approve GMO apple that won't brown is pretty well researched and includes quotes from Neal

Carter, president of Okanagan Specialty Fruits. Ms. Dininny writes: "the company licensed the non-browning technology from Australian researchers who pioneered it in potatoes."

I think the Australian researchers that Ms. Dininny refers to are from CSIRO (the Commonwealth Scientific and Industrial Research Organization, which is Australia's national science agency), but they haven't published anything specifically about polyphenol oxidase silencing either. They have published a lot of papers about their efforts to use RNAi, though, which leads me to believe that the gene for the polyphenol oxidase enzyme was silenced in the non-browning apples with RNA interference - RNAi for short.

Some viruses carry their genetic material as double stranded RNA. Plants (and animals, and fungi) use a special mechanism in their cells to recognize the double stranded RNA and cut it to pieces. Whenever that genetic sequence appears again, even if it is single stranded, it will be cut up as well. Scientists can use that natural defense mechanism to effectively shut off a gene by adding a double stranded version of the corresponding RNA with genetic engineering.



RNA can bind to itself and form a "hairpin loop", creating double stranded RNA. This structure is key to RNA interference.

RNAi can be used to change characteristics in existing plants, such as turning off the genes in onions that make you cry, turning off the genes in wheat that make gluten (great for people with celiac disease!), and turning off other allergens (such as in peanuts and apples). RNAi can also be used to add new characteristics in plants such as nematode resistance or virus resistance (both of which have been done in multiple species). It's a very versatile tool that I expect we'll see much more of as researchers and companies figure out new ways to use it.

Of course, shutting off a gene can cause unintended effects. For example, Cornell researchers found that the potato plants that had reduced polyphenol oxidase due to RNAi also had reduced disease resistance (Thipyapong, 2004). Polyphenol oxidases seem to play a role in helping plants protect themselves and recover from disease. Note that this experiment reduced the expression of all polyphenol oxidases, not just one, and they turned the genes off in all plant tissues, not just the tuber. An earlier study, also from Cornell, only turned off polyphenol oxidases in the potatoes, not in the rest of the plant, and the researchers didn't find any adverse affects on disease resistance (Bachem, 1994).

Sometimes the unintended effects of genetic engineering can be positive. The J. R. Simplot Company has also created reduced browning potatoes using RNAi. In a study that evaluated their potatoes compared to wild-type potatoes, the RNAi potatoes

were found to have not only reduced browning but french fries made from the potatoes also tasted better, smelled better, and had greatly reduced accumulation of acrylamide, a toxin naturally produced in potatoes and other foods during high temperature cooking (Rommens, 2004).

Will these non-browning apples have negative unintended effects, positive unintended effects, or both? The truth is, we just won't know until we have more information from Okanagan Specialty Fruits. We'll just have to wait for that APHIS risk assessment for petition 10-161-01p to see the details of the non-browning apples, but we have a hint in the review Plant Regeneration and Transformation in the Rosaceae (pdf, Rosaceae is the family of plants that includes apples):

> Multiple years of field testing of this material confirmed the stability of the non-browning phenotype and have identified no negative impacts on horticultural traits, or on resistance to diseases and insects when grown under field conditions. The non-browning technology developed at [Okanagan Specialty Fruits] has been incorporated into a new enabling platform that:
> (i) eliminates the selectable marker,
> (ii) removes all interfering [intellectual property],
> (iii) uses only plant derived gene sequences and control elements, and
> (iv) improves the efficiency of gene silencing.
> Plants arising from this series of transformations are now entering field trials*.

Remember that fresh fruit tray that this post started with? Which would you prefer - apples treated with chemicals or heat, apples bred to brown a little more slowly, or apples engineered to silence the enzyme that causes browning?

* This information was from a seminar given at the 1st International Symposium on Biotechnology of Fruit Species, 1-5 September 2008 in Dresden, Germany by J Armstrong and N Carter titled "A new addition to the buffet". Unfortunately, I was unable to find the full text.

Fruit tray by Tim Inconnu via Flickr.

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