New rapeseed variety reduces formation of unwanted fatty acids

Better oil for **better health**

Plant genetics: markers on the rapeseed DNA show Ir. Benjamin Laga (above right) which of the genes in the rapeseed plant (right) are responsible for the fatty acid composition of its oil.

Researchers at Bayer CropScience in Ghent, Belgium, are concentrating on making food healthier. They have succeeded in breeding new varieties of high-yielding rapeseed with a modified fatty acid composition. The benefit for consumers: this rapeseed oil is naturally stable and can be used to make healthier margarines, baking and frying fats.

Rapeseed oil is a good item for health-conscious consumers to put on the grocery list. It contains a large proportion of essential polyunsaturated fatty acids which the body requires and cannot produce itself. Polyunsaturated fatty acids in all vegetable oils can, however, pose a problem when the liquid oil is hardened and converted into products such as margarine. This process stabilizes the oil, but also creates unwanted byproducts such as trans fatty acids. Benjamin Laga, a rapeseed expert at Bayer CropScience in Ghent, Belgium, explains: "We are breeding new varieties of rapeseed plants custom-tailored to the requirements of major food producers, which includes ensuring that trans fatty acids are kept to a minimum in stable rapeseed oils – an advantage for healthy nutrition."

According to the Food and Agriculture Organization of the United Nations (FAO), rapeseed is the world's second most important oil-producing plant after soy, with 46 million tons of grain produced annually worldwide. No other major agricultural commodity crop provides oil with such low levels of saturated fatty acids, the oil components that are most responsible for harmful low-density lipoprotein (LDL) cholesterol in the blood. LDL cholesterol has a tendency to build up in the walls of blood vessels, which can lead to atherosclerosis. Rapeseed oil, on the other hand, contains an especially high proportion of mono-unsaturated and polyunsaturated fatty acids, which ensure plenty of "healthy" high-density lipoprotein (HDL) cholesterol in blood.

On a chemical level, unsaturated fatty acids have one or more double bonds which often cause the elongated, straight fatty acid molecules to curve to the side. This keeps neighboring molecules at a distance and thus increases molecular mobility. As a result, a high saturated fatty acid content causes fat to solidify while a high unsaturated fat content keeps it liquid.

Healthy rapeseed oil arrived in the kitchen 30 years ago

The current healthy fatty acid composition of commodity rapeseed oil is a relatively new development. Rapeseed of old was long regarded as inferior in quality because the high levels of erucic acid cause bitterness and may also have a negative health impact. In 1974, however, new varieties of rapeseed oil containing very low levels of erucic acid were launched. These were known as zero rapeseed varieties (0-rapeseed), or 0 erucic acid varieties. In the mid-1980s, double-zero rapeseed (00-rapeseed oil) varieties were introduced. In these varieties the meal (non-oil seed fraction) was free of glucosinolates - the ingredient which gives mustard its sharp taste. Since then, it has been possible to use the meal, a byproduct of the oil extraction process, as a form of high-protein animal feed, further adding value to the crop. In North America and Australia, the 00-rapeseed varieties are now known as Canola (a term derived from "Canadian oil").

Bayer CropScience has been developing and marketing Canola hybrids



Molecular rapeseed breeding: Marijke van Mansart (left) harvests rapeseeds (second left) after cross-breeding. To prevent accidental pollination caused by airborne rapeseed pollen, the rapeseed flowers are wrapped in plastic covers. The scientists prevent self-pollination by removing the stamens (third left). Robots provide support in the molecular search for rapeseed plants that possess the genetic variations required for the desired oil composition (right).

for almost a decade under the InVigor® brand. With over 30 percent market share, the North American market is the most important region for Bayer. "Our InVigor® Canola hybrids have consistently yielded significantly higher than rival products. This, together with our dedication to Canola research and breeding, has made us leaders in the field," says Garth Hodges, Canola general manager at Bayer CropScience in Calgary, Canada.

Hydrogenation produces unhealthy trans fatty acids

Today's Canola and rapeseed oil is ideally suited for cooking and is especially healthy in products such as salad oil and marinades thanks to its fatty acid profile. When the oil is heated to high temperatures or repeatedly re-used for deep frying, however, the polyunsaturated fatty acids tend to oxidize. Oils with a high level of these healthy – but unstable - fatty acids have a short shelf-life, quickly becoming rancid. Consumers would also notice smoke and a fishy odor when frying or deep-frying foods. Oxidized fatty acids are also unhealthy as they, like saturated fatty acids, promote atherosclerosis.

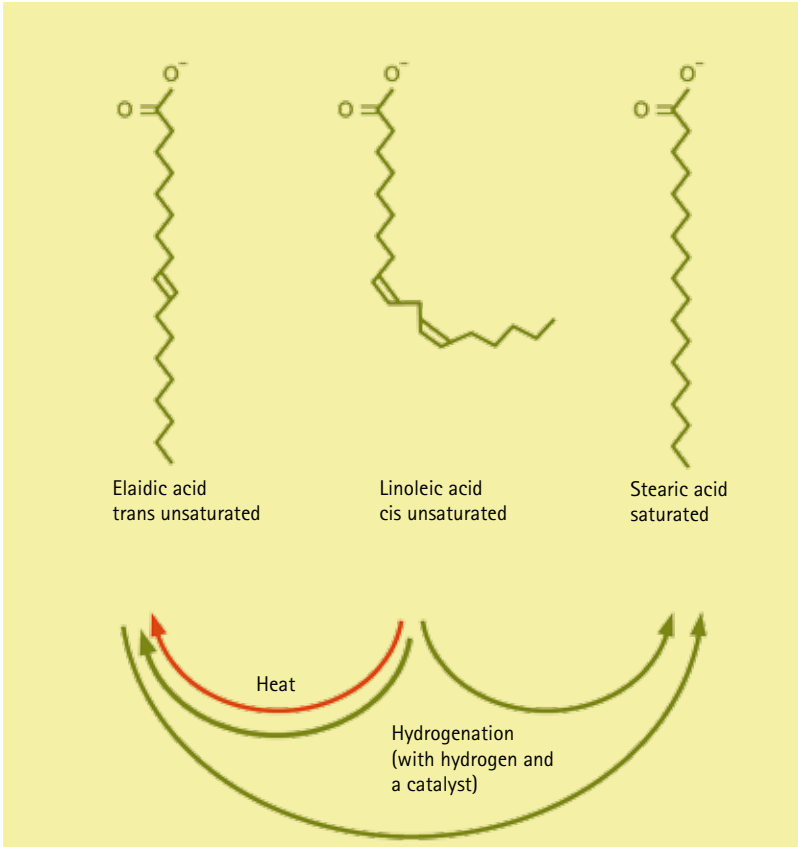
In order to prevent this oxidation from happening, processors of edible oils have long relied on industrial hydrogenation of the oil, which saturates the double bonds in fatty acids by the insertion of hydrogen atoms. The conversion process makes liquid oils more solid and more stable. This is what first made it possible to use vegetable oils as raw materials for producing margarine and as a replacement for animal fats.

This process of hydrogenation, however, results in increased levels of saturated fats and trans fatty acids, both of which are currently the subject of health warnings. In North America, manufacturers of processed foods have to indicate the amount of trans fatty acids on packaging when it exceeds 0.2 grams (Canada) or 0.5 grams (United States). The controversy surrounding

How rapeseed is cultivated

Rapeseed (Brassica napus) is a member of the mustard family. The plant has 19 chromosomes and is a hermaphrodite – 65 to 70 percent of its offspring are produced by self-pollination. This autonomous style of reproduction long made it difficult to breed high-performance varieties. Around the world, fields of corn, rice and increasingly rapeseed are now dominated by hybrids – plants whose parents were genetically very different from one another. Such hybrids can produce especially high yields because they combine a variety of positive characteristics.

Today, Bayer uses the SeedLink™ system for cultivation of hybrid Canola seeds. This is a molecular-genetic technique to keep rapeseed plants pollen-free (sterile) or female during the production phase, guaranteeing that the offspring is always the result of pollination by a different pollen-producing, male plant. The InVigor® hybrids from Bayer include technology which further provides the plant with tolerance to the herbicide Liberty® (glufosinate).



Fat hydrogenation

During fat hydrogenation, hydrogen is attached to the double bond of unsaturated fatty acids in order to turn oils which contain many unsaturated fatty acids into solid fats with mostly saturated fatty acids. In this way, for example, cis unsaturated linoleic acid is converted into saturated stearic acid. As a byproduct of hydrogenation or when heated to high temperatures or repeatedly re-used, oils with a high content of unsaturated fatty acids can form unhealthy trans fatty acids such as elaidic acid. Many makers of margarines and processed foods have significantly reduced the proportion of trans fatty acids in their products over the course of the past few years, but consumers still find plenty of snacks and French fries with well over ten grams per portion.

healthy foods combined with mandatory food labeling has led to a huge demand for alternatives in the food sector. "Manufacturers would prefer to dispense with fat hydrogenation and thus with trans fatty acids entirely," says Laga. "It is important, however, that saturated fats are not used to replace trans fats, as we need to keep the levels of both as low as possible for a healthy diet."

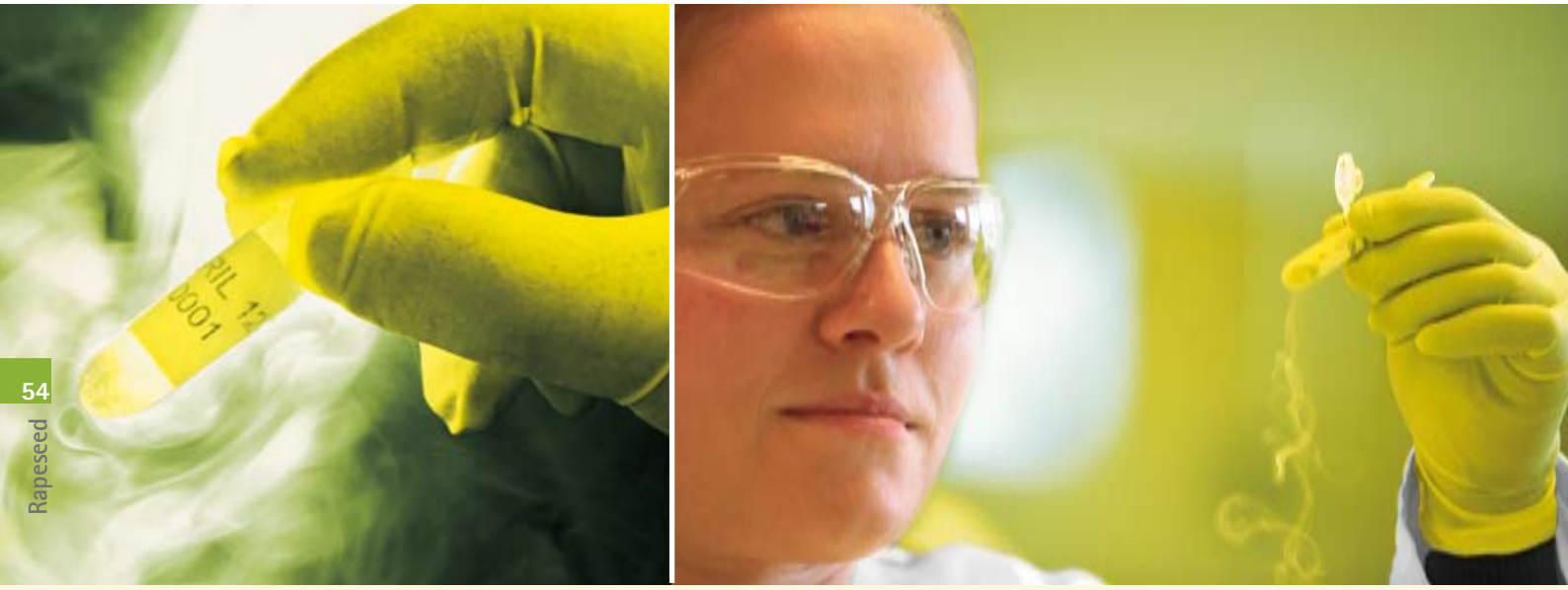
The opportunity to do just that is presented by plants which already produce more stable oils while growing in the field – oil that ideally doesn't have to be hydrogenated at all. The industry often refers to these oils as "HOLL" or high oleic, low linolenic oil. Oil with this fatty acid profile is naturally stable, does not require hydrogenation and therefore does not produce trans fatty acids during further processing.

Molecular fingerprint accelerates breeding

Several seed companies offer HOLL varieties. "Until now, the yields from these varieties have been unable to keep pace with the high-performance hybrids such as InVigor®," says Laga. But that is set to change very soon.

In 2000, the Canola research team launched a project where the goal was to develop certain high yielding Bayer CropScience InVigor® hybrids with HOLL characteristics. The resulting hybrids will be marketed together with Cargill, a leading international provider of food, agricultural and risk management products and services with its head office in Minneapolis, Minnesota, USA.

"Breeding," explains Laga, "is crossing plants with desired characteristics with each other." Ideally, some of their offspring will display the characteristics of both parent plants. "People have has been breeding plants and even animals using this principle ever since they abandoned their nomadic lifestyle and began raising crops and livestock," adds Laga. "Since the genes which determine the different characteristics often occur in new combinations in each genera-



Analysis from the ice: Tessa van der Merlen freezes leaf fragments from young rapeseed plants in liquid nitrogen at minus 196 degrees Celsius. This preserves the DNA for subsequent genetic analysis.

tion, breeders needed to be patient and wait until the desired combination appears."

Laga and his colleagues were able to save time with the help of a kind of molecular fingerprint, however. Previously, a chemical analysis of the seed oil was always required to identify which plants had the best characteristics for the next round of crossbreeding. "But the plant is usually long dead by that time," says Laga. "The plants then have to be newly cultivated from stored seeds for the next crossbreeding. We wanted to find an easier and faster way of doing things." What was needed was a gene test for HOLL that would make it possible to determine which of these plants would be the best candidates for the next round of crossbreeding based on leaf material taken while a generation of plants was still growing.

Even today, the hunt for genes is still like searching for a needle in a haystack. Researchers must first break down an organism's genetic information into tiny pieces, separate them and make them visible by attaching special markers. This genetic fingerprinting has long been a standard technique in the world of medicine and forensics.

In order to use this process to track down the "HOLL genes", the researchers in Ghent produced a large number of fingerprints with different markers for HOLL plants, for plants with a conventional fatty acid composition and for the joint offspring of the two. This is arduous work: "It can take eighteen months in greenhouse and laboratory," notes Laga. The final stage of analysis was handled by the computer, which compared variations in the fingerprints against minute differences in the characteristics of the plants, ultimately creating a genetic map which pinpointed the exact location of the genetic factors responsible for HOLL characteristics.

Mutations reduce the need for hydrogenation

As it turns out, these factors are attributable to mutations in three genes for enzymes which create double bonds in fatty acids: one enzyme that converts oleic acid into linoleic acid and two other enzymes which turn linoleic acid into alpha-linolenic acid. If the genes for these three enzymes are blocked, the plant forms only oleic acid. This finding enabled the group to develop the desired genetic identification test. Plants with different oil profiles were found to contain these mutations and selected for cross breeding with InVigor®. All that extra effort spent on research paid off. "The test has reduced the time needed to breed new hybrids by several years," says Laga.

The new InVigor® high-performance hybrids with HOLL characteristics are expected to be available on the market in Canada from 2008 onwards. Laga is convinced that "changing the oil profile right in the plant is an intelligent solution." And one that will enable consumers to avoid a considerable amount of saturated and trans fatty acids.

www.canola-council.org/
Information about Canola from the Canadian Canola Council