

ARCTIC APPLES



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PREFACE

Fruits and vegetables are an important part of our nutrition. Apples, for example, are a very popular fruit and are consumed all around the world. There is just one big problem with apples. Once they are cut into pieces, they turn brown if one doesn't eat them immediately. That makes the apple seem unappetizing. Therefore, Okanagan Specialty Fruits Inc. has developed non-browning "Arctic Apples" with the help of genetic engineering.

The questions we asked ourselves were

Why do apples turn brown in the first place?

How do we prevent them from doing so? What techniques are used?

What other genetically modified apples may be used in the future? What other research is going on concerning apples?

In the following, we will explain the process that is used to create the Arctic Apples and discuss the relevance, the advantages and the disadvantages of this product. We will also have a look at research concerning apples that is being done in Switzerland.

INTRODUCTION

Arctic Apples are genetically modified apples that are non-browning. If the cells are damaged in a non-genetically modified apple, for example from a bite, polyphenol oxidase (POO) starts a chemical reaction. This reaction makes the flesh of the apples turn brown. The technique to make the apple non-browning is to reduce the POO. A biotechnical company in Canada called Okanagan Specialty Fruits Inc. produces the Arctic Apples, and they are the first genetically modified apples on the market. They became available for consumers in the US and Canada in 2017. The method of POO-inhibition was first developed for potatoes.



Illustration 1: Bag of arctic apples

The consuming of kinds of apples is not allowed in Switzerland. In Switzerland, only research concerning genetically modified apples (and food in general) is allowed. Current research focuses mainly on creating new sorts of apples that are resistant against wreckers, e.g. fire blight or scab. The process most commonly used is accelerated flower development (Blühverfrühung), using the method of cisgenesis. Unlike the method of transgenesis, where the gene added is taken from a non-crossable species, in cisgenesis the gene added is from a species that is crossable with the original species. This cisgenesis plant can now be crossed over with the original plant so that none of the features of the previous plant get lost.

ALTERNATIVE TREATMENTS

There are no alternative procedures to grow non-browning apples that are as effective and non-harming as the one used for Arctic Apples. But there are some treatments that have also been used. The most used anti-browning treatments are:

CALCIUM ASCORBATE

Calcium ascorbate is used in fresh cut apples. The Apple slice is dipped into a calcium ascorbate solution. Depending of the concentration of this solution, it has different effects. The higher the concentration, the less enzymatic browning occurs. But with increasing calcium ascorbate concentration, the aroma intensity of the apples decreases. This process is often very expensive and isn't able to keep the aroma of the apple while being non-browning.

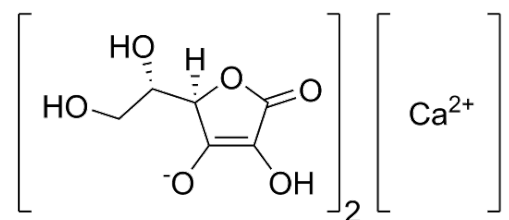


Illustration 2: Calcium ascorbate

NATURE SEAL

Nature Seal can also be used to prevent apples from browning. The sliced apple is dipped into the Nature Seal solution, and this keeps the apple fresh for about two weeks. The solution is made of vitamins and minerals that allow the apples to stay fresh for a longer time. As with calcium ascorbate, the disadvantage of Nature Seal is that the solution has to be applied manually on the slice, or the apples have to be bought already sliced and packed. There are no disadvantages like loss of aroma intensity, but since the nature seal solution has to be bought and applied it is more expensive and time consuming than Arctic Apples.

ENGINEERING TECHNIQUES

ARCTIC APPLES

Why does an apple turn brown in the first place when it is cut? When an apple is cut, its cells are damaged and because of that the apple's phenolic compounds comes in touch with the enzyme polyphenol oxidase. With the presence of oxygen, they join together and the apple starts to turn brown. The Arctic Apples do not turn brown because the enzyme polyphenol oxidase (POO) is reduced. This is done with a biological process called RNA interference (RNAi). In the process of RNA inference, gene expression is inhibited. It serves as the mechanism for gene silencing and is also known as co-suppression or gene silencing. It is initiated by a double-stranded RNA (dsRNA)

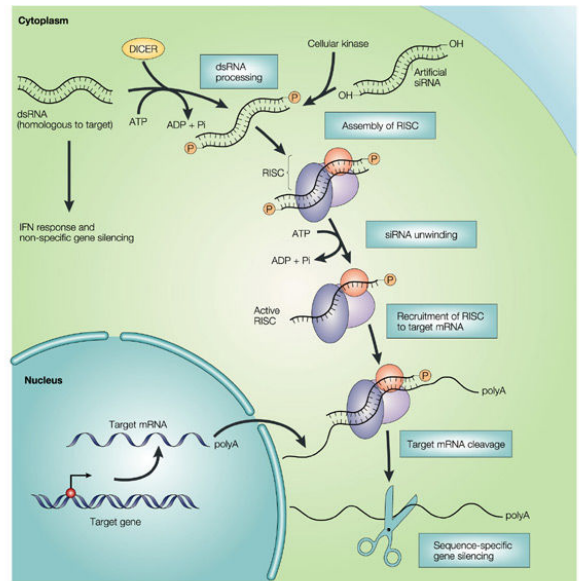


Illustration 3: RNA Interference

[1]. An enzyme (III endoribonuclease), called Dicer, turns the dsRNA into RNA duplexes, for example, short interfering RNA (siRNA) [2] or microRNA (miRNA). The siRNA activates a RNA-induced silencing complex (RISC), which then bonds with the mRNA. The mRNA is destroyed and translation is blocked. Therefore, the protein that should have been produced is not produced. Antisense RNA works the same way, except that it is a single strand of RNA that is complementary to the mRNA. Therefore, it also blocks translation.

In the Arctic Apple, certain apple genes are added to the other genes (as the enzyme POO is present in more than one gene). This gene then activates the RNAi, which silences the production of the POO enzymes. Silencing means that the gene's expression is reduced. This is not the same as gene knockout, which means that the gene is erased. A precise gene modification silences the enzyme but nothing else is changed. In the end, the Arctic Apple produces less than 10% of POO compared to non-Arctic Apples.

ACCELERATED FLOWER DEVELOPMENT

Every time a new trait, for example resistance against a virus, should be added, the genetically modified plant is crossed with the original one. This must be done several times, and normally this process would take 25-30 years. To speed up this process, accelerated flower development (Blühverfrühung) was developed. Scientists added a birch gene into the apple gene pool because the birch reproduces a lot faster. In the end, this method takes around 5 to 6 years. The gene of the birch disappears over many crossings, and the apple is resistant against the virus but still has all the traits of an apple.

[1] Its original strand and its resistant one

[2] It has 21–23 nucleotides with a 3' two-nucleotide overhang

DOCUMENTATION

As our topic is Arctic apples we thought it would smart to ask Okanagan Specialty Fruits Inc., the company behind the apple for an interview. When we contacted them and they send us a MediaKit, which already answered a ton of our questions. Therefore, we only asked a few questions, which the MediaKit didn't answer. The questions were answered by the President Neal Carter and we stood in contact with Denise Everett, the communications specialist from Okanagan Specialty Fruits Inc. The Interview was conducted per e-mail. The Info graphs are taken from the MediaKit and websites since they couldn't send us any exact pictures on the procedures.

1. How exactly, biogenetically, does the process of RNA interference work? How does it turn down the genes that produce POO?

Apples typically brown when they're bitten, sliced or bruised due to an enzyme called polyphenol oxidase (PPO). Using an innovative biotechnology-based approach, we use an apples' own genes to silence the production of PPO. As a result, Arctic® apples have too little PPO to experience superficial browning while still being just as healthful as their conventional counterparts. However, Arctic® apples will still turn brown if they have a fungal or bacterial infection resulting in rotting fruit. The same technique can be applied to other apple varieties as well, which we are currently in the process of developing.

2. Were (in previous models/ at the early stages of development) there any dangers or disadvantages? Are there today?

Arctic® apples are one of the most studied foods on the planets. They have gone through rigorous regulatory reviews by Health Canada, the Canadian Food Inspection Agency, the U.S. Food and Drug Administration and the United States Department of Agriculture's Animal and Plant Health Inspection Service, and all have concluded that Arctic® apples are just as safe as any other apple.

3. What are the next steps concerning the artic apples as well as other projects?

Our Arctic® Goldens were the first variety available commercially this past fall and we anticipate Arctic® Grannies will be available shortly after this year's fall harvest. We plan to start putting Arctic® Fujis in the ground this year. We have also done some preliminary work with other crops such as pears and cherries. While these projects are promising, they will be several years away. Our primary focus will remain on developing other Arctic® apple products and new nonbrowning varieties.

Arctic

MAKING THE PERFECT FRUIT EVEN BETTER

HOW APPLES BROWN
When an apple is cut, bitten or bruised, an enzyme called Polyphenol Oxidase (PPO) triggers the browning reaction.

CONVENTIONAL APPLE → **PPO ENZYME RELEASED** → **ARCTIC APPLE**

40% LESS BROWNING (About 60% of apples previously needed)

71% MORE (Time for 100 new apples when they're picked)

HOW DO WE STOP BROWNING?
STOP

One way to think of it
We replace one piece of a "railway track" (the PPO genes) on a coast-to-coast railway (genetic code) with a slightly different piece of track.

CONSUMERS WANT A NONBROWNING APPLE!
After learning that the browning reaction can be turned off, the majority of consumers expressed interest in buying these apples.

62% of consumers expressed interest in buying these apples

ARCTIC APPLES ARE JUST AS SAFE & HEALTHY AS ALL APPLES

- Convenient snack
- Doesn't brown when bitten, sliced or bruised!
- The same nutrition

Another way to think of it

- We introduce apple genes that produce less PPO into apple leaf tissue.
- A successful transformation is confirmed by growing the tissue under special conditions.
- Once the tissue has grown into plants, we graft it onto rootstock.
- Arctic® trees can then be planted and grown just like any other apple tree!

www.arcticapples.com

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Illustration 4: Arctic apple simply explained.



Illustration 5: Arctic apples growing

DISCUSSION

RNA interference is a biological process that occurs naturally and has always been there. But as we see with the Arctic Apple, researchers have found out how to use it to their advantage. RNAi is also used to protect potatoes from viruses. Since the process was only discovered in the 1990s, it is a new topic and there still is a lot of research to be done. In 2006 Andrew Fire and Craig C. Mello won the Nobel Prize due to their work with RNAi. Scientist set great hopes on RNAi because it allows to target individual genes. In medicine, viral infection, cancer and neurological diseases may be able to be treated. With RNAi viral RNAs can be targeted and it has been done so successful. Viral RNA includes diseases like HIV and hepatitis A and B. RNAi may also be able to target cancer tumors more specifically than chemotherapy. In agriculture, RNAi technology is an emerging field, especially in improving crops in quality as well as disease/insect resistance.

Arctic Apples are a start of GM food in our supermarkets and only time will tell if any more GM food make it there.

ETHICAL ASPECTS

There is still a lot of worry and uncertainty around genetically modified food and the Arctic apples as well. Since RNAi relatively new method many people do not trust it yet. Of course there are also many people who say that nature is nature and that it shouldn't be changed or still do not trust GM food.

ADVANTAGES

The Arctic Apples keep their flavor even after being cut, because oxidation does not take place.

The nutritional value is protected, because vitamin C and antioxidants, which are normally lost through oxidation, are still present in Arctic Apples.

Almost 50 % of the fruits and vegetables grown in the USA are wasted, often because of their browning. With non-browning apples, the worldwide food waste can be reduced.

Arctic Apples aren't any different from non-treated counterparts in terms of agricultural and nutritional aspects.

On the molecular level, the transgene genetic material of Arctic Apples is quickly degraded, so that it's indistinguishable from that of untreated apples.

The protein NPTII, which confirms if genetic engineering was successful, was undetectable in Arctic Apples and was also found to be non-toxic and non-allergenic.

There was no evidence that genetic engineering had a negative effect on humans or the environment.

Arctic Apples rot like untreated apples and are therefore not harmful for the environment.

DISADVANTAGES

Not all consequences of genetic modification can be foreseen, and it could therefore be a danger for humans and the environment.

SUMMARY

In summary, genetic engineering opens a new door for the development of apples. With techniques like RNA interference and accelerated flower development, apples can become resistant against viruses and their overall quality can improve, for example the non-browning. Food waste can be decreased, and farmers can make more profit since their apples won't become unusable because of viruses. In the future, the technique of RNAi may also be a method to cure diseases like HIV. Arctic apples have a lot of advantages the original apples do not have and have successfully made their way to US and Canadian supermarkets.

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