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# **BIOSENSORS**

## **AN EVOLVING APPROACH TO DETECTING, MONITORING AND TREATING DISEASES**

By Riccardo Sbarbati, Dan Lucian Tihon & Maria Torres Vadillo

### **Introduction**

The continuous aging of the population and the raising expectations for a better quality of life require modern medicine to intervene in a pre-symptomatic phase, acute or chronic of an illness.

Diseases like Parkinson's, Alzheimer's or cardiovascular diseases are very difficult to deal with if not treated in a timely manner.

Advances in biotechnology have resulted with a potential way to overcome these issues: biosensors.

A biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells in order to detect chemical compounds usually by electrical, thermal or optical signals (definition given by IUPAC).

In essence, a biosensor is a device that can detect a specific molecule and transmit a recognizable signal (like electrical, thermal or optical signals).

This technology allows us to detect a disease as early as possible and to act in time.

### **Working principle**

Biosensors are composed of two main parts: a biological component and a transducer.

The biological component (or bioreceptor) uses biomolecules from organisms or receptors to interact with the specific analyte of interest to produce an effect that can be measured by the transducer, which outputs a signal proportional to the presence of the target analyte.

The different types of transducers define the classification of biosensors; in fact we can differentiate electrochemical biosensors, optical biosensors, electronic biosensors, piezoelectric biosensors, gravimetric biosensors (which use the basic principle of a response to a change in mass) and pyroelectric biosensors (which generate an electric current as a result of temperature change).

### **Biomarkers are essential in the detecting process**

Biosensors themselves cannot detect the specific disease; in fact, the first step in detecting the presence of an illness is to find out its biomarker(s). This word stands for "biological marker", a measurable indicator of some biological state or condition, which in medicine is a substance that can be at abnormal levels in disease conditions.

Once we have determined the biomarker, we can start creating a proper biosensor especially made for a specific substance.

The concept of biomarker is largely used in oncology: in fact, tumor cells usually produce tumor markers or stimulate normal cells to produce them in response of their presence. Based on their chemical nature, tumor markers can be proteins, conjugated proteins, peptides or carbohydrates. Proteins or conjugated proteins may be enzymes, hormones or fragments of proteins.

Another important factor is the accuracy needed for the correct detection of these markers (in order to avoid false positive values): some analytes give small or moderate changes in

concentration or activity, thereby requiring high accuracy and precision to be useful. Others, which show large differences between normal and pathological values, may be useful even with low precision and accuracy.

### **Monitoring a disease evolution**

As well as for detecting the presence of a disease, biosensors are also used to control and monitor the evolution of pathologies that may aggravate at an uncertain moment, like diabetes or cancer. For example, 70% of tumors can be cured but it is important to perform a correct follow-up in order to avoid a recurrence of the pathology, because some tumor cells may survive and start growing again even after many years.

Other important uses for biosensors in medicine are the evaluation of pharmacological treatments used during the prevention or the treatment of a disease (indeed, the use of some drugs may cause some side effects, e.g. hepatotoxicity or nephrotoxicity) and the analysis of the concentration of the drug itself in the patient's blood or its excretion in urine. Some types of biosensors are also used in endoscopic procedures; especially in the oesophagus, stomach and bowel endoscopy where it is important to check the mobility and acidity. In this case it is possible to use manometer or pH-meter probes equipped with sensors which can remain in the organ up to 24 hours.

### **Why is it an evolving technology?**

While a few years ago we could only use biosensors as tools for analysing body fluids (e.g. blood and urine), recent development allowed us to create nanoscale sensors. The size of these latter can be less than 100 nm, which enables implantation inside the patient's body and eventually inside a single cell. Therefore, we are now able to study very clearly all the different processes that happen at intramolecular level. The small dimensions of these devices also permits to instantly detect thousands of different biomarkers within the same patient.

A research carried out by Russian scientists<sup>(1)</sup> defined a way to predict cardiovascular diseases thanks to a biosensor implanted in the body. Through analysis of saliva it is possible to detect the presence of a specific biomarker (C-reactive protein) that characterized the development of cardiovascular diseases which could lead to heart attacks or stroke. The benefit of using a body-implanted biosensor is the possibility of a constant monitoring an immediate alert in case of a positive response.

A special type of these biosensors are linked with therapeutic compounds, so that we can observe and treat an illness at the same time (theranostic approach).

An innovative approach in the use of biosensors is the *quantum dots* technology, which uses particles made by an inorganic core coated by molecules, allowing penetration inside the tissue. Quantum dots contain compounds operating as sensors and therapeutic agents; therefore, they represent the most developed example of theranostic agent.

### **Conclusions**

In summary, biosensors have always played an essential role in the diagnosis, treatment and follow-up of a disease. They have been used since long before the development of modern medicine and technology; in fact, the first biosensor in history is considered to be a little bird in a cage, used by miners to detect leaks of toxic gasses.

Amongst all the new discoveries and researches, it seems that the most notable are the ones concerning implantation of devices and nanoscale sensors, which allow an empowerment of the human body and provide a better expectation of life.

### **References**

<sup>(1)</sup> Victor Aleksandrovich Stupin, Ekaterina Vladimirovna Silina, Dmitry Valerievich Kolesov, Igor Vladimirovich Yaminsky and Valery Semenovich Shalygin: *“Development of a Biosensor for the Prediction and Early Detection of Cardiovascular Diseases Based on Saliva Composition Analysis”*, Biosciences Biotechnology Research Asia, 2015.

# BLOOD GLUCOSE BIOSENSORS

## WHAT ARE BIOSENSORS?

The term 'biosensor' is short for 'biological sensor'. A BIOSENSOR IS AN ANALYTICAL DEVICE THAT IS USED TO DETECT A SUBSTANCE OR COMPOUND AND CAN BE IDENTIFIED AS A 'COMPACT ANALYTICAL DEVICE OR UNIT INCORPORATING A BIOLOGICAL OR BIOLOGICALLY DERIVED SENSITIVE RECOGNITION ELEMENT INTEGRATED OR ASSOCIATED WITH A PHYSIO-CHEMICAL TRANSDUCER'. The device is made up of a transducer and a biological element that may be an enzyme, an antibody or a nucleic acid. The bio element interacts with the analyte being tested and the biological response is converted into an electrical signal by the transducer.

## HOW DO BIOSENSORS WORK?

A biosensor typically consists of a bio-recognition component, bio transducer component, and electronic system which include a signal amplifier, processor, and display. Transducers and the recognition component, often called a bio receptor, uses biomolecules from organisms or receptors modelled after biological systems to interact with the analyte of interest. This interaction is measured by the bio transducer which outputs a measurable signal proportional to the presence of the target analyte in the sample. The general aim of the design of a biosensor is to enable quick, convenient testing at the point of concern or care where the sample was procured.

### Bio receptors

In a biosensor, the bio receptor is designed to interact with the specific analyte of interest to produce an effect measurable by the transducer. High selectivity for the analyte among a matrix of other chemical or biological components is a key requirement of the bio receptor. While the type of biomolecule used can vary widely, biosensors can be classified according to common types bio receptor interactions involving: antibody/antigen, enzymes, nucleic acids/DNA, cellular structures/cells, or biomimetic materials.

### Antibody/antigen interactions

An immunosensor utilizes the very specific binding affinity of antibodies for a specific compound or antigen. The specific nature of the antibody antigen interaction is analogous to a lock and key fit in that the antigen will only bind to the antibody if it has the correct conformation. Binding events result in a physicochemical change that in combination with a tracer, such as a fluorescent molecule, enzymes, or radioisotopes, can generate a signal.

### Enzymatic interactions

The specific binding capabilities and catalytic activity of enzymes make them popular bio receptors. Analyte recognition is enabled through several possible mechanisms:

- 1) the enzyme converting the analyte into a product that is sensor-detectable,
- 2) detecting enzyme inhibition or activation by the analyte
- 3) monitoring modification of enzyme properties resulting from interaction with the analyte.

The main reasons for the common use of enzymes in biosensors are:

- 1) ability to catalyse a large number of reactions;

- 2) potential to detect a group of analytes (substrates, products, inhibitors, and modulators of the catalytic activity);
- 3) suitability with several different transduction methods for detecting the analyte.

### **Nucleic acid interactions**

Biosensors that employ nucleic acid interactions can be referred to as Geno sensors. The recognition process is based on the principle of complementary base pairing, adenine: thymine and cytosine: guanine in DNA. If the target nucleic acid sequence is known, complementary sequences can be synthesized, labelled, and then immobilized on the sensor.

### **WHY ARE BLOOD GLUCOSE BIOSENSORS USED?**

Blood glucose monitoring has been established as a valuable tool in the management of diabetes. Since maintaining normal blood glucose levels is recommended, a series of suitable glucose biosensors have been developed. During the last 50 years, glucose biosensor technology including point-of-care devices, continuous glucose monitoring systems and non-invasive glucose monitoring systems has been significantly improved.

Previously testing of blood sugar level was a tedious process but now glucose biosensors have evolved to be more reliable, rapid, accurate, more compact and easy to use. The majority of the current glucose biosensors are of the electrochemical type, because of their better sensitivity, reproducibility, and easy maintenance as well as their low cost.

The basic concept of the glucose biosensor is based on the fact that the immobilized glucose oxidase catalyses the oxidation of  $\beta$ -D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide. The hydrogen peroxide is then oxidised at a platinum anode. The anode recognises the number of electron transfers that occur during oxidation. This electron flow is proportional to the number of glucose molecules present in the blood.

A prime example of blood glucose biosensors being used is the self-management of diabetes for adjusting medications, dietary regimes, and physical activity. Regular and frequent measurement of blood glucose may provide data for optimizing and/or changing patient treatment strategies.

Self-monitoring of blood glucose (SMBG) has been established as a valuable tool for the management of diabetes. The goal of SMBG is to help the patient achieve and maintain normal blood glucose concentrations in order to delay or prevent the progression of microvascular and macrovascular complications (stroke and coronary artery disease).

The findings of the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) showed that intensive control of elevated levels of blood glucose in patients with diabetes, decreases the frequency of complications and may reduce the occurrence and severity of large blood vessel disease.

## Red biotechnology Stem cell biotechnology by Dustin and Mairo

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell. In addition, in many tissues they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions.

We may divide stem cells: totipotent stem cells that are one of the most important stem cell types because they are the only cells that are able to become all tissues and a placenta, pluripotent embryonic stem cells originate as inner mass cells within a blastocyst these stem cells can become any tissue in the body, excluding a placenta, multipotent stem cells are unspecialized cells that have the ability to: self-renew for long periods of time and differentiate into specialized cells with specific functions. A multipotent stem cell can give rise to other types of cells but it is limited in its ability to differentiate. There are embryonic stem cells, which can become any cell or organ in the body; however, each organ in the body also contains non-embryonic stem cells that are specific to that organ. These organ-specific stem cells control reproduction and growth of the organ through replenishing damaged or aged cells, as well as regeneration of tissues.

Embryonic stem cells are pluripotent stem cells derived from the inner cell mass of a blastocyst. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized *in vitro*—in an *in vitro* fertilization clinic—and then donated for research purposes with informed consent of the donors. They are *not* derived from eggs fertilized in a woman's body.

An adult stem cell is thought to be an undifferentiated cell, found among differentiated cells in a tissue or organ. The adult stem cell can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Scientists also use the term somatic stem cell instead of adult stem cell, where somatic refers to cells of the body (not the germ cells, sperm or eggs). Unlike embryonic stem cells, which are defined by their origin (cells from the preimplantation-stage embryo), the origin of adult stem cells in some mature tissues is still unknown.

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to



be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal. Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body like a heart muscle cell, and it cannot carry oxygen molecules through the bloodstream like a red blood cell. However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

When unspecialized stem cells give rise to specialized cells, the process is called differentiation. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment. The interaction of signals during differentiation causes the cell's DNA to acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division.

Adult stem cells typically generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to the many types of blood cells. It is generally accepted that a blood-forming cell in the bone marrow—which is called a hematopoietic stem cell—cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue.

## **Cancer**

Cancer is characterized by uncontrolled cell proliferation and diffusion into intact tissues of the body and the formation of transient distal organs. Multiple studies have shown that even within the same lesion, not all cancer cells are the same. Researchers also often find cancerous stem cells within a lesion, and many believe these cells are the key to preventing the initiation and relapse of cancer.

The genetic information in a cell or DNA of various carcinogens (such as UV radiation, tobacco carcinogens, etc.) in a continuous impact on the outside. Every day, several thousand cell DNA-s are damaged and preserving the integrity of the genetic information of a living organism is one of the main priorities. For most of the cell damage caused by different mechanisms to enhance, or removed from such defective cells with apoptosis or programmed cell death in the course of the organism.

According to the classical approach developing cancer tumors can be started from a normal healthy cell becoming malignant because the damage there is left unrepaired as a result of mutations. Altered cell begins to divide more quickly than the surrounding healthy neighboring cells and forms a tiny nodule. After achieving a certain size tumor cells are going to spread to the remote parts of the body in the blood, through the body, thereby providing metastasis. Tumor Node appearance of the body is always preceded by various precancerous conditions.

The truth is that the complete picture and understanding of the functioning of complex cancer is, at this moment still inadequate. Nor could the mutation theory of cancer based on having assumed that the removal of the tumor of the patient must be healthy treat, but even that is not backed lives presumption and, despite some progress in the early detection and diagnosis of cancer, current treatments for patient survival, however, have radically improved. Chemotherapy may be less aggressive enough to kill tumor cells and reduce tumor mass in this way, but the remaining cure-resistant cells will soon be back at the end of treatment, and grow even faster, by canceling the initial positive effect of treatment.

Some breast and prostate cancer cases have fueled the cancer stem cell theory. Often years after the organ or the cancerous lesions are removed and the patient is declared cancer-free, breast or prostate cancer can return in other organs, indicating the cancer had metastasized before it was originally detected. Cancerous stem cells may be the reason for this.

It is believed that the formation of malignant tissue growth is caused by cancerous stem cells who may trigger reproduction and growth of cells within a cancer. These cancerous stem cells lurking within the cancer, under the radar of cancer drugs that target cell proliferation, may underlie the relapse of tumors after surgery of the primary tumor or other cancer treatments. The cancer stem cell removal would thus be one of the only effective (and sufficient) ways to treat cancer. The cancer is characterized by very different genomic changes. Wherein each of the cancer, the tumor tissue of each patient is different, and a unique.

## **The Role of FGF in Normal Cell Communication**

Nearly every cell in the body expresses the FGF (fibroblast growth factor) protein, but there are 22 different types, so researchers have struggled to understand their role in cell communication. Until recently it has been a mystery as to how one of the 22 different types of FGF were sent out by cell expressers and taken in by cell receivers.

Fen Wang and Wallace McKeehan discovered the specific pathways FGF uses to activate stem cells or to keep them dormant. This discovery has major implications for future cancer therapies. It could be used to hold cancer at bay and stop it before it would be dangerous. In their studies released in 2015 July, the team traced the life cycle of multiple generations of cells to observe the normal pathways of FGF and what happens when a miscommunication occurs.

Research has shown that FGF appears to play a major role in breast and prostate cancer, which is why the duo decided to focus on the protein's role.

Researchers liken an FGF miscommunication between cells to a game of "telephone." In this game, FGF miscommunication activates previously dormant stem cells in one organ, and proceeds to miscommunicate with other cells in the same system, enabling the cancerous stem cells to reproduce and spread, impacting other systems in the body.

While Wang and McKeehan's research is specific to prostate stem cells and prostate cancer, it could have implications for cancers in other organs as well. Current cancer therapies such as chemotherapy and radiation only target actively proliferating cancer cell. Controlling how cancerous stem cells remain dormant and how they are activated, can cure cancer.

## Using stem cells for treatment

The most well-established and widely used stem cell treatment is the transplantation of blood stem cells to treat diseases and conditions of the blood and immune system, or to restore the blood system after treatments for specific cancers. The US National Marrow Donor Program has a full list of diseases treatable by blood stem cell transplant. More than 26,000 patients are treated with blood stem cells in Europe each year.

Since the 1970s, skin stem cells have been used to grow skin grafts for patients with severe burns on very large areas of the body. Only a few clinical centres are able to carry out this treatment and it is usually reserved for patients with life-threatening burns. It is also not a perfect solution: the new skin has no hair follicles or sweat glands.

A new stem-cell-based treatment to repair damage to the cornea after an injury like a chemical burn, called Holoclar, it has recently received conditional approval in Europe. In Estonia the first hematopoietic stem cell transplant was performed in 1993 at the University of Tartu Hospital. 1995 performed the first living-related allogeneic origin hematopoietic stem cell transplantation and in 2005, the first from registered donor hematopoietic stem cell transplantation.

[https://www.sm.ee/sites/default/files/content-editors/eesmargid\\_ja\\_tegevused/Tervis/Muud\\_infot/uus\\_eesti\\_transplantatsioon\\_arendukava\\_2010-2015\\_18.pdf](https://www.sm.ee/sites/default/files/content-editors/eesmargid_ja_tegevused/Tervis/Muud_infot/uus_eesti_transplantatsioon_arendukava_2010-2015_18.pdf)

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**green  
biotechnology**

## Genetically modified maize

Genetically modified maize (corn) is a genetically modified crop. Specific maize strains have been genetically engineered to express agriculturally-desirable traits, including resistance to pests and to herbicides.

One example of the traits that modified corn can express is the resistance to herbicide: it can be resistant to glyphosate herbicides, so it tolerates the use of Roundup, which is an herbicide that kills especially annual broadleaf weeds and grasses that compete with crops. This herbicide is widely used by farmers.

Then lots of other kind of maize, resistant to other problems, have been produced; as of 2011, herbicide-resistant GM corn was grown in 14 countries. By 2012, 26 varieties herbicide-resistant GM maize were authorised for import into the European Union., but such imports remain controversial, because this maize is really usefull for farmers, but lots of people think it is not safe for our healt.

Another example is the “insecticide producing corn”: it is a kind of maize, as his name suggests, that can produce itself an insecticide; in fact, this maize produces one or more proteins from the bacterium *Bacillus thuringiensis* (because of this, the protein produced by the corn is called “Bt protein). This protein is poisonous to certain insect pests and is widely used in organic gardening.

Upon sporulation, *B. thuringiensis* forms crystals of proteinaceous insecticidal  $\delta$ -endotoxins (called crystal proteins or Cry proteins).

Cry toxins have specific activities against insect species of the orders Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), Coleoptera (beetles), Hymenoptera (bees and ants) and nematodes. When insects ingest toxin crystals, their alkaline digestive tracts denature the insoluble crystals, making them soluble and thus amenable to being cut with proteases found in the insect gut, which liberate the toxin from the crystal. The Cry toxin is then inserted into the insect gut cell membrane, paralyzing the digestive tract and forming a pore. The insect stops eating and starves to death.

In 1996, the first GM maize producing a Bt Cry protein was approved, which killed the European corn borer and related species; subsequent Bt genes were introduced that killed corn rootworm larvae.

There is general scientific agreement that food from genetically modified crops is not inherently riskier to human health than conventional food, but should be tested on a case-by-case basis. The scientific rigor of the studies regarding human health has been disputed due to alleged lack of independence and due to conflicts of interest involving governing bodies and some of those who perform and evaluate the studies.

GM crops provide a number of ecological benefits, but there are also concerns for their overuse, stalled research outside of the Bt seed industry, proper management and issues with Bt resistance arising from their misuse.

Critics have objected to GM crops on ecological, economic and health grounds. These controversies have led to litigation, international trade disputes, protests and to restrictive legislation in most countries.

# Golden Rice

By Swaantje Heinrichsdorf and Ellis van Keulen

## Introduction

In Europe it's hard to imagine that there are countries where people don't live in luxury like we do. The government takes care of the poor people in our countries. But that is something you don't have everywhere! For example there is a huge problem with vitamin A deficiency. Vitamin A is found in animal products such as meat and eggs. But in many countries they don't eat varied. Children are fed with rice. Which creates a huge problem. According to UNICEF 1,5 million children per year die of a vitamin A deficiency and 500.000 children lose their sight. And how can this happen? The countries where there is a huge problem with vitamin A deficiency are very poor. People can't eat varied just because they don't have money and there is not enough food for everyone. Scientists came with a potential solution. Since 1997 they have been developing golden rice. Golden rice is a genetically modified organism also called a GMO. They have put carotene in the rice and now it's containing vitamin A. Could this be the solution and why is it not already produced?

## How it's made

The history of golden rice:

- 1990 scientist Swapan Datta developed transgenic breeding by using a method of direct gene transfer.  
How?: He used protoplasts (plant cells without cell walls), they were able to take up foreign genes respectively DNA and could be drawn again to rice plants.
- Charles Sanford developed the "bambastische transformation method". This is a method that allows us to put foreign genes respectively DNA directly into plant cells. For this method you have to use stem cells. A few of the affected cells installed the foreign DNA into their own genome and could be regenerated into a plant
- A project started to increase the vitamin A content in rice by a laboratory of the Technical University Zurich in the early 90's. The problem: four genes - whose enzymes should perform a biochemical synthesis - were missing. It seemed impossible to transform them. Later they finally managed to divert for one of the missing genes an existing synthesis. Now the other three genes should be transformed individually, to cross the transgenic plants with each other, to unite the whole synthesis route in the entire rice plant.
- In 1997 the scientist Xudong Ye did instead of the previous shooting on stem cells, he insert agrobacterias for transformation. This was the big breakthrough!

**What did he do?:** The bacterium *Agrobacterium tumefaciens* has a so called Ti-plasmid with genes for mobilization and transfer of a DNA fragment, called Transfer-DNA or t-DNA. The ability to transfer foreign genes in plants was a great technology development. Ti-plasmids and t-DNA could be modified so that a transmission of desired DNA was possible – meanwhile the transmission of bacteria was stopped. Using this method, it seemed easier to transfer all four genes together for the

carotene-synthesis in a single "co-transformation."

In fact, with this approach 500 independent transgenic rice lines could be produced in a short time. Of the 60 tested lines contained 12 all four genes. Even the polished seeds clearly showed that this rice produces Vitamin A: Golden color could clearly be seen.

### **How can you produce it and what are the problems for the scientist?**

When we talk about GMOs we also talk about risks! If you want to do this in the field you have to be careful. In the EU there are strict rules about what you can produce and what not. But in other continents they are not so strict. In 2004 they started a test with golden rice in the field. This happened in Louisiana. The reason was that Louisiana State University is one of the few places where field trials can be done. This is because of the laws and they have the right fields for it! They can isolate the plants. But how can you produce GMOs safely? The problem is that we don't know what the effects are from GMOs. That means we only know potential problems. One thing we know for sure! When you plant GMOs there is a chance that the genes are mixed with the original plants. The possible consequence: you lose the original plants. A whole ecosystem can be over turned. Because you lose diversity. That can create huge problems with plagues. So if you want to produce this in the field you have to make a situation where the plants can't propagate. But this is almost impossible! For example also the biologic maize are 0.9 % GMO.

With coexistence measures they try to prevent this. Think of buffer zones. This are zones where they don't plant the same crops! So you have less chance to mix the GMO and the non-GMO. With other GMOs it's also possible to create plants that won't survive out of the lab. But with plants you want to produce on a large scale it's not possible. They also had to make sure that golden rice has no side effects. They tested on allergies and what happens in your body. It's also tested on humans. But this is a discussion point for many scientists. GMOs can come with allergies. This is also with the genetic modified maize. The effects of GMOs in the long term are also unknown! There is a research that claims that you die earlier if you eat GMOs. This research is criticized.

They try to produce golden rice in India and Vietnam, but the problem is that the governments don't want it. And many people don't want it either.

### **The pros and cons**

But why? We already produce GMOs. Not in Europe but in other countries we do and if we don't trust them, why do we use them? If you buy custard there is a good chance there is genetic modified maize in it. 77% of all the soya is a GMO, 49% of the cotton and 26% of all the maize. But why not golden rice? There is a huge resistance against golden rice and that's because we don't know what the effects are. But do we know that of the other GMOs? Let's put what we know about golden rice in a list.



Pros	Cons
Golden rice can save people. Vitamin A in their normal food can keep many children alive	Farmers are scared of the costs. But the companies have provided access to the required technologies free of charge, for humanitarian purposes
They used carotene instead of pure vitamin A. Too much vitamin A can be dangerous but too much carotene and nothing happens.	People are scared but scientists believe that GMOs are digested just as normal plants. They contain amino acids that our body can handle. That's the reason we are allowed to eat GMOs in Europe. But we don't know the effects in the long term
	the effects on our ecosystem. With GMOs we are going to lose species. Because even with buffer zones and trying to isolate GMOs on the field you can't stop that.
	Greenpeace claims that this project is used by the companies to get GMOs accepted. A Trojan Horse!
	The project cost millions and Greenpeace is very angry about this! They think it is not the solution.

### Conclusion

The fact that after more than ten years of research golden rice is still not produced on a large scale says something about this project. The farmers don't want it. The people are scared of it! The governments are against it! It cost millions to develop it, but why not simply give vitamins? Or give people knowledge to produce vitamin A. Golden rice is an amazing idea, but it's way too complicated! Maybe in a different time, when we are used to GMOs, but for now it's not the solution. In Bangladesh they give the children vitamin supplements. That works! Maybe in the future?

## Uses of meristematic material

### Biodiversity

In recent decades, the whole world has been increasingly talking about biodiversity. Biological diversity or biodiversity is known as the abundance of life forms on Earth. Biodiversity includes both the diversity of species, the genetic variability within species and the diversity of the communities. Biodiversity is vital to countless human activities. Nature provides us everything necessary for life: oxygen, clean drinking water, food, heating materials, medicine, etc. In materialistic world, need for all these things are often taken for self-explanatory and put upon it. Biodiversity at all levels is important because life on Earth would be sustainable. Because human is part of biodiversity, it is essential for the preservation of one's own existence. Increasing urbanization, over-exploitation of natural resources, all possible pollution, introduction of alien species to ecosystems - all of these factors are extremely harmful to biodiversity. Biodiversity is considered nowadays as a natural resource and to protect it, variety of measures have been implemented.

Genetic diversity and biodiversity (in terms of species diversity) are dependent upon each other—that diversity within a species is necessary to maintain diversity among species, and vice versa. Genotypic and phenotypic diversity have been found in all species at the protein, DNA, and organismal levels; in nature, this diversity is nonrandom, heavily structured, and correlated with environmental variation and stress. Changes in species diversity lead to changes in the environment, leading to adaptation of the remaining species. Changes in genetic diversity, such as loss of species, leads to a loss of biological diversity.

### Gene banks

Among different measures, protection of different species, including varieties bred for conservation is organized. There are different types of food products gene banks in the world: in some of them genes will be stored as seeds. In others, parts of the plants are preserved by freezing tissue in liquid nitrogen. seeds. In Estonia its own culture of plants in vitro gene banks have been created according to a plant genetic resource collection and preservation programme. One of these is in Saku. EVIKA gene bank is one of these, where food grain varieties are preserved in vitro; culturing microplugly renewed plants by the meristem method. In research centre EVIKA they are preserving plant genetic resources as meristem plants in vitro for medium term storage.

In vitro gene banks, plants cultivated from meristematic tissues and regenerated from plants microplugs are preserved on phytotron flasks and test tubes filled with feed mixture. Mostly domestic virus-free crops (potatoes, strawberries, cherries, plums, etc.) are preserved by this method. It is important because this plant varieties have been bred just to grow in our climate. In nowadays world, it is a major problem to cultivate foreign origin monocultures in a wide-range areas. If case of the whole vegetation is destroyed by climate cathastrophe or by any other reason, it is possible to use the plants of a gene bank to recover the plants destroyed.

At EVIKA, they have been preserving potato varieties, breeding lines and land-races in vitro as meristem plants for more than 30 years. Various experiments have been conducted to determine the effects of medium components, growth conditions and other factors on regeneration and the sub-culturing interval of in vitro plants. Based on these experiments, the optimal preservation medium and long-term preservation conditions in vitro for many varieties have been developed. The interest in varieties as genetic resources and in those with coloured flesh tubers is increasing.

The global seed banks' aim is to maintain the natural biodiversity and to preserve as many plant species seed stocks, ensuring the survival of species in case of natural disasters, wars and plant diseases. Svalbard Global Seed Vault (located in Norway Spitsbergen island) seed bank is backup storage for the world's cultivated plants

### **What is meristem?**

On tops of shoots (growing parts) the plants have meristem buds, in other words meristem. Meristem cells are not differentiated and by using different growth factors of them, it is possible to cultivate the whole plant. A group of identical cells which are in a continuous state of cell division. Some of the cells from the meristematic tissue stops dividing and exhibit certain changes to become permanent tissues of the plant. The rest of the cells in the meristematic tissues carry on their meristematic activity.

A small Meristem-tip (often less than 1mm in length) is removed from the donor plant by sterile dissection under the microscope. Virus-free plants, however, are more beefed-up blossoming and give more abounding harvest. The meristematic tissue can be excised from the sprout or shoot; it can be lateral or apical.

Meristem cultures is the in vitro culture of a generally shiny special dome like structure measuring less than 0.1mm in length and only one or two pairs of youngest leaf primordia, most excised from the shoot apex.

### **Where are meristems found?**

Apical Meristems - Found at the tips of roots and shoots. Plants get taller, and roots get longer, from their tips. Increase in length as the apical meristems produce new cells (primary growth)

Lateral meristems are thin cylinders of tissue that form in mature regions of shoots and roots of many plants, especially those that produce woody tissue. Vascular cambium and cork cambium are examples of lateral meristem. The thin walled vascular cambium is highly vacuolated unlike other meristems and become meristematic for long period even throughout life.

Intercalary meristem – between the tip and base of stems and leaves Intercalary meristems are capable of cell division, and they allow for rapid growth and regrowth of many monocots.. They occur at the base of node or at the base of internodes or at the base of leaf or sheathing leaf of monocots. In dicots, leaf sheath is replaced by stipule. The intercalary meristems are also responsible for increase in length.

## **Meristem method**

Cloning means the creation of genetically identical progeny individual from an individual object. Meristematic method has been created as plants newest and technologically more developed cloning type. The aim of the method is to preserve the species and varieties which are threatened to become extinct and rescue the plants from diseases. Through the meristem method, it is possible to clean the plant from a variety of different viruses, and other pathogens. The method is principally the following: a slice of meristematic tissue is cut from the bud of a plant and cultivated in culture medium in the conditions that are favorable for the regeneration of the whole new plant by sterile dissection under the microscope. Meristematic cloning is based on growing a plants meristematic tissue in a test tube. The plant is operated under a microscope, the meristematic tissue is then placed into a test tube onto a feeding mixture, where it will start to grow.

The survival of the meristem tips, under the controlled condition, is determined by the size of the explant. Explants should be small enough to eradicate viruses and large enough to be able to develop into a complete plant. In a culture medium containing essential growth regulators, the excised meristems domes develop bipolar axes very quickly during reorganization.

Meristem method was first used in mid-1950s in The Netherlands, France, Canada and Belgium. In Estonia, the method was first practiced for potatoe and dianthus plants in 1966 at Estonian Crop Research Institute. During the 1960s it also turned up that meristem tissues are mostly virus free. The genetical resource of potatoes are preserved in science centre EVIKA as vitro meristem plants. Besides the potatoes, other plant varieties such as strawberries, raspberries, dianthus, chrysanthemum and other ornamental plants; berries; forest trees; cherry-, plum-, pear- and apple trees are cloned and cured by using meristem method. Meristem clones with improved traits would be helpful for potato growers disease resistant meristem clones with uniform tuber shape and size and with higher yield capacity enable to reduce the use of chemicals and others costly inputs.

**white  
biotechnology**

# Biogas plants in Germany

## Which idea lead to the emergence biogas plants?

People were on the lookout for alternative forms of energy which would work exclusively on wind and water in order to supply enough energy for the electrical grid. In addition, this power source should be carbon neutral. The most important requirement for this new form of energy was: Not to emit more CO<sub>2</sub> than is bound. This goal turned out to be very ambitious.

## What is biogas?

Biogas is produced from renewable raw materials, which produce methane from the fermentation. The gas is then burned in a cogeneration plant. The gas is then used to drive a generator, which then generates electricity. It also generates heat, which can be used for district heating. Biogas proves a power / heat source that does not use fossil fuels.

## How is a biogas plant build up?

The fed into the biogas plant materials are transported once. First the organic plant material for the biogas plant is loaded into the reception pit. There, all substrates are mixed together. Later they are transferred to the most important part of a biogas plant, the fermenter. Here the substrates are heated to a temperature of 38 °-40 ° and stirred continuously. This produces the desired methane. From the main fermenter the substance is pumped to the secondary fermenter, where slight methane formation is still taking place. The gas which has formed, rises and can thus be pumped. The gas now passes to a cogeneration system, which drives a generator. The generator converts the energy of motion (kinetic energy) into electricity. The combustion of methane produces heat that is partly used for heating the digester or as district heating. The biogas can be send to a biogas upgrading plant and then be fed into the natural gas electricity-grid or be refueled at the gas stations as fuel.

The leftovers from the fermentation are transferred into a storage room that is exclusively used for remains of fermentation. They can later be used as valuable fertilizer.

## Which substrates are used?

There are many raw materials that can be used. A first association, thinking of biogas plants, would be corn, since you can see corn on the fields almost throughout the whole year. However, there are many other renewable raw materials which are well suited as a substrate for biogas plants. Whole crop silage (GPS), sugar beet, grass, manure and even organic waste can be used.

## What are the components of biogas?

Biogas consists mainly of methane and carbon dioxide. However, it is subject to other by-products such as ammonia, water vapor, nitrogen, oxygen and hydrogen.

The desired methane has the largest share of biogas, it makes up 40-75% of the biogas. Carbon dioxide is the second biggest component of biogas with 25-55% it should therefore also be taken into consideration.

## Which substrates emit the most methane?

The following substrates have different percentages of methane. However, please note that you can win a different number m<sup>3</sup> of biogas per ton from different substrates.

## Comparison of biogas raw materials

material	biogas yield in m <sup>3</sup> per ton of fresh mass	methane content
corn silage	202	52%
grass silage	174	54%
Rye-GPS	163	52%
mangel	111	51%
biowaste	100	61%
chicken manure	80	60%
beet pulp	67	72%
pig manure	60	60%
cattle manure	45	60%
Distillers	40	61%
pig manure	28	65%
cow manure	25	60%

### How does biogas develop?

The substrates go through several phases, before biogas is produced.

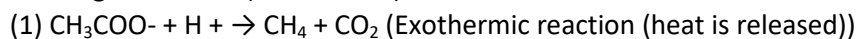
The first stage is the hydrolysis. In this phase the proteins, carbohydrates and lipids are broken down into their components, such as amino acids, monosaccharides and peptides, using exo (amylase, protease and lipase). This process takes is the longest.

In the second phase (Acidogenesis) the products of the hydrolysis are converted into low fat and carboxylic acids, and various other acids or alcohols, by acid-forming microorganisms. This also produced acetic acid (acetate), hydrogen and carbon dioxide.

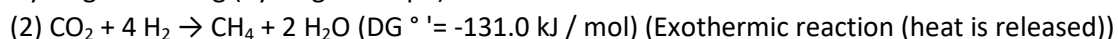
In the third phase, the Acetogenesis or vinegar-forming phase, the low fat and carboxylic acids are converted into acetate.

In the fourth and last phase the acetate is converted to methane and carbon dioxide.

Dividing acetic acid (acetoclastic):



Hydrogen utilizing (hydrogenotroph):



### Are there losses during the combustion of the methane?

In the combustion of methane, there are no relevant losses.

However, only 40% of the energy can be supplied as power to the grid.

The other 60% will released as heat, which can be used to heat the digester and surrounding homes, swimming pools and greenhouses. This district heating is only profitable in a radius of two kilometers. However, this applies only, if the purchaser buys greater amounts of heat.

**What advantages and disadvantages are associated with biogas plants?**

One disadvantage is the excessive application of remainders of fermentation as fertilizer on the fields, but there are conditions that determine accurate application of manure. If these conditions are complied with, there are no problems with too much fertilizer on the fields. Large parts of the population see a problem in the increase of planting large growing crops. The main focus of this discussion is corn. The reason for this is that on average corn stays on the field for a long time its size gives privacy. If the corn finally harvested, the remains are still in the field, they are not considered beautiful. This is why there is the subjective impression of an increased cultivation of maize as crops. Looking at it objectively, this trend is evident only to a small extend, small in proximity of large biogas plants.

A big advantage is that biogas plants can feed electricity into the network, regardless of wind and sun. Even in a windless night energy will be produced. In addition, the biogas production can be regulated to a certain point. Another advantage is that the production of biogas can be done with waste. This decreases the amount of CO<sub>2</sub> released, because the waste must not be burnt.

**Is the production of biogas sponsored by the state?**

The German Renewable Energy Act (EEG) guarantees operators of biogas plants a fixed payment over the next 20 years. This has the positive effect that rate of return from the investments can be planned very detailed. The returns are easy to calculate, because the electricity that comes from renewable energy sources, when fed into the grid by law have priority to the electricity derived from fossil power.

## **The following questions were answered by a biogas plant operator in North Germany:**

**How big your plant and how many employees you employ?**

The plant has an annual output of 700kW and is operated by an employee with 8 hours a day and a temporary worker.

In addition there are people who help harvesting the substrates.

**Which substrates do you mainly use?**

We use corn, GPS, sugar beets, grass and manure.

**How do you use the waste heat generated during the combustion of methane?**

10% of the waste heat is used for heating the fermenter and to keep the temperature of 38 °-40 °. The remaining 90% goes to surrounding households that are supplied with heat all around the year.

**What was the most important reason to open a biogas plant?**

There is a real chance to earn money, because of the EEG. If this law did not exist, it would an unpredictable business.



**Why are funds from the state needed at all?**

The funds are very important, because the plant needs to be cultivated and maintained. These costs must not be issued when fossil fuels are used. This is because nature has taken care of its formation and maintenance for many decades for. Thus, one can gain fossil fuels much cheaper, but much CO<sub>2</sub> is released.

**How do you see biogas in the future?**

The highlight was in the period between 2004 - 2009. So the new construction of biogas layers will decrease. Since the promised funds by the state decrease, more and more biogas plants will be opened within the next 10 years.

**My conclusion:**

In my opinion, the idea has been implemented very well. I find it very important that biogas can be produced climate neutral. The CO<sub>2</sub> which is released during combustion originates in the carbohydrates which have before been created within the plant using CO<sub>2</sub> from the air as well as water and oxygen. So no additional CO<sub>2</sub> is released. I also think it is good that fluctuations caused by weather can be balanced.

## Food from waste

The European and Indian fruit and cereal processing industries generate several million tons per year of by-products that are mainly disposed of through landfills. In this way, many sources of valuable biobased compounds, potentially profitable in the preparation of food ingredients or new food products with improved and healthy properties, are unavoidably lost. The opportunity to design novel strategies to turn citrus and wheat processing byproducts into food ingredients and food products via sustainable processes was the main objective of the NAMASTE-EU project.

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*Europe & India* The European and Indian food industries generate many millions of tons per year of processing by-products in the fruit and cereal processing sectors. In the fruit sector, Europe is the third major citrus producing area in the world with about 20% of production that goes for processing. The residue resulting is mainly constituted by peels and pulp, corresponding to about 50-60% of the original mass processed. A major part of the by-product is applied for cattle feed or disposed of as waste. India, conversely, produces about one third of the global mango production with the generation of a solid side stream (peels and kernels) accounting for 40 to 60% of the original mass processed and a liquid juice and wash water). Kernels are used to obtain mango kernel fat (mainly used for cosmetics), whereas dried peels are used for animal feeding or disposed of as waste.

In the cereal sector, the European Union is, by far, the main world producer of wheat and accounts for over 20% of the production, while Indian production of rice accounts approximately 20% of the global production. The bulk of these grains are refined and used for food production, generating a wheat bran fraction, which is currently used predominantly as animal feed or disposed of as waste.

The current valorization of these by-products is thus very limited; as a result, a large portion of these materials are disposed of in landfill, which itself is becoming increasingly restricted (e.g. EU Land-fill Directive and related international legislation) in order to reduce pollution and health hazards.

Nevertheless, prior to disposal, such food processing by-products can be considered sources of valuable food ingredients: after specific pretreatments with physical and biological agents, they might provide specific natural antioxidants, antimicrobial agents, vitamins, etc., along with macromolecules (bioactive oligosaccharides, oligopeptides, and pigments). Further, some of the compounds occurring in the hydrolyzates resulting from the by-products pretreatment can be transformed into more sophisticated molecules like flavors and fermentation products. Deconstruction of these by-products into their polymeric, oligomeric and individual components, through mechanical and/or (bio)chemical means, could provide valuable streams for exploitation in a number of different applications in the food industry.

### PHASES of the PROJECT

- Novel protocols for the selection, characterization and stabilization of citrus and wheat processing by-products (WP2);

- Assessment of physical/chemical/ biological procedures to obtain food ingredients from the stabilized by-products (WP3);
- Protocols and technologies to exploit natural ingredients and pretreated by-products in the formulation of new food products and procedures for the assessment of the quality, chemical and microbial safety of developed new food products and the environmental and economical sustainability of the processes employed (WP4);
- Analysis of risks, economical benefits and new market opportunities (WP5);

The two co-product classes of interest for NAMASTE-EU are wheat bran and citrus peels. Currently the major use of citrus by-products is limited to cattle feeding, with up to a 24 %, used for biofuel production. Similarly bran, and in particular wheat bran, is currently used for the production of low-value products, like composts and livestock feed, and significant amounts are disposed in landfills as waste. Both citrus peel and wheat bran are a potentially rich of ingredients of interest to the food processing industry.

Fibres from citrus are currently considered of higher quality than those from cereal due to a better balance of soluble and insoluble dietary fibre (DF) content and, also, due to their higher water and oil retention capacities. The additional advantage of citrus DF is their content of associated bioactive compounds with antioxidant properties, which may have health promoting effects better than DF. Bran is rich in DF and contains significant quantities of starch, protein, vitamins, and minerals. The presence of phenolic acids esterified to arabinoxylans gives this fraction the potential to be exploited as a source of polymers and oligosaccharides with anti-oxidant properties for food use and also a potential substrate for the biotechnological production of vanillin.

### **WP2 - Novel protocols for the selection, characterization and stabilization of citrus and wheat processing by-products**

At first the project was dedicated to identifying and quantifying target value-added compounds of citrus and wheat processing by-products and to identify constraints on proposed innovative valorization pathways (statistics on wheat bran has shown that geographical origin and wheat type have little effect on the composition of the bran). Wheat bran has low moisture content citrus peels are highly hydrated. This affects the strategy for by-product stabilization. Drying costs are critical, as in the case of citrus by-products. Different innovative drying technologies were assayed for citrus by-products, like microwave drying (MW) or combined conventional drying with thermo-mechanical procedures were assayed as alternative to conventional drying methods MW technology was also selected as the preferred drying method for stabilizing citrus peels since it was effective in maintaining microbiological safety.

### **WP3 - Natural ingredients for new foods: pre-treatment of by-products and waste and recovery and production of natural molecules**

In WP3 the major objectives were to characterize ingredients obtained from wheat bran and citrus pomace in terms of composition, bioactive properties and technological functionality.

*Wheat bran* is a by-product of the flour milling industry and for it the aim was to develop a range of hybrid processes at the laboratory scale to convert and recover one or more of the following products: Oligosaccharides with potential prebiotic activity from component arabinoxylans (AX) - A bran product pre-fermented with carbohydrases and including probiotic agents - Ferulic acid for bioconversion to bio-vanillin - Enzyme-recalcitrant wheat bran fibre residues - A peptide- / maltose-rich product suitable a growth / feed medium.

Wheat bran is potentially a good source of bio-vanillin, produced through bioconversion of ferulic acid.

*Citrus pomace* is a by-product of juice extraction from the citrus fruits and has two principle components: whole peel (rind) and 'rag'. The rind is principally the flavedo or epicarp and the albedo or mesocarp, whilst 'rag' is the residue left behind after juice extraction and comprises cores, segment walls or membranes, juice vesicles and seeds. The scope for protocol development to exploit citrus pomace (orange and lemon) was to provide processes at the laboratory scale to produce: - functional dietary fibres; - polyphenols and carotenoids; - clouding agent.

The objective was to develop processes to obtain citrus peel extracts and a fibre fraction as intermediate products or ingredients with a defined functionality that offers added value to the precedent step. Innovative process technologies, involving MW drying for product stabilization and DIC for product expansion were used to obtain dietary fibre. The protocols developed have demonstrated that dietary fibre extracts with defined functional properties can be directly produced from citrus pomace. The extracts are available with bittering agents and colour removed and the components removed can also be exploited as commercial ingredients.

#### WP4 - *New foods formulation and production*

The natural extracts obtained in WP3 from citrus peels were used in the production of fruit juices or fruit juice-based beverages. The product prototypes developed were three: an orange juice enriched with citrus fibre, a polyphenol-rich fruit juice-based beverage, and a soft drink with the clouding agent obtained from orange peels. Two prototypes were finally selected, produced at pilot scale and sent to Hungary (CCH) for a consumer's acceptance test: a citrus fibre enriched fruit juice and fruit based beverage using clouding agent.

The food products were formulated and prepared at lab-scale level: 1) several snacks from citrus by-products 2) a citrus-based monodose beverage 3) two citrus paste-based filled bakery products 4) a bakery product based on pre-fermented wheat bran 5) two citrus-based instant desserts.

Several pre-treatment and operation conditions were evaluated in order to stabilize the raw materials prior to their processing. In particular, to choose the orange-ingredient form, various trials were done to avoid citrus by-product colour and bitterness. High hydrostatic pressure (HHP) treatments of orange paste were used to evaluate its effect on bitterness, but also on its texture and mouthfeel properties. In particular modifications in the viscosity and in the consistency of the citrus were observed thus

making the treated products suitable to be used as a filler for: 1) a sweet product made of two soft bakery layers filled with the HPH-citrus; 2) crunchy biscuits containing bran, citrus as filler and covered by chocolate.

In order to understand consumer acceptance of ingredients and food products developed in the NAMASTE project, different consumer tests were carried out. As some of the products could not be tested with large number of consumers, the acceptance of the different product concepts was assessed by using an online survey. The online survey was based on a questionnaire consisting of three parts. A first part with the description of the products developed in the NAMASTE project. The participants evaluated their preferences, buying intent and consumption frequency of each product type. The second part of the questionnaire explored consumers' attitudes towards sustainability and use of by-products. The third part contained buying intent regarding the NAMASTE products with the information shown about the by-product based ingredients. The NAMASTE products selected for the survey were: • Snack bar • Citrus paste biscuits • Yoghurt dessert with HPH citrus paste • Fibre enriched cake based on pre-fermented whole and concentrated wheat bran fibre and citrus paste • Instant citrus dessert

In order to prevent possible nutritional losses due to processing, special attention must be paid to the processing conditions adopted, and the mildest temperature/chemical conditions should be used when possible to preserve bioactive molecules or technologically important compounds. In fact, from a chemical point of view it is well known that both whole grains and fruit processing by-products represents a potentially valuable resource that can be developed into high value products. Particularly, citrus peels and their extracts have been reported to have potent health and preventive activities due to the abundance of flavonoids, while whole grains are rich in fibre and antioxidants, including trace minerals and phenolic compounds, which have been linked to disease prevention.

The use of NAMASTE ingredients can improve their nutritional characteristics of the final products with the wheat bran contributing to recommended fibres intake for 10.4 - 45.68 %. Similarly, the NAMASTE beverage contribution to the optimal fibres intake is near 8%, while the contribution of the same amount of conventional citrus beverage is 0%. *Fibre* has beneficial effects on the intestinal function and high-fibre diets also tend to be less "energy dense". *Cloud* provides better texture and improves the quality of the drink product, while it is still a natural product obtained from citrus peel, which is an advantage. *Antioxidant* extracts obtained from peel are natural flavonoids and carotenoids that have potential health benefits and ability to scavenge free radicals. *Prebiotic oligosaccharides* and *pre-fermented bran* with antioxidant properties also have beneficial intestinal function.

### WP5 - Economic assessment

We'll now give an example of a market study of one of the new products and make comparisons between prices of this one and actual food on the market. For the HPH citrus paste a strategy was developed to scale up the NAMASTE research results to a hypothetical industrial scale and four alternative production processes were hypothesized:

- A. 50 % Fibre 50 % Cloud;
- B. 50 % Fibre 50 % Paste;
- C. 50 % Cloud 50 % Paste;
- D. 100 % Fibre, 100 % Cloud, 100 % HPH Paste

The results of scenarios A, B, C for the economic analysis of the three NAMASTE processes have been compared with the reference market prices of 6 EUR/kg for fibre and a range between 1 EUR/kg to 4.785 EUR/kg for cloud. The market price for dry Citrus Fibre is 6 EUR/kg whilst according to our calculation the Namaste price was approximately 6.88 EUR. The gap between these two can be reduced either by modifying the hypotheses used or by improving the production process. The results for the cloud agent are economically sustainable referring to a market price which ranges from 1 EUR/kg to 4.78 EUR/kg. In light of the market prices, the economic sustainability of the processes is attainable in the production hypotheses A, C and D.2 (with A as the most affordable one).

### **European Food Quality and Safety regulations**

#### **Possible use of the new food products within a balanced human diet**

Possible risks for the consumers related to the presence of pathogenic and/or toxigenic microorganisms, allergens, heavy metals and pesticides in the bran and citrus derived ingredients are taken into consideration: the critical analysis of the information collected evidenced that no microbiological risk is associated to citrus and wheat. It was established that ingredients made of by-products such as citrus peel and wheat bran do not represent an increased health risk for the consumer than those main food products with which they are generated during processing. This estimation is valid until the raw materials from which are generated comply to the actual EU food legislation and appropriate control measures based on Good Agricultural Practices and Assured Systems are applied during their production.

### **CONCLUSION**

The technologies developed in the NAMASTE project provide novel solutions for the management of the high amounts of by-products generated by the current food processing practices and contribute to the improved exploitation of available biological resources generated by raw material production; thus they can reduce the environmental impact associated with food production. These technologies and ingredients will be used as demonstration examples of the viability of the concept of valorization of by-products of food processing.

The results and experiences of the NAMASTE project (the EU and the Indian parts both) highlighted that the current food processing, by-product exploitation and waste handling practices should be reconsidered and reevaluated.

*Until edible parts of the food grade raw material are handled as food, they are considered food components, not wastes. Therefore, neither the food hygiene nor the emotional concerns "on eating waste" are realistic.*

# Removing Oestrogen from drinking water

## Introduction

The anticonception pill is a well-known pill that uses hormones to prevent children from being born. This pill has synthetic hormones in it which let the menstrual cycle stop. Most of these hormones are being used for stopping this process, but any excess comes out of the human body when a woman is going to the toilet. This water with hormones is being filtered and used again after it has been cleaned, for example as drinking water. However, modern technics for cleaning water, do not take the hormones out of the water, which means that hormones from the pill get into our drinking water.

Although the hormones are in the water with a very low concentration, most scientists are concerned that these hormones can do harm to the human body. They say, for example, that these hormones can cause allergies, damage to the brain and even cancer.

The hormones which scientists believe are harmful, are called estrogens. Estrogens are aromatic structures. The estrogens are part of the hormone regulation of women. They cause the uterine lining to break down, so if women take the anticonception pill containing these estrogens, their body won't grow any uterine lining. Unfortunately, the pill contains too many of these hormones and the female body is not able to use them all, so a part of the hormones leave the body again and when this urine is being filtered to become drinking water again, modern techniques do not filter these hormones.

Laccases are copper-containing enzymes. They are able to catalyze the oxidation of aromatic structures. In this case, the estrogens do not fit on the receptor protein anymore and so, the structure isn't harmful anymore.

## The danger of hormones in drinking water

Scientists do not agree on the direct effects of hormones in drinking water. Some scientists think that hormones in drinking water do not do any harm, others are convinced that these same hormones can cause cancer. One thing scientists do agree on is that the synthetically produced hormone called ethynyl-estradiol has the best chance of being most harmful. It is a hormone the birth-control pill contains. Technically speaking, this hormone is not dangerous, because millions of women take the Pill every day and much research on this hormone has been done, but the question we should ask ourselves is whether we want to get these hormones in our body even when we do not take the Pill. This is what is happening now, since the hormones aren't taken from the water before we drink it again.

Besides from negative side-effects to human, many scientists believe that these hormones can be harmful for other organisms, too. Water people dump into rivers can contain hormones. When some fish species get too many of these hormones in their body, they can change sex, they can change from being man to woman. This can have terrible effects on the environment in a river: once there are only female fish in the water, they can't reproduce, causing them to extinct.

### Common ways of filtering water

With modern ways of filtering water, only garbage and big chemical molecules can be removed. Once water comes at a water filtering center, the water goes through what's been called the first sedimentation. Big pieces of chemicals and other stuff goes to the bottom of a big waterbasin and are removed from here. In the next step, air is blown through the water, which gets rid of the smaller parts which are harmful. After this, the water will be filtrated. Most of the time, sand is used to filtrate the water. Water flows through the sand, from the top to the bottom. All the particles which are smaller than the space between the grains of sand will be hold down and stay behind. The water keeps flowing and many particles are removed. Still, the hormones remain. Even in the last step, the second sedimentation, the hormones are not removed and thus they can still be harmful for human health.

### How it is done

That something should be done to remove these hormones from water may be clear, but still it is not done on a big scale. For the iGem-project, students from Bielefeld have find a way to create a bio-filter, which is able to break down hormones.

To create a bio-filter, a filter which gets out the harmful hormones out of the water in a natural, not-chemical way, bio-bricks which contain genes for the production of the laccases-enzyme have to be build. Pieces of genes that can produce laccases are taken out of the DNA from an organism with genes for the production of laccases and are placed into a bacterium which then is able to break down the hormones in the drinking water. Plasmids from bacteria can be used to put these genes in. Once the plasmids are put back into a bacterium, chances are that the bacterium will produce the enzyme laccases and will break down hormones in our drinking water.

The laccases which are needed for this process can be find in fungi. Many different species of fungi can be used for the building of the bio-bricks. Also, many different species of bacteria can be used, but E. Coli is the most common one. We know most about E. Coli and this is why scientist prefer to use E. Coli. Apart from that, E. Coli is not harmful.

Once a bacterium is created which produces laccases, the cleaning of drinking water can start. When bacteria with the gene for the production of laccases are put into water with feminine hormones, the produced laccases will bind on the hormones and due to the enzyme, which works as a catalyzer, the hormones will be broken down.

### Conclusion

Even though scientist do not agree on the fact whether the hormones from the anticonception-pill are really harmful, it is good to find ways to filter the hormones from drinking water, in case they are harmful or even in case they do harm to water life. With modern techniques, we are able to create bacteria which can filter drinking water and remove the hormones. In the future, this may help us cleaning drinking water on a big scale.



**bioethics**

## Ethics and laws.

### What are GMOs?

A genetically modified organism is an organism whose genetic material has been altered using genetic engineering techniques. GMOs can have a both positive and a negative effect on humans, animals and the environment, that's why The EU has introduced regulations and laws which manage the distribution and cultivation of GMOs. In addition to that, some countries in the EU have introduced extra laws and regulations concerning GMOs.

### Positive sides of GMOs

Genetically modified organisms are used in biological and medical research, pharmaceutical drugs, experimental medicine and agriculture. Using GMOs in these fields, researchers are trying to solve problems that face humanity .

For example golden rice was created with the intention of bringing in vitamin A to the areas where the everyday food lacks vitamin A, which leads to the death of 670 000 children annually . So in this case golden rice could replace regular white rice which lacks vitamin A. Another famous GM crop is Corn. There are different variation of GM corn, such as herbicide resistant corn, insecticide-producing corn , insect resistant sweet corn and drought resistant corn. These different variations of corn were made so it would be easier to farm them, since there are different environmental problems in different areas.

### Negative sides of GMOs

Even though GM crops are made with good intentions, there are always negative sides to them. The biggest concern is that they can alter the biological diversity of nature by replacing non-GM crops completely. A good example of this comes from both North- and South-America where both growing and consuming GMOs is allowed: Most of the corn grown there is now Genetically modified, which is pesticide and herbicide resistant. Due to that insects and weeds have started to develop a resistance to the crops and that is forcing the researchers to make stronger and more expensive pesticides and herbicides to battle them. This is also hurting the farmers who aren't growing GM crops because the now resistant pests and weeds have spread to their fields as well.

Another problem that has come up is the growing corporate power in the GMO industry. The companies that have researched and developed the GM crops have a lot of power over the cultivation of the crops. Farmers have to buy the seeds from these companies(i.e. Monsanto) and they also have to use the herbicides and pesticides produced by the same company. Worst of all the farmers can't use the last years seeds in order to plant more crops, they have to buy the seeds again every year from one of the companies that have the rights to the seeds. This leads to the farmers having a really hard time returning a profit from their hard work.

## Regulations In the EU

The EU has implemented numerous regulations concerning GMOs with the intentions to:

- Protect the environment and the health of humans and animals
- **Ensure clear labelling of GMOs** placed on the market in order to enable consumers as well as professionals (e.g. farmers, and food feed chain operators) to make an informed choice.
- Ensure the traceability of GMOs placed on the market
- Put in place procedures for **risk assessment** and authorisation of GMOs that are efficient, time-limited and transparent.
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The EU demands a pre-market authorization for any GMOs to enter the market and a post-market environmental monitoring. A risk assessment must show that the food or feed is safe for human and animal consumption and it also has to be safe for the environment.

As of September 2014, 49 GMO crops, consisting of eight cottons, 28 GM maizes, three GM oilseed rapes, seven GM soybeans, one GM sugar beet, one GM bacterial biomass and one GM yeast biomass have been authorized in the EU. So far only 1 GMO has been authorized for cultivation- MON810, a GM maize which is resistant to the European corn borer. It was authorized in 1998. The largest producer of GM maize as of 2014 is Spain where 20% of corn grown there is genetically modified.

## Regulations In the EU member states

Each member state in the EU has the right to put in place additional laws to prevent the cultivation or sale of Genetically modified crops. So far 19 member states have issued bans on various GM crops:

Austria, Belgium, Croatia, Cyprus, Denmark, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Malta, The Netherlands, Poland, Slovenia, The United Kingdom (excluding England). The most common banned GM crop is maize among the member states with 1/3 of them banning all cultivation and sale of all GM crops.

# **Bioethics**

By Emma, Jordan and Kees

## **Introduction**

Ethics is a philosophical discipline pertaining to notions of good and bad, right and wrong. Bioethics is the application of ethics to the field of medicine and healthcare. Bioethics is becoming more important due to the advances in biology and medicine, creating new situations and possibilities.

## **What does it accomplish?**

Bioethics applies the foundational disciplines of philosophy and theology, and incorporating perspectives from various other disciplines including sociology, medicine, nursing, anthropology, and law. Significant questions are addressed in bioethics such as the ends and purposes of the life sciences and healthcare, the meanings and implications of distributive justice, and issues in global healthcare. Bioethicists explore even deeper issues such as the meaning of life and death, pain and suffering, and rights and responsibilities.

## **Religion**

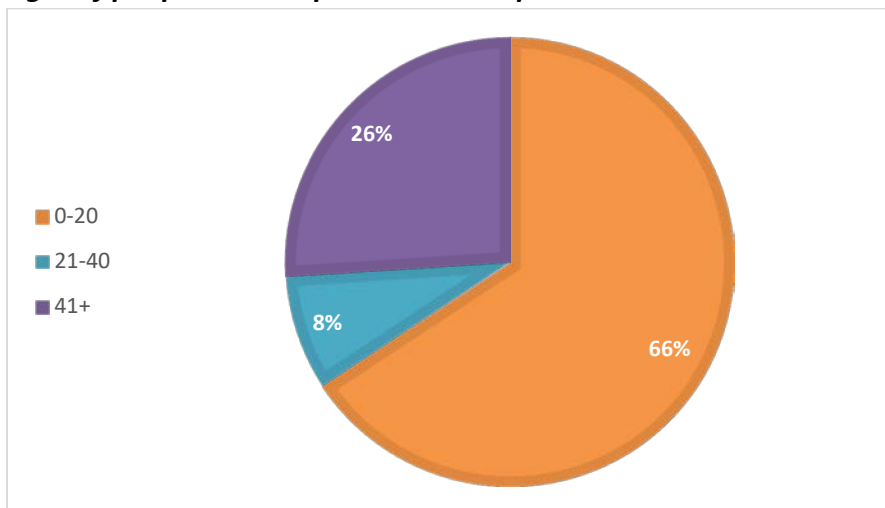
Many religious communities have their own histories of inquiry into bioethical issues and have developed their own rules and guidelines on how to deal with these issues from the viewpoint of their faiths. The Jewish, Christian and Muslim faiths have each developed a large amount of literature on these matters for their religions. Buddhist bioethics, in general, is characterised by a naturalistic outlook that leads to a rationalistic, practical approach.

## Questionnaire

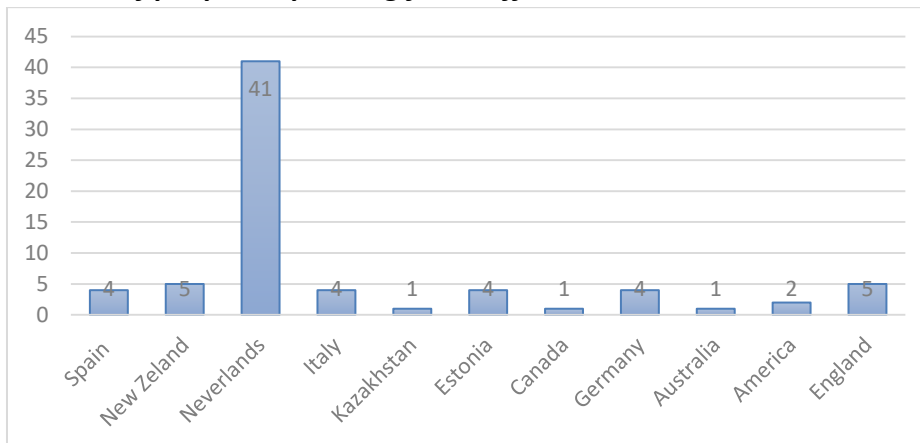
We compiled a questionnaire to get public views on bioethics from different ages and countries to analyse the difference and similarities in their views towards different questions on bioethics.

We composed 10 questions and these are our results.

### *Ages of people who responded to the questionnaire*



### *Number of people responding from different countries*



We had 11 countries from 4 continents take our survey giving us a wide range of responses from different nationalities and beliefs.

**Question 1: Do you know what biotechnology is?**

65 people said they know what it is, 6 believe they did but not the specifics of biotechnology, and 1 said they didn't know what it was.

**Question 2: Do you think biotechnology is a positive thing?**

Before this question we had a paragraph describing what biotechnology was and some of the things it is used for, this then improved the understanding and gave a more accurate reply to this question. 38 people said yes, 14 said no and 22 said maybe. With those who responded maybe most had a comment such as 'as long as they don't go over the limits' and 'there are risks to it'. This suggests that they do believe it is a good thing as long as it doesn't get taken too far.

**Question 3: Do you believe that stem cell research is ethical?**

We had a paragraph before on what stem cell research is to give more information before the response. Only 1 person said no. Many said maybe again that there are limits to it, like it should be used for diseases and that embryos should not be created for this sole reason as this would be unethical. A few people replied with just yes - they believe it is ethical.

**Question 4: Do you believe that stem cell research could be good for us?**

65 people said yes, 2 maybe and 5 no. These results show that even though in the question before people said they had doubts on it being ethical and being taken too far, they do believe that it would be beneficial to humans.

**Question 5: Do you believe there are risks to using stem cells?**

Most people replied with yes and maybe, and giving strict conditions for it being ok. Very few people believe there will be no risks. For those who said they believe there will be risks, these range from side effects to people being put under pressure to produce embryos.

**Question 6: Do you believe that it is ethical to genetically modify organisms?**

A paragraph on genetic modification was put before the question. 60 people responded with yes as long as it didn't cause pain. 5 said maybe for the same sort of reasons. 8 people said no. Many people said it depends on the situation and use it is being done for.

**Question 7: Do you believe that genetically modifying organisms will be beneficial to people?**

6 people said no, the rest said yes or maybe depending on the cause for it. Such as if it was to modify crops to feed those with no food then it would be beneficial.

**Question 8: Do you believe there are risks to genetic modification of foods?**

Almost everyone replied to this with yes. Some reasons are 'it could be used to set straight the widening gap between rich and poor', 'there can be people that could be allergic to the modified products but they weren't to the original products.' This shows that there are many potential risks to genetic modification of food, but they are not tested so it is unknown how many will actually happen.

**Question 9: Do you have any further comments about Biotechnology?**

Finally we asked for any further comments to see any extras that people have to add to our questionnaire responses and analysis.

“We should never lose sight of the purpose of biotechnology: to improve the health of humans, animals, and the natural world.”

“It's a door which leads to a better future: research in this sector is not enough valorized and researchers mustn't be blocked by ethical questions.”

“All new technology will have positive and negative outcomes. Embrace the future, initiate and fund research but make sure that usage and implementation is properly addressed in regulations. And evenly important, visible to the general public when modified products are used, so people can decide if they want to use them.”

“Biotech developments cannot be stopped, whether it is ethical or not. I sincerely believe that such developments can be used in a positive way, or negative way depending on the people applying biotech. It is not a black or white discussion. Biotech should be accepted, but with strict parameters.”