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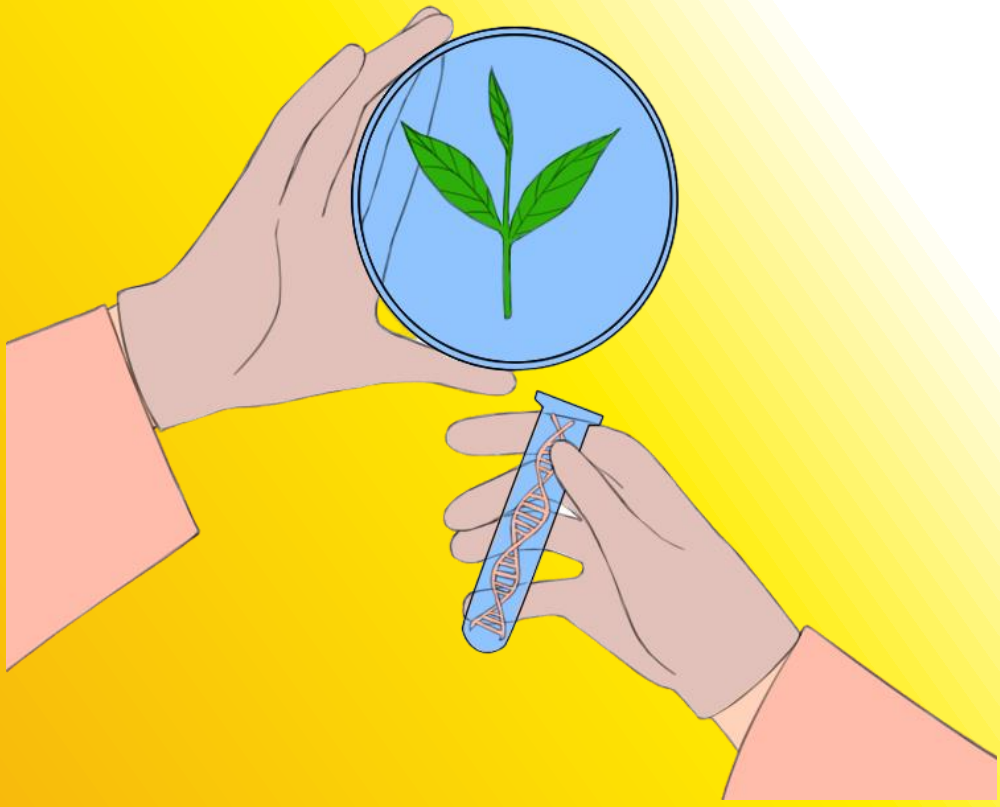

PROCEEDING

of the

1st Autumn School-Conference on Food Biotechnology for Candidates, Students and Young scientists

Yekaterinburg, 9th–12th November, 2022

PROCEEDINGS
OF
THE 1ST INTERNATIONAL AUTUMN SCHOOL-CONFERENCE ON FOOD BIOTECHNOLOGY FOR CANDIDATES, STUDRNTS AND YOUNG SCIENTISTS,
URAL FEDERAL UNIVERSITY,
9TH -12TH NOVEMBER, 2022

Editors:
Elena G. Kovaleva, Yulia O. Savlukova

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2023

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The Proceeding of the 1st Autumn School-Conference on Food Biotechnology for Candidates, Students and Young scientists in English (*foodbiotech.urfu.ru*) includes Lectures and Master Classes from Senior Scientists in Food Biotechnology, Molecular Biology and Food Chemistry. The School-Conference was held in the Ural Federal University named after the First President of the Russian Federation B. N. Yeltsin (UrFU) in face-to-face and on-line modes from November 9 to November 12, 2022.

It is addressed students of the last and penultimate years of undergraduate and graduate studies studying in biological, chemical and chemical-technological areas of Russian and foreign universities and preparatory departments of leading universities in Russia.

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PROGRAM

for face-to-face and online communications (via BigBlueButton Platform)

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November 9th, 2022

Moderator: Prof. Elena G. Kovaleva

10.00 – 13.00 **(Yekaterinburg time, UTC +5)**

Plenary session **Co-Working Center** (Lenina 66 Prospect, the building of the UrFU dormitory, entrance from Lenina prospect, Yekaterinburg, Russia) **& Online**	
10.00 – 10.15	**Opening Ceremony**
10.15 – 11.30	**Dr. Svetlana V. Merenkova,** Ass. Prof. Department of Food and Biotechnology, South Ural National Research University, Ph. D. (Food Technology), Chalyabinsk, Russia (online) ***Microemulsions: Unique Properties, Biotechnological Applications***
11.40 – 13.00	**Dr. Ranga Rao Ambati,** Ph. D. Associate Professor & Senior Scientist Department of Biotechnology (NBA Accredited) Vignan's Foundation for Science, Technology and Research, Deemed to be University, Accredited by NAAC 'A', India ***Microalgal pigment production for commercial applications***

Master Class **ХФ-403** (Scientific, Educational and Innovation Center of Chemical and Pharmaceutical Technologies, 21a Mira, Yekaterinburg, Russia) **& Online**	
14.00 – 16 00	**Dr. Kinan Darkazanli,** Ph. D. Biology, Ass. Prof. (part time), Department of Technology for Organic Synthesis, Ural Federal University, Russia ***DNA isolation: basics of sample preparation methodologies and isolation procedures***

November 10th, 2022

Moderator: Prof. Maxim A. Mironov
10.00 – 13.00 **(Yekaterinburg time, UTC +5)**

Round Table Meeting **Room T-212** (Sof'i Kovalevskoy 5, second Floor, Yekaterinburg, Russia) **& Online**	
10.00 – 13.00	***Biotechnological education for foreigners in Russia: trends and prospects***

Master Class **ХФ-403** (Scientific, Educational and Innovation Center of Chemical and Pharmaceutical Technologies, 21a Mira, Yekaterinburg, Russia) **& Online**	
14 00 – 16 00	**Dr. Ol'ga S. Koptyaeva,** Ph. D. Chemistry, Ass. Prof., Department of Technology for Organic Synthesis, Ural Federal University, Russia ***Chemostat without optimization***

November 11th, 2022

10.00 – 13.00 **(Yekaterinburg time, UTC +5)**

Master Class **ХФ-207** (Scientific, Educational and Innovation Center of Chemical and Pharmaceutical Technologies, 21a Mira, Yekaterinburg, Russia) **& Online**	
10.00 – 13.00	**Prof. Elena G. Kovaleva,** Ph. D. Chemistry, Department of Technology for Organic Synthesis, Ural Federal University, Yekaterinburg, Russia ***Production of all grain beer of ale fermentation by infusion method***

Moderator: Prof. Elena G. Kovaleva
14.00 – 17.00 **(Yekaterinburg time, UTC +5)**

Plenary session **Co-Working Center** (Lenina 66 Prospect, the building of the UrFU dormitory, entrance from Lenina prospect, Yekaterinburg, Russia) **& Online**	
14 00 – 15.20	**Dr. Tarek M. Itani,** Ph. D, Molecular Biology, Head of Laboratory of Enteral Viral Infections, Research Institute of Viral Infections, Rospotrebnadzor, Yekaterinburg, Russia ***Viruses of foodborne origin: a review***
15.40 – 17.00	**Dr. Kinan Darkazanli,** Ph. D. Biology, Ass. Prof. (part time), Department of Technology for Organic Synthesis, Ural Federal University, Russia ***A journey in Molecular Genetic Fundamentals of Food Biotechnology***

November 12th, 2022

11.00 – 14.00 **(Yekaterinburg time, UTC +5)**

Master Class **ХФ-207** (Scientific, Educational and Innovation Center of Chemical and Pharmaceutical Technologies, 21a Mira, Yekaterinburg, Russia) **& Online**	
11.00 – 14.00	Assistant Lecturer **Mustapha Kamel**, Ph. D. Student in Chemical Technology, Scientific, Educational and Innovation Center of Chemical and Pharmaceutical Technologies, Ural Federal University, Yekaterinburg, Russia ***Production of semi-hard cheese in mini-cheese maker***

Moderator: Prof. Maxim A. Mironov
15.00 – 17.00 **(Yekaterinburg time, UTC +5)**

Plenary session **ХФ-110** (Scientific, Educational and Innovation Center of Chemical and Pharmaceutical Technologies, 21a Mira, Yekaterinburg, Russia) **& Online**	
15.00 – 15.45	**Prof. Elena G. Kovaleva,** Ph. D. Chemistry, Professor, Department of Technology for Organic Synthesis, Ural Federal University, Russia ***Natural Deep Eutectic Solvents in Extraction of Biologically Active Compounds***
16.00 – 16.45	**Prof. Antioine Nsabimana,** Ph. D, Biotechnology, Professor, Department of Biology, National University of Rwanda, Kigali, Rwanda ***Methodologies for the Characterization of Germaplasm collection originated from Rwanda***
16.45 – 17.00	**Conclusive Remarks and Closing of the 1st International Autumn School-Conference on Food Biotechnology for candidates, students, graduate students and young scientists in English**

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PREFACE

The Proceeding of the 1st Autumn School-Conference on Food Biotechnology for Candidates, Students and Young scientists in English (*foodbiotech.urfu.ru*) includes Lectures and Master Classes from Senior Scientists in Food Biotechnology, Molecular Biology and Food Chemistry. The School-Conference was held in the Ural Federal University named after the First President of the Russian Federation B. N. Yeltsin (UrFU) in face-to-face and on-line modes from November 9 to November 12, 2022. It was organized by the Institute of Chemical Technology and its new structural subdivision «Scientific, Educational and Innovation Center for Chemical-Pharmaceutical Technologies» at UrFU.

The main goal of the school-conference was to raise the awareness of both Russian and foreign students about the possibilities of studying at the Ural Federal University in the direction of the master's program 19.04.01 Biotechnology (Master's programs Food biotechnology, Molecular biotechnology and bioengineering, Living systems. Promising chemical-pharmaceutical and biotechnologies: research and development) and in the direction of postgraduate study 1.5.6 Biotechnology and related areas, to demonstrate laboratories facilities, competence and professionalism of the teaching staff, with the involvement of specialists conducting scientific research in the field of food biotechnology, developing new innovative products and technologies, organizing modern industrial production, participating in import substitution and promoting new generation competitive biotechnological products in the market.

The target audience of the school-conference were students of the last and penultimate years of undergraduate and graduate studies studying in biological, chemical and chemical-technological areas of Russian and foreign universities and preparatory departments of leading universities in Russia.

The school-conference program includes lectures by leading scientists on the main aspects of food and biotransformation technologies, master classes in various areas of food biotechnology, a round table meeting entitled as «Biotechnological education for foreigners in Russia: trends and prospects» with the invitation of both leading scientists as well the UrFU International Service and the UrFU department of International Education.

Experts in biotechnologists from partner institutes and universities were involved in the school-conference. They were as follows: Yekaterinburg Research Institute of Viral Infections, SRC VB VEKTOR, Rospotrebnadzor, South Ural State (National Research) University, Chelyabinsk, Republican control and testing complex for food quality and safety RUE «Scientific- Practical Center of the National Academy of Sciences of Belarus for Food», Republican Unitary Enterprise «Scientific and Practical Center of the National Academy of Sciences of Belarus for Food», Minsk, Belarus, Damanhour University, Egypt and School of Natural Sciences and Applied Technology Vignan's Foundation for Science, Technology and Research (Deemed to be University, Accredited by NAAC 'A'), Guntur, Andhra Pradesh, India; University of Rwanda (Kigali, Rwanda), and production companies Ural Biopharm OJSC, Tabletta, Zoobioprom, Beersfan Ltd.

Prof. Elena G. Kovaleva
Chair of the 1st Autumn School-Conference on Food Biotechnology
for Candidates, Students and Young scientists in English

LECTURES

LECTURE 1
NATURAL NANOCRYSTALS

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1. General characteristics of natural nanocrystals

Crystalline nanoparticles of inorganic substances are quite widespread in nature. Most often, they are distributed in the atmosphere, forming nanoaerosols. In rocks, nanoparticles are formed as a result of chemical weathering processes of silica, aluminosilicates, magnetites and other types of minerals.

An example of a layered nanomaterial is mica, a group of aluminosilicate minerals having a lamellar appearance, characterized in that the layers representing them are capable of splitting into extremely thin leaves (nanoobjects) that retain flexibility, elasticity and strength.

Mineralized natural materials such as shells, corals and bones: Many of these materials are formed by calcium carbonate crystals, which self-organize together with other natural materials such as polymers to form three-dimensional architectures.

For example, a layer of cell proteins grows, preserved by a polysaccharide polymer such as chitin. **Proteins occur as a nano-assembly mechanism to control the growth of calcium carbonate crystals.** Around each crystal remains a honeycomb structure of protein and chitin. The size of each crystal is about 100 nm. As a result, mother-of-pearl, for example, shellfish acquires unusual physical properties (strength, resistance to compression, etc.).

In the role of changing colors of the chameleon, large cellular pigments appear - iridocytes or guanophores, which detect guanidine crystals. These crystals are silver or golden in color. Under pressure and a high concentration of compounds, the crystals change position in the chromatophore.

The deviations and the reflection spectrum of the skin color are counted among themselves. The higher the gap, the higher the shift from the blue part of the spectrum to green and then to red.

The nanostructure of lotus and nasturtium leaves is responsible for their unusual surface properties and their ability to "self-cleanse". The hydrophobicity, as in the "lotus effect", is due to the micro / nano structure of the leaf surface.

The self-cleansing properties of the lotus plant are produced by the combination of leaf microstructures and epidermal cells on their rough formations covered with waxy crystals. These crystals cause a water-repellent layer that competes with the rough surface, provoking its super-hydrophobic surface with a contact angle of about 150°.

The consequence of this is that water drops roll off the surface of the leaf and at the same time remove dirt from it. This "self-cleaning" effect makes the lotus leaf clean and resistant to dirt.

2. Zeolites

The term "zeolite" (translated as "boiling stone") was proposed by F. Kronstedt, who in 1756 discovered that the aluminum silicate mineral swells when heated - it increases in volume with the release of water.

Subsequently, it turned out that other minerals of this family also have the same property to release and absorb water: mordenite, faujasite, chabazite. It should be noted that crystalline hydrates, which include crystals of a number of compounds containing water molecules held in crystals due to chemical bonds, have a similar ability to absorb and release water.

Unlike crystalline hydrates, zeolites absorb and release not only water, but also other substances, moreover, without changing the crystal structure.

The absorption capacity of zeolites is due to adsorption - the concentration of a substance from a gas or liquid phase on the surface of a solid body (adsorbent) or in the volume of pores formed by its structure. Usually, the absorption capacity of natural zeolites is low, but it reaches high values for synthetic and natural modified zeolites.

Due to this unique property, zeolites are promising to be used as adsorbents and catalysts. Zeolites are crystalline aluminosilicates of alkali or alkaline earth metals.

The structure of zeolites is characterized by the presence of a highly developed network of pores (pore channels), which leads to the formation of a large inner surface, which ranges from 10,000 to 100,000 values of the outer surface. Pores (pore channels) have molecular dimensions, so zeolites can be considered as special molecular sieves.

Zeolite cations are easily exchanged for cations of other types. For example, in zeolites, sodium cations can be replaced by calcium cations, which leads to an increase in the pore diameter to 0.5 nm, or by potassium cations, which leads to a decrease in the pore diameter to 0.3 nm.

The main structural element of these types of zeolites is an almost regular tetrahedron, in which the bond angle is about 109°. The connection of tetrahedra through oxygen atoms leads to the formation of various secondary structures: rings, prisms and more complex polyhedral (Figure 1).

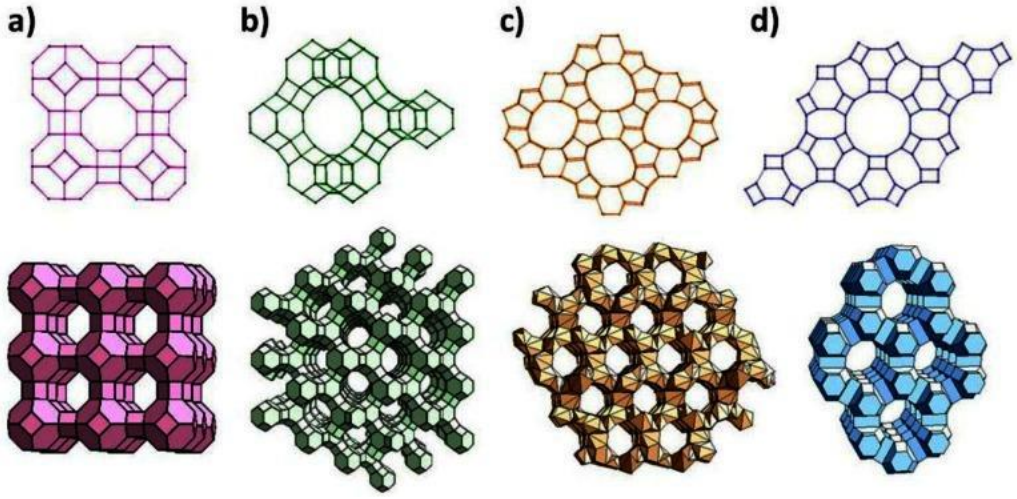

Figure 1. Representative zeolite skeletons (with pore openings). (a) Zeolite A 3 D, 4.2 Å); (b) Zeolite Y 3 D, 7.4 Å); (c) Zeolite X 1 D, 7.1 Å); (d) ZSM 5 silicalite) 2 D, 5.3 × 5.6 Å, 5.1 × 5.5) D dimensions of the channelsystem

For pentasil-type zeolites, the main structural element is a fragment of five- and six-membered rings. The combination of such fragments gives chains that form layers. At the same time, a system of channels of various types is formed in pentasils, including straight channels with a circular cross section (0.54–0.56 nm) and zigzag (sinusoidal) channels with an elliptical cross section (0.51–0.55 nm). Zeolites of various types are characterized by different pore sizes: from 0.3 to 1.5 nm.

By varying the synthesis conditions, it is possible to obtain zeolites with different types of secondary structures and spatial lattices. Thus, it is possible to purposefully create zeolites having different pore sizes.

Usually zeolites are unstable in acidic and alkaline environments and do not withstand heating to high temperatures - 600-800°C. The thermal stability of zeolites can be influenced by changing the nature of the cations. In particular, the presence of a cation in the center stabilizes the structure. Thermal stability also increases with increasing Si/Al ratio.

The structure of zeolites has a decisive influence on their adsorption capacity. Typically, the outer surface of a small adsorbent granule is less than a square centimeter, while the inner surface due to a highly developed pore system can be hundreds of square meters per 1 gram of adsorbent.

Openwork structure of zeolites creates a large adsorption volume, and its geometry determines the molecular sieve properties. Moreover, the presence of cations, which play the role of specific acceptor centers, determines the strong interaction of the adsorbed molecules with the adsorbent. Zeolites are able to selectively extract various ions from solutions and ensure their concentration. These qualities determine the widespread use of zeolites as ion exchangers.

Natural zeolites form relatively large agglomerates, so their use in industry is difficult, while synthetic zeolites are obtained in the form of small crystals with a particle size of about a few microns (usually 1-6 microns).

Due to the special properties of zeolites, such as the ability for reversible adsorption, the presence of pores with inlets of strictly defined sizes, and a large internal surface, zeolites can be use in the following areas: separation of substances depending on the size of molecules; separation of substances depending on polarity. At the same time, their possible fields of application include: drying and cleaning of gas or liquid; separation of mixtures of hydrocarbons of various structures; softening of water flows from heavy metal cations and absorption of radionuclides in nuclear power engineering.

Among the various examples of industrial use of zeolites are: the separation and purification of hydrocarbons, the catalytic reactions of hydrocarbons, the drying of refrigerants, the separation of air components, the production of carriers for catalysts, the extraction of radioactive isotopes from liquid nuclear waste, the separation of carbon dioxide and sulfur compounds from natural gas, the separation enzymes, removal of impurities polluting the atmosphere (Figure 2).

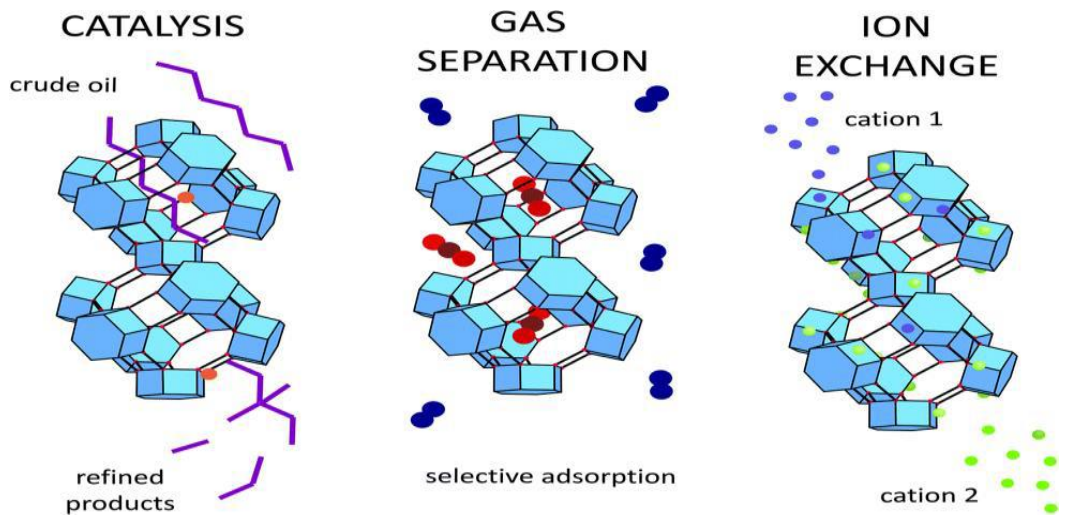

Figure 2. Areas of zeolites application

In agriculture, zeolites are used as a feed additive, for the prevention of animal diseases, to improve fertility, and for many other purposes.

The term zeoponics means growing plants on any artificial soil, a significant part of which is natural or artificial zeolites.

The production volumes of zeolites and the products produced with their participation are constantly growing: at present, chemicals and motor fuels are produced using zeolites for 1 trillion dollars per year.

In many cases, zeolites are much more effective than well-known adsorbents such as activated carbon or silica gel.

Application to improve human health. Currently, zeolites are raw materials for the production of soft drinks, specialized food products and biologically active food supplements. Zeolites, adsorbing toxic substances, not only remove them from the body, but also increase the pH value.

Zeolites also influence the course of inflammatory processes and the activity of the immune system in various acute and chronic disorders of human health.

The use of zeolites can have the following positive effects on the human body:

- reduction of tumors and improvement of tolerability of chemotherapy;
- rapid healing of wounds and burns;
- improving the effectiveness of the treatment of infectious diseases);
- prevention of gastrointestinal diseases;
- treatment and prevention of a huge number of other diseases and pathologies - thyroid pathology, diabetes treatment.

3. Microcellulose

Cellulose is the most abundant natural polymer available on Earth and is an important structural component of the cell wall of various plants. In addition to plants, cellulose is also present in a wide variety of living species such as algae, fungi, bacteria, and even some marine animals.

Cellulose is a fibrous, rigid and water-insoluble polymer and plays an essential role in maintaining the structure of plant cell walls. In addition, cellulose is a biodegradable, biocompatible and renewable natural biopolymer and is therefore considered an alternative to non-degradable fossil fuel based polymers.

According to the chemical structure, cellulose is a polymer formed by polycondensation is composed of monomers linked together by glycosidic oxygen bridges. Cellulose consists of β-1,4-linked glucopyranose units, which form a high-molecular linear homopolymer in which each monomeric unit is twisted 180° relative to its neighbors. The repeating unit of this natural polymer is the glucose dimer, known as cellobiose. The degree of polymerization of cellulose can vary depending on the source and is about 10,000 units of glucose for wood-based cellulose and 15,000 units for cotton cellulose (Figure 3). Each unit of glucopyranose has three hydroxyl groups, which give cellulose some characteristic properties such as hydrophilicity, the ability to biodegrade, which are initiated by the high reactivity of hydroxyl groups. The ability of these hydroxyl groups to form strong hydrogen bonds is the main reason for some other properties, such as the multiscale microfibrillated structure, hierarchical organization (crystalline and amorphous fractions) and highly cohesive character.

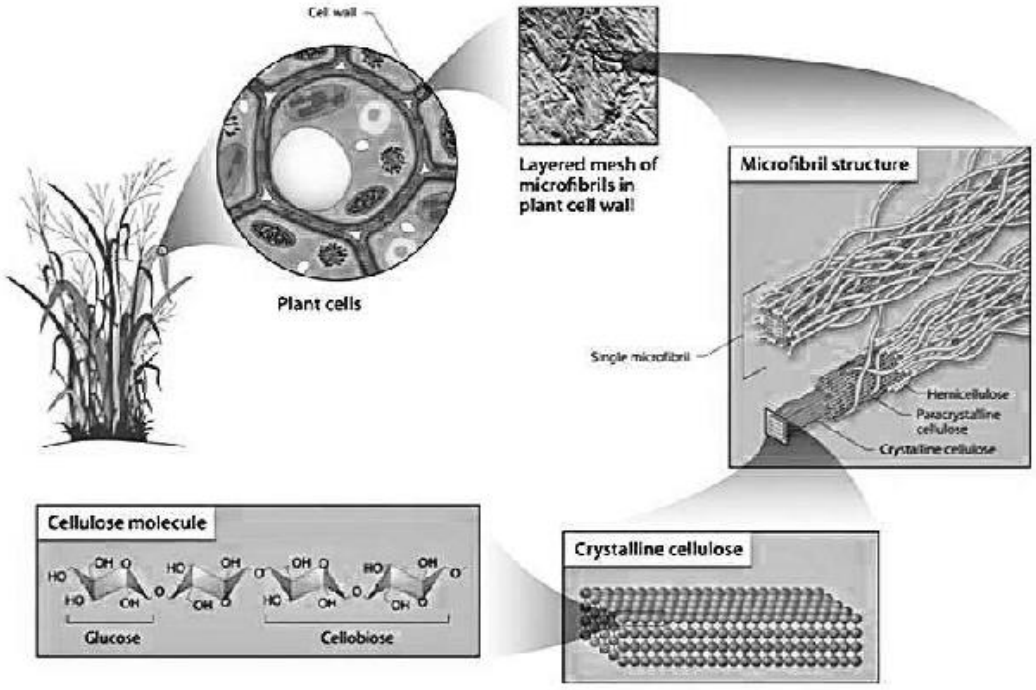

Figure 3. Chemical structure of the cellulose

Many individual cellulose chains come together to form elementary fibrils (protofibrils) having an approximate diameter of 3.5 nm. Depending on the source, the diameter of elementary fibrils can vary in the range of 2-20 nm, and they occur in different packages depending on the conditions that regulate biosynthesis. Microfibrils are formed by aggregation of elementary fibrils by coalescence, which is used as a mechanism for reducing the free energy of surfaces. This aggregation phenomenon is carried out due to Van der Waals forces, as well as intra- and

intermolecular hydrogen bonds. Cellulose molecules that are aggregated to form microfibrils have different orientations.

Depending on the intramolecular interactions and molecular orientations present, cellulose can exist as different polymorphs or allomorphs. Natural bulk cellulose consists of highly ordered crystalline regions along with some disordered (amorphous) regions in different proportions depending on its origin (Figure 4).

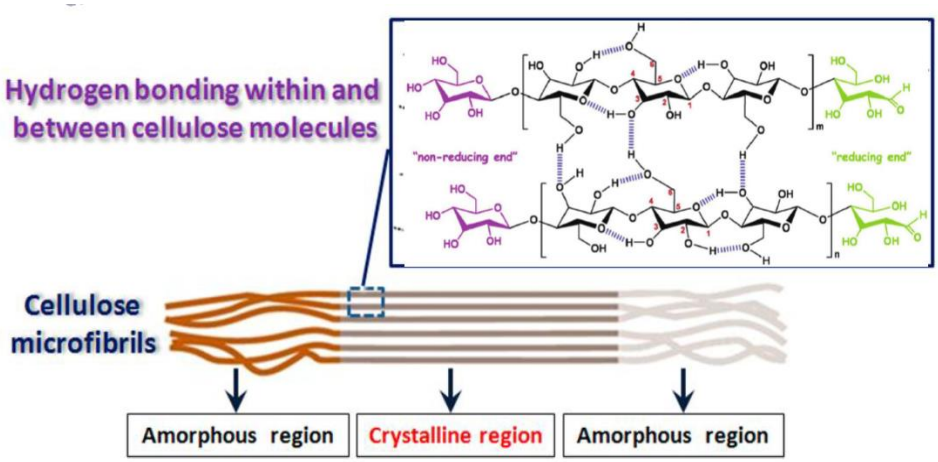

Figure 4. Molecular structure of the natural cellulose

When these microfibrils are subjected to a combination of mechanical, chemical and enzyme treatments, highly crystalline regions can be extracted, resulting in the formation of cellulose nanocrystals. Nanocrystal cellulose (NCC) is rigid rod-shaped particles consisting of segments of a cellulose chain with an almost perfect crystal structure.

Compared with bulk cellulose, which has large amorphous fractions, these nanocrystals have high specific strength, elastic modulus, high surface area and unique liquid crystal properties.

Several mechanical processes are used to extract cellulose microfibrils, such as high-pressure homogenization, high-intensity ultrasonic processing, microfluidization. These mechanical processes provide sufficient shear force to separate the cellulose fibers along the longitudinal axis and help extract the cellulose microfibrils. The chemical method of converting cellulose microfibrils into NCC is better than mechanical methods, since it reduces energy consumption and also produces rod-shaped short nanocrystals with improved crystallinity.

Strong acid hydrolysis is usually used to remove amorphous domains that are regularly distributed along microfibrils. Strong acids can easily penetrate into amorphous regions with a low level of order and hydrolyze them, leaving the crystalline regions unaffected.

The geometric dimensions of the NCC, such as length and width, may vary depending on the origin of the cellulose microfibrils and acid hydrolysis conditions, such as time, temperature, purity, etc. The average length of a particle in the form of a rod can vary from tens of nanometers to several micrometers, and the width - from 3 to 50 nm.

Nanocellulose obtained from wood had a diameter and length in the range of 3-5 nm and 100-300 nm. NCCs derived from cotton have been found to have a diameter of 5-10 nm and a

length of 100-150 nm. Marine algae produce NCCs with sizes in the 20 nm range and 1000-2000 nm in length.

Under suitable conditions and at critical concentrations, all asymmetric rod-like or lamellar particles spontaneously form ordered structures, which leads to the formation of a nematic phase. Rod-like NCCs, when dispersed in water, self-align to form chiral nematic phases with liquid crystal properties. Their rigidity, proportions, and ability to align under certain conditions make them ideal for exhibiting liquid crystal behavior.

Various factors such as size, charge, shape, dispersion, presence of electrolyte, and external stimuli can also affect the liquid crystallinity of NCC. The liquid crystallinity of nanocrystals, combined with their birefringent nature, leads to interesting optical phenomena.

NCC obtained by hydrolysis with sulfuric acid often has a negatively charged surface, which contributes to uniform dispersion in water due to electrostatic repulsions.

Despite the fact that interactions between nanocrystals are strong, highly sulfonated NCC is easily dispersed, which leads to the development of lyotropic behavior. NCCs with sulfuric acid and phosphoric acid usually give a chiral nematic structure, while NCCs with hydrochloric acid with post-reaction sulfonation give a birefringent glassy phase.

NCCs have a high surface to volume ratio as well as a high amount of hydroxyl groups, making it suitable for many types of surface functionalization. By introducing any chemical functionality on their surface, it is possible to modify the type of interactions that the material exhibits with its environment. Commonly used surface functionalizations for NCC are esterification, oxidation, amidation, nucleophilic substitution, polymer grafting.

These nanomaterials offer several potential advantages as drug delivery vehicles due to their properties such as smaller size, hydrophilicity, biocompatibility. Due to their large surface area and the possibility of obtaining a negative charge upon hydrolysis, a large number of drugs can be bound to the surface of these materials for optimal dosing.

NCC-based aerogels are also of great interest in biomedical and pharmaceutical applications due to their open pore structure and high surface area, which can provide increased drug bioavailability and better drug loading capacity.

NCC/polymer based nanocomposites have many applications. A polymer nanocomposite is a multiphase material in which the polymer phase is reinforced with a nanomaterial. These polymer nanocomposites have unique properties due to their nanometer size and increased surface area of the reinforcing material, NCC is used as a carrier component in many polymer nanocomposite systems as it can significantly improve mechanical properties even at very low concentrations.

In addition, its high aspect ratio, good dispersion in hydrophilic systems, and the ability to form loose network-type architecture in a polymer matrix make it a widely preferred reinforcing agent. Various functional molecules, such as fluorescent molecules, DNA, can also be attached to the surface of the NCC using the chemical grafting method, which can be used for fixing in a biological environment. Given the biocompatibility of NCC and the possibility of chemical modifications, NCC can be used in such biomedical applications as the creation of biosensors, biotransducers, fluorescence bioanalysis, bioimaging applications.

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The full text of presentation can be accessed on https://foodbiotech.urfu.ru/en/presentations-of-plenary-speakers/

LECTURE 2
A JOURNEY IN MOLECULAR GENETIC FUNDAMENTALS OF FOOD BIOTECHNOLOGY

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Introduction

Molecular genetics has been defined as the field of biology and genetics that studies the structure and function of genes at the molecular level, and it employs the methods of genetics and molecular biology to elucidate molecular function and interactions among genes. The beginning of this field dates back to mid-1800 s. Here three major events in the mid-1800s led directly to the development of modern genetics {Journal of Crop Improvement [1]**.** Firstly Charles Robert Darwin published The Origin of Species, which describes the theory of evolution by natural selection of course this theory requires heredity to work [2], after that Gregory Mendel published Experiments in Plant Hybridization, which lays out the basic theory of genetics that widely ignored until 1900, then Friedrich Miescher isolated "nucleic acid" from the nuclei of leukocytes obtained from pus, the nucleic acid was named nuclein, the nuclein was slightly acidic and rich in phosphorus and nitrogen. In 1900 Carl Erich Correns, Hugo de Vries, and Erich von Tschermak rediscovery of Mendel's work and they came to the same conclusions about inheritance as Mendel. After ten years exactly in 1910 Thomas Hunt Morgan proved that genes are located on the chromosomes (using Drosophila) [3].

In the 20th century there are some events that are no less important than what happened previously, for example in 1926 Hermann J. Mueller showed that X-rays induce mutagenesis (discovery of the mutagenic effects of X-rays), except that in 1953 James Watson and Francis Crick determined the structure of DNA molecule (Diagram 1,2), which directly leads to knowledge of how it reproduces, this discovery was an important turning point in the history of genetic science [4], where the nitrogenous bases involved in the synthesis of DNA were identified [5].

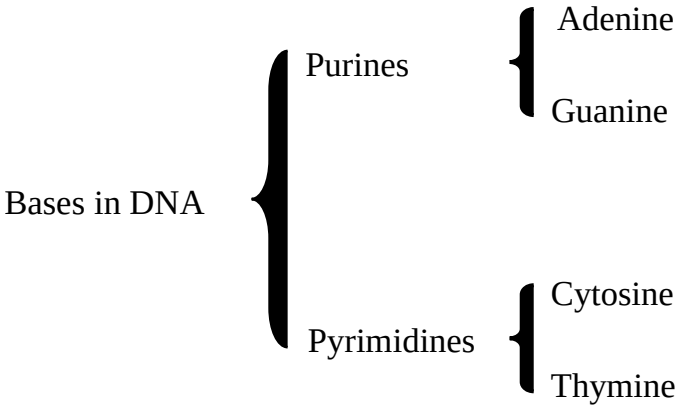

Diagram 1. The nitrogenous bases of DNA

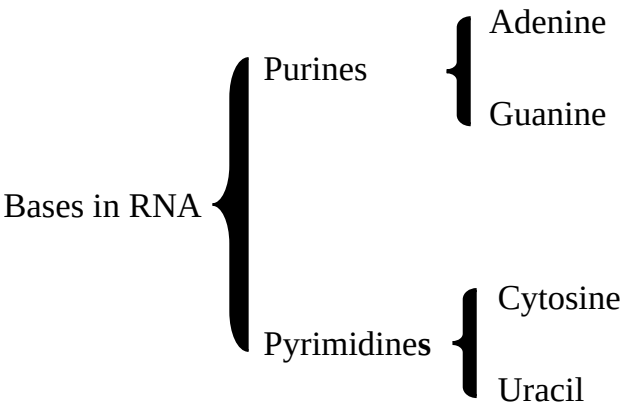

Diagram 2. The nitrogenous bases of RNA

The important event, which was the starting point in genetic engineering in 1973 Stanley Cohen and Herbert Boyer combined DNA from two different species in vitro (Figure 1), then transformed it into bacterial cells, this was the first DNA cloning [6].

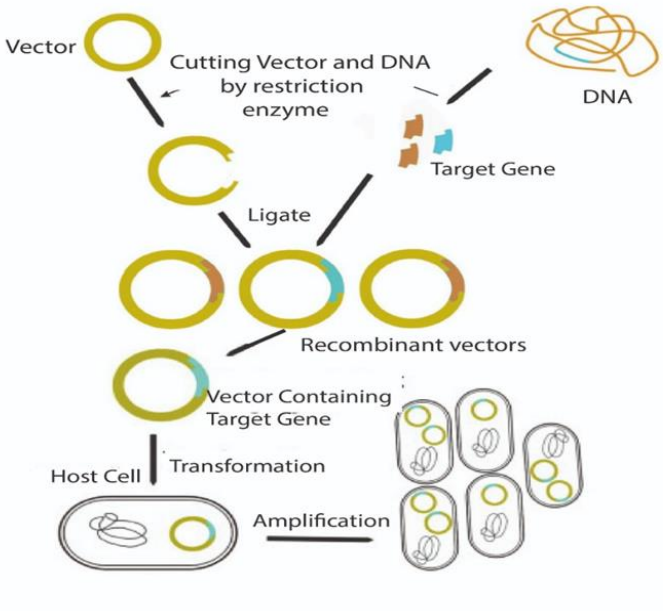

Figure 1. The first experiments in genetic engineering

Events continued over time and discoveries followed in probing the depths of the genetic material until the DNA molecular markers were finally reached.

Molecular genetic markers one of the most strong tools for the analysis of genomes of different living organisms. Molecular marker technology has developed rapidly over the last years. Two sequence based marker, Simple Sequence Repeats (SSRs), also known as microsatellites, and Single Nucleotide Polymorphisms (SNPs) now predominate applications in modern and fundamentals genetic analysis [7].

All manipulations at the molecular level in this field can be applied to diversity analysis, genetic trait mapping, association studies, and marker assisted selection. These markers are inexpensive; require minimal labor to produce and can frequently be associated with annotated genes. Except that not subject to environmental influences and Potentially unlimited in number

but here we need to know that all application in genetic markers need for technically more complex equipment (Thermocycler, Gel documentation system, etc.) and this the major disadvantage of these markers.

Based on the above, and to understand the nature of the action of genes, we must understand the mechanism of action of genes in prokaryotes and eukaryotes, by which I mean how to regulate gene expression; we must first understand how a gene codes for a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different ways than we understand.

Prokaryotic organisms are single-celled organisms that lack a defined nucleus; therefore, their DNA floats freely within the cell cytoplasm. To synthesize a protein, the processes of transcription (DNA to RNA) and translation (RNA to protein) occur almost simultaneously. When the resulting protein is no longer needed, transcription stops. Thus, the regulation of transcription is the primary method to control what type of protein and how much of each protein is expressed in a prokaryotic cell. All of the subsequent steps occur automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of gene expression is mostly at the transcriptional level (Figure 2).

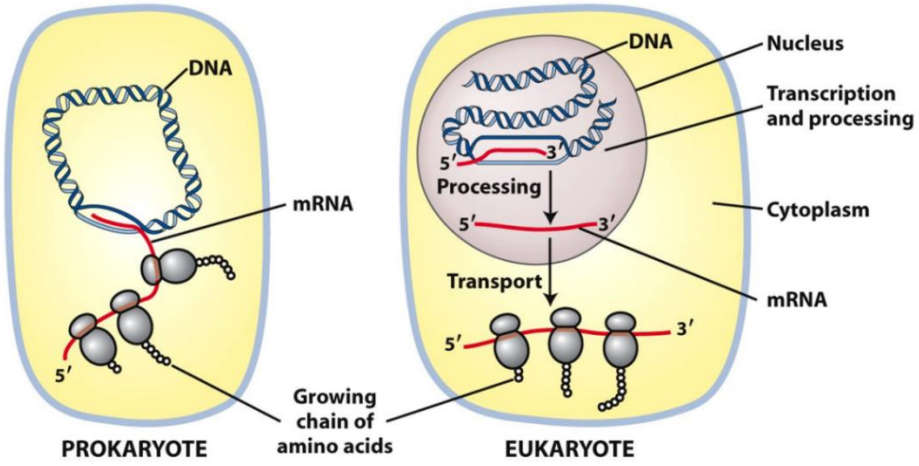

Figure 2. Transcription and translation comparison between prokaryotes and eukaryotes

Eukaryotic cells, in contrast, have intracellular organelles (Figure 2) that add to their complexity. In eukaryotic cells, the DNA is contained inside the cell's nucleus where it is transcribed into RNA. The newly-synthesized RNA is then transported out of the nucleus into the cytoplasm where ribosomes translate the RNA into protein. The processes of transcription and translation are physically separated by the nuclear membrane; transcription occurs only within the nucleus, and translation occurs only outside the nucleus within the cytoplasm. The regulation of gene expression can occur at all stages of the process. Regulation may occur when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors (epigenetics), when the RNA is transcribed (transcriptional level), when the RNA is processed and exported to the cytoplasm after it is transcribed (post-transcriptional level), when the RNA is translated into protein (translational level), or after the protein has been made (post-translational level).

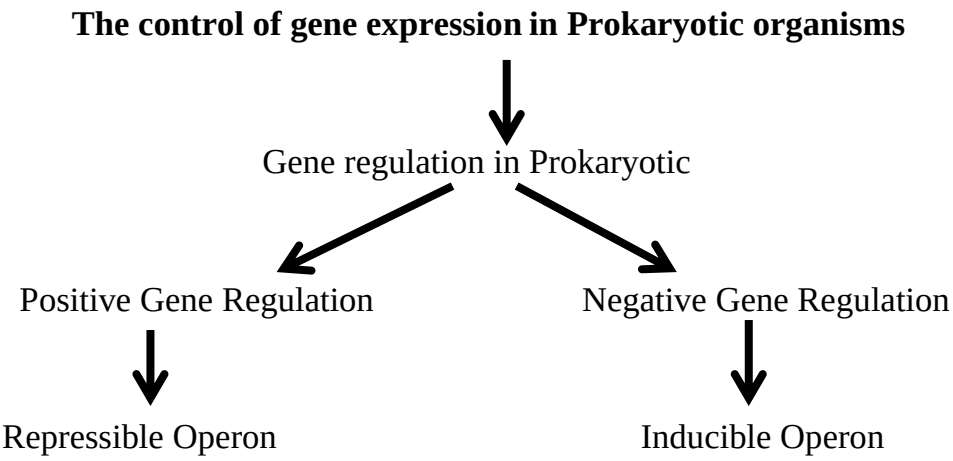

Diagram 3. The control of gene expression in Eukaryotic organisms

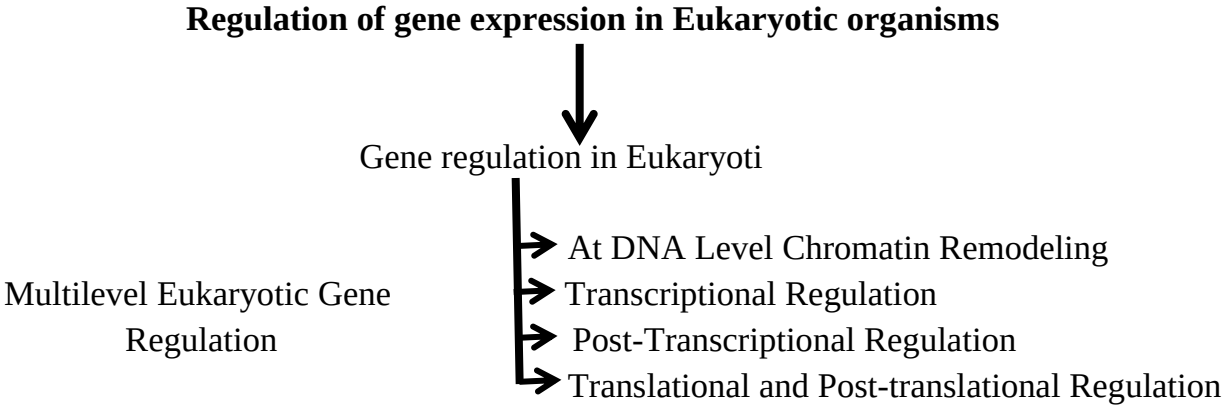

Diagram 4. Regulation of gene expression in Eukaryotic organisms

Prokaryotic transcription and translation occur simultaneously in the cytoplasm, and regulation occurs at the transcriptional level (Diagram 3). Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, and during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins [8].

Operons in general a group of genes that are transcribed at the same time, usually control an important biochemical process, they are only found in prokaryotes. In other words operon is cluster of genes in which expression is regulated by different factors (genes) as:

- Inhibiting Site: Regulatory gene that regulating the operon
- Controlling site: Promoter, Operator
- Coding sequences: Structural Genes witch code the protein
- Terminator: Stop gene

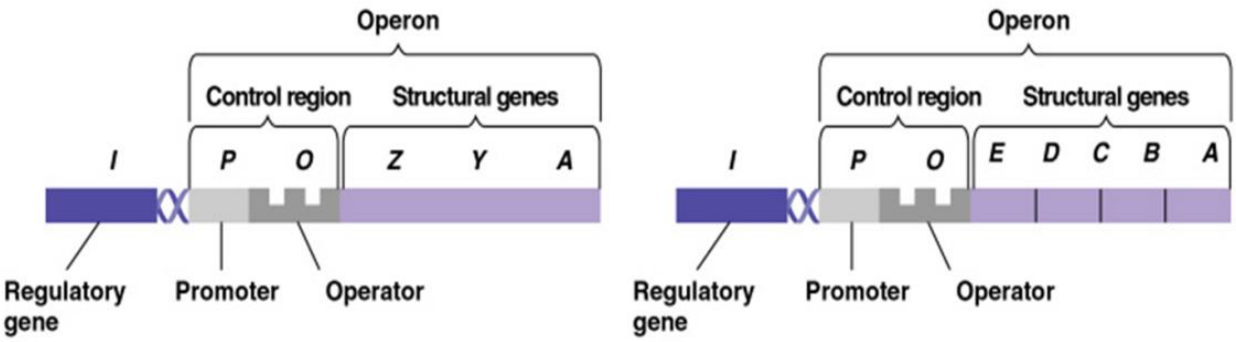

Figure 3. Structure of operon

The most studied model of the operon is the lactose operon in *E.coli* (Figure 3), in this operon has a law of gene regulation by Lac operon in *E.coli.* These bacteria can't use the lactose sugar as a disaccharide which consists from galactose and glucose. For that *E.coli* can discriminate low glucose concentration through enzymes as Adenylcyclase to analyzing ATP to a cAMP (Figure 4)after then cAMP bind to catabolite activator protein (CAP) and form a complex (CAP-cAMP) (Figure 5) to start transcription of the structural genes (lac Z-lac Y-Lac A), and also *E.coli* can discriminate a high concentration of glucose with the help of enzymes such as phosphodiesterase, analyzing cAMP, which in turn will lead to decomposition (CAP-cAMP), after which transcription of the structural genes (lac Z-lac Y-Lac A) will be stopped. All these procedures will be through following rules to break lactose sugar by β-galactosidase:

Low glucose --> High cAMP

High glucose --> Low cAMP

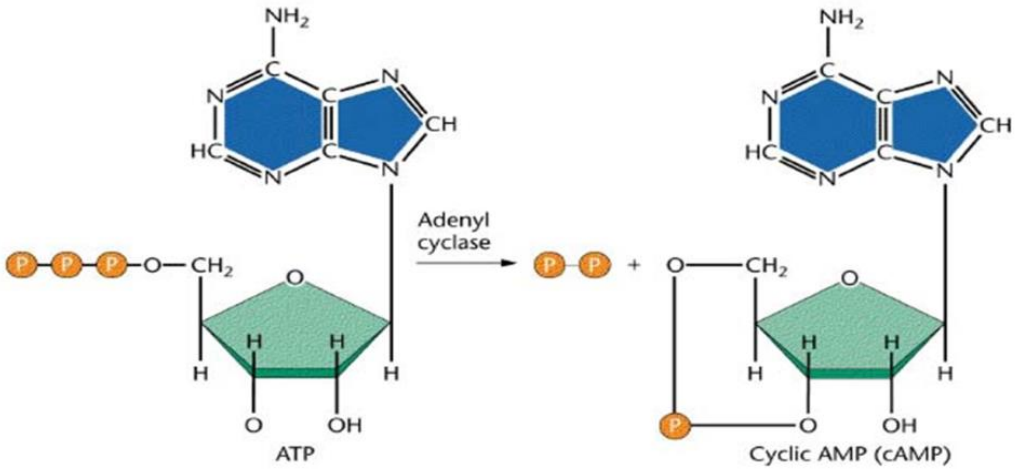

Figure 4. Chemical structure of cAMP

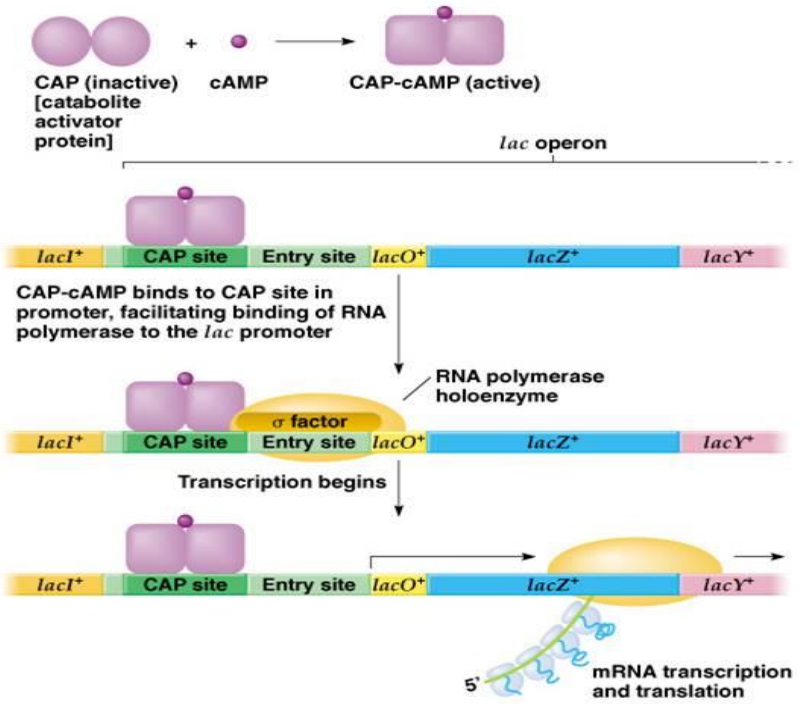

Figure 5. Positive control of the lac operon with catabolite activator protein (CAP)

As for eukaryotic organisms, the matter is different there many factors affect the transcription and translation process in these organisms, from these factors:

1- Chromatin structure: Chromatin a nucleoprotein complex, includes DNA, histones, and various non-histone proteins and chromatin compaction influences activity of DNA in transcription (Figure 6).

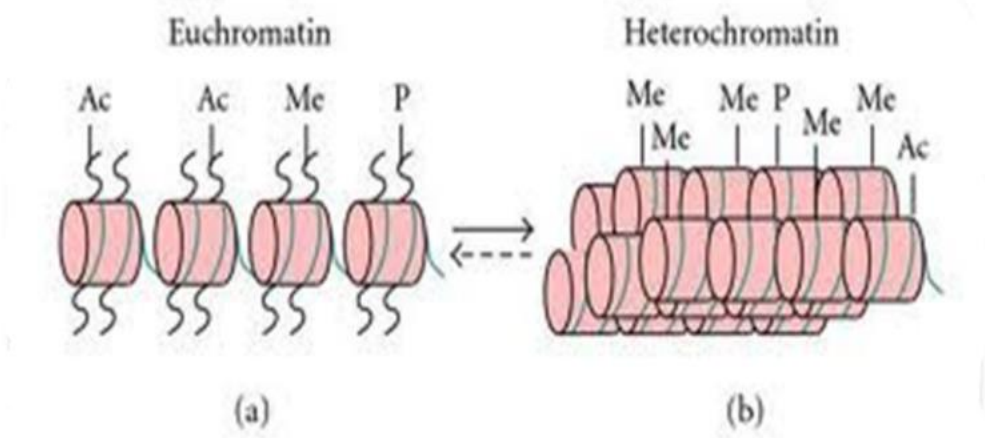

Figure 6. Chromatin structure

In Figure 6 shown in the section (a) the euchromatin – active DNA-histone acetylation transcriptionally active (Euchromatin uncondensed stains less densely, contains the majority of eukaryotic genes), but in the section (b) shown heterochromatin (Heterochromatin condensed and stains densely), inactive DNA-histone deacetylation and DNA methylation-here the DNA transcriptionally silent.

Acetylation of histones enhances access to promoter region and facilitates transcription (Figure 7).

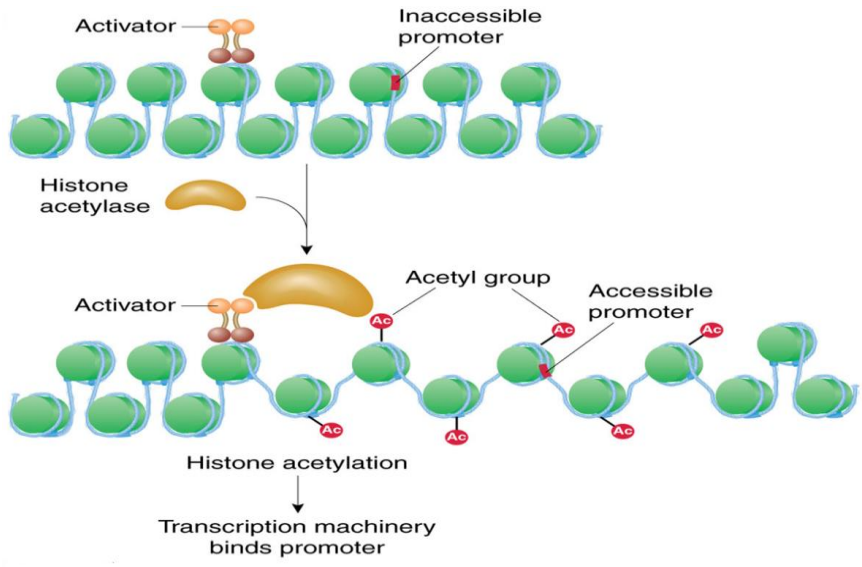

Figure 7. Acetylation of histones in chromatin

2- Promoters: Where transcription begins (e.g., TATA box transcription start).

TATA box is a DNA sequence that indicates where a genetic sequence can be read and decoded. It is a type of promoter sequence, which specifies to other molecules where transcription begins, Except that TATA box located 25-35 base pairs before the transcription start site of a gene and is able to define the direction of transcription.

3- Enhancers: a small piece of DNA that, after binding to it by transcription factors, stimulates transcription from the main promoters of a gene or group of genes (Diagram 5). Each promoter and enhancer possesses a specific set of proteins (coactivators) that determines expression

4- Insulators-Silencers-Mediators: These proteins increase or decrease the frequency of gene expression are copied from their genes.

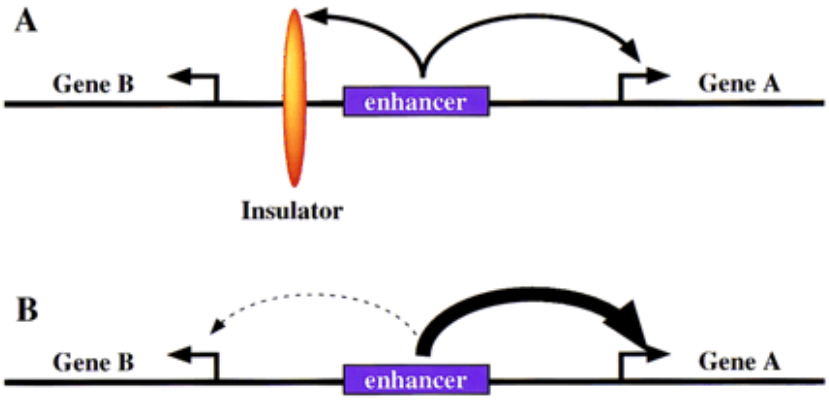

Diagram 5. Regulation of enhancer–promoter interactions

The diagrams depict two divergently transcribed genes, A and B, with a common enhancer located in the intergenic region. (A) An insulator DNA is located between gene B and the enhancer. In principle, this blocks interactions of the enhancer with gene B, without altering the activation of gene A. (B) Promoter competition.

The enhancer can activate both gene A and gene B, but prefers the promoter region of gene A. Enhancer–gene A interactions preclude activation of gene B.

Detection GMO in foods

The presence of ingredients derived from genetically modified organisms (GMOs) in food products on the market is governed by a number of rules in various countries in the world, and for this purpose a variety of GMO detection methods based on DNA molecular markers have been developed to screen of the presence of modified DNA in foods and processed foods.

The most common method of GMO detection is based upon the amplification of GMO-specific DNA amplicons using the polymerase chain reaction (PCR).

Of course the invention of the PCR (Polymerase Chain Reaction) by American scientist (Kary Mullis) in 1983 led to a quantum leap in the development of genetic researches at the molecular level. The principle of the method is very simple just a doubling (amplifying) of a DNA region is delimited by the primers (Molecular markers) using DNA polymerase enzyme (Figure 8).

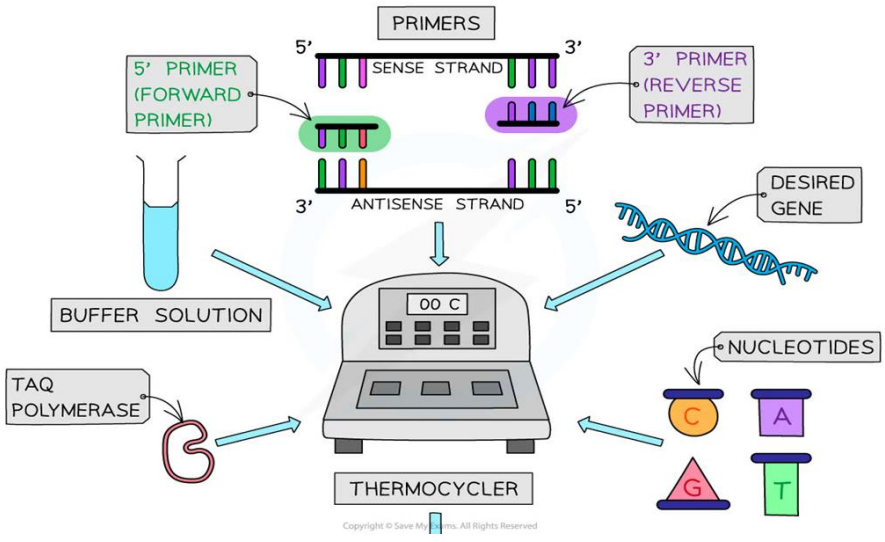

Figure 8. In vitro method of DNA amplification

The reaction components

For the PCR, in the simplest case requires the following components:

1. DNA Template
2. Two primers (Forward – Reverse)18-26 bp
3. DNTPs: dATP, dGTP, dCTP, dTTP
4. Cations: Mg^{++}, Mn^{++}, K^{+}
5. Buffer solution or master mix which Provides the necessary reaction conditions
6. Thermostable DNA polymerase: DNA Taq polymerase or other polymerase
7. Nuclease-free water

Table 1. PCR Reaction setup

Component	25 µl reaction	Final Concentration
2X Standard Reaction Buffer containing following	12.5 µl	1X
Each dNTP (dATP, dCTP, dGTP, dTTP)	0.4 mM	0.2 mM
DNATaq Polymerase	0.05 µl	0.025 units/ µl PCR
Mg^{++} as MgCl2	4 mM	2 mM
Forward Primer	0.5 µl	0.25 µl
Reverse Primer	0.5 µl	0.25 µl
Template DNA	200 ng	8 ng/ µl
Nuclease-free water	to 25 µl	

PCR Steps:

The first step - Denaturation (Tm=melting temperature) (96°C)

The second step - Annealing (Ta=annealing temperature) (55 – 65 °C)

The third step - Primer Extension (72°C)

The fourth step - Detection by using gel electrophoresis to visualize the results of PCR.

This cycle repeats 25 - 35 times in a typical PCR reaction, which generally takes 2 - 4 hours, depending on the length of the DNA region being copied. If the reaction is efficient (works well), the target region can go from just one or a few copies to billions.

For detection GM genes (Soybean lectin gene and related genes) in some product by using in PCR reaction, need to use in this reaction molecular markers (Primers) for these genes as in Table 2.

Table 2. Sequencing of primers

Primer	Sequencing	Gene	bp*	Ta*
GMO3/ GMO4	5’-GCCCTCTACTCCACCCCCATCC-3’ 5’-GCCCATCTGCAAGCCTTTTTGTG-3	Lectin gene*	118	63
P35s-cf3/P35s-cr4	5’-CCACGTCTTCAAAGCAAGTGG-3’ 5’-TCCTCTCCAAATGAAATGAACTTCC-3’	35S promoter*	123	60
HAnos-118-f/ HAnos-118-r	5’-GCATGACGTTATTTATGAGATGGG-3’ 5’-GACACCGCGCGCGATAATTTATCC-3’	Nos terminator*	118	62

*Lectin gene: Soy bean lectin gene

*35S promoter: Cauliflower mosaic virus (CaMV) 35S promoter - for transcriptional regulatory mechanisms

*Nos terminator: Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*

bp*: Molecular length the target gene

Ta*: Annealing temperature

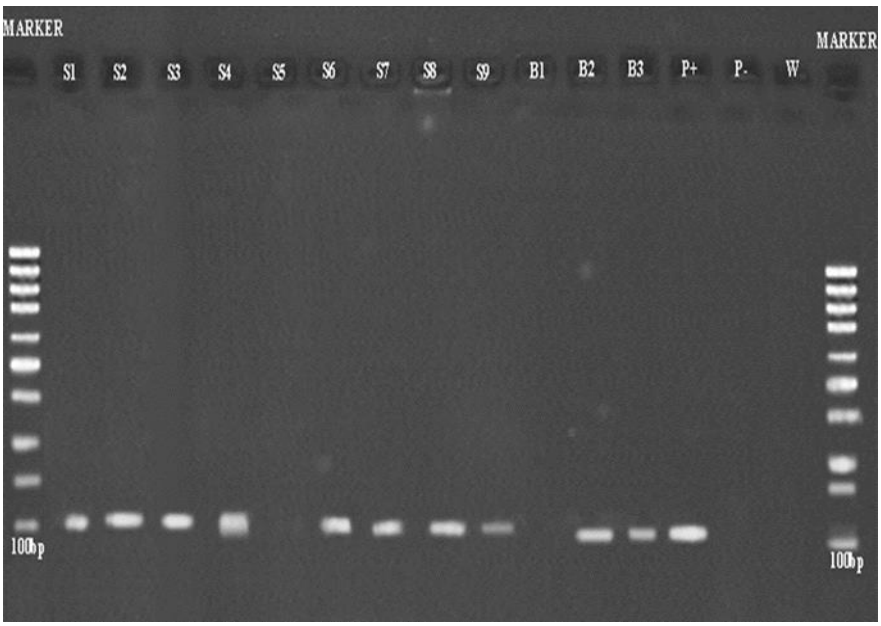

Figure 9. Detection of cauliflower mosaic virus (CaMV) 35S promoter using P35s-cf3/P35s-cf4 primers

S-B: Different samples of processed foods

P-: Negative control (0% CRM- GMO certified reference materials)

P+: Positive control (2% CRM-GMO certified reference materials)

W: Water (no template DNA)

Recently for detection the genetic biodiversity in organisms are widely used microsatellite and between microsatellite or ISSR-markers (Inter Simple Sequence Repeats). Some primers for polymorphism analysis ISSR-PCR using dinucleotide.

Trinucleotide and tetranucleotide microsatellite repeats:

Dinucleotide: In animals – (CA) n, In plants – (TA) n, (GA) n

Trinucleotide: (GTG) n, (CAG) n, and (AAT) n, these sequencing Related to disease and cancers

Tetranucleotide: (GATA) n, (GACA), these sequencing have highly polymorphic to detect the mutations

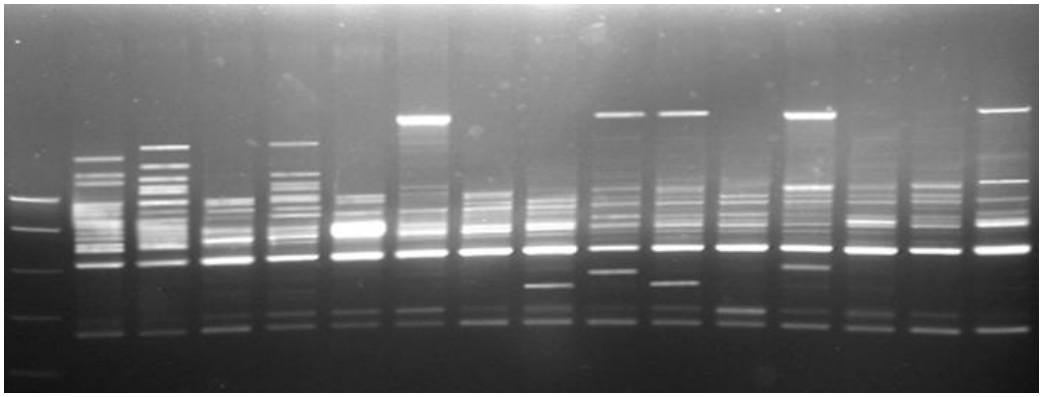

Figure 10. Polymorphism rate for the alfalfa (*Medicago sativa*) genotypes using ISSR primers

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LECTURE 3
VIRUSES OF FOODBORNE ORIGIN: A REVIEW

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On the 13th January 2012, The Hickory daily record reports 40 people have complained to officials at Catawba Country Public Health (North Carolina) that they got sick after eating a meal at Harbor's Inn Seafood restaurant. Health officials worked to determine what the illness is and what caused it. Catawba County Public Health received reports of illness after visiting the Harbor Inn Seafood Restaurant located in Hickory, North Carolina. Eight staff members were identified to have worked on January 12th while experiencing gastrointestinal symptoms. Altogether, 166 illnesses were identified [1].

What went wrong? **Norovirus** is often called the "stomach bug" or "stomach flu" Not related to respiratory flu. Employees who work with symptoms of diarrhea and/or vomiting will spread the virus. Concerning diarrhea: Hands may have virus after bathroom use and improper hand washing. As for vomiting: Virus particles may be spread to hands, clothes, and other surfaces.

1- Sources of exposure to Enteric viruses:

The main sources of exposure to enteric viruses consist of drinking water, food, person-to-person direct transmission (fecal) or indirect (vomitus), fomites (door handles, phones…), and recreational waters.

The at-risk foods are molluscan shellfish, fresh-produce, and ready-to-eat foods. Fresh produce are raw and minimally processed fruits and vegetables, typically sold to the consumer in a ready-to-use or ready- to-eat form. These products do not generally contain preservatives or antimicrobial agents and rarely undergo any heat processing prior to consumption.

For many years, raw fruits and vegetables have been implicated as vehicles for transmission of infectious micro-organisms. Viruses cannot grow in or on foods but may sometimes be present on fresh produce as a result of fecal contamination.

This contamination can arise:

- at source in the growth and harvesting area: polluted water and inadequately or untreated sewage sludge used for irrigation and fertilization.
- fruits or vegetables handled by an infected person might become contaminated with virus and transmit infection.

Molluscan shellfish can be contaminated by growing waters in which human feces have been dumped or otherwise deposited. While, fresh produce become contaminated by the hands of pickers or by irrigation waters contaminated with human fecal matter. Foods with extensive human handling are usually contaminated by poor personal hygiene of infected food handlers, or by aerosolization and deposition of virus due to vomiting events.

2- Human Enteric viruses

Human enteric viruses are obligate intracellular parasites (not alive, require a host cell to propagate) and most of them cannot be propagated *in vitro*/no animal model. They are inert in foods (cannot grow in the food matrix).

Most enteric viruses have a simple structure, single-stranded RNA genome, positive sense RNA, protein coat, and no lipid envelop. They are transmitted by humans and by animals for some (Noroviruses). They are highly transmissible between people. Table 1 shows a non-extensive list of human and animal viruses related to food-borne outbreaks [2].

The food borne enteric viruses of known epidemiological significance, which will be discussed in this review, are: Astrovirus, Rotavirus, Norovirus, the hepatitis A virus, and the hepatitis E virus.

Table 1. Human and Animal viruses (potentially) related with food-borne outbreaks [2]

Viruses			*Evidence* [a]	Clinical features
Norovirus	NoV	(+)sRNA	yes	gastroenteritis
Hepatitis A virus	HAV	(+)sRNA	yes	enterically hepatitis
Rotavirus		dsRNA	yes	severe gastroenteritis (children)
Astrovirus	ASV	(+) RNA	yes	mild gastro-enteritis (children)
Adenovirus	Ad	dsRNA	possible [b]	respiratory, eye, gastroenteritis infections
Enterovirus	EV	(+)RNA	less common	respiratory, eye, central nervous infections
Hepatitis E virus	HEV	(+)RNA	yes	enterically hepatitis
Tickborne encephalitis	TBE	(+)sRNA	yes, less common	encephalitis via unpasteurized milk of TBE infected animals
Parvovirus		ssDNA	little evidence	gastroenteritis
Coronavirus		(+)sRNA	linked to sewage-(water)	respiratory and gastroenteritis infections
Torovirus		(+)sRNA	role unknown	gastroenteritis in humans?
Picobirna virus		(+)sRNA	role unknown	gastroenteritis in immunocompromised

[a] see also: Greening GE. Human and Animal viruses in food(including taxonomy of enteric viruses). In Viruses in Foods. *Ed.* S.M. Goyal. Springer Science+Business Media, LLC, New-York. Pg 5-42.
[b] no documented evidence for food-borne transmission or disease resulting from consumption of contaminated shellfish

2.1- Astroviruses

Astroviruses have a star-like appearance with five or six points. Their name is derived from the Greek word "astron" meaning star. They are non-enveloped RNA viruses with cubic capsids, approximately 28–35 nm in diameter with T=3 symmetry.

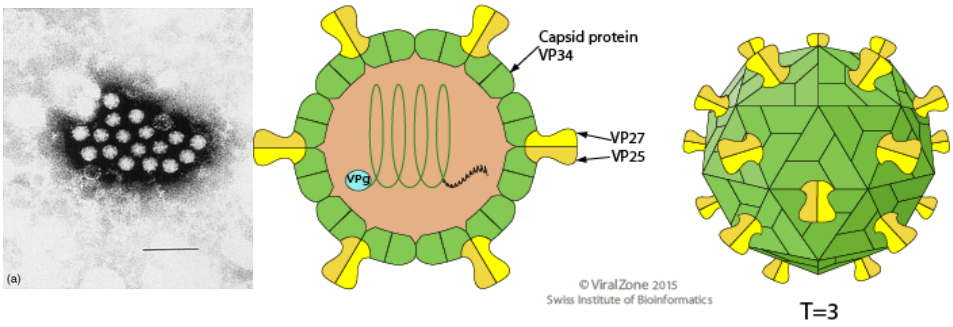

Figure 1. Astroviruses structure (ViralZone 2015, Swiss Institute of Bioinformatics)

They are Endemic in most communities worldwide Mostly infect young children and babies, although they have been associated with disease in the elderly. Very low pathogenicity for adults. Hosts: man, cattle, sheep, pigs and felines Mainly direct or indirect fecal-oral transmission through food, shellfish and contaminated water. Sporadic or epidemic gastroenteritis, especially **during winter** Often observed at extreme ages of life and in immunocompromised people After incubation from 1 to 4 days, **watery diarrhea with nausea**, vomiting, rarely febrile Direct diagnosis on stool samples: electron microscopy, viral antigen detected by ELISA or genomic detection by **RT-PCR (most important).**

2.2- Rotaviruses

«Rota» in Latin means wheel. First detected in April, 1973 by Rose Bishop et al from a biopsy of an Australian child with severe gastroenteritis.

Figure 2. Rose Bishop and Rotavirus discovery. Photo Credit: F.P. Williams, U.S. Environmental Protection Agency; Adapted from Parashar et al, Emerg Inft Dis 1998,14(4) 561–570

Rotavirus is the most common diarrheal pathogen in children worldwide [3], infecting virtually every child within the first 5 years of life3, irrespective of race or socio-economic status.

Worldwide, rotaviruses account for more than 125 million cases of infantile dehydrating gastroenteritis [4] and 440,000 deaths per year (5), mainly in developing countries of the Indian subcontinent, sub-Saharan Africa and South America (6). An estimated 1,205 children die from rotavirus disease each day (the equivalent to approximately a child every minute), and 82% of these deaths occur in children in the poorest countries [5]. An additional hazard in developing countries is that malnutrition makes children more prone to severe rotavirus disease and, in turn,

repeated episodes of diarrhea may lead to malnutrition [4]. Infections that cause endemic/epidemic gastroenteritis and infantile diarrhea are second only to respiratory infections in infants less than 2 years old, and are a major cause of death in the developing world.

Globally, rotaviruses are the most common cause of severe diarrhea with a similar incidence of disease in both developed and developing countries [5]. The landmark review of the global prevalence of rotavirus disease published by De Zoysa and Feachem (1985) (7) indicated that in developing countries, rotavirus accounted for 6% of diarrhea episodes and 20% of deaths among children less than 5 years old [3]. Despite improving trends in mortality rates and relatively stable diarrhea morbidity over the past three decades, current estimates of the global burden of disease suggest diarrhea is still responsible for a median of 21% of all deaths among children aged under 5 years [3, 8].

The similar incidence of rotavirus disease between developed and developing countries suggests that improvements in water supply, hygiene and sanitation will not impact the control of the disease. This conclusion, along with the dramatic disease burden associated with rotavirus, underscores the urgent need for intervention measures such as vaccination, particularly to prevent childhood deaths in developing nations [3].

Rotavirus is usually transmitted from the feces of an infected person, mainly by contaminated water, but also by contaminated hands, fomites, flies and food.

The most common cause of acute gastroenteritis in infants and toddlers. The peak season is in the cooler fall and winter months (year-round) and the peak age incidence is 3 to 24 months. Usually the virus has an incubation period of 24-48 h.

Vomiting is the first symptom in 80-90% of patients, followed within 24 h by low-grade fever and voluminous watery diarrhea and non-bloody. In most cases, a satisfactory diagnosis can be made on the basis of clinical and epidemiologic features. Specific identification of rotavirus is not required in every case, especially in outbreaks. PCR is the gold standard for diagnosis. While ELISA, offers approximately 70-80% sensitivity and 71-100% specificity.

Treatment is symptomatic and consists of rehydration (oral rehydration salts) and oral adsorbents (activated charcoal). Two lively attenuated vaccines exist: RotaTeq® (RV5) and Rotarix® (RV1).

2.3- Human Noroviruses

Human noroviruses are responsible of approximately 700 million illnesses annually worldwide, 56,000 to 71,000 hospitalizations in U.S. and 200,000 related deaths worldwide. It is the most common cause of foodborne illness in U.S. (~58%).

Some Norovirus infections occur without symptoms (asymptomatic). Infected people can shed large numbers of Norovirus in feces and vomit, while the virus can persist on surfaces up to 6 weeks. The commonly available, alcohol-based hand sanitizers are not effective against norovirus.

The main symptoms are vomiting (projectile), diarrhea, nausea, and abdominal cramps. The foods at-risk are the typical ready to eat Foods contaminated by infected person, fresh produce (e.g. cut fruit, salads), shellfish, and contaminated water.

Places at risk are places with large amounts of people in close quarters (nursing homes, daycare centers, schools, hotels, cruise ships, airplanes). 70% of Norovirus outbreaks are caused by food handlers. Measures to control these outbreaks are: to monitor employee health, to exclude employees with vomiting and diarrhea and confirmed Norovirus infection, to enforce personal hygiene (wash hands after bathroom), to rinse produce with potable water, to clean and disinfect kitchen utensils and counters, and to clean and disinfect any area with vomit. Of the enteric viruses, has the HuNoV have the greatest epidemiological significance.

Norovirus is member of the Caliciviridae family ("calic" means "cup", due to cup-like indentations on the virus surface. Five genera in this family but only two (Novovirus and Sapovirus) have strains that cause human disease [2].

Norovirus is the most significant genus with respect to human disease; the genus consists of 10 genogroups and 49 genotypes. Human infection is caused by genogroups I, II and IV; while Norovirus GII.4 (Genogroup II, genotype 4) is responsible of more than 75% of all outbreaks. About 10% of cases associated with GI, 90% with GII. Within any one genogroup, there are multiple genotypes which differ in nucleic acid sequence of the RNA genome.

Disease is mainly gastrointestinal, vomiting is hallmark symptom, may be accompanied by diarrhea. The incubation period is 18-36 hours, duration about 2 days, recovery usually complete. No vaccine against noroviruses is in sight and no sensitive cell line is currently available to culture noroviruses. It is highly transmissible between people in close proximity to one another.

Norovirus is a "perfect" pathogen. It has a tendency to evolve/emerge yields new strains, many co-circulating strains Complex, incomplete and short-lived immunity means constant pool of susceptible persons. Norovirus infection requires a low infectious does and high levels of virus in feces facilitates transmission. Shedding occurs in vomitus exacerbates the problem. Persistence in the environment results in long-term exposure. Difficulties in inactivation exacerbates the problem.

2.4- Hepatitis A virus

Hepatitis A virus is a member of the *Picornaviridae* family, genus: *Hepatovirus*.

Hepatitis A infection does not cause chronic liver disease and is rarely fatal, but it can cause debilitating symptoms (in less than 0.1%).

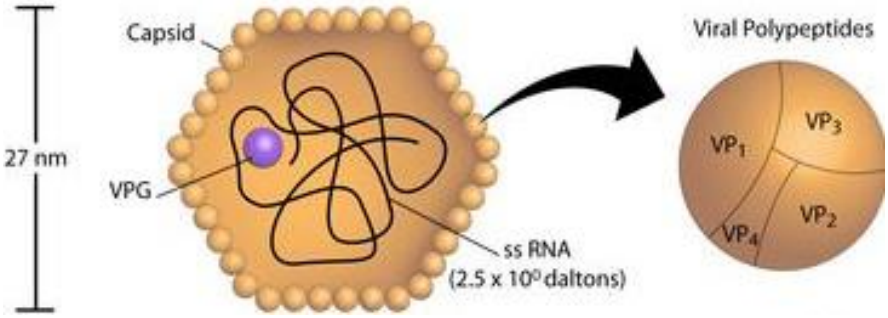

Figure 3. Hepatitis A virus structure

Hepatitis A is a non-enveloped virus contains a single-stranded RNA packaged in a protein shell "capsid". There is only one serotype of the virus, but multiple genotypes exist at the 5' end of the RNA strand is a viral protein called VPg.

Hepatitis A is transmitted by digestive or by fecal routes. Poor hygiene and poor sanitary conditions in some countries lead to high rates of infection. Some areas of the world are particularly prone to hepatitis A virus e.g. India, South America, Bangladesh, and Central America 1/3rd of people in the U.S of America have been exposed to the hepatitis A virus (9). Foods at risk include ready to eat foods, shellfish harvested from contaminated waters, and any food contaminated by an infected employee. The spread occurs through fecal-oral transmission. As for the replication mechanism: HAV enters the bloodstream through the epithelium, reaches to its target, the liver, where it multiplies within hepatocytes and Kupffer cells. The incubation period is 15–50 days and mortality is less than 0.5% [9].

Symptoms usually develop between 2 and 6 weeks after infection. Most common symptoms are nausea, vomiting, diarrhea (especially in children), low-grade fever, loss of appetite, rash, tiredness, fatigue, jaundice, urine (dark brownish in color), and pain in area of liver.

In hepatitis A, the presence of anti-HAV IGM is detectable about 3 weeks after exposure, its titer increases over 4 to 6 weeks, then declines to non-detectable levels generally within 6 months of infection. Anti-HAV IGA and IgG are detectable within a few days of the onset of symptoms. IgG antibodies persist for years after infection and provide lifelong immunity.

Vaccine protects against HAV in more than 95% of cases for longer than 20 years.

It contains inactivated hepatitis A virus providing active immunity against a future infection. Vaccine was first phased in 1996 for children in high-risk areas, and in 1999 it was spread to areas with elevating levels of infection. An initial dose provides protection starting two to four weeks after vaccination; the second booster dose, given six to twelve months later, provides protection for over twenty years.

Control consists to exclude employees with symptoms or confirmed hepatitis A. Good personal hygiene, washing hands after using the bathroom, cooking shellfish are all measures to reduce transmission [9].

2.5- Hepatitis E virus

The *Hepeviridae* includes enterically-transmitted, small, non-enveloped positive-sense RNA viruses. Members of the family are assigned to two genera and five species.

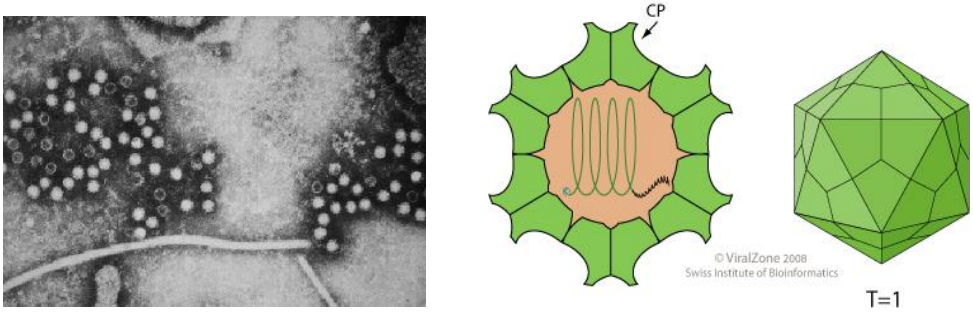

Figure 4. Hepatitis E virus structure (ViralZone 2008, Swiss Institute of Bioinformatics)

Virions of HEV are icosahedral, non-enveloped, spherical particles with a diameter of approximately 27–34 nm. Spikes and indentations can be seen on electron micrographs of virions. The capsid is formed from capsomeres consisting of homodimers of a single capsid protein, forming the virus shell [10].

HEV is a major cause of hepatitis transmitted by the fecal-oral route. It is thought to be more common than HAV in many developing countries. It is a common cause of waterborne epidemics of hepatitis in Asia, Africa, India, and Mexico but is uncommon in the United States [10].

In immunocompetent patients, HEV-3 rarely causes symptomatic hepatitis but can result in acute fulminant hepatitis with decompensation in patients with underlying chronic liver disease.

HEV genotype 3 and genotype 4 may lead to chronic infection in patients with an impaired immune response due to illness or medication. Both acute and chronic HEV infections have been associated with extrahepatic manifestations.

Currently no vaccine is available other countries than in China. There are no data to suggest whether such a vaccine would protect against HEV (re)infection in immunocompromised patients. Severe disease, occurs especially in pregnant women [10].

2.6- Other viruses

Perhaps causes of food borne disease of "unknown" etiology. Other members of the *Picornaviridae* family (human enteroviruses, coxsackieviruses), enteric adenoviruses.

And many others (parvoviruses, enteric coronaviruses, Bocaviruses) are capable of causing food-borne infection. Current data based on analysis of fecal specimens of patients presenting with acute gastroenteritis.

3- Control of viruses: where we are?

Increased recognition of the role of viruses in food borne disease. Food handling appears to be the most common source of contamination. We should avoid human fecal contamination. Hand-washing (soap and warm water) is best control to date if implemented appropriately and frequently. Food handlers with suspicious symptoms should be excluded from work. Rigorous surface disinfection (high concentrations of chlorine) for vomiting incidents or fecal contamination.

When to wash hands:

- Before
  - Starting work
  - Touching raw or TCS food
  - Touching ready-to-eat food
  - Putting on new gloves
- During
  - Food preparation as frequently as required to maintain clean hands and prevent cross-contamination
  - Switching between raw and cooked food

- Switching between raw and ready-to-eat food

- After
  - Handling raw food
  - Using bathroom
  - Coughing, sneezing, blowing nose
  - Touching handkerchief, hair, face.
  - Cleaning/sanitizing
  - Removing garbage
  - Changing protective gloves
  - Breaks
  - Eating, drinking, smoking
  - Touching money

4- Reporting illness/exclusion

Employees must report to "person in charge".

If diagnosed with hepatitis A, *E.coli 0157:H7*, norovirus, *Salmonella species*, *Shigella species*. Have had a past illness from above or live in household where someone has had above or have traveled within last 50 days to an area with an epidemic from above.

Exclusion of an employee with following symptoms: diarrhea, vomiting, fever, jaundice, sore throat and fever, if the employee has a draining boil or open infected wound, or suspected of causing or being exposed to confirmed illness outbreak.

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LECTURE 4
BIOACTIVIES FROM MICROALGAE FOR FUNCTIONAL FOOD APPLICATIONS

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Abstract

The world population is increasing day to day and it is expected to outreach 9.7 billion by 2050. Demand for bioactivities from natural sources rather than synthetic ones has increased in the global market. Microalgae produce various bioactivities such as lipids, fatty acids (EPA and DHA), carotenoids, amino acids, vitamins, and proteins etc. and they are used in food, feed, pharmaceutical, and nutraceutical applications. These bioactivities from microalgae can be produced under various algal culture conditions such as autotrophic, mix-trophic, and heterotrophic.

Microalgae are grown in raceways and photobioreactors for the production of larger quantities of bioactivities. Bioactivities from microalgae for functional foods are well accepted by consumers. However, the number of products that are launched into the market is limited compared to the potential it has for expansion. Among the industrially important species, the prominent ones are *Spirulina, Chlorella, Haematococcus* and *Dunaliella* which are cultivated for commercial applications. Extensive research was carried out by us on *H. pluvialis* is green microalga as it accumulates astaxanthin and astaxanthin esters which are used as food ingredients and food supplements. This presentation covered the importance of microalgal forms for functional food applications. The aspects of constituents of microalgae, cultivation methodologies, challenges in a scaleup, downstream processing, purification as well as evaluation of biological activities and product formulations will be described to highlight the industrial production scenario for present and future applications.

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LECTURE 5
NATURAL DEEP EUTECTIC SOLVENTS IN EXTRACTION OF BIOLOGICALLY ACTIVE COMPOUNDS

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Abstract

The valorization of agricultural and fishery by-products by extracting their bioactive compounds is a very interesting alternative to their incineration or even composting. Bioactive compounds have been studied intensively due to their biological properties, capable of providing multiple health benefits. They can be extracted from by-products of agricultural, food, and fishing industries, such as crustaceans' shells, plants, algae, or microalgae by-products, which can correctly contribute to a circular economy based on zero waste.

For applications, mainly in the food, cosmetic and pharmaceutical industries, these compounds are generally extracted using organic solvents or also known as conventional solvents (methanol, acetone, benzene, chloroform, petroleum ether, and hexane), which many times are rejected for being flammable, explosive, poorly biodegradable and for the toxicity they can produce to the final consumer. Green technologies such as supercritical fluid extraction, ultrasonic extraction and microwave field extraction can increase process selectivity, increase productivity and increase efficiency, but they are costly and energy intensive. Alternative solvents, sometimes referred to as "green solvents", such as ionic liquid (IL) solvents, deep eutectic solvents (DES), and natural eutectic solvents (NADES) can be used as a solution to this problem [1]. In this review, developments related to the use of deep eutectic solvents for the extraction of different bioactive compounds and our own studies on ultrasound-assisted extraction of isoflavones of kudzu roots and flowers, red clover flowers and soy molasses as well as piperine from black pepper are briefly discussed.

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The full text of presentation can be accessed on https://foodbiotech.urfu.ru/en/presentations-of-plenary-speakers/

LECTURE 6
METHODOLOGIES FOR THE CHARACTERIZATION OF BANANA GERMAPLASM COLLECTION ORIGINATED FROM RWANDA

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Banana and Plantain (Musa spp.) are important staple and income generating fruit crops for millions of people in the tropical and subtropical regions of the world. They are classified into genomic groups such AA, AAA, AAB etc. Exact knowledge of the genomic group and the ploidy of a variety are important to breeders for manipulation of a multi-ploidy crop such as banana.

The Highland bananas from Rwanda were classified using heritability characters of fruit, into five clone sets. Using flow cytometry, the ploidy level of a banana germplasm collection was investigated and revealed that all highland bananas investigated were triploids. While investigating the genomic groups of some highland bananas using RAPDs as molecular markers using a primer OPA 18 which is a marker for B. genome, all highland bananas from Rwanda examined did not show the presence of the B genome. Based on these results, all highland bananas examined were triploid and belong to the genomic group AAA.

Key words: Musa spp, Ploidy, genomic groups.

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MASTER CLASSES

MASTER CLASS 1
DNA ISOLATION: BASICS OF SAMPLE PREPARATION METHODOLOGIES AND ISOLATION PROCEDURES

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Introduction

Friedrich Miescher conducted the first DNA extraction in 1869 from the nuclei of leukocytes obtained from pus, hence coined the term “nuclein” (the first name of nucleic acid) was slightly acidic and rich in phosphorus and nitrogen [1], making scientists exceptional progress in designing extraction methods that were more reliable, easier and faster to perform, more cost-effective, and produced a higher yield [2].

The extraction of nucleic acids is the starting point in any molecular biology study and is considered a crucial process. DNA can be extracted from sources as diverse as soil, plant, animal tissue, insects, protozoa, bacteria, yeast, food and processed foods.

DNA isolated from various biological samples can be used for a vast array of downstream applications, namely DNA sequencing, polymerase chain reaction (PCR), quantitative PCR (qPCR), southern blotting, random amplification of polymorphic DNA (RAPD), preparation for genomic libraries as well as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), short tandem repeat polymorphism (STRP), single nucleotide polymorphism (SNP) and a variable number of tandem repeat (VNTR) applications, detection GMO in foods and processed foods [2].

The principle of DNA extraction consists of the following steps:

1- Disruption of cytoplasmic and nuclear membranes
2- Separation and purification of DNA from other components of the cell lysate such as lipids, proteins, and RNA
3- Concentration and purification of DNA.

Here it must be mentioned to optimize the DNA extraction method including the time, cost, potential toxicities, yield, laboratory equipment, and expertise requirements, as well as the required sample amount for the protocol [3].

From different methods for extraction DNA, we will use (CTAB) Extraction method (Cetyltrimethylammonium Bromide method).

The CTAB extraction method was introduced by Doyle et al. in 1990 [4]. In this method, DNA-containing samples (in our case we will use lettuce leaves) are added to 2% CTAB at pH=8. The extraction buffer precipitates DNA and acidic polysaccharides from the rest of the cellular components in a solution of low ionic strength. Solutions with high salt concentrations are then used to remove DNA from the acidic polysaccharides which form a precipitate with CTAB. Hence, this method benefits DNA extraction from plants that produce high amounts of

polysaccharides [5]. DNA is then purified using various organic solvents, ethanol, and isopropanol, including chloroform.

Materials and Procedures

Microfuge tubes	20g/l CTAB
Mortar and Pestle	1.4M NaCl
Centrifuge	0.1M Tris-HCl
pH meter	20mM Na2EDTA
Weighing balance	dH2O
Pipette tips	Boric acid
Spatula	Tris base
Weigh dishes/Paper	EDTA
Pipettors	DNA size standards (Ladder DNA)
Beakers-Flasks	Sample (Lettuce leaves)
6x Gel-loading buffer	Agarose
Ethidium bromide (0.5 ug /ml)	1X TBE buffer

A. Preparation of a Stock Solution of 0.5 M EDTA (500 ml)

The mass of 93.05 g EDTA was weighed and Dissolved in 200 ml dH_2O while stirring with a magnetic. The pH was adjusted to 8.4 with NaOH. The volume was adjusted to 500 ml with dH_2O.

B. Prepare a Stock Solution of TBE 10X TBE (1 liter)

The masses of 108g of Tris and 55 g boric acid were dissolved using a magnetic stirrer in 800 ml dH_2O. Then 40 ml 0.5 M Na_2EDTA was added and volume was adjusted to 1 Liter. The solution was autoclaved and stored at room temperature.

C. Preparation of CTAB buffer (100ml)

i. Preparation of 1.4M sodium chloride solution

The solution was prepared by dissolving 8.1816 g of Sodium chloride in 100ml distilled water.

ii. Preparation of 0.1 M Tris-HCl solutions

The solution was prepared by weighing 1.5764 g of Tris and dissolving it 100ml distilled water. The pH was adjusted to pH 8.0 with concentrated hydrochloric acid. The solution was allowed to cool to room temperature before making the final adjustments to the pH=8.

iii. Preparation of 20 mM EDTA

The solution was prepared by pipetting 0.4 ml of EDTA from the stock solution (0.5M EDTA) into the 100ml flask and adding water up to 1ooml mark.

iv. Preparation of 5g/l CTAB reagent

CTAB solution was prepared by measuring 25 ml of the stock solution into a 100ml flask, followed by adjusting the volume to the mark.

All reagents that make up the CTAB buffer were adjusted to pH 5.0 with HCl, mixed and made up to 100 ml with dH_2O.

D. DNA extraction

- Leaves of lettuce were collected and ground using a mortar and pestle for 15 min.
- The mass of 0.3g of the crashed sample was transferred into an eppendorf tube.
- The volume of 600µl of water was added to the sample followed by 600µl of CTAB extraction buffer and vortex mixed for 2min.
- The solution mixture was centrifuged at 6000rpm for 15 minutes and the supernatant was collected with a pipette.
- Chloroform was added to the supernatant up to 2ml and shaken for 1 min. The mixture was again centrifuged for 15 minutes at 6000rpmfor 15 min and the supernatant was collected. This step was repeated twice.
- The supernatant was again collected and the precipitation buffer was added up to 2ml.
- The solution was heated in a warm water bath with intermittent shaking for 1 hr.
- The solution was centrifuged at 600rpm for 15min and the supernatant was discarded.
- The volume of 500µl NaCl and Chloroform solutions were added to the eppendorf tube containing DNA and centrifuged at 6000rpm for 15 min.
- The upper layer was transferred to a new microcentrifuge tube to which 1ml isopropanol was added and the sample was placed in a freezer at -20o for 7days.
- The solution was centrifuged at 6000rpm for 15 minutes followed by discarding of isopropanol.
- A volume of 1ml ethanol was added and centrifuged again for 15min at 6000rpm. Ethanol was discarded and the pellets were dried and re-dissolved in 100 µl TBE (Tris HCL – Borate-EDTA). The DNA solution is stored in the refrigerator (-20) for further processing.

CTAB buffer comprises CTAB, sodium chloride, Tris-HCl, and Na2EDTA. CTAB is a cationic detergent that facilitates the separation of polysaccharides during purification. CTAB exploits the different solubilities of polysaccharides and DNA in CTAB depending on the concentration of sodium chloride. At higher salt concentrations insoluble polysaccharides could not mix with the DNA. Sodium EDTA helped to chelate metal ions such as magnesium ions which could activate DNase and RNase enzymes that could denature DNA.

After treatment with the CTAB buffer, the sample was subjected to solvent treatment and centrifugation. Solvents played an essential role in cleaning up the sample through a series of centrifugation steps. Chloroform protects genomic DNA and the removal of proteins and increases the efficiency of protein denaturation by allowing proper separation of the organic phase and aqueous phase and keeping DNA protected into the aqueous phase. Isopropanol was used in the last stage to precipitate DNA from the solution by removing phosphate water hydration shells from DNA. This was done at room temperature to prevent core precipitation of DNA with salts. In order to prevent it from degradation and maintain its structure, DNA was stored in the refrigerator at a very low temperature.

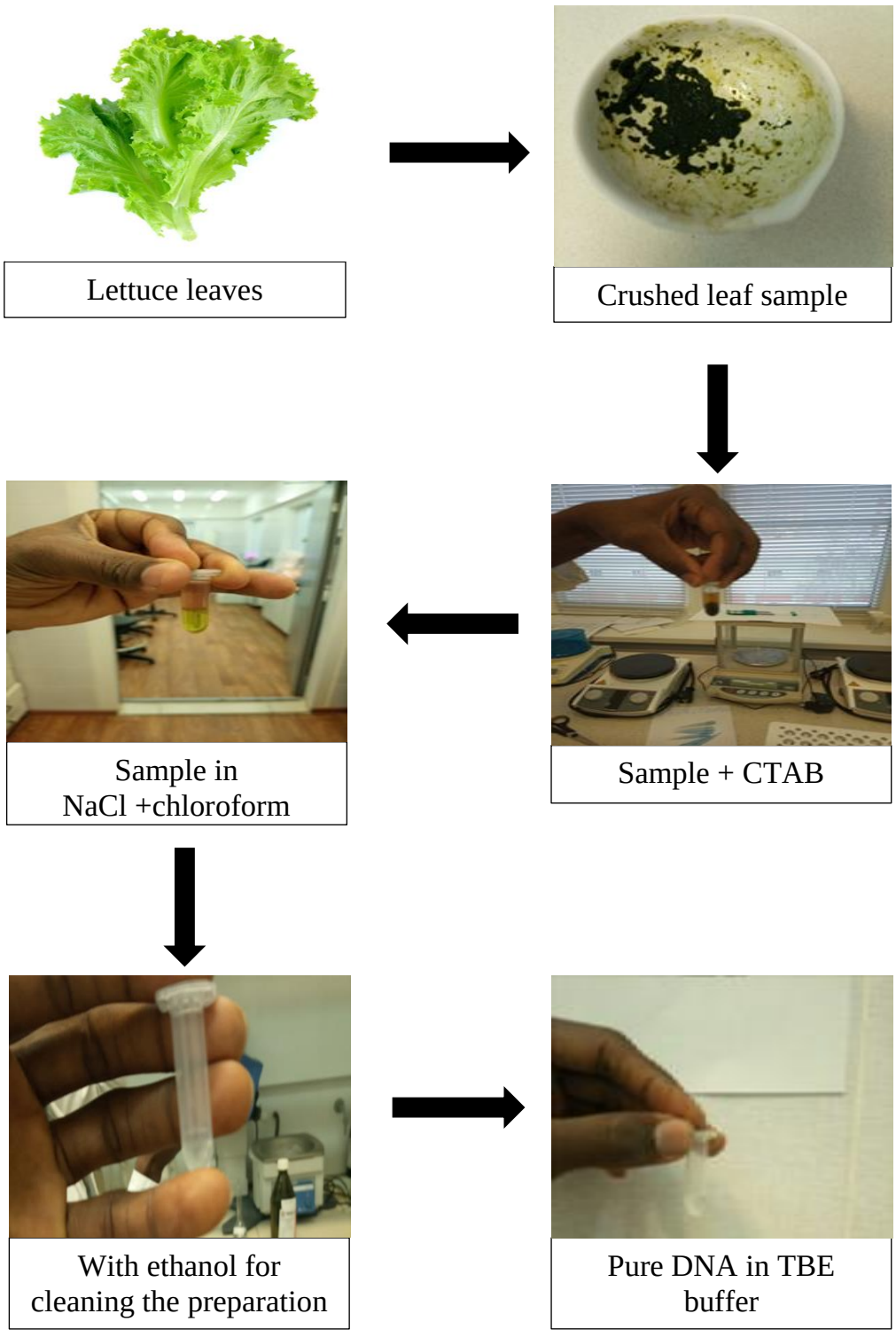


Figure 1. Extraction of DNA from Lettuce leaves

After DNA extraction it is necessary to perform electrophoresis to check the results, for that need to prepare the agarose gel to use in horizontal gel electrophoresis.

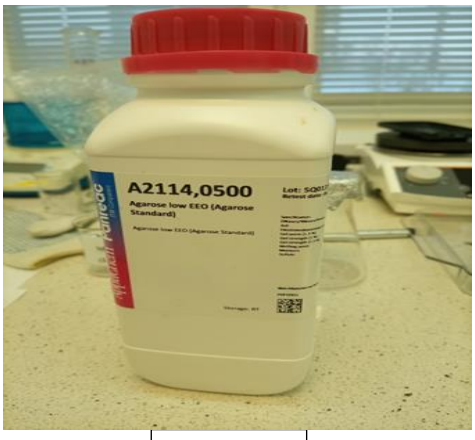
Gel electrophoresis lab procedures

1. About 0.7 g of agarose was dissolved in 100 ml x1 TBE and heated in water bath to sterilize and dissolve to obtain a clear solution.

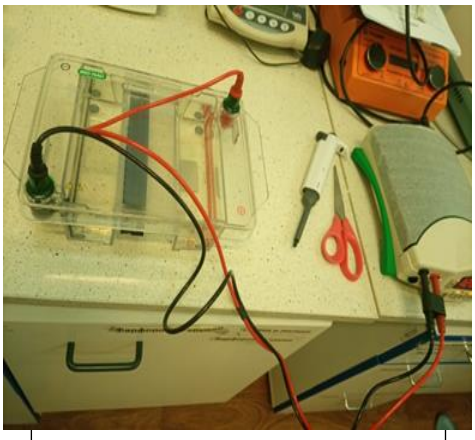
2. On cooling (Temperature the gel about 55 ° C, about 4 µl of ethidium bromide (for the intercalation of the DNA between bases) was added and mixed gently.
3. The solution is then gently poured into the mold with the comb inserted to form wells and allowed to solidify into a gel.
4. The TBE buffer was then poured into the electrophoresis tank to cover the gel and the comb, and the tape gently removed.
5. The samples were prepared in the following amount (table 1) and loaded using the micropipette into the wells gently.

Ladder DNA and dyes

Tris-borate EDTA buffer



Agarose



Gel box and power supply

Figure 2. Horizontal Electrophoresis Set up and Materials

Table 1. Sample preparation

	Marker –DNA Ladder	DNA sample
Volume	3 µl	10 µl
Loading bffer	5 µl	5 µl
Total volume	8 µl	15 µl

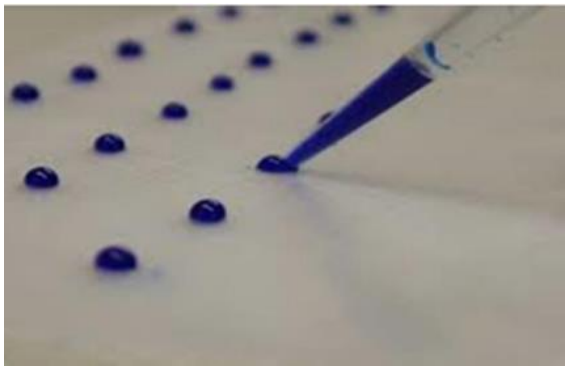


Figure 3. Preparation of DNA for loading

6. The lid of the gel tank was closed, and the electrical fields connected to the power supply. (Voltage was 120 V for few minutes and reduced to later reduced to 90 V).
7. The results were visualized using the gel documentation system.

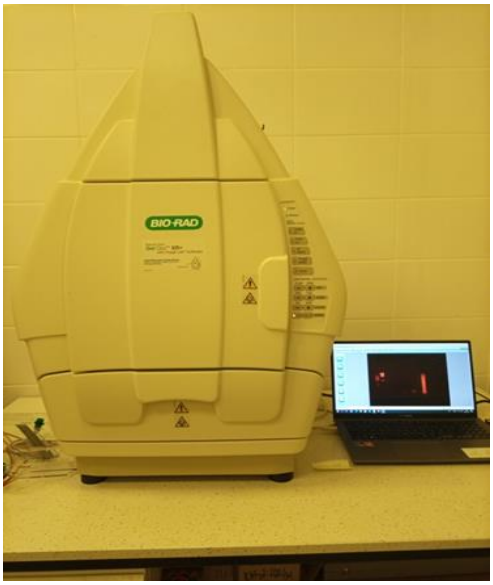


Figure 4. Bio Rad gel documentation system

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MASTER CLASS 2
PRODUCTION OF SEMI-HARD CHEESE IN MINI-CHEESE MAKER

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Introduction

Dairy products averagely are made up of about 20-30% of an individuals' diet. Milk and dairy products such as cheeses and yoghurts are main sources of proteins and fats which support growth and provides the body with energy [1]. Fermented diary promotes improve the availability of the nutrients contained in the milk. There is an increase in the demand for foods containing naturally occurring biological molecules that help promote body nutrition and health [2]. Milk and fermented dairy products have the capacity to improve the antioxidant activities especially when fortified with carotenoid which is a naturally occurring biological molecule [3].

Cheese is a dairy product produced in wide ranges of flavors, textures, and forms by coagulation of the milk protein casein. It comprises proteins and fat from milk, usually the milk of cows, buffalo, goats, or sheep. During production, milk is usually acidified and the enzymes of either rennet or bacterial enzymes with similar activity are added to cause the casein to coagulate. The solid curds are then separated from the liquid whey and pressed into finished cheese. Some cheeses have aromatic molds on the rind, the outer layer, or throughout.

The fermentation of cheese is accomplished by lactic acid bacteria (LAB) strains. These bacterial strains may help remove some toxic and antinutritional factors such as lactose and galactose, from the fermented milks to prevent lactose intolerance and galactose accumulation [4]. The commonly used strains of LAB for milk fermentation are *Streptococcus thermophilus*, usually in association with Bifidobacteria, such as *Bifidobacterium breve* C50, *Bifidobacterium lactis*, *Bifidobacterium longum* and *Bifidobacterium animalis*, or with Lactobacilli such as *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus johnsonii* and *Lactobacillus casei* [5].

Types of Cheese

In the world exist around 600 types of cheese; they can be classified by different attributes as detailed below:

Type of milk:

- Cow's milk cheese
- Goat's milk cheese
- Sheep's milk cheese
- Buffalo milk cheese

According to Maturation:

- Fresh cheese: product is ready to consume after the manufacturing process.
- Matured cheese: this cheese undergoes the necessary and characteristic biochemical and physical changes of each variety of cheese, since it is kept for a certain time in certain conditions of humidity and temperature until it is consumed.

According fat content:

- Extra fat: it has a minimum of 60% of the dry extract
- Fat: between 60 and 45% of the dry extract
- Semi fat: Between 45-25% of the dry extract
- Lean: between 25-10% of the dry extract
- Skimmed: maximum of 10% of the dry extract

According to the percentage of humidity:

- Low humidity up to 35.9%
- Medium humidity: between 36-45.9%
- High humidity between 46 and 54.9%
- Very High humidity: not less than 55% [1]

The purpose of Master class is to demonstrate production of semi-hard cheese in mini-cheese maker.

Materials and Methods

Materials

Raw milk, mini cheese maker, rennet enzyme, resveratrol nano emulsions, strainer, calcium chloride, curd knife, starter culture, curd cutter, cheese wax, cheese mold, cheese brush, incubator pipette, Petri dishes.

TREMAS-MIX 3.0
cheese maker

Whole milk
3.5-4.5%

Calcium Chloride

Starter culture

Figure 1. Equipment and Materials

Methods

Preparation of cheese

1. Pasteurize 5 ltrs of milk at 65^0C and hold the temperature for 15 minutes.
2. Cool down to 40^0C and then add 5ml of Calcium chloride (100mg/ml) to milk.
3. Cool down to 30^0C and then add starter culture.
4. Stir and wait for 20-30 minutes and add 500ml encapsulated resveratrol.
5. After 5 minutes add rennet enzyme (0.078g/l).
6. Stir and wait for 30 minutes to observe the state of the clot.
7. Slice through the cheese and wait for 5 minutes to release whey.
8. Continue cutting with the cheese cutter until the desired grain size is obtained.
9. Second heating up to 40^0C for 30 minutes.
10. Stir for 10 minutes and look at the condition of the grains.
11. Wait for 20 minutes for the grains to settle.
12. Separate the whey from the grains using a strainer.
13. Place the grains in a mold and press.
14. Store the cheese in the chamber at 12^0C until next day.
15. Carry out salting by putting the cheese in 20% saline solution for 2 hours and turn over.
16. Dry the cheese on racks for 2 days at 10^o-12^oC.
17. Cover the head with cheese wax (parafilm)
18. Leave the cheese to ripen at 8-12^oC for a month

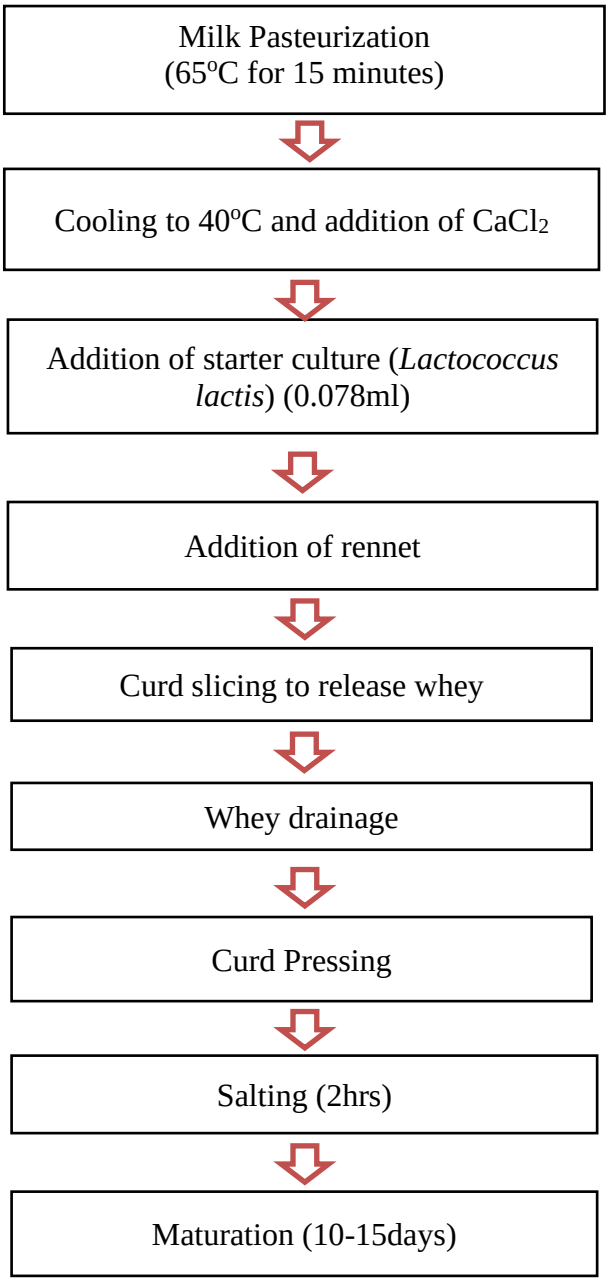

Figure 2. Flow chart for cheese production

Description of cheese

Cheese was produced using a TREMAS-MIX 3.0 cheese maker. The net weight of the final semi-hard cheese produced is 600g from an initial milk of 500 liters. The cheese obtained was standard and had good organoleptic properties such as flavor, aroma, and appearance. This characteristic of the cheese is due to the milk we used since it was obtained from a trusted source. Also, the moisture significantly imparted the compact body of the cheese with a standard moisture content for semi-hard cheeses from 34-45%. In other to coagulate and make the milk become solid, rennet enzyme was used followed by addition of $CaCl_2$ a crosslinking agent.

Figure 3. Semi-hard cheese coated with parafilm

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MASTER CLASS 3
PRODUCTION OF ALL GRAIN BEER OF ALE FERMENTATION BY INFUSION METHOD

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Introduction

Beer is the most popular and most consumed alcoholic drink around the world, and it is also one of the oldest. Beer drinking and brewing have been part of human activities since the dawn of civilization. The first beer was basically made from grain, water, and spontaneous fermentation due to wild yeast present in the air, just before bread was invented [1]. It has been reported that the Egyptians were the first to document the brewing process around 5000 B.C, it is also believed that the first brewers were part of primitive cultures of Mesopotamia. The birth of modern beer was during the early Middle Ages, when German monks introduced hop as a bittering and flavoring agent. Beer brewing used to be on a domestic scale, but with the emergence of the Industrial revolution, its production moved to mass manufacture, allowing beer to be produced on a much larger scale [2].

Beer is produced commercially by the controlled fermentation of wort, a liquid-rich in sugars, nitrogenous compounds, sulfur compounds and trace elements extracted from malted barley. Fermentation is the process by which glucose is converted to ethanol and carbon dioxide and is expressed chemically as:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Behind this simplified chemical reaction is a series of complex biochemical reactions. These reactions (known as the ‘Glycolytic pathway’ or ‘Embden-Meyerhof-Parnas pathway’) involve a number of enzymes and the reactions take place anaerobically inside the cells of brewing yeast [1]. Breweries carry out this fermentation by a continuous process in which the beer moves through a series of stirred vessels for a period of 40 to 120 hours. After the ethanol has formed, the beer is transferred to maturation vessels and the flavor is naturally refined. Following this the product is developed into a variety of different brands [1]. Brewing beer involves microbial activity at every stage, from raw material production and malting to stability in the package. Most of these activities are desirable, but others represent threats to the quality of the final product and must be controlled actively through careful management [3].

Barley beer is said to have many health benefits if it is consumed in moderate amounts. Some of these interesting health benefits include anti-cancer properties, reduced risk of cardiovascular diseases, increased bone density, diabetes, prevention of anemia, hypertension,

anti-aging properties, gallstones, prevention of dementia and coronary disease, aids digestive system, kidney stones and osteoporosis, stress buster and diuretic.

The purpose of this Master class is to demonstrate the production of all grain beer of ale fermentation by infusion method.

Theoretical aspects

Steps involved in beer production are:

Malting

Beer is produced from malted barley, wheat, rye grains as well as malt extracts. Barley is the most popular cereal for beer production.

Barley grains are first cleaned and then soaked in water for about 2 days. Then excess water is drained away and the barley are incubated for 4-5 days to allow germination.

The germination steps allow the formation of highly active α-amylase, β-amylase and proteases enzymes as well as various flavor and color components.

Kilning

The germinated seed are then killed by slow heating at 80° This process is called kilning. The kilning temperature must not harm amylase enzyme. Furthermore, if kilning temperature is higher, darker will be the beer produced.

Miling

The dried barley grains are then crushed between rollers to produced coarse powder called grist.

Mashing

There are two Mashing Methods: Infusion and Decoction.

In **Infusion method** the entire mash is heated up (with appropriate rest periods) to the final mashing temperature. Decoction is the method based on increasing the temperature by moving part of the mash from the mash converter to the mash cooker where it is boiled (possibly also with carbohydrate rest periods in the mash cooker). By pumping it back to the remainder of the mash in the mash converter, the temperature of the total mash is increased to the next higher rest temperature.

Following **Infusion method** grist is mixed with warm water and the resulting materials is maintained at 65°C for about 1 hour. In doing so, starch is hydrolyzed by amylase enzyme to produce single sugar, maltose, dextrose etc. similarly, protein is hydrolyzed by proteolytic enzymes into small fragments and amino acids. The degree of enzymatic hydrolysis is strongly depends on pH and temperature. β-amylase has optimum activity at temperature 57-65°C whereas α-amylase has optimum activity at temperature 70-75°. The liquid obtained by mashing is called wort. The husks and other grains residue as well as precipitated proteins are removed by filtration.

Boiling of wort

The filtrate is then boiled with stirring for 2-3 hours and hop flowers are added at various interval during boiling. Boiling coagulate remaining protein and partially hydrolyze protein and help in removal of protein. Boiling inactivates enzymes that were active during mashing, otherwise causes caramelization of sugar. Boiling also sterilize and concentrate the wort.

Hops

Hops are added to wort during boiling to provide the following properties.

Provide beer with its pungent and aromatic character.

Provide tannin which helps in coagulation of remaining protein.

Contains α-resin and β-resin which gives bitter flavor as well as preservative action against gram positive bacteria.

Contains pectin which is responsible for foam characteristic of beer.

Fermentation

Beer production utilize strain of *Saccharomyces carlsbergensis* and *S. uvarum* which are bottom yeast and S. cerevisiae which is a top yeast.

"Bottom-fermentation yeast" beers, which are fermented at a low temperature (8 to 12°C) with a yeast that sinks to the bottom of the beer; originated in central Europe and spread to the rest of the world. "Top-fermentation yeast" beers, fermented at 15 to 25°C, with a yeast which rises to the surface of the beer after fermentation, mainly brewed in England. Approximately half of the top yeast beer comes from Belgium. In France, the top yeast method is rare.

During fermentation yeast converts sugar mainly into ethanol and CO_2 plus some amount of glycerol and acetic acid. For fermentation open tank fermenter can be used however closed fermenter tank is preferred, so that CO_2 liberated during fermentation can be collected for later carbonation step.

Finishing, Ageing, Maturation and Carbonation

The young and green beer is stored in at 0°C for several weeks to several months. During this period, precipitation of protein, yeast, resin and other undesirable substances take place and beer become clear. Ester and other compounds are also produced during ageing which gives taste and aroma. After ageing, the beer is carbonated by carbon dioxide of 0.45-0.52%. The beer is then cooled, clarified, filtered and packed in bottles, barrels and cans.

With the genetic manipulation of yeasts, numerous varietal strains have been bred, this, along with modifications in the brewing process have led to different types of beers. Those most often seen beers include:

- ***Lager:*** Beers made with yeast that settle on the bottom (*Saccharomyces carlsbergensis*) of the container used. Thus, all the yeast and other material settles on the bottom which results in a clear beer.
- ***Pilsner:*** A colorless lager beer originally brewed in the city of Pilsen. Water used for this style of beer tends to be harder, with a higher calcium and magnesium content than water used for lager. The color of pilsner is also lighter than that of lager beer.
- ***Ale:*** Beers made with yeast that floats (*Saccharomyces cerevisiae*) to the top of the brewing vats, resulting in a cloudier beer. They tend to have a higher alcohol content than lagers.
- ***Porter:*** A very dark ale. The darker color and special flavor come from toasting the malt before brewing. This usually results in a stronger taste and higher alcohol content.
- ***Stout:*** A very dark, almost black ale. The dark color and roasted flavor are derived from the roasted barley, and/or roasted malt [4].

Figure 1. Beer types

Materials and Equipment

The mash ingredients and other ingredients for the beer processing are indicated in table 1 below. Below are some apparatuses used:

Mash equipment (Easy Brew, 40 L, brewery, Mash paddle, Siphon, Hydrometer, Refractometer, pH meter, Thermometer, Water, Peracetic acid.

Belgian Amber Ale beer recipe

Table 1. Mash Materials Needed for Beer Production

Malt	**Color (EBC)**	**Amount (kg)**	**% IBU**
Château Pale Ale	8.5	5.00	66.7
Château Munich	25.0	1.00	13.3
Château Cara Gold®	120.0	0.50	6.7
Château Cara Ruby®	50.0	0.50	6.7
Château Melano	80.0	0.30	4.0
Château Special B®	300.2	0.20	2.7
Hops	**-**	**Amount (g)**	**% IBU**
Centennial	-	10.00	6.5
Cascade	-	30.00	6.9
Citra	-	30.00	6.1
Yeast	**Volume (mL)**	**Amount (pkg)**	**% IBU**
Safale American (DCL/Fermentis #US-05)	50.28	1.00	-

Table 2. Boil ingredients

Quantity (g)	Type of hops	Boiling time (min)	IBU (%)
10.0	Centennial (10.0%)	30.0	6.5
30.0	Cascade (5.5%)	15.0	6.9
30.0	Citra (12.0%)	5.0	6.1

Table 3. Mashing malt

	Boiling temperature (°C)	Holding time (min)
Add water (25 L)	55	20
Continue heating by changing temperature	63	60
	72	20
	78	5
After malting, rinse with water about 15 L and heat to	80	

Table 4. Wort boiling

Hops	Boiling time (min)
Hops 1	30
Hops 2	15
Hops 3	5

Beer quality indicators

The indicators are the essential parameters that guided and ensured a good production of beer at the laboratory scale. The quality indicators for the beer production are outlined below:

- **Boil Size**: 40.78 L
- **Boil time**: 90 mins
- **Final Bottling Volume**: 29.00 L
- **Fermentation**: Ale, two stage
- **Brewhouse efficiency**: 70.00 %
- **Brix (%)**: 13.00%
- **Color**: 31 EBC
- **pH**: 5.5

Procedure

Mashing Protocol and Wort Production

1. The simple infusion method was employed for this practical work. All equipment and apparatus used were sterilized prior to the mashing using ethanol and acetic acid.
2. After that, about 28 L of water (Kirovsky) was put into the mash tun. The mash, 7.50 kg was added to the water in the mash tun and then allowed to boil at 55°C for 20 mins.
3. After 20 mins, the first rest period was reached, and the temperature was increased to 63°C and allowed to boil for 60 mins.
4. The second rest period was attained after 60 mins of boiling and the temperature was increased to 78°C and allowed to boil for 5 mins. Filtration of the mash was carried out

to obtain the final wort. The pH of the mash obtained with pH meter was 5.6. The specific gravity of the mash was also found to be 1.048 with a Brix of 12.40 using the refractometer.

5. The residue was discarded, and the filtrate (wort) boiled at a temperature of 100°C for 90 mins. During the wort boiling, proteins that coagulated on top of the wort were collected and discarded.

6. During the wort boiling, the process of sparging began, where 12 L of water (Kirovsky) was boiled at same temperature and later added to the wort.

7. After the wort boiling, the first hops for bitterness were added and allowed to boil at same temperature for 30 mins. The two different hops (hops 2 and 3) were added afterwards and allowed to boil for 15 mins and 5 mins, respectively.

8. After boiling, a portion of the samples were collected and stored for further analysis.

Pitching and Fermentation

1. Prior to fermentation of the wort into beer, the Clean sterilized copper wort chiller was then lowered into the boiled wort for cooling. With the aid of sterilized syphon, the cold wort was transferred into a clean fermentation bucket equipped with airlock bubbler.

2. A top fermenter yeast (Safale American) was added to the wort after cooling to a temperature of 18°C and allowed to ferment for about 2 weeks.

Beer Carbonation

1. All equipment was sterilized before use.

2. Parameters such as volume, pH, specific gravity, and temperature of beer were measured prior to carbonation.

3. Based on these key parameters, the amount of sugar to be used for carbonation was determined by using the “Priming Sugar Carbonation Calculator”.

4. In a 1.5 L bottle, 10.71g of glucose was put in with the addition of the green beer. Also, in a 1 L bottle, 7.12g of glucose was added together with the green beer.

After bottle conditioning, the bottles were stored for about 2 weeks after which the beer was analyzed.

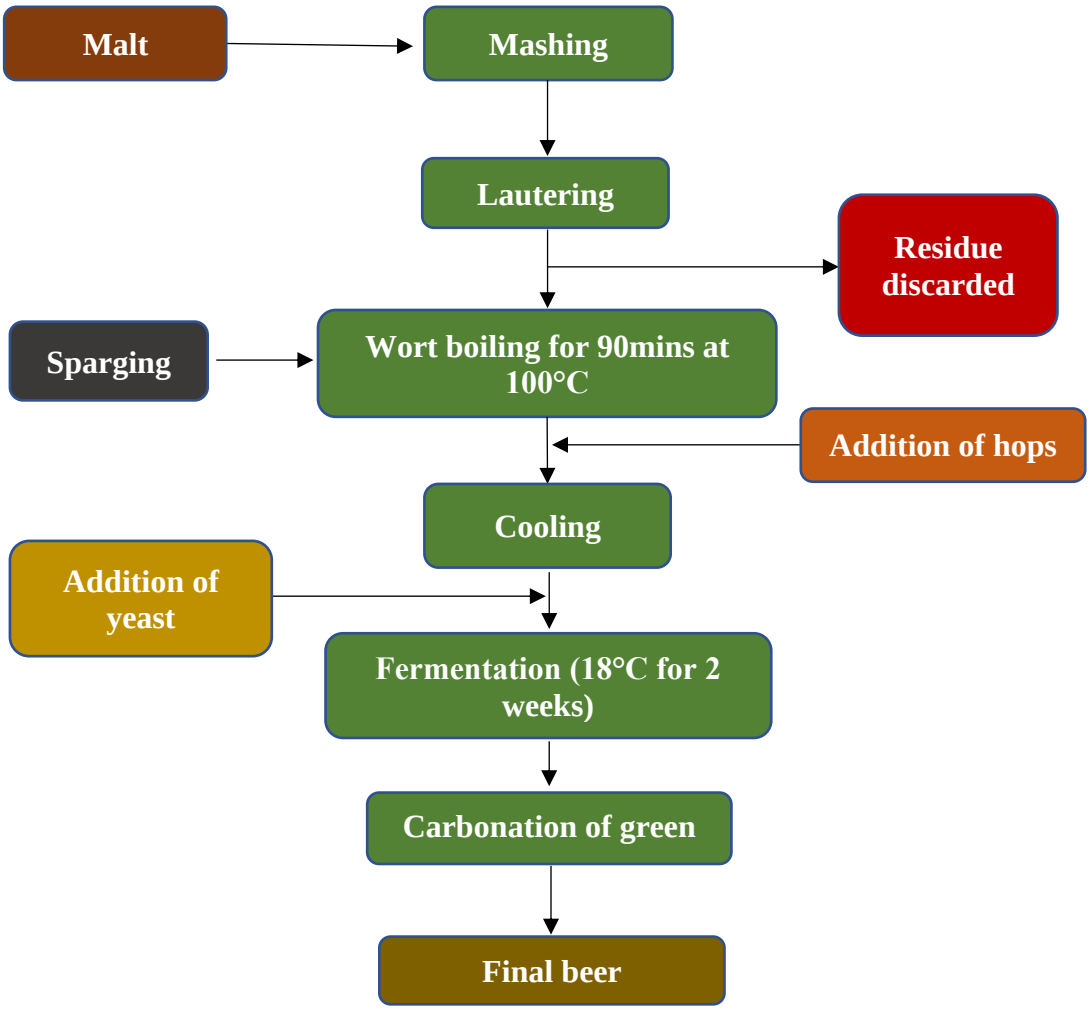

Figure 2. Flowchart of beer processing

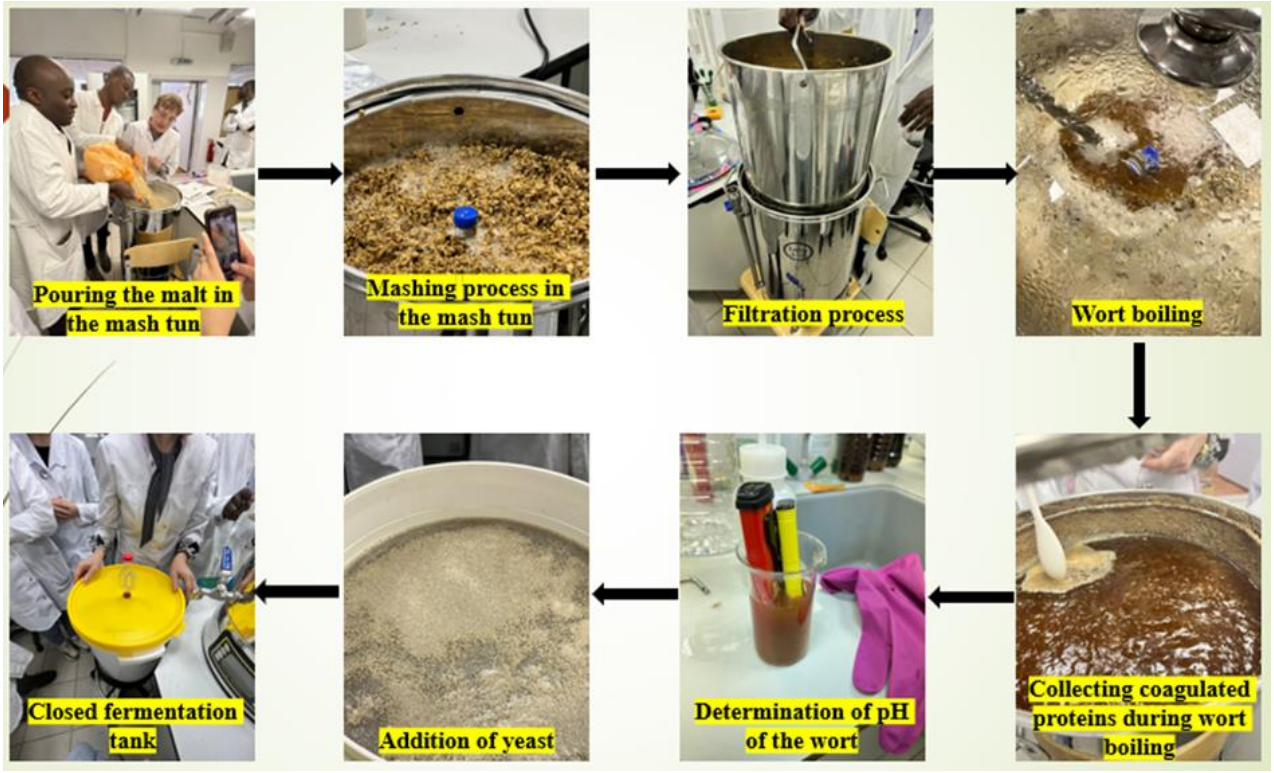

Figure 3. Flowchart of beer processing

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MASTER CLASS 4
CHEMOSTAT

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Introduction

Cells regulate their rate of growth in response to signals from the external world. As the cell grows, diverse cellular processes must be coordinated including macromolecular synthesis, metabolism and ultimately, commitment to the cell division cycle.

The chemostat, a method of experimentally controlling cell growth rate, provides a powerful means of systematically studying how growth rate impacts cellular processes - including gene expression and metabolism - and the regulatory networks that control the rate of cell growth. When maintained for hundreds of generations chemostats can be used to study adaptive evolution of microbes in environmental conditions that limit cell growth [1].

One of the most important features of chemostats is that microorganisms can be grown in a physiological steady state. In steady state, growth occurs at a constant rate and all culture parameters remain constant (culture volume, dissolved oxygen concentration, nutrient and product concentration, pH, cell density, etc.). In addition, environmental conditions can be controlled by the experimenter [2].

Chemostats in research are used for investigations in cell biology, as a source for large volumes of uniform cells or protein. The chemostat is often used to gather steady state data about an organism in order to generate a mathematical model relating to its metabolic processes. Chemostats are also used as microcosms in ecology and evolutionary biology [3-7].

Baker yeast *Saccharomyces cerevisiae* is an important microorganism for biotechnology because it is eukaryotic organism, which grows well in bioreactor, produces important metabolites, proteins or performing important processes, can be relatively easy transformed to express heterologous proteins, enzymes, whose biochemistry and molecular biology are relatively well studied.

The main purpose of this Master class is to analyze a process of growing of yeast *Saccharomyces cerevisiae* in a chemostat by comparing 5 different flow rates (5rpm, 7rpm, 9rpm, 11rpm, and 13rpm).

Theoretical Aspects

The method of continuous culturing using a chemostat was independently described by Monod [8] and Novick & Szilard [9] in 1950. A chemostat operates by maintaining a growth rate through continuously feeding a growth-limiting nutrient and withdrawing part of the fermentation broth at the same rate, thereby achieving steady-state growth. The growth-limiting nutrient may

be carbon, nitrogen, phosphorus, or any other essential nutrient, which influences the specific growth rate. A significant advantage of chemostat mode over batch mode is that by changing the feed rate of the growth-limiting nutrient, the growth rate can be varied (Fig.1) [10].

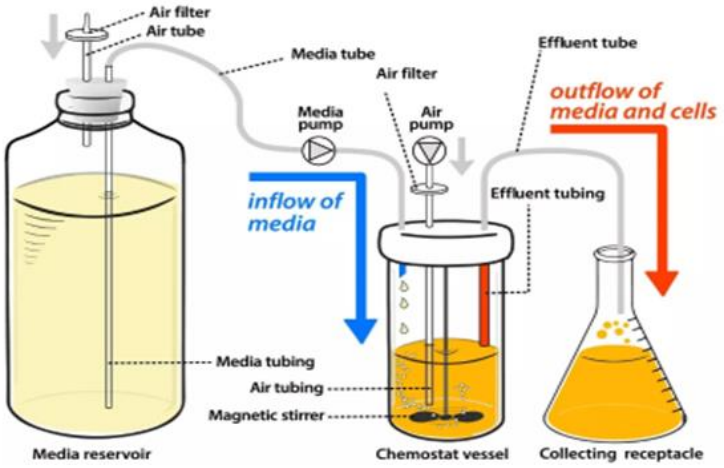

Figure 1. Chemostat assembly

Each microorganism growing on a particular substrate has a maximal specific growth rate μ_{max} (the rate of growth observed if growth is limited by internal constraints rather than external nutrients). If a dilution rate is chosen that is higher than μ_{max}, the cells cannot grow at a rate as fast as the rate with which they are being removed so the culture will not be able to sustain itself in the bioreactor and will wash out.

However, since the concentration of the limiting nutrient in the chemostat cannot exceed the concentration in the feed, the specific growth rate that the cells can reach in the chemostat is usually slightly lower than the maximal specific growth rate because specific growth rate usually increases with nutrient concentration as described by the kinetics of the Monod equation [8]. The highest specific growth' rates (μ_{max}) cells can attain is equal to the critical dilution rate (D):

$$D = \mu_{max} \frac{S}{K_s + S}, \quad (1)$$

where S is the substrate or nutrient concentration in the chemostat, and K_S is the half-saturation constant (this equation assumes Monod kinetics).

The concentration of microorganisms within the chemostat does not vary with time, but the rate of growth of the microorganism varies. The growth rate is lower at lower dilution rates. The generation time is inversely proportional to the growth rate, that means that the shorter the time the higher growth of the culture.

The amount of substrate metabolized by microorganisms can be calculated using the next equation:

Added nutrient = Nutrient consumed + Nutrient not consumed (not metabolized) (2)

Experimental part

Reagents

- Distilled water (H_2O)
- Yeast (*Saccharomyces cerevisiae*)
- Glucose ($C_6H_{12}O_6$)
- Potassium chloride (KCl)
- Sodium chloride (NaCl)
- Magnesium sulphate ($MgSO_4$)
- Potassium hydroxide (KOH)
- Potassium ferricyanide ($C_6N_6FeK_3$)

Equipment

- Bioreactor (Winpact system)
- Vials (400 mL and 200 mL)
- Volumetric flasks
- Beakers 1L, 50mL
- Pipettes 1mL and 10mL
- Analytical balance

Procedure

1. Preparation of nutrient media, inoculum, buffer and feeding solutions

a) The mass of 333.3 g of glucose and 0.067 g of each salt (KCl, $MgSO_4$, NaCl) were weighed and dissolved in 1 L of dH_2O. The volume was adjusted to 2 L with dH_2O.

b) The mass of 33.3 g of baker's yeast was weighed aseptically and dissolved in 100 mL of sterile H_2O. The volume was adjusted to 200 ml with sterile H_2O.

c) The solutions of 200 ml of 1% NaOH and 200ml of 1% HCl were prepared.

d) The mass of 64.0 g of glucose was weighed and dissolved in 200 mL of dH_2O. The volume was adjusted to 400 mL with dH_2O.

2. Assembly of the reactor

The assembly of the reactor was carried out in accordance with the instructions. The nutrient medium was poured into the reactor.

3. Sterilization

The reactor with nutrient media and vials (buffer and feeding solutions) were sterilized in an autoclave (121°C, 20 min). After sterilization, the bioreactor and vials were allowed to cool, then the vials were connected to the bioreactor.

4. Inoculation

The conditions for the chemostat to run were set at a speed of 300 rpm, 37°C, pH 5.7. Them inoculum was aseptically loaded into bioreactor and was cultivated for 15 min a batch mode. Then a zero sample was taken from the reactor for analysis.

5. Chemostat

Then the processes of inflow of sterile feeding solution and outflow of media and microbial cells to collection vessel were started. These processes proceeded at the same flow rate, 5 rpm for 5 min. Then rate of the peristaltic pumps was changed to 7 rpm, 9 rpm, 11 rpm, and 13 rpm respectively. Thus, 5 samples were collected at different flow rates.

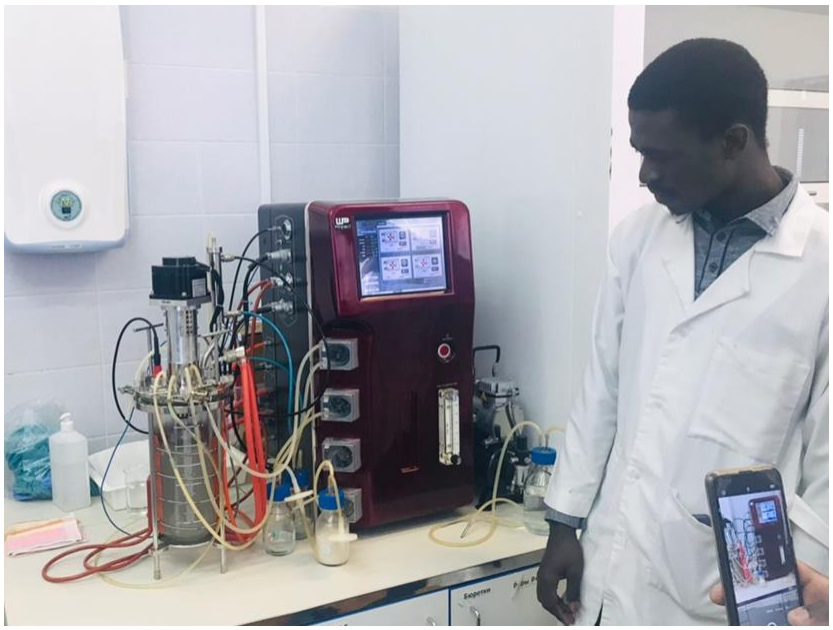

Figure 2. Chemostat set up

The further steps to be done are colorimetric analysis by UV Spectrophotometry Procedure as well as counting of the number of cells by optical microscopy.

The main goal of the experiment is to analyze the influence of medium flow rate in the growth rate of *Saccharomyces cerevisiae* in a continuous system (chemostat) by comparing 5 different flow rates (5 rpm, 7 rpm, 9 rpm, 11rpm, and 13 rpm) in a constant volume (2L).

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