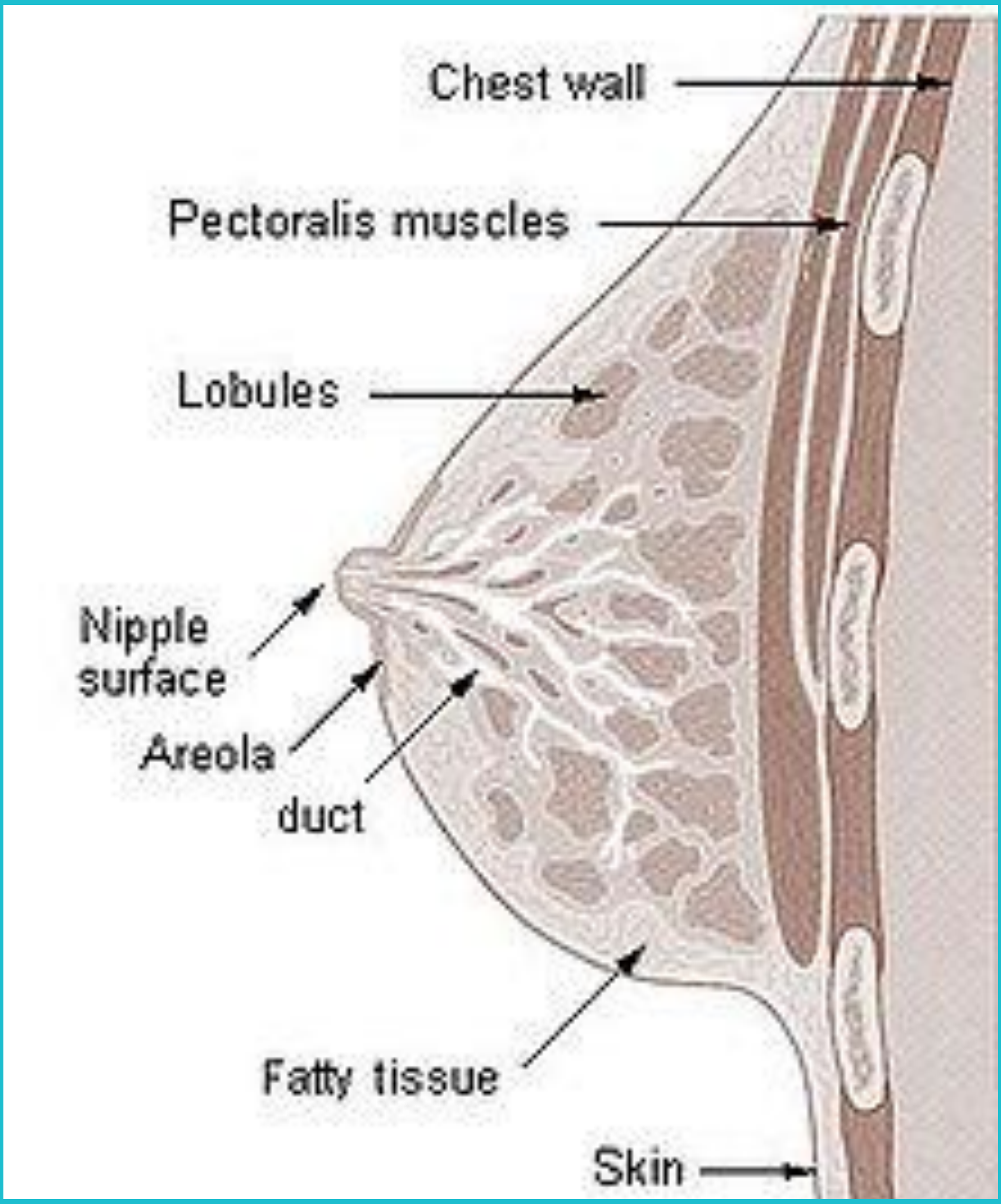
Carbohydrates in breast milk

a better health with smart carbohydrates



Colophon

IRRESISTIBLE is a project on teacher training, combining formal and informal learning focused on Responsible Research and Innovation. It is a coordination and support action under FP7-SCIENCE-IN-SOCIETY-2013-1, ACTOVITY 5.2.2. Young people and science: Topic SiS.2013.2.2.1-1 Raising youth awareness to Responsible Research and Innovation through Inquiry Based Science Education. The project IRRESISTIBLE is funded by the EU as FP-7 project number 612367

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SEVENTH FRAMEWORK PROGRAMME

1

Engage – Secrets of Breast Milk

“Why don’t babies just drink milk from the supermarket?”

Humans are (also) mammals

Human beings, apes, cats, dogs, cows, goats; they all belong to the class of Mammalia: mammals. The common characteristics of mammals is that they ‘nurse’ their offspring, that is, feed them by means of breast milk. All mammals produce milk after giving birth that has the ideal composition to help their offspring grow optimally. Goat’s milk is made for goats, cow’s milk is made for calves, and human milk has its own special composition that optimally help babies to develop.

How long animals are nursed is dependent upon the kind of animal: cats do this approximately 8 to 12 weeks, a walrus two to three years. And this is different for human beings. Originally, mothers nursed their children for about five years, eventually mainly as additional nutrition. This is still the case in developing countries. In a modern country, such as the Netherlands, where women often start working again after giving birth, women don't breastfeed as long.

table 1.1

%of women in the Netherlands that breastfeed/give formula

	birth	1 month	2 m	3 m	4 m	5 m	6 m
Breastfeeding	80	57	53	47	45	42	39
Mixed		12	11	11	13	11	12
Formula	20	31	36	42	42	47	49

Source: http://www.borstvoeding.com/aanverwant/maatschappij/borstvoedingscijfers.html

The World Health Organization (WHO) advises mothers to completely breastfeed up to six months, and to continue breastfeeding until the age of two in addition to giving other food. Not a lot of mothers in the Netherlands do this. Breastfeeding a toddler of two is often considered to be strange and controversial (the cover of the magazine to the right created a lot of commotion)). In the Netherlands, this doesn't happen often. Unfortunately it isn't measured, but estimations vary that only 2-5% of the mothers still breastfeed their toddler.

Breast milk: superfood?

If the WHO advises breastfeeding until age two so passionately, it must be really good. But why is that the case? Isn't milk just milk? And couldn't you just give your baby milk from a carton? The answer is no. Breast milk differs from regular milk in various ways. First, breast milk adapts to the needs of the child through time. Breast milk just after birth (colostrum) contains a lot of antibodies and has a laxating effect. After three weeks, the milk is 'ripe', this milk contains less antibodies but is full of materials that are needed for growing. Within one session of breastfeeding, the first milk is more watery, and the last part contains more fat. After three months, milk is different again, especially attuned to what the baby needs at that particular moment. The ratios of the components of breast milk are also different than those of cow's milk.

Figure 1.1 Cover Time Magazine, May 2012

Figure 1.2 This is milk too, right?

Milk mainly consists of water, with proteins, fats, carbohydrates, and vitamins and minerals. The table below presents the ratios of these components in milk of various mammals. As you can see, there are major differences between the various mammals, and therefore also between breast milk and cow's milk.

table 1.2

composition of milk of various mammals

	human	cow	goat	sheep	horse	buffalo	donkey	reindeer
protein	1.5 g	3.5 g	3.8 g	5.2 g	2.1 g	4.0 g	n.a.	n.a.
fat	4.0 g	3.4 g	4.1 g	6.2 g	1.3 g	8.0 g	1.4 g	18.0g
lactose	6.9 g	4.6 g	4.4 g	4.2 g	6.3 g	4.9 g	6.3 g	2.8 g
residuals	0.3 g	0.8 g	1.9 g	0.9 g	0.4 g	n.a.	0.4 g	1.5 g

Source: Wikipedia: http://nl.wikipedia.org/wiki/Melk_(drank)

Especially notice the major difference in proteins and lactose between breast milk and cow's milk. But not just the amount, also the type and composition of lactose in breast milk is very different than those in cow's milk. The sugars are important for the health of babies and children. You can learn more about this in sections 3.1.3. and 3.2.

There are a great many varieties of these special sugar-like molecules in breast milk that are collectively referred to as *Human Milk Oligosaccharides* (HMOS). It is notable that these molecules are hardly broken down by the human digestive system. This gives rise to the question why they exist in breast milk. This was unclear until recently.

Experiments

With various experiments, you can determine the protein, fat, and carbohydrate level of various types of milk. Follow your teacher's instructions when you conduct these experiments. See the appendix Experiments

Mothers who don't want to or can't breastfeed completely (anymore) give their children *'breast milk substitutes'* (the official term), or formula. Formula is made of cow's milk, while, as we've just read, the composition of that is not optimal for human babies.

For the production of formula, cow's milk is processed and adjusted in a factory to make it resemble breast milk more closely. Formula was first made in 1884 by diluting cow's milk with water and by adding cream and sugar, but the insight into the composition of breast milk and the production process have greatly improved. For example, special milk sugars that mimic the complex sugars in breast milk are added to cow's milk. These must first be synthesized in a factory. You can read more about this in section 3.3.

Babies that are given formula also grow up, without any major health issues. But population studies conducted in the last decades have provided increasing evidence that babies who are breastfed are better protected from diseases such as diabetes, asthma, and other allergies. Furthermore, breastfeeding (longer) provides protection against developing obesity later in life. This evidence mainly comes from epidemiological studies, in which groups of people with or without a certain affliction are compared. This is discussed in further detail in section 3.2. The underlying biological mechanisms, so how milk sugars advance health, are still not entirely clear. After all, proper research in this area has only been possible recently. This is discussed in more detail in section 3.3.

Figure 1.3 Different types of formula (source: bol.com)

So exactly how breastfeeding can have a protective function is not entirely clear yet. But there is increasing evidence that breast milk has an advantageous effect on the development of good intestinal bacteria in babies and that these good bacteria help in the optimal functioning of the immune system. How this works, you will learn in section 3.1.3.

Through knowledge and technological innovations, formula is improving and resembles breast milk more and more. Because of these adjustments, formula also increasingly stimulates the development of good intestinal bacteria. But it's still not the same. And will it ever be? Do we want it to be? Should manufacturers be allowed to advertise their powdered milk as 'resembles breast milk'? These ethical aspects of breast milk substitutes are discussed in Chapter 4. As an introduction to the ethical aspects, the next part of this chapter is about Responsible Research and Innovation.

Responsible Research and Innovation

Many people think of science and innovation as activities that are carried out by smart people, far away at universities and companies. Sometimes, it seems as if scientific research does not properly match with what is important to society and that 'ordinary people' have no influence on it. The European Union has created the term "*Responsible Research and Innovation" (RRI)* to achieve that people can more easily converse with scientists and the business world. In this way, society becomes more involved in scientific research and innovation.

A major part of scientific research is paid by tax money, and people are eager to see some feedback. Where does this money go? What is researched and what is not? The EU and other governments have formulated key objectives: scientific topics society benefits from if these were researched. These topics are determined together with all manner of societal organizations, in order to ensure that the results of scientific research better matches the wishes of society. Additionally, innovations are also looked at. You can think of a lot of improvements to current life (innovations), but are all these improvements actually useful? Does it benefit us as a society? Should you actually do everything that's possible?

This is what *Responsible Research and Innovation* is about: people working on scientific discoveries and innovations, must do so in a responsible way.

RRI has six components:

Engagement:

Researchers, industry, policy makers, and citizens have to collaborate in the research and innovation process. In this way, social, economic, and ethical interests of all groups can be included to find a joint solution for societal problems.

The European Union, the United Nations, and other governmental institutions have identified a number of 'Grand Challenges' of modern day society that science could answer. These challenges are worldwide problems such as clean drinking water, proper food production, poverty, hunger, and climate change, as well as aspects that focus more on Western society, such as healthy aging, Internet safety, and sustainable transportation. Good education and reducing child mortality are also on these lists.

In the Netherlands, the government has established the 'Science Vision 2025' in November 2014. This vision states the challenges for Dutch society. These are: quality of life, circular economy, resilient society, building blocks of life, complexity: coping with unpredictability and big data. For society, it's important that exactly these problems are solved by means of scientific research and innovations, by engaged researchers.

Gender equality

Men and women must be equally involved in research and innovation; the full potential of the population must be used.

In 2011, over half of the people graduating from university in the Netherlands were women, but less than 15% of professors is female (Monitor Vrouwelijke Hoogleraren, 2012). People mainly think of men when talking about professors (do a Google Images search for '*scientist*'). The aforementioned Science Vision 2025 also showed this; it contained a page of pictures of Dutch top scientists, all of whom were men (NRC, 2 dec 2014). Furthermore, top positions in the business world are still mainly occupied by men (NRC, 4 sept 2014).

However, gender should also not be a determining factor in other professions, such as healthcare, daycare, or technology, for hiring an applicant. The EU thinks that it will take (too) long before this changes without giving it a little push, and therefore this is important for *Responsible Research and Innovation*.

Science education

Through better education on science, more people will understand how science works. Only then will 'ordinary' citizens be able to participate in and think about research and innovation.

Young children need to be encouraged to pursue science and technology to make them tomorrow's scientists. This is already happening in various ways; in science centers such as NEMO and Science LinX, through science programs on TV, and through activities in schools. You yourself are examples of this. With the project IRRESISTIBLE, that this lesson module is part of, several thousands of students from ten countries throughout Europe come into contact with scientific research in three years.

Open access – availability of scientific results

Many research results (published in scientific articles) are only available by subscribing to a science journal. Universities often have these subscriptions, but the EU believes that citizens should also have free access to scientific results in order to allow them to form an opinion and participate in discussions about research results.

This is going to change. In the Netherlands in 2024, all research financed by the government will be published in so-called *open access* journals that are available to everyone with an Internet connection, so citizens can also benefit from the results of this research.

In Europe and the US, most universities do have subscriptions to journals, but this is usually not the case for universities in developing countries. Consequently, scientists in these areas have less access to the latest discoveries. For *Responsible Research and Innovation*, in which everyone can participate, *open access* is paramount.

Ethics

People and animals have fundamental rights, and research and innovations must respect these rights. Therefore, scientific research must be relevant and acceptable to society and must not violate the fundamental rights of both people and animals.

Science and new innovations must help to solve societal problems. However, this should be done in a responsible way. You can't just build a factory to produce useful things anywhere you like, when this factory causes pollution and the people living in the vicinity will get ill. That is what happened in India in 1984, for instance, where a gas leak in the city of Bhopal caused thousands of deaths, the largest industrial disaster worldwide. But such things also happen closer to home, for instance in the Volgermeerpolder (near Amsterdam). Toxic waste was dumped at this location for years, causing it to now be the most polluted location of the Netherlands.

Animal testing is also not always allowed; researchers have to be able to properly justify the use of animals in their research. So, in order to ensure responsible research and innovation, we have to consider what the consequences are (or could be) of the results, and whether the process does not violate the rights of citizens and animals.

Governance

Policymakers and politicians are responsible for overseeing that no harmful or unethical things are being done during research and innovation, and to react if this does occur.

This will be achieved because governments, such as the EU, the UN, and the Dutch government in its Science Vision 2025, consider the scientific goals of the country or region. The National Science Agenda that must be drafted in 2015 in the Netherlands is an example of this. For this Science Agenda, Dutch scientists will formulate themes that are important to our society, together with entrepreneurs, societal organizations, engaged citizens, and the government. These themes will resemble the EU's Grand Challenges, but will focus more on Dutch society, and on the strengths of Dutch scientists (also see point 1).

Citizens shouldn't determine what scientists should do, but they will have more influence on it than before through organizations.

Sources:

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Assignments 1-Engage – Secrets of Breast Milk

(students can use the internet to answer the questions)

1. Can you read in the first table when women have to get back to work?
2. Would women in countries that have longer maternity leave also breastfeed longer?
3. It says that breastfeeding adapts to the needs of the child. Are there various types of formula for different ages?
4. Discuss the different aspects of Responsible Research and Innovation in a small group or with the teacher. How would these aspects relate to the topic of breast milk?

2

Explore – Unravelling the Secrets

“Do you have the Guts?”

These last years, it has become possible to unravel a few of the secrets of breast milk thanks to new research techniques. When we look at table 2.1, we can see that a mother passes on a lot of things to her child. In addition to the nutritional components that consist of building blocks and fuel, we find protective substances, but also the aforementioned oligosaccharides that can hardly be broken down by human digestive enzymes. This module mainly discusses these mysterious substances.

table 2.1
Composition of breast milk

Component (per liter)	Breast milk
Energy (kcal)	677
Carbohydrates (g)	70-85
Lactose (g)	67-70
Oligosaccharides (g)	**5-15**
Glucose (g)	0.1-0.2
Lipids (g)	35-48
Triglycerides (g)	34-47
Fatty acids (g)	30-42
Cholesterol (g)	0.1-0.2
Cholesteryl-esters (g)	0.01
Phosphollipids and Sphingolipids (g)	0.25-0.30
Nitrogen (g)	1.9
Non-protein bound	0.45
Protein bound (g)	1.45
Protein (g)	8-11
β-Casein (g)	3-4
κ-Casein (g)	1-2
α-Lactalbumin (g)	2-3
IgA (g)	0.5-1.0
IgM (g)	0.01
IgG (g)	0.05
Lactoferrin (g)	1-3
Lysozyme (g)	0.1
Serum albumin (g)	0.3

Source: http://nl.wikipedia.org/wiki/Moedermelk

The meaning of the Human Milk Oligosaccharides, or HMOS, that used to be so mysterious is becoming increasingly clear the last few years. This is due to the fact that it has only been recently possible to discover that a great many bacteria live in our intestines by means of DNA techniques. Before, we didn’t know these existed. These bacteria include types that are beneficial to our health and that live off these oligosaccharides.

n this way, human oligosaccharides that are present in breast milk but not in regular formula provide an optimal ratio between the various groups of bacteria in the intestines of the infant (see figure 2.1). In section 3.1.2, you will learn more about these bacteria.

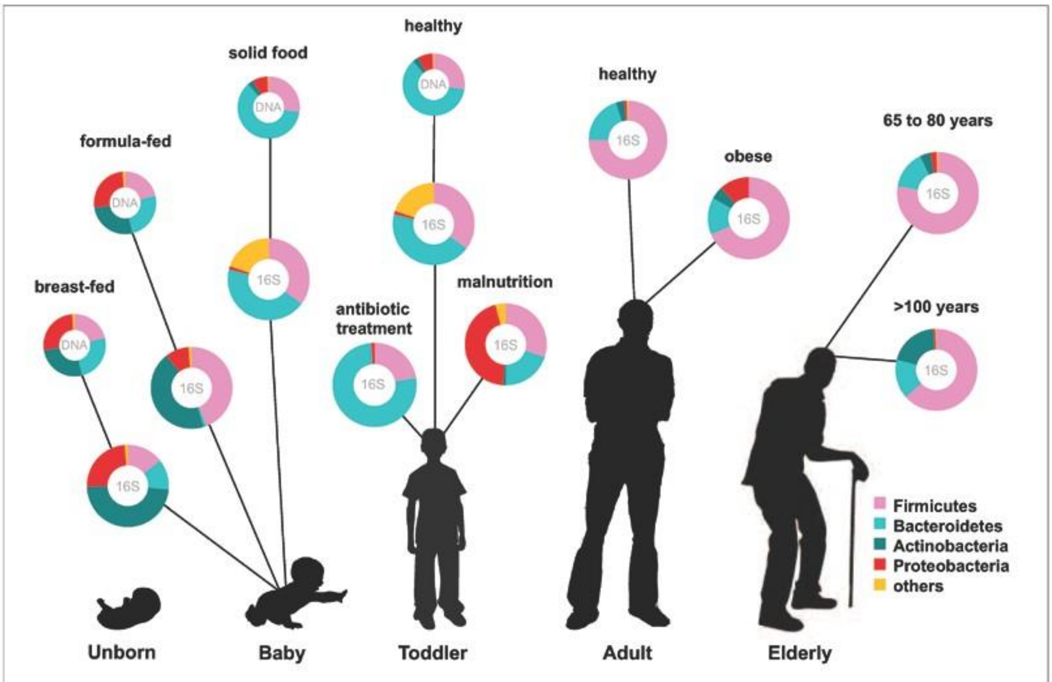

FIGURE 1 | Human microbiota: onset and shaping through life stages and perturbations. The graph provides a global overview of the relative abundance of key phyla of the human microbiota composition in different stages of life. Measured by either 16S RNA or metagenomic approaches (DNA). Data arriving from: Babies breast- and formula-fed (Schwartz et al., 2012), baby solid food (Koenig et al., 2011), toddler antibiotic treatment (Koenig et al., 2011), toddler healthy or malnourished (Monira et al., 2011), adult, elderly, and centenarian healthy (Biagi et al., 2010), and adult obese (Zhang et al., 2009).

Figure 2.1 - The composition of several important groups of bacteria (phyla) in the human intestines. Note the differences in infants, the influence of antibiotics and malnutrition in toddlers, and the changes when growing up (Source: Ottman et al, 2012)..

Most intestinal bacteria are found in the large intestine. Medics consider this community as a separate organ, and no longer refer to it as *gut flora*, but the *microbiome*. These last years, there have been indications that the composition of this microbiome influences our health and plays a role in a properly functioning immune system, allergies, obesity, diabetes, asthma, irritable bowel syndrome, and much more.

A lot of this research in which a connection is made between the presence of certain types of bacteria and a good health, or the absence of diseases and health issues, respectively comes from population studies of large groups of people. Connections can be made between health aspects and the presence of bacteria, or health effects and breastfeeding in early childhood. This concerns just *correlations*. A lot of new research with advanced techniques is needed to determine the *causal* relationship between breastfeeding and the presence of bacteria A and the absence of decease B, for instance. Yet the found connections are strong enough for the nutrition industry to add oligosaccharides to baby formula. But also a lot of research is still required to discover the exact mechanisms that lead to the fact that oligosaccharides from food lead to a good health.

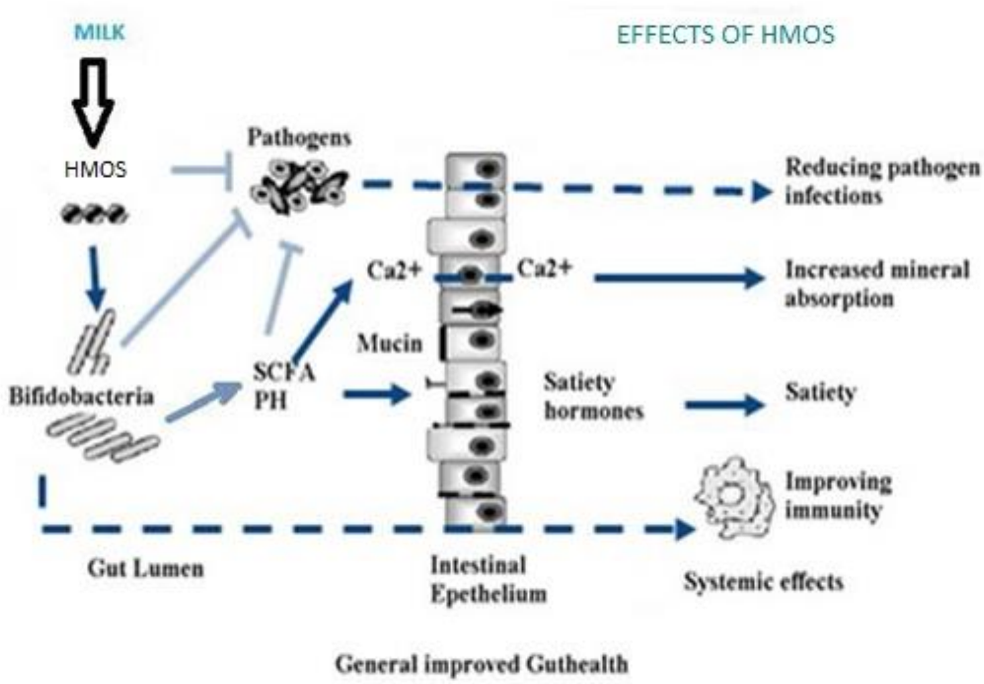

Figure 2.2 - Possible effects of human milk oligosaccharides (HMOS). Source: adapted from Saulnier et al, 2009

Figure 2.2 shows that adding oligosaccharides to powdered milk for infants is important. Human Milk OligoSacharides (HMOS) that are present in breast milk have a direct inhibitory effect on pathogenic bacteria in the intestines and in this way prevent infections through the intestines. Furthermore, oligosaccharides stimulate the growth of 'good' Bifidobacteria' in the gut.

Also, research has shown that there might be benefits between healthy ageing and receiving breastmilk (or the best breast milk substitutes) in early years. Evidence suggest that developing a healthy microbiome at that age can play an important role for further health benefits.

In this module, we look at what research is being conducted to discover the causal relationships between human milk oligosaccharides and health, and how such oligosaccharides are factory-produced to be added to infant nutrition. For instance, manufacturers have succeeded in producing two types of artificial oligosaccharides (GOS made from lactose and FOS made from inuline) and adding them to formula. In figure 2.3 you can see that adding GOS and FOS indeed leads to a lower number of infections in newborn babies.

GOS will be discussed in further detail in Chapter 3.2.5, also it will be explained how you can study if certain additions to milk powder can have an effect on the health of babies.

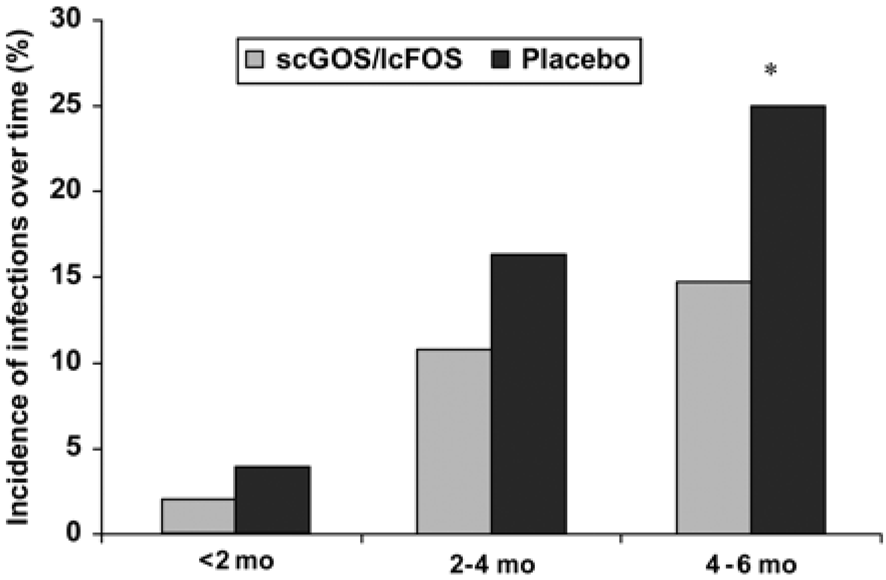

Figure 2.3 - This graph shows the relationship between the presence of GOS/FOS (gray bars) in milk for babies and the number of times babies suffer from infections. For milk without GOS/FOS, traditional formula, more infections occur (black bars). Source: Arslanoglu et al. 2007

Sources:

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Assignments 2-Explore – Unravelling the Secrets

(students can use the internet to answer the questions)

1) Name the fueling substances, the building substances and the protecting substances in table 2.1. To which group do the oligosaccharides belong, according to you?
2) In chapter 1 we spoke about the biological class. In chapter 2 we speak about Fyla. Look up in which systematical groups organisms are subdivided.
3) Name the differences in fyla of gut bacteria (from figure 2.1) from babies that have and have not had milk with oligosaccharides.
4) Which major changes occur in the microbiome with aging? (figure 2.1)
5) In most schoolbooks, the term 'gut flora' is still used. Why did people talk about 'flora' and not 'fauna'?
6) What can be demonstrated from epidemiological studies, and what cannot be concluded?
7) Name at least three positive effects of the presence of Bifidobacteria in the gut (figure 2.2)
8) Figure 2.3 is the result of epidemiological research. Explain that figure 2.2 suggests the causal explanation for the beneficiary effect seen in figure 2.3.
9) What is a community? Why is the term 'microbiome' currently used to describe the intestinal bacteria and not 'gut flora'?

3

Explain – Gathering Knowledge

3.1. Biology

3.1.1 Digestion

What happens to milk in the baby's intestines?

Just milk?

All mammals, including human beings, only feed on milk in the first phase of their lives. In Chapter 2, you've learned that milk contains various substances. A major part of these substances have to be digested before the baby's body can take energy from them. The first part of the digestive process takes place in our digestive tract by means of enzymes. You will learn more about this below. We will first discuss digestion in general, and subsequently look at what happens to (breast) milk in babies' intestines.

The digestive tract

We can view our digestive tract as a tube that runs through our body from top (the mouth) to bottom (the anus). Only when substances from the digestive tract have been absorbed into the blood this has really become part of the internal environment.

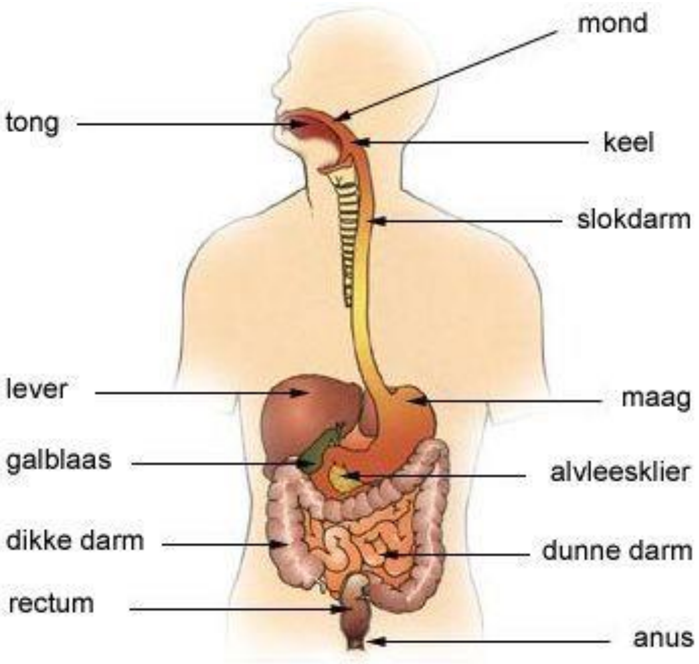

Figure 3.1 The human digestive tract
Source: https://obhw.wordpress.com/biologie/spijsvertering-en-meer-bio/spijsverteringsstelsel/

In the mouth, food is ground and mixed with saliva, after which it travels to the stomach via the esophagus. The stomach excretes gastric juices that are very acidic. How long food remains in the stomach depends on the composition of the food. Every now and then, part of the stomach contents are passed on to the duodenum. There, food is mixed with bile (from the liver) and pancreatic juice (from the pancreas). The duodenum becomes the small intestine, where most nutrients are digested and subsequently absorbed. The small intestine is very long and has a large surface area (150-200m^2) because of the intestinal villi and microvilli. After digestion, the nutrients have become so small that they can be absorbed into the blood. The undigested remains are transported to the large intestine where they are thickened and where part of the fibers are digested by bacteria. The thickened mass gathers in the rectum after which it can be disposed of via the anus.

Digestive enzymes

Digestive enzymes are produced in various places in the digestive tract. These enzymes speed up the breakdown of proteins, carbohydrates, and fats. In Chapter 3.4, you will learn more about enzymes.

The enzymes that help you to digest the abovementioned nutrients are added to the food in various places. In this way, the nutrients concerned are digested in steps until the reaction products are small enough to be absorbed into the blood (absorption).

The baby's intestines

In babies, the digestive tract is largely the same as that of adults in terms of buildup, but especially the small and large intestines are still developing in the first years of life. Initially, the digestion of complex molecules has not been developed well yet and the microscopic build of the small and large intestines are different.
The outer layer of the intestine, the epithelial cells that come into contact with the intestinal contents have two functions: absorption of nutrients and to stop pathogens. In babies, the space between the epithelial cells is fairly large because the number of tight junctions is low. These tight junctions are formed by proteins that increase especially after birth and that 'close the bowels' (the epithelial cells) in this way. Because few tight junctions are present at first, absorption of large molecules from breast milk is easier. This is advantageous for the baby, because their own immune system doesn't function sufficiently yet. Through the openings in the epithelial cells, antibodies from the mother can also be absorbed and help the immune system. However, this accessibility also creates a disadvantage: pathogens can enter more easily, and the same is true for 'ordinary nutrients' that can subsequently activate the specific immune system. When this is deployed against innocent substances, a (food) allergy will develop.

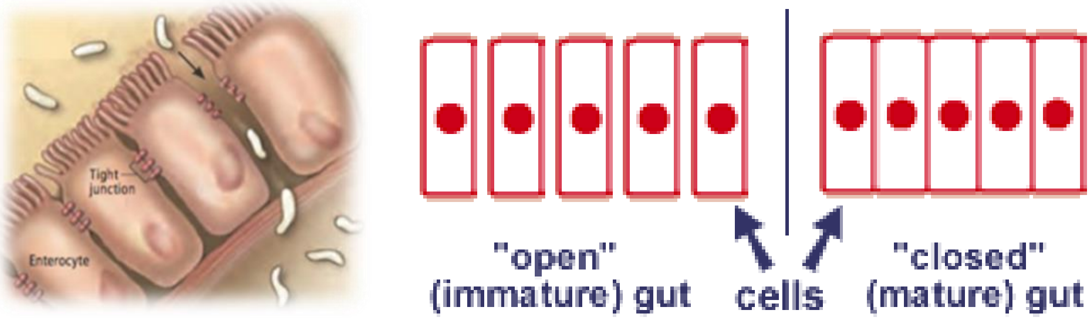

Image 3.2
Tight junctions in intestinal epithelial cells (left) and the open versus closed intestinal epithelial (right)

After birth, the permeability quickly reduces, partly influenced by hormones and growth factors from breast milk. The above explains why allergies and infections occur less frequently in babies who have been breastfed compared to babies who were raised with breast milk substitutes. This effect is noticeable into adulthood, because once an allergy has developed, it is hard to counter.

Digestion of (breast) milk in babies

The first months, babies only consume liquid food. Liquid food generally passes through the stomach quickly. Why is it that babies who only drink milk still feel full after having been fed? To understand this, you can do the experiment 'making cheese with acid'.

Experiment

With acid, you can mimic what happens to milk in the baby's stomach. Follow your teacher's instructions when doing this experiment.
See appendix Experiments

Breast milk and formula contain, amongst others, milk sugar (lactose), protein, and fats; substances that need to be digested before they can be absorbed. Right after birth, part of the necessary enzymes come with breast milk, which is why we cannot completely compare digestion of these substances to adults.

In the baby's stomach, the the enzyme rennin (chymosin) contributes to the clotting (coagulation) of proteins, because the baby's stomach produces a lower amount of acid in the first 2 months. Due to this coagulation, the proteins from the milk (largely casein) remain in the stomach longer where they can be digested by the enzyme pepsin. The acidity level in the stomach is not optimal yet, which inhibits the pepsin activity, so a longer presence in the stomach is also useful. In the small intestine, digestion is followed by (chymo)trypsin, after which amino acids and small peptides are absorbed by the intestinal epithelial.

In contrast to adults, babies also produce the enzyme lipase in the stomach. This helps them to digest fat from the (breast) milk which is necessary because the liver produces very few bile acids and the pancreas is not yet sufficiently developed to produce lipase.

In the small intestine, the most important carbohydrate lactose (milk sugar) is broken down by the enzyme lactase. For a lot of people, the production of this enzyme decreases after the third year. This is usually not the case for Europeans and North-Americans; this is why lactose intolerance is much less common in these areas (see box 3.1.). For complex carbohydrates like HMOS, no digestive enzymes have been detected, neither in babies or adults, that can digest these carbohydrates. Until recently, it was thought that the large intestine's only function was to remove fluids from the mush of undigested food. But it has become clear that there is a large community of bacteria in the large intestine, and that there is a complicated but important symbiosis between these bacteria and the host (see also chapter 3.1.2).

In the small intestine, digestion is completed and the digestive products are absorbed. The milk protein casein is important for the minerals, in addition to being a source of amino acids. In the following chapters, we will learn that the intestinal epithelial also plays an important role in the continuous further health, in addition to playing a role in absorption.

Box 3.1: Lactose intolerance

We talk about lactose intolerance when people cannot (completely) digest lactose (milk sugar). Lactose is a disaccharide consisting of glucose and galactose. Lactase is the enzyme that breaks up the connection between these mono-sugars, after which they can be absorbed by the blood. In case of lactose intolerance, not enough lactase is produced due to which too much lactose remains present in the intestine. This is subsequently fermented by intestinal bacteries; the gases and stimulating substances that are released create a stomach ache, cramps, a bloated feeling, and nausea. Moreover, lactose withdraws a lot of water from the body and can lead to watery stool. Reducing the amount of milk products in one's diet can alleviate the symptoms. It is often unnecessary to stop taking milk products completely.

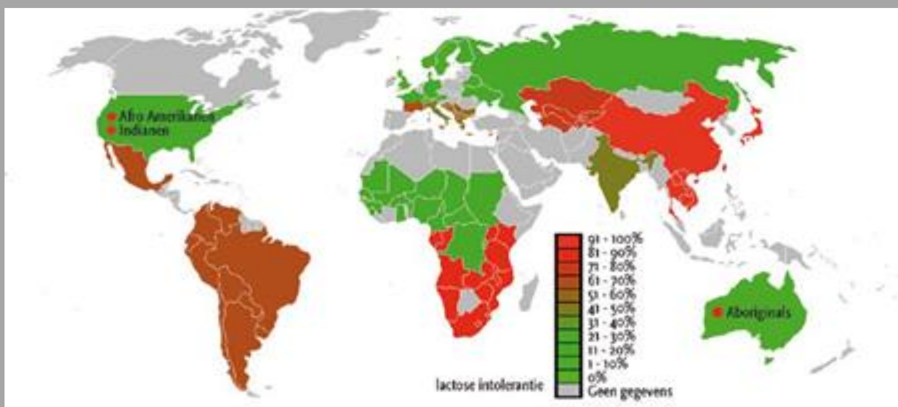

Image 3.3 Percentage of the population with lactose intolerance in various parts of the world. Source: Chemische Feitelijkheden Melk

Box 3.2: Neonatal heel prick

In The Netherlands, a little bit of blood is taken from all babies 3-5 days after birth. This happens in the heel and is therefore referred to as the heel prick. The blood is used to test for 17 hereditary diseases that are incurable, but can be treated when caught on time. Fourteen of these are (rare) metabolic disorders that require a special diet. One of these disorders is Galactosemia (GAL), due to which galactose (from lactose) cannot be broken down and accumulate which may damage the liver and crystalline lenses. These symptoms can be prevented when the baby follows a diet. In this diet, lactose (and therefore milk products) must be avoided entirely, so these babies receive nutrition on the basis of soy (lactose-free) and breastfeeding is not an option in this case. The diet must be followed the rest of the child's life.

Sources:

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- Image human digestion http://obhw.wordpress.com/biologie/spijsvertering-en-meer-bio/spijsverteringsstelsel/
- http://www.thevisualmd.com/health_centers/child_health/infant_nutrition
- http://www.rivm.nl/dsresource?objectid=rivmp:75690&type=org&disposition=inline (galactosemie)
- http://www.voedingscentrum.nl/encyclopedie/lactose-intolerantie.aspx

Assignments 3.1.1 – Digestion

Explain – Gathering Knowledge

1. Indicate which organs are involved in the digestion of fats, proteins, and carbohydrates respectively (use Binas 82E).
2. By means of the above text, explain where digestive products are absorbed in the body.
3. Formula is based on cow's milk. Compare the composition of cow's milk and breast milk: which nutrients will especially be added to the cow's milk to make sure that the composition resembles breast milk more closely?
4. Describe how babies' digestion deviates from that of adults.
5. Explain by means of table 2.1 which role protective substances in breast milk play in new born babies.
6. What does 'symbiosis' mean?

3.1.2 Microbiology.

How does a sterile baby accumulate 2 kilograms of bacteria?

A new view on bacteria.

Watch the movie "The Invisible Universe of the Human Microbiome" by NPR - https://youtu.be/5DTrENdWvvM

Anthoni van Leeuwenhoek is considered the discoverer of micro-organisms. He was the first to see them through his cut lenses, that enlarged the image more than the composed microscopes of that time did. In 1676, he was the first to observe in a pepper drip. After Anthoni van Leeuwenhoek observed bacteria, the role and function of these small "animalcules" remained a mystery for approximately 200 years. It wasn't until the end of the 19th century that bacteriologists Cohn, Pasteur, and Koch were able to grow bacteria to research the characteristics of these micro-organisms. This especially revealed their role as pathogens.

It was also discovered that oxygen is lethal to certain bacteria. This are called *anaerobic* bacteria. Growing these bacteria is difficult and requires special techniques. Consequently, research into these bacterial species was very fragmented. During the study of gut flora, aerobic and facultative anaerobic bacteria were mainly studied, such as *E.coli*, that were able to grow in the presence of oxygen. Even today, most (>90%) bacteria on earth are unknown and not yet researched.

Figure 3.4 Anthoni van Leeuwenhoek. From: G.A. Lindeboom; Geschiedenis van de medische wetenschap in Nederland (1972)

Thanks to the development of new DNA techniques, it is possible to recognize bacterial species by fragments of their DNA. However, this does not mean that it is known how these bacteria live and function. Bacteria often live in clusters of various species. In these clusters, they are dependent upon each other, for instance because they jointly break down certain substances they live on. They can also fight each other by excreting substances that are toxic to other species.

Bacterial species in nature, in your mouth, and in your intestines, are never found individually but in communities, so-called *biomes*. DNA techniques have shown that *E.coli*, a bacterial species that is often considered an indication of fecal contamination, only occurs in the intestines in low densities. However, given the fact that this species can live in an oxygen-rich environment, it was the only useable indicator in the past.

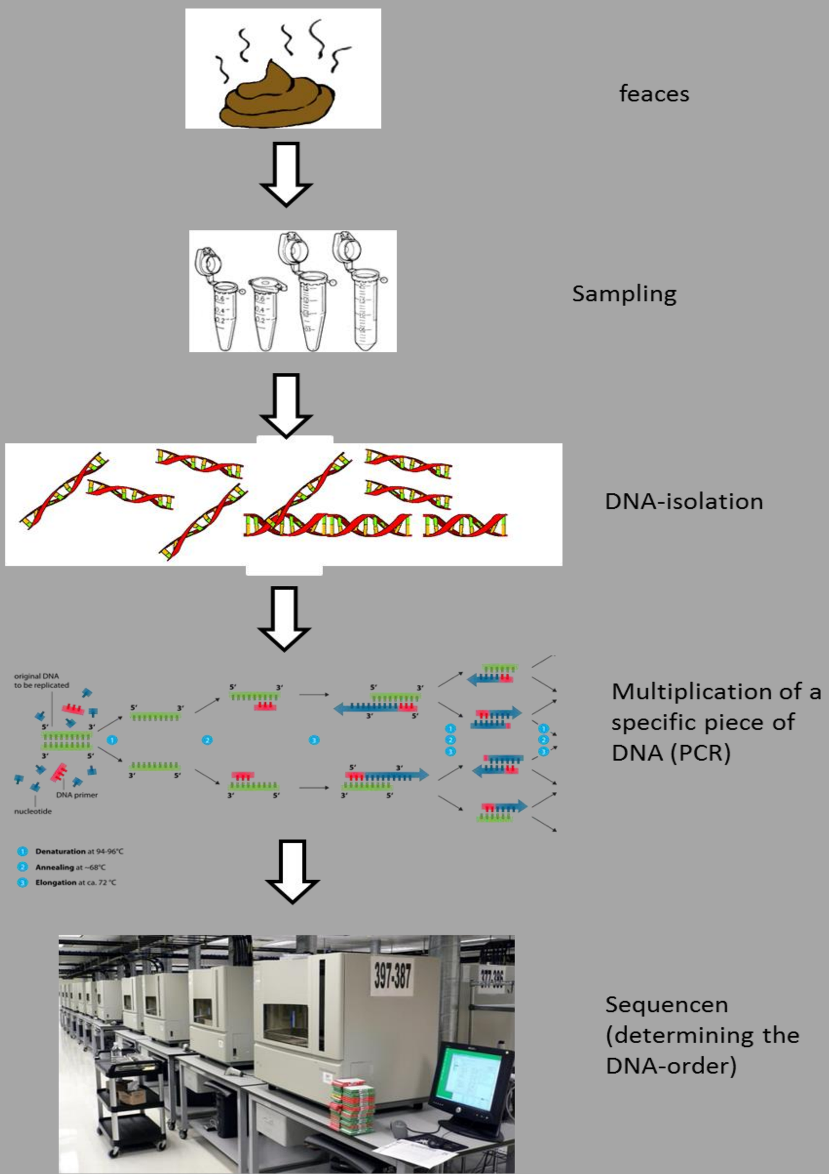
New research of intestinal contents by DNA analysis

The use of DNA techniques has developed on a large scale these last decennia. This is partly due to the Human Genome Project, for which many new analysis techniques were developed. These techniques were also used to study bacterial DNA in different ecosystems. This technique is called *meta-genomics*, see box 3.3.

This technique has made it possible to research which (groups, types of) bacteria occur in a certain environment and also how and which conversions these bacteria cause. Instead of growing intestinal bacteria and identifying the different types with a microscope, researchers can now work much more quickly with the abovementioned DNA techniques and can make more determinations. Consequently, it has been discovered that human beings are walking ecosystems of bacteria. Instead of DNA, also RNA can be used as molecule to determine what species are present.

Box 3.3 Metagenomics

In this figure, you can see the technique of metagenomics applied to feces.



Metagenomics is ‘the application of modern DNA-techniques (genomics) in the study of communities of micro-organisms in their natural environment without the necessity to isolate and grow individual species’.

- Samples from nature are treated with different chemicals to isolate DNA from cells. Most DNA-molecules will have fallen apart in small pieces
- The amount of DNA is too low to be detectable, so a technique of DNA-multiplication is used. This is called PCR (polymerase chain reaction).
- By using sequencing, the DNA-sequence can be determined
- With a computer you can re-construct the original DNA-sequence.

By comparing the sequence with a large database of well-known DNA-molecules, the bacterial species, order or family can be determined.

Classifying bacteria

Just like other living beings, bacteria can be classified according to a taxonomy based on kinship. For instance, the similarities and differences of a piece of ribosomal RNA (the so-called 16S rRNA), that show a limited variation between various large groups of bacteria, are observed. In the classification of 2007, the domains of *Bacteria* and *Archaea* have been subdivided into 27 phyla. These phyla are further subdivided into classes and subsequently orders, families, strains, genera, and species. The largest groups (phyla) also show the largest differences in DNA and RNA. In Figure 3.9, various phyla of bacteria are mentioned, whereas figure 3.6, 3.7 and 3.8 shows various genera that sometimes differ much less from each other. Figure 3.10 provides a complete overview of what human beings carry around.

How does a sterile baby accumulate 2kg of bacteria as an adult?

During pregnancy, the fetus is well protected in the uterus; there is no direct contact with the outside world. Consequently, the baby develops in a virtually sterile environment. The first contact with the outside world occurs during birth. During the passing through the birth canal, the first contamination with bacteria takes place. Recent studies also showed a presence of a small number of bacteria in mother's milk.

Eventually, adults carry approximately 1.5 – 2 kilos of bacteria in their intestines. Most live in the oxygen-free environment of the large intestine – the colon in figure 3.6. There are approximately 1000 different types of bacteria in our large intestine. Approximately half of our stool consists of bacteria.

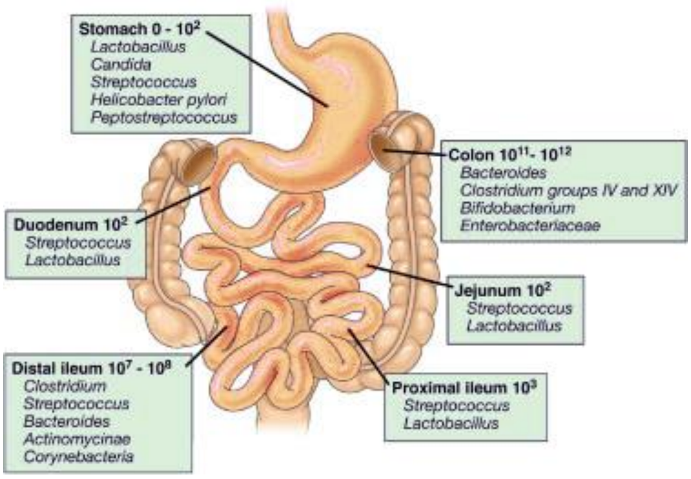


Figure 3.6 Numbers and several types of bacteria in the adult human gastrointestinal tract (per ml of intestinal contents)

In the first week after birth, colonization of the colon mainly occurs with *E.coli* and *Streptococcus.* Subsequently, anaerobe species from the *Bacteroides, Bifidobacterium, Clostridium, and Ruminococcus* genera settle in the large intestine. These bacteria stem from the immediate environment of the child, mostly by body contact with the mother. When children are breastfed, the number of *Bifidobacteria* and *Lactobacilli* greatly increase at the cost of the other types. When children are bottle-fed, this does not happen. At a very young age, there is a clear difference in the composition of the microbiome between breastfed and bottle-fed babies.

Once children get (more) solid food, the adult microbiome slowly starts to develop. The number of *Bacteroides, Clostridium*, and *peptococcus* increase, but other species can also be found. As of the third year of life, a stable bacterial community has established itself. The moment when breastfeeding is stopped greatly influences the composition of this bacterial community.
When children are around the age of 3, there are no detectable differences in the gut bacteria between children that were and were not breastfed. At younger ages, there is a difference (see figure 2.2).

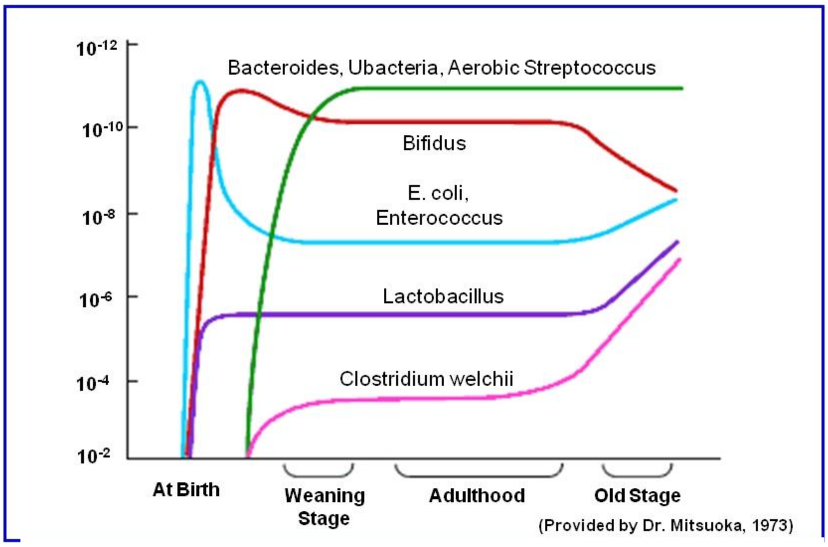


Figure 3.7 Changes in the composition of the microbiome during aging (weaning = the moment when solid foods are introduced).

In 2011, scientists discovered that the composition of the microbiome of human feces is independent of age, sex, body weight, and nationality. Yet, people can be subdivided into 3 groups, the so-called "enterotypes", named for the dominant bacteria genera that were found in the intestinal biome. We now know the enterotypes *Bacteroidetes*, *Prevotella*, and *Ruminococcus*.

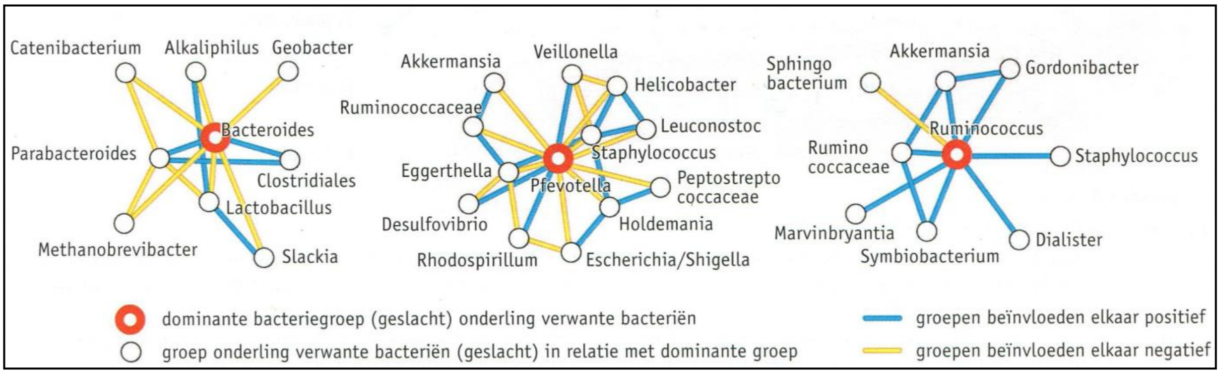


Figure 3.8. Composition of the three intestinal enterotypes. The various bacteria genera are mentioned here. From: Biologie voor jou 4vwo – 5^e^ druk.

Bifidobacteria, the good bacteria

Bacteriological research has shown that the feces of infants who are breastfed contain high concentrations of *Bifidobacteria*. This in contrast to infants who receive formula. One of the most important differences between formula and breast milk is the presence of high concentrations of soluble oligosaccharides, the Human Milk OligoSacharides (HMOS).

The obligate anaerobic *Bifidobacteria* and the facultative anaerobic *Lactobacillus* can grow with HMOS as sole source of carbon in their nutrition. Obligate anaerobic means that these organisms can only live in an oxygen-free environment. Breakdown of nutrients rich in energy therefore occurs without oxygen; this is referred to as fermentation. Because of the fermentation of oligosaccharides that are difficult to break down, these bacteria are able to outcompete other species.

These bacteria break down the oligosaccharides into smaller molecules: gasses such as CO_2, H_2, and CH_4, plus the so-called Short Chain Fatty Acids (SCFA's). The latter are substances such as acetate, propionate, butyrate, lactate, and succinate. The SCFA's that are released in the large intestine have a positive effect on the host's health. Moreover, the host can get up to 10% more energy from his/her food due to the absorption of these SCFA than without bacterial 'aid'.

Research has showed that the presence oligosaccharides in food resulted in more *Bifidobacteria* in newborns. Also, oligosaccharides influence which specific species of *Bifidobacterium* will be most present (see figure 3.9).

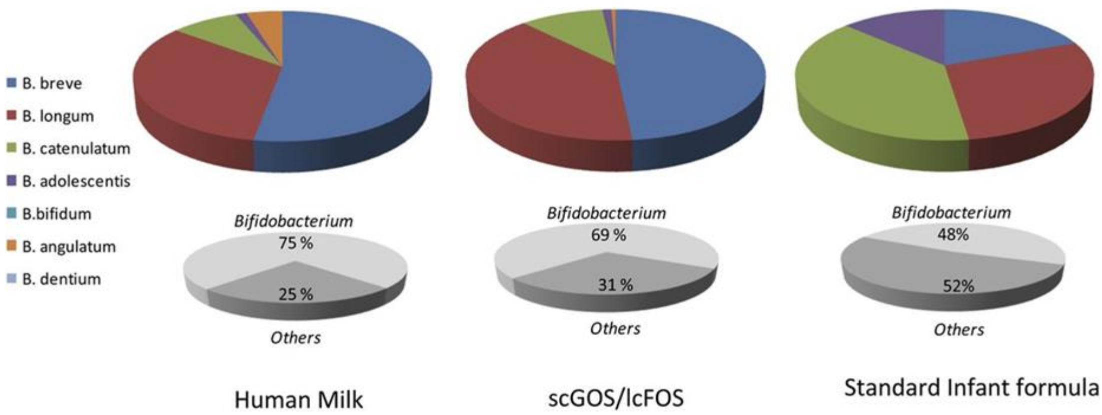


Figure 3.9 Comparison of the microbiome of newborns that receive different types of milk. The 3 graphs below show the distribution of the bacterial genus, the colored graphs show the distribution in species of Bifidobacterium. (from: Am J Clin Nutr 2013;98(suppl):561S-571S

Nowadays, oligosaccharides are added to formula, mostly Galacto-OligoSascharides (GOS) and Fructo-OligoSaccharides (FOS). These have a beneficial effect on the microbiome of infants, which starts to resemble the microbiome of infants that were breast-fed. So, human Milk Oligosacharides (HMOS) and GOS stimulate the metabolism and so the growth of *Bifidobacteria* and *Lactobacilli*, and so stimulate a healthy microbiome for the host.

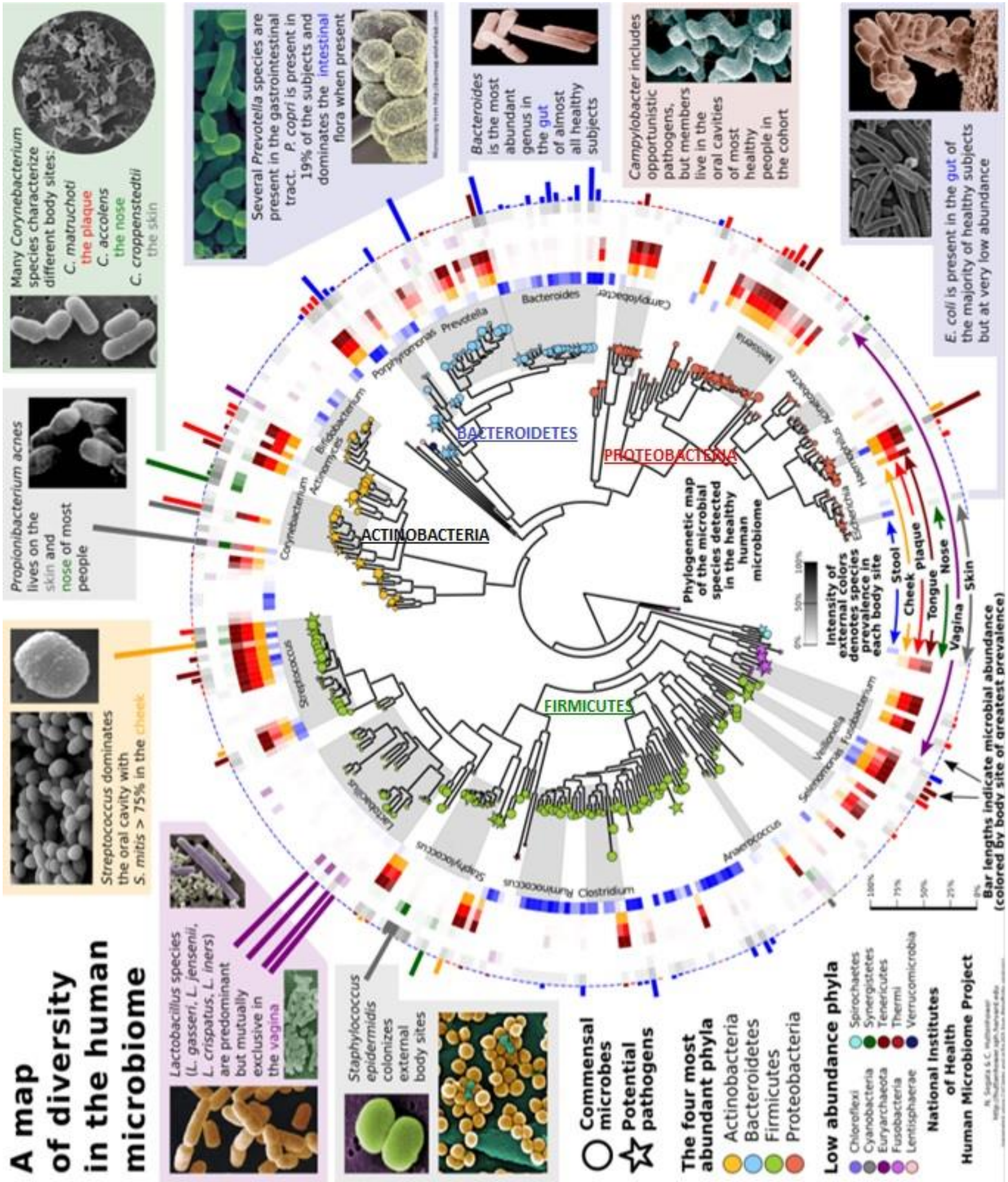
Box 3.4: Intestinal content transplantations

Because of the explosive development of DNA research, the interest in how the microbiome influences the functioning of the human body has increased considerably.

How does the microbiome influence the body's resistance against pathogens? How does the microbiome influence diseases such as diabetes, rheumatoid arthritis, muscular dystrophy, multiple sclerosis, and some forms of cancer? And how does the microbiome influence obesity?

The custom to treat intestinal diseases with feces is thousands of years old. The Chinese already knew yellow soup, a mixture of feces and water, that was drunk by the patient. The first scientific publication appeared in 1958. Surgeons from Colorado treated patients with serious colitis ulcerosa (an inflammatory bowel disease) with enemas containing feces of healthy people. This treatment led to a speedy recovery. This treatment is also referred to as fecal bacteriotherapy (FBT). Since 2013/2014, FBT has been an accepted treatment in the US.

Look at this movie for more information about fecal transplants:
https://www.youtube.com/watch?v=Dim7YXYlRm0



You never walk alone

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Assignments 3.1.2 - MicrobiologyExplain – Gathering Knowledge

1. What is meant by a ‘new’ view on bacteria?
2. During a normal birth, the child is contaminated with bacteria from the mother’s birth canal. How will a child be contaminated with bacteria when it is born via C-section?
3. Name a few bacterial species from this chapter that are aerobe, facultative anaerobe or obligatory anaerobe
4. Is the citric acid cycle present in Bifidobacteria, Lactobacilli or both? Why?
5. Bacteria digest sugars in different ways. Find out how Lactobacillus delbruecki and Escherichia coli do this.
6. What is the increase in % of Bifidobacteria in the microbiome of newborns when synthetic oligosaccharides are added to formula (see figure 3.9).
7. Use figure 3.10 to determine the genus to which bifidobacterium belongs
8. Look in figure 3.10 to find out which bacterial phyla and genera are present in the colon
9. Look up what pre-and pro-biotics are and explain
10. Explain why in older studies, only bacterial phyla are shown (figure 2.1) and in newer studies also genera and species (figure 3.7 and 3.9)
11. Why does colonization of the large intestine first occus with Streptococcus and E. coli and only later with anaerobe species?
12. Which techniques were necessary before the significance of bacteria could be understood?
13. Look up and name the four postulates of Koch. Why are these important?
14. Explain what PCR is.
15. List the characteristics of the bacterial cell and name the differences with eukaryote cells.
16. What does Gram Positive mean?

3.1.3 The influence of intestinal bacteria on health

Healthy yoghurt in 1904

Ilja Metchnikoff already won a Nobel prize when he started studying yoghurt. Mountain farmers in Bulgaria sometimes reached ages of 100 or more, and Metchnikoff thought that might have to do with the leather bags they used to transport the milk of their cows. After milking, the farmers had to walk a long way home, so when they arrived, frequently their milk had turned into buttermilk or yoghurt, because the leather bag contained bacteria. Metchnikoff was convinced that eating these bacterial products was the key to the old age of these farmers.

He wrote about the importance of bacteria in yoghurt in his book 'The Prolongation of Life'. But, unfortunately for him, these insight appeared at the wrong moment: just when bacteria were discovered as disease-causing agents and the effect of antibiotics were found. So people were convinced that less bacteria was always better. Only now, Metchnikoffs ideas begin to be recognized again.

Although antibiotics have saved countless lives in fighting bacterial infections, we now know that there are also species of bacteria that contribute to a good health. During the fermentation of oligosaccharides by intestinal bacteria, substances rich in energy, SCFAs, become available to the host as extras (see 3.1.2.). Consequently, the pH-value of the intestine is lowered which is disadvantageous for harmful bacterial species. Furthermore, the absorption of minerals is improved, and the growth of intestinal epithelial cells is stimulated. Additionally, the intestinal biome produces vitamin K, folic acid, and biotin.

The microbiome also plays a major role in adjusting the immune system in order to find a balance between the intense reactions against harmless substances and insufficient resilience against pathogens. The composition of the microbiome of the infant in the first months and years of his/her life is important in this. So it is not surprising to see that there is a relationship between the composition of the microbiome and many different diseases – see box 3.5.

Interaction between gut bacteria and the immune system

Recently, it is becoming clear that the composition of the microbiome has an important influence on our immune system. Mostly, these data stem from epidemiological studies. But also experimental studies have been performed in which mechanisms are unraveled of how some bacteria help our immune system, whereas our immune system fight others.

It is clear that our microbiome has a much greater influence on our physical and mental health than was assumed before. But some claims are too far-reaching: that our microbiome can directly influence our mental condition and that diseases as autism can be treated with a fecal transplant. However, correlations have been found and will be studied in more detail to unravel the underlying mechanisms.

In the uterus, unborn babies have (almost) no contact with bacteria. The immune system, the general defense, learns to recognize the own body cells. This way, the immune system is prevented from attacking the baby’s body. If this does happen, for instance because the system is out of balance, we speak of an auto-immune disease such as MS.

As we have been able to see in section 3.1.2, the first time a baby comes into contact with bacteria is during birth, namely the mother’s bacteria. Breast milk already contains antibodies against harmful bacterial species. Just like the mother, the child has to coexist with these bacteria his/her entire life. The specifically developing immune system of the child adjusts to this.

Around our digestive tract is an extensive, special lymphatic system, the so-called GALT (gut-associated lymphoid tissue). This consists of lymph vessels and lymph nodes (see figure 3.11), in which many types of white blood cells “perceive” and influence our intestinal bacteria. Many white blood cells are stored in the lymph nodes as assault troops. These assault cells take action when they receive a signal from T-helper cells. Other white blood cells travel in between the intestinal wall cells as scouts.

Box 3.5 The microbiome and diseases

Examples of a number of diseases and their correlation with the microbiome

Irritable bowel syndrome:

A study about irritable bowel syndrome showed clear differences in the composition of the microbiome of people with this disease and healthy people. The differences consisted of a reduced presence of *Bifidobacteria* and *Bacteroides* and an increase in the bacterial genera *Clostridium, Coprococcus,* and *Coprobacillus.*

Bowel cancer

Differences are found between the microbiome in patients with bowel cancer and the pre-stages of this disease and healthy people. These differences especially concerned the numerous occurrence of *Fusobacterium sp.* and a decrease of the phyla *Firmicutes* and *Bacteroidetes*. *Clostridium leptum* and *C. coccoides* also occurred more often.

Colitis caused by *Clostridium difficile*

Clostridium difficile occurs in low amounts in many people without it causing any symptoms, but it can cause serious inflammations after an antibiotics treatment, for instance. Recovery was difficult, until people began to replace the microbiome in 2013 (a fecal transplant – watch the movie from box 3.4).

Inflammatory Bowel Disease (IBD)

Inflammatory bowel diseases, like Crohn´s Diseas and Colitis Ulcerosa, show very clear infections of the tissue in the gut. At the same time, a loss in diversity of the microbiome is observed, just as an increased number of *E.Coli* and *Clostridium.*

Obesity

In general, there is a slightly lower chance of children that were breastfed to become obese later in life that children that were bottle-fed.

Experiments with mice showed that transferring the microbiome of an obese mouse (with a lot of *Clostridium coccoides, Bifidobacterium longum* and *Bifidobacterium adolescentis*) to a normal mouse results in weight gain. Vice versa, mice that received a microbiome transplant from skinny mice (with a lot of *Bacteriodes fragilis* and *Lactobacilli*) lose weight. However, it is questionalble whethe the same effect would occur in humans.

In livestock-breeding, it is known that animals gain weight faster when they are fed low doses of broad-spectrum antibiotics. This might be caused by an increase in *Firmicutes* and *Lachnospiraceae* phylas.

During the first years of life, mutual influencing occurs: the intestinal microbiome influences the development of the GALT, and the immune system in development influences the composition of the microbiome.

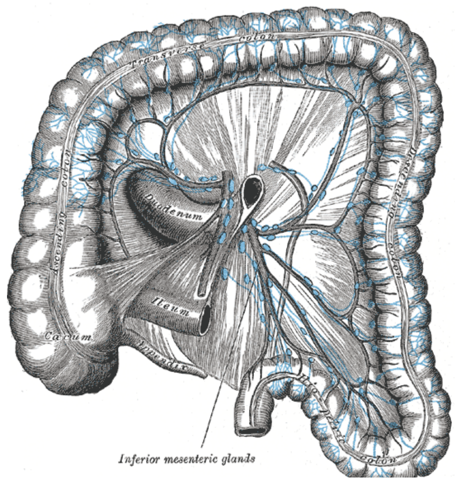
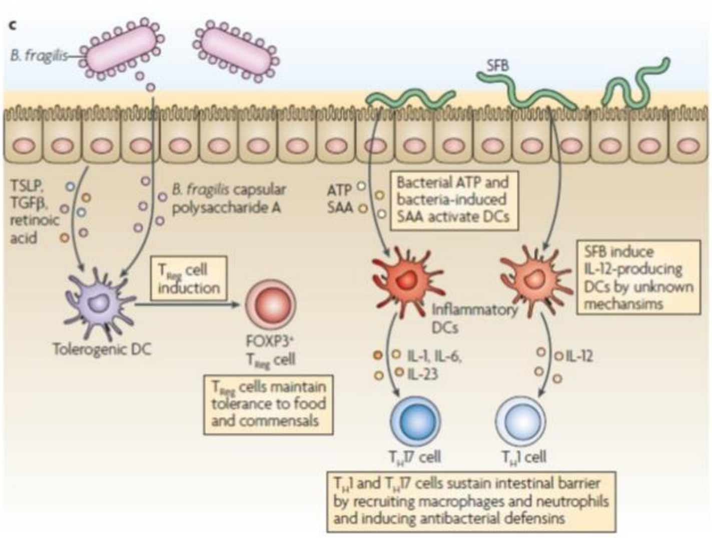


Fig 3.11 Large intestine with lymphatic tissue, part of the GALT. From Wikipedia

In case of a correct balance, there is a symbiotic (sym = together, bios = living, Greek) relationship. Epithelial cells of the intestinal wall excrete small amounts of antibacterial substances due to which bacteria hardly come into contact with the wall. Bacterial growth and human defense are in balance. In the first years of life, the specific defense system has to learn to recognize that the intestines contain a lot of innocent, foreign antigens that do not require a defense response! For example, good bacteria in the mucus layer of the intestinal wall are recognized because of their secretion products. An inflammatory reaction of the immune system is suppressed by the stimulation of so-called regulatory T-cells in the GALT (see figure 3.12). In that case, we speak of homeostasis.

However, when the composition of the microbiome is disrupted by a strong growth of harmful bacteria, we speak of dysbiosis (dys = bad, difficult, Greek) and the epithelial cells give stimulating signals to the dendritic cells that immediately activate the immune system to start an inflammatory reaction via T-helper cells. This leads to an extra supply of blood with antibodies (IgA) and killer T-cells. In this way, the immune system determines whether a bacteria is tolerated in the intestines or is fought as an enemy.



- Bacteria in lumen, benign, (purple) and malign (green).

- Epithelial cells in the intestinal wall

- Dendritic cells (white blood cells).

T_{reg} cells repress reaction.	T_{helper} cells lead to inflammatory reaction

Figure 3.12 Reactions to good and bad bacteria by immune system in the large intestine.
From: Cerf-Bensussan N, Gaboriau-Routhiau

Box 3.6: Mechanisms of the interaction between bacteria and the immune system

The bacterium *Bacillus fragilis* appears to excrete a carbohydrate (polysaccharide A) that stimulates, via a number of steps, regulating white blood cells (T_{reg}) due to which an inflammatory reaction is inhibited. If these *good* bacteria are given to mice that suffer from Crohn's disease (a disease that is caused by the bacteria *Helicobacter hepaticus*), the inflammation decreases greatly. This shows how important a correct composition of the microbiome is. The molecule "polysaccharide A" itself also inhibits this type of intestinal inflammation that often is accompanied by blood loss, so this seems to be the signal molecule in this interaction.

The occurrence of an opposite reaction is also important (see figure 3.3.2); a bacteria that activates an inflammatory response such as *SFB* (that has no name because it can't be cultivated yet), takes care of the production of certain T_{helper} cells in mice. If these cells are not produced, later infections with certain other pathogenic bacteria cannot be fought!

Relationship between the microbiome, the immune system, and general health

The above experimental examples indicate that a proper adjustment of the immune system in early childhood is important for future health. People who did not have a varied microbiome in their early childhood appear to be more susceptible to all sorts of allergies later in life, because the immune system responds too intensely. On the other hand, an immune system that has been too weak in childhood may increase the susceptibility to infectious diseases. However, it is not yet clear what the direct link between certain bacteria in the microbiome and an optimal immune system is. First, it is not yet possible to accurately determine what an optimal immune system exactly consists of. Too much is still unknown. Furthermore, too little is known about the exact influence of important bacterial species from the microbiome to make definitive statements. It is clear, however, that research of the microbiome is developing very fast in the 21st century.

Assignments 3.1.3 –

Influence of intestinal bacteria on health

1. Why is a contamination with Clostridium difficile difficult to fight?
2. Why and where are “intestinal biome” transplantations performed in the Netherlands?
3. List a disadvantageous effect of the use of antibiotics in cattle food.
4. Which two defense mechanisms in the intestines are part of the nonspecific defense?
5. What is the verdict of the Voedingscentrum (Nutrition Center) on probiotics?
6. Think of two reasons why the daily use of prebiotics has more effect than the use of probiotics (see Wikipedia for this, among others).
7. Which role do the dendritic cells in figure 3.2.2 have?
8. Where does activation of B- and T-cells take place? And by which cells are these activated?

3.2 (Bio)chemistry of milk

3.2.1 Composition of milk

Milk mainly consists of water. Additionally, it contains nutrients such as fats, proteins, carbohydrates, and minerals. In this chapter, we discuss the chemistry of these nutrients in further detail.

Milk is an emulsion of oil in water. In third grade, you've learned that oil and water mix poorly. When you add an emulsifier, a stabile emulsion arises. In milk, the balls of fat are so small that they occur in milk finely divided. Part of the present proteins in milk provide an emulsifying functioning. In figure 3.4.1, you can see a micro image of milk and a schematic presentation of the various particles in milk. The particles are mixed on a micro scale. These particles are called colloids. They are larger than a molecule, but not visible with the naked eye. The size of the particles varies; for instance, the balls of fat are 5 micrometers and the casein micelles are 0.1 micrometers in diameter.

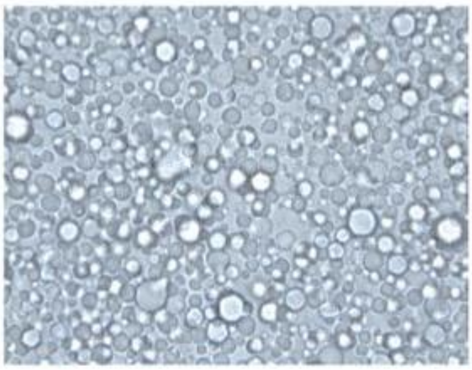


Figure 3.13 a Micro-image of milk

(Source: http://www.hielscher.com/nl/ultrasonication-and-its-manifold-applications-in-food-processing.htm)

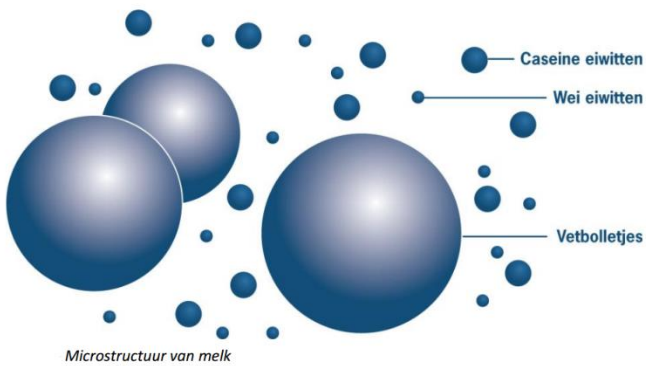


Figure 3.13b Microstructure in milk (Source:

http://www.wageningenur.nl/upload_mm/b/4/7/d8dea611-0f83-4cfd-be42-04301a0688cb_v218_Moleculaire_Gastronomie_ev_ll_21092010_11.pdf)

3.2.2 Fats and fatty acids

Milk contains an average of 4 to 5% of fat (see table 1.1, chapter 1). Fats are triglycerides. This is a glycerol molecule with three fatty acids connected to it, via an ester bond. The carbon chains of these fatty acids have a length of 12, 14, 16, or 18 carbon atoms.

Figure 3.14 shows the general structure formula of a fat.

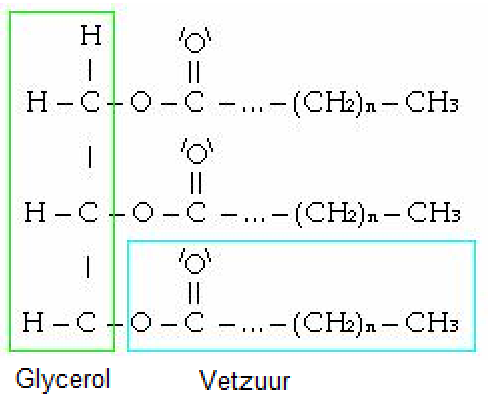


Figure 3.14

General structural formula of a fat

Raw milk is not a stable emulsion. The balls of fat have a layer around them, due to which they don't dissolve but flow through the water. The thin surface layer partly consists of an adhesive. This adhesive is a protein and can cause the balls of fat to cling together. Consequently, the balls of fat form larger clusters that float on the milk. This layer forms the cream. The surface layer has a second function; the protection against the enzyme lipase (see figure 3.15). This enzyme naturally occurs in milk and can divide fat. Free fatty acids arise. In chemistry, this process is referred to as a hydrolyser reaction. This gives the milk a deviating and usually acidic taste.

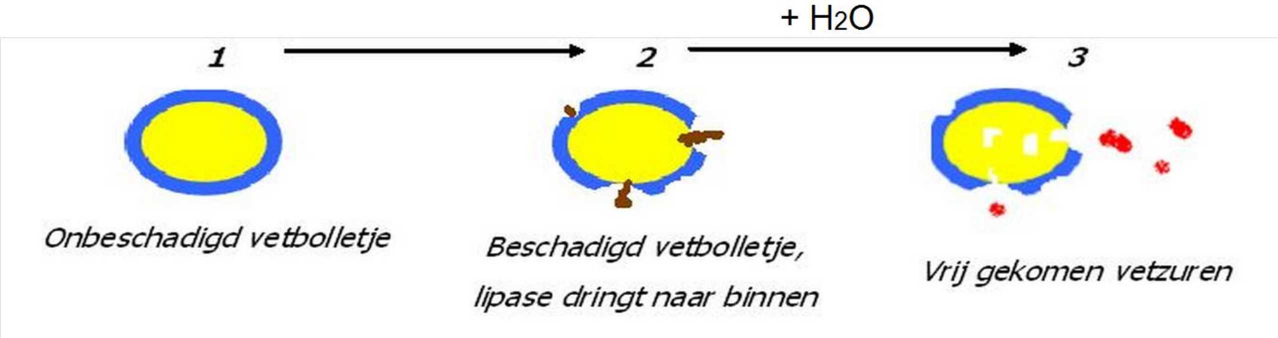


Figure 3.15 – Digestion of fats by lipase

In one ball of milk fat, billions of triglycerides can be found. In addition to long chains (see table 3.4.1), extra short fatty acids with a length of 2 to 10 carbon atoms also occur in milk. Approximately two-thirds of the fatty acid chains in milk are saturated, one-third unsaturated.

Because of the presence of double bond in an unsaturated fatty acid, the fatty acid can enter into a trans or cis configuration, see figure1. In milk (and in general in nature), the cis form mainly occurs, see table 3.4.1. The cis orientation of the chains causes glitches in the chain. Consequently, the chains are less easily stackable. And the less stackable, the weaker the van der Waals connections between the fat molecules and the lower the melting point are. Fatty acids that cannot be produced by the body are referred to as essential fatty acids. From the table, you can deduce that especially unsaturated fatty acids should be absorbed via food.

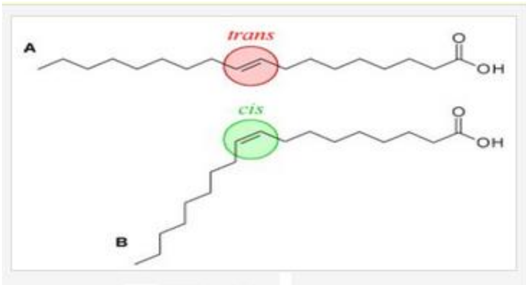


Figure 3.16
Trans and cis bonds in fat

table 3.4.1

types of fatty acids in cow's milk

Fatty acid	**C: n : m cis x** *n = length of chain* *m = number of double bonds* *x = location of double bonds*	**Mass percentage in total fat level**	**Source of fatty acid: made by cow or via food**
Butyric acid	C:4	3.9	udder
Capron acid	C:6	2.5	udder
Caprylic acid	C:8	1.5	udder
Capric acid	C:10	2.9	udder
Lauric acid	C:12	3.6	udder
Myristic acid	C:14	11.1	Udder sometimes food
Palmitic acid	C:16	27.9	udder and food
Stearic acid	C:18	12.2	Food
Myristoleic acid	C:14:1 cis 9	0.8	unkown
Palmitoleic acid	C:16:1 cis 9	1.5	food
Oleic acid	C:18:2 cis 9	17.2	food
Linoleic acid	C:18:2 cis 9 cis 12	1.1	food
Linolenic acid	C:18:3 cis 9 cis 12 cis 15	1.0	food

Source: [text]

Now complete assignments 1 to 4 (see end of chapter)

3.2.3 Proteins

Milk contains approximately 3.5% proteins (see table 1.2). The protein level in milk has a high biological value because it contains practically all needed amino acids. Milk consists of casein micelles, serum proteins (also referred to as whey proteins), and other protein-like substances. The most important proteins are casein, β-lactoglobulin, and olactalbumin.

Proteins are macro-molecules that consist of amino acids. In nature, 20 different types of amino acids occur. All amino acids, excluding glycine, have an asymmetrical carbon atom. Consequently, two stereo isomers can be made of every amino acid. However, only one form occurs in nature. This is called the L-form.

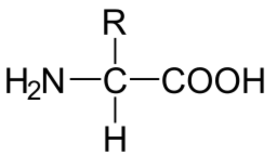


Figure 3.17 General structure of an amino acid

Source: http://nl.wikipedia.org/wiki/Categorie:Alfa-aminozuur

A protein arises due to the connection of amino acids. This is a condensation reaction between the acid group of the one amino acid and the amino group of the other amino acid. The binding that arises is referred to as a peptide binding. It has been agreed that the amino group is on the left end and the carbon acid group on the right.

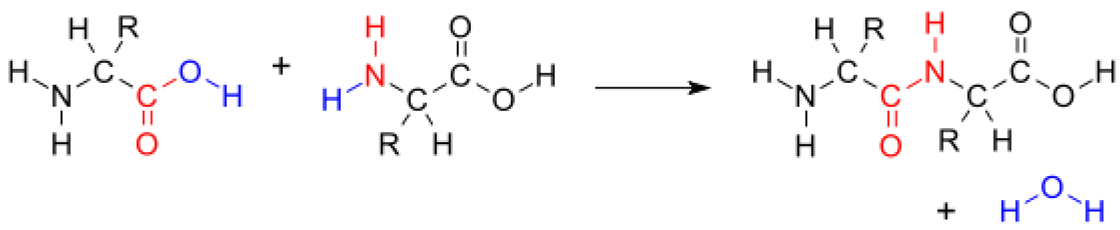


Figure 3.18 Condensation reaction of two amino acids

Source: http://nl.wikipedia.org/wiki/Condensatiereactie

Proteins are often complex molecules consisting of multiple amino acid chains. The primary structure is determined by the cell's DNA; this indicates the order of the amino acids. This primary structure is often presented by means of letter codes of each amino acid; Gly-Tyr-Cys. You can find these letter codes in table 67H of Binas.

The amino acids do not form straight chains. In 1951, Pauling and Corey published a number of articles on the structure of proteins. They concluded that the amino acid chains can order themselves in various ways. There appeared to be two important structures that can be formed: the alpha-helix and the beta-sheet. They were awarded the 1954 Nobel Prize for chemistry for this work. Subsequent research confirmed their ideas.

Such structures can develop due to the fact that the amino acids are exclusively L-amino acids. Consequently, there is one orientation possibility of the amino acid. Hydrogen bonds form between the peptide bindings in a chain. The oxygen atom of the carbonyl group forms a hydrogen bond a few positions further along with the hydrogen atom of the amide group in the peptide binding. This appears to be a regular pattern. As a result, helix structures develop. These hydrogen bonds form the spine of the helix structure.
Because proteins are built from hundreds of amino acids, it is possible that parts of the chain run parallel to each other. Here, hydrogen bonds also form between the peptide bindings of the various chains and, as such, a beta-sheet structure arises. A beta sheet often consists of multiple chains that are both parallel and anti-parallel to each other.

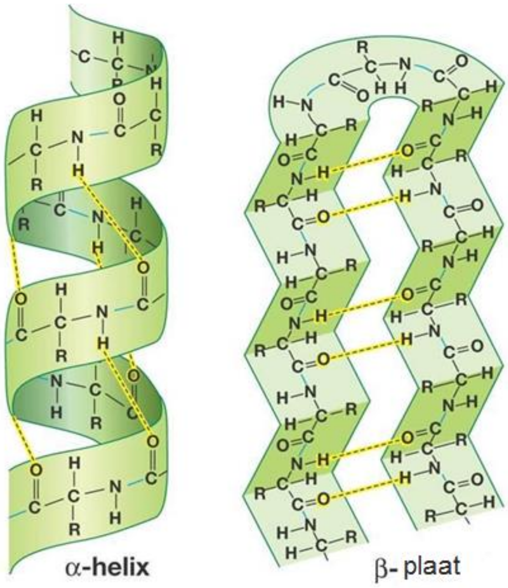


Figure 3.19
Alpha-helix and beta-sheet structure of a protein

The alpha-helix and beta-sheet structure both occur in a chain alternately. On average, a protein consists of such structures for 60%. The remaining 40% is formed by parts that do not have a three-dimensional structure or curves that are present in the chain.

Now complete assignments 5 and 6

The secondary structures can be folded further. The side groups of the amino acids play a large role in this. Depending on the side group, hydrogen bonds, electrostatic interactions (ionogenic interactions), dipole forces, disulfide bonds, van der Waals forces, or hydrophobic interactions

may occur. This tertiary structure is determined by the protein and consequently determines the function of the protein.
Most proteins consist of multiple amino acid chains, each with their own tertiary structure. Together, these various chains form the specific protein; the quaternary structure.

Proteins are sensitive to the environment they are in. Their three-dimensional structure is especially dependent upon the temperature and pH. When the protein loses its tertiary structure, its characteristics also disappear and it no longer functions. Even a small change in the structure can already lead to changes in its function. This process is referred to as denaturation. The best known example of denaturation is boiling an egg. The protein becomes hard and opaque and it gets a different function, an irreversible process. Not all denaturation processes are irreversible. For example, the protein hemoglobin will unfold in case of a pH change, but will fold back when it is returned to its normal pH state. This is referred to as renaturation.

Under influence of changes in their environment, proteins may also coagulate which causes the proteins to clot. In the normal situation, proteins have been dissolved in a colloidal solution. Here, proteins may partly or completely denature.

Milk consists of proteins for 3.4%. The most important proteins are casein 2.8% and serum proteins (whey proteins) 0.6%. There are four different types of caseins: αs1-, αs2-, β, and κ-casein. Table 3.4.2 shows the composition of the various proteins in cow's milk. This composition and the level of casein is dependent upon the type of milk. For example, human milk contains relatively little casein, whereas sheep's milk contains a lot.

table 3.4.2
Casein in cow's milk

	α_{s1}-casein	α_{s2}-casein	β-casein	κ-casein
Total amino acids	199	207	209	169
Molecular mass	23.614	24.350	23.982	19.023
% of skim milk protein	34-40	11-15	25-35	8-15
gram/litre skim milk	12-15	3-4	9-11	2-4

Source: http://www.tigweb.nl/TIG19_1_03_Van_Wijk_-_Melk.pdf

Most casein molecules consist of relatively a lot of hydrophobic amino acids. These molecules form casein micelles (see figure 3.4.8), with often κ-casein molecules on the outside that usually contain hydrophilic groups. Enclosed in these micelles are calcium ions. These micelles have a particle size ranging from 0.1 to 0.001 μm and occur in colloidal condition. The hydrophilic κ-casein molecules give the balls a somewhat hairy structure. At the same time, these 'hairs' ensure that the casein micelles repel each other and to prevent clustering from happening.

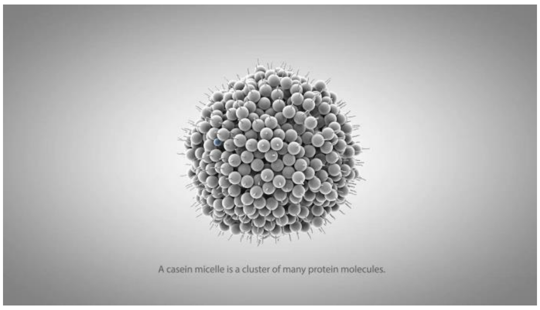


Figure 3.20 Casein micelles

The white color of milk is the result of the presence of these micelles. When milk becomes acidic or is acidified, these micelles clot and flocculate. This leaves a liquid with a yellow-green color. When making cheese, this same characteristic is used. However, the enzyme chymosin is used for this purpose. This enzyme cuts the micelle's hairs due to which the micelles start to clot (see figure 3.4.9). The protein casein itself hardly changes when acid is added to milk, though clotting does occur. In this way, cheese or yoghurt can be made from milk. Take a look at the following animation that shows the transformation from milk to yoghurt: http://vimeo.com/41245310

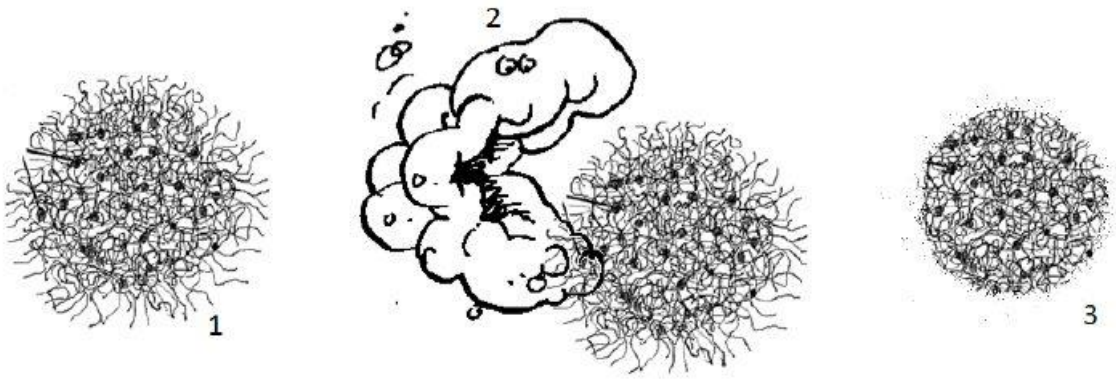


Figure 3.21

Working of the enzyme chymosin

The serum proteins occur in a dissolved state. Their particle size is smaller than 0.001 μm. These proteins exist in the watery solution that often was a by-product in the preparation of cheese. This watery solution is referred to as whey, hence the name whey proteins. Nowadays, whey is the most important raw material for formula for babies. This is not so strange given the composition of breast milk (see figure 3.4.10). The main proteins present in whey are β-lactoglobulin, α-lactalbumin, blood serum albumin, and immunoglobulins. They mainly occur as molecules and have a high hydrophobicity and a strongly folded peptide chain. The characteristics of these proteins are highly dependent upon their environment.

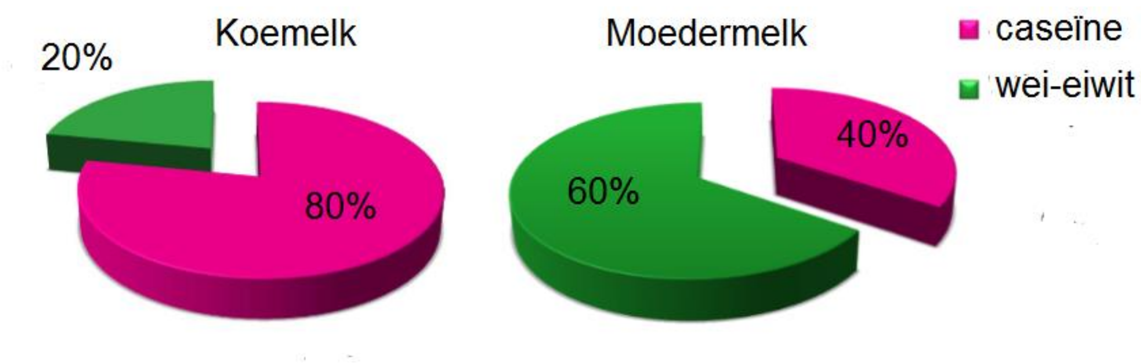


Figure 3.22
Ratios casein and whey proteins in cow's milk and breast milk

β-Lactoglobulin is most common. The solubility is highly dependent upon the pH and the ion strength. In milk, it occurs as a dimer where van der Waals forces play a large role. The protein is compactly folded. The α-lactalbumin is practically spherical and practically insensitive to the pH and salt level. It has an important biological function as co-enzyme in the synthesis of lactose. It is considered as the most important protein in breast milk due to the nutritional characteristics for infants. Blood serum albumin binds insoluble fatty acids that are released in the stomach during the digestion of food in order to transport these in the blood. Immunoglobulins are antibodies.

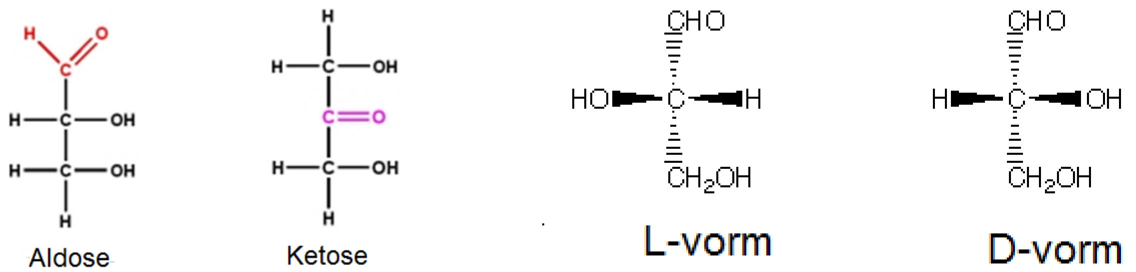
When milk is heated, these proteins denature and become insoluble. However, they do not flocculate, but descend on the casein micelles and remain in solution. Whey proteins contain high concentrations of dendritic amino acids such as valine, leucine, and isoleucine.

Now complete assignments 7 and 8

3.2.4 Carbohydrates

Cow's milk contains 4.5% carbohydrates. Carbohydrates are also referred to as sugars or saccharides. Carbohydrates have various functions in the body. The most important function is the storage of energy. Additionally, they play a role in the communication within a cell and in recognition processes.

The general formula for a saccharide is $(CH_2O)_n$. The basic structure consists of an aldehyde and a hydroxide group on the remaining carbon atoms. The total number of carbon atoms in a monosaccharide varies from 3 to 6. The carbon with the aldehyde group becomes number 1. We then speak of an aldose. If the oxygen group is not attached to the first carbon atom but to a secondary carbon atom, we speak of a ketone group. In that case, the saccharide is referred to as ketose. In the aldose-form, there is an asymmetric carbon atom. Consequently, this form can take on two stereo isomers, presented in figure 3.4.11. The various isomers can be indicated by means of a prefix D- or L-.

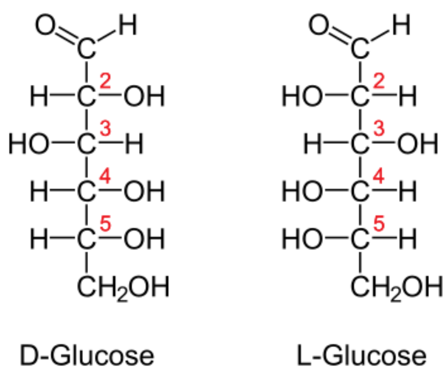


Bron: https://chelseaharripersad.wordpress.com/2013/03/30/ bron: https://chelseaharripersad.wordpress.com/2013/03/30/

Figure 3.23: Aldose, ketose, L- and D-forms of carbohydrates

The most frequently occurring saccharide form is hexose. It consists of a chain of 6 carbon atoms. In this chain, there are four asymmetric carbon atoms. Theoretically, there can be 2^4 (16) stereo-isomers. Glucose is the best known form of this. The L- and D-forms can be found in figure 3.24 below.

Note the difference in the orientation of the OH groups. This plays a major role in the metabolism of all living organisms. D-glucose is the energy source for most cells in organisms. In the rest of the chapter, we will therefore only discuss the D-form.



Bron: http://nl.wikipedia.org/wiki/Glucose

Figure 3.24 L- and D-forms of glucose

D- and L-forms of glucose

In an environment rich in water, the linear structure changes into a cyclic structure. Due to a difference in the position of the OH group in the first C-atom, two varieties per isomer arise: the α- and β-variety. In the α-variety, the OH is oriented downwards in the field and upwards in the β-variety. The following animation shows the creation of these two varieties: http://www.stolaf.edu/people/giannini/flashanimat/carbohydrates/glucose.swf

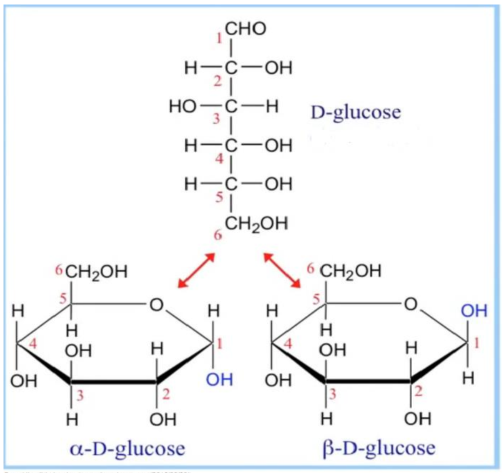


Figure 3.25 : Cyclic shapes of glucose

The alpha and beta varieties can easily merge into each other. Enzymes in the body can distinguish between both varieties; they have different biological functions.

Now complete assignment 9

The cyclic units can be linked by means of a condensation reaction. In addition to the chain that is formed, water develops as a by-product. The link is called a glycosidic bond (-O-). This glycosidic bond is formed between the C1-atom from the one monosaccharide and the C4-atom from the other monosaccharide. The length of the chains can vary. Based on their length, the chains are subdivided into three groups: mono-, di-, and polysaccharide. This classification is presented schematically below. When two monosaccharides are linked, this is referred to as a disaccharide. Very long chains are referred to as polysaccharides. Examples of these are starch and cellulose. Oligosaccharides form the interim group. The length of the chains varies from two to seven monosaccharides. It is exactly this group that plays an important role in the biochemistry of living organisms.

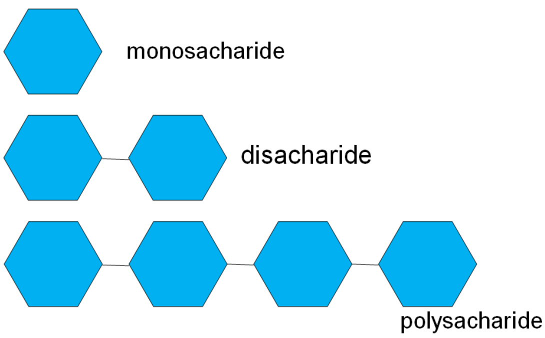


Figure 3.26

Schematic presentation of mono, di and polysaccharides

Because of the occurrence of various stereo-isomers, multiple links are possible. In the figure below, you can see the links between two alpha forms of D-glucose and between two beta forms of D-glucose.

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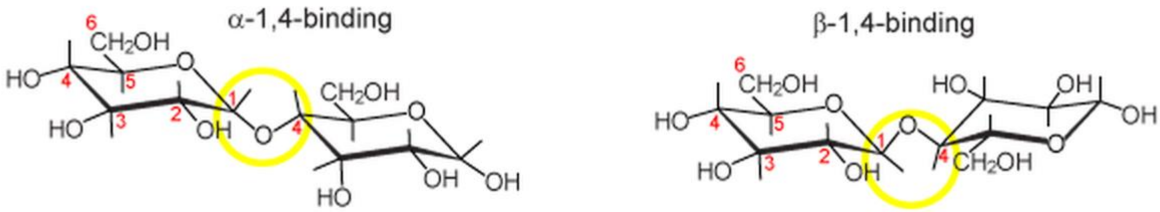

Figure 3.27

Glycosidic bond in two glucose molecules

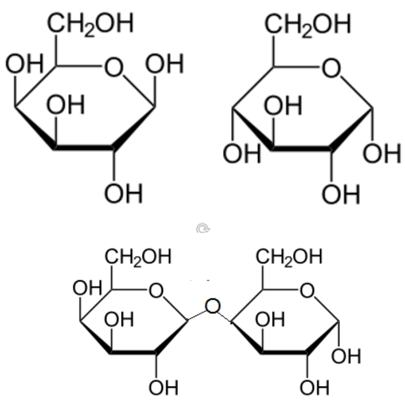

Figure 3.28 D-galactose (top left) and D-glucose (top right); lactose (bottom)

3.2.5 Human Milk Oligosaccharides (HMOS) and galacto-oligosacharides (GOS)

The sugar level in breastmilk is much higher than in cow's milk. Additionally, the milk sugar composition is quite different between the two types of milk. The sugar molecules are exactly what gives breast milk its remarkable characteristics. In this section, we will discuss these Human Milk Oligosaccharides, or HMOS.

The carbohydrates in milk mainly consist of the disaccharide lactose. Lactose arises due to a β-1,4 bond between D-galactose and with D-glucose. D-galactose is an epimer of glucose, where the orientation of the OH group and the hydrogen atom on C4 is the reverse of D-glucose.

By adding one to eight extra galactose-units to lactose, so-called galacto-oligosaccharides appear. A large variety of molecules can be made, because not only the length of the chain, but also the type of glycoside-connections can vary. In figure 3.29, a number of these possible GOS are shown. The structure is simplified, by only presenting the hexagon of glucose (blue) and galactose (yellow).

HMOS resemble the GOS as described above. However, there is even more variety possible than with GOS. The composition per women differs, but the composition also changes during the period of breastfeeding.

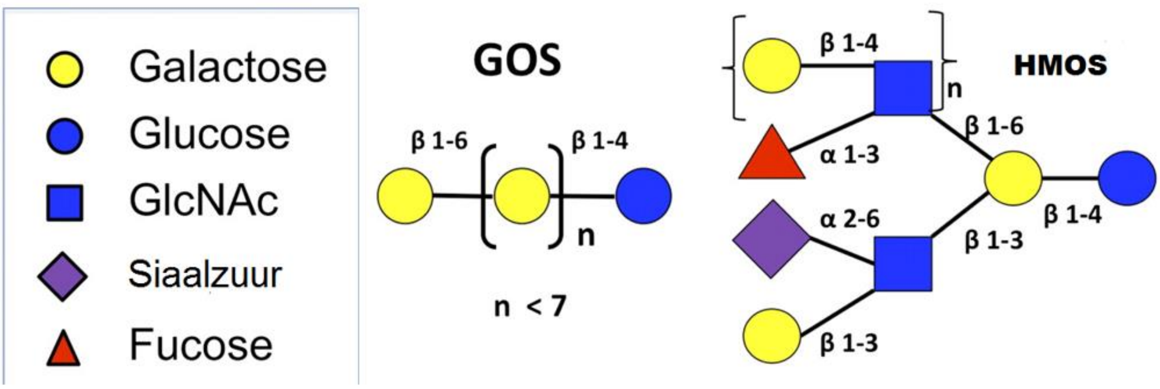

Figure 3.30 Possible structures of HMOS

HMOS consist of five monosaccharides; glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc), and sialic acid (Sia). Over 100 different HMOS have been identified. The basis of the HMOS is formed by a glucose and galactose monosaccharide that have been linked via a β-1,4 bond. The other groups, GlcNAc, Fuc, and Sia, are linked to this in different combinations. Here, chains of 8 monosaccharides can be formed, possibly dendritic. Figure 3.4.19 schematically presents which possible structures can be formed of HMOS.

HMOS are known for their positive effect on the health of newborn babies. They stimulate the growth of good bacteria in the gut, like *Lactobacilli* and *Bifidobacteria*. They stimulate the immune system and improve the overall health of the baby by lowering cholesterol and ameliorating liver function.

These sugars are also referred to as glyconutrients. In addition to the five aforementioned sugars that occur in breast milk, three other sugars belong to this group; mannose, xylose, and inulin. These sugar molecules appear to provide an essential contribution to the development of the gut flora and the immune system of new born babies. The developments in the field of knowledge of these sugars, both biological and chemical, are united in the field of *glycobiology*.

The detection of HMOS from mother's milk

As you have read in the previous section, there are a lot of possible combinations of sugar molecules in HMOS. At the moment, already more than 150 different HMOS have been identified, but new ones are still being found. The detection of HMOS happens in many labs over the world, but also at the Carbohydrate Competence Center at the University of Groningen.

The detection of HMOS is a time-consuming and complicated process. First, small amounts (1 milliliter) of mothers milk are collected. This milk has to be treated before the oligosaccharides can be detected: all proteins and fats have to be removed from the milk. This is done by centrifugation, that results in an easy-to-remove layer of fat floating on top of the milk. Following, the milk is poured over a so-called carbon-column (like Norit), which leads to flushing out the salts and the mono-saccharides. The proteins stick to the column, in this way a solution containing only the oligo-saccharides remains.

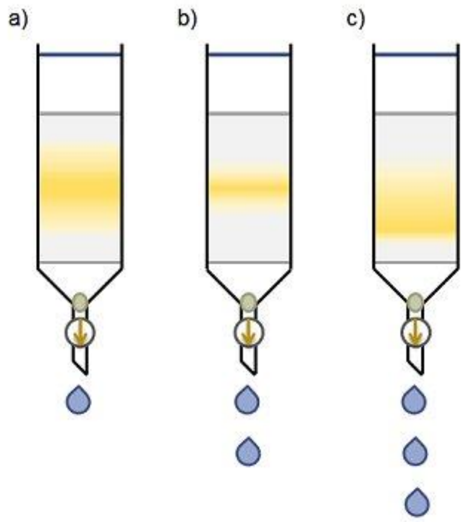

Figure 3.31. The basis of a column. By the type of fluid and the material in the column, one can determine which substances stay in the column and which flow through. Source: http://www.chemistryviews.org/details/education/2345141/Tips_and_Tricks_for_the_Lab_Column_Troubleshooting_and_Alternatives.html

This mixture is studied with a combination of two analysis-techniques: Nuclear Magnetic Resonance (NMR) and Liquid Chromatography/Mass Spectrometry (LC-MS).

NMR (or Nuclear Spin Resonance) is based on the fact that atomic nuclei are a bit magnetic. By placing molecules in a magnetic field, these nuclei can absorb magnetic radiation, and subsequently release this radiation. This can be measured. Different atoms have different magnetic properties, therefore this technique can be used to measure the relative amount of atoms in a mixture. This leaves the scientist with a general overview of the structural information of the substances in the mixture, milk in this case. NMR is also the basis of the frequently used medical examination technique MRI.

With LC-MS, a mixture is squeezed through a small column under high pressure. By chromatography, the fragments that come out of the column are separated by size. The mass spectrometer can determine the mass of the fragments. By comparing information from well-known molecules (like lactose), the scientist can predict which other molecules were present in the mixture.

It is not yet known how exactly these various HMOS are formed by the body. In the current models of the biosynthesis of HMOS, it is assumed that the forming of the bond Glc-GAL occurs in the first step and subsequently the addition of the other units or extra galactose units. A number of genes have been identified that code for the enzymes that perform these additions, like the Fucosyltransferases FUT2 and FUT3. But there has not been enough studies to know whether these genes can influence the number of oligosaccharides in breast milk or the health of the baby.

The production of oligosaccharides for formula

Because of the positive effects of HMOS on the health of newborn babies, researchers started to try to synthesize these molecules in the lab. That has not worked so far for the majority of HMOS (especially not on a large scale), but researchers from the University of Groningen did succeed in producing Galacto-Oligosaccharides with lactose from cow´s milk. FrieslandCampina, a large Dutch producer of formula, has transferred this knowledge to start a large-scale production of GOS, and is now one of the world's largest GOS-producers. Since 1998, to many brands of formula GOS is now added.

In our body, lactose is converted into galactose and glucose by means of enzymes. This is a hydrolysis reaction (see figure 3.4.18). The enzyme involved in this reaction is referred to as lactase. Lactase belongs to the β-galactosidase-family of enzymes. In food technology, it has been discovered that the enzyme β-galactosidase can convert lactose from cow's milk into galactose-oligosaccharides under specific conditions. You will read more about this in chapter 3.5 which is about process chemistry.

Now make assignments 10 and 11

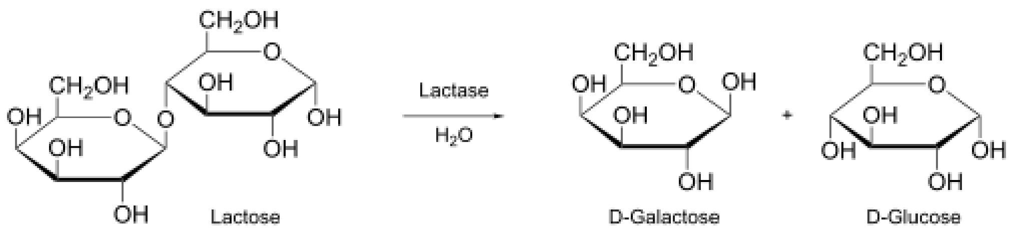

Figure 3.32
Division of lactose by means of the lactase enzyme

3.2.6 Enzymes

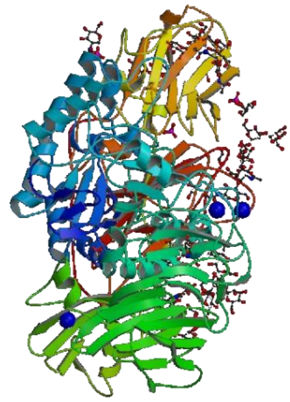

Figure 3.33
The enzyme lactase

Most chemical reactions in biochemistry occur under the influence of enzymes. Enzymes are biocatalysts. They speed up the reaction without being used themselves. Additionally, enzymes work very specifically. They can only convert one (or at most several related substances). Enzymes always end in an –ase. The name of the enzyme often shows which substance is converted. For example, the enzyme lactase converts lactose (in D-galactose and D-glucose).

The enzyme works in accordance with the lock-key principle. The substance that is converted by the enzyme is called a substrate (the key). The enzyme that activates the conversion is the lock. Enzymes are large proteins. The substrate fits in the active place of an enzyme like a key in a lock. This creates an enzyme-substrate complex. The figure below presents this schematically.

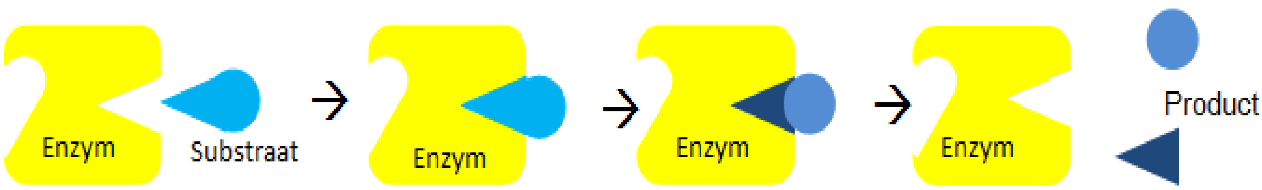

Figure 3.34 - An enzyme-substrate complex

The way an enzyme works and the reaction speed are dependent upon a number of factors. A chemical reaction will occur much more quickly under a higher temperature, which is also true for the reactions in biochemistry. The figure below depicts this with curve A. However, under a higher temperature, proteins, and therefore also enzymes, lose their spatial structure (denaturation) and with that their functioning. This is depicted by curve B. This sensitivity to temperature differs greatly per enzyme. There are enzymes that still function at a temperature of 95°C. These are enzymes that occur in bacteria living in hot springs. Other enzymes already lose their functioning at 40°C. Lactase from yeast works optimally at a temperature of 48°C. Similarly, every enzyme has its own optimum curve (curve C).

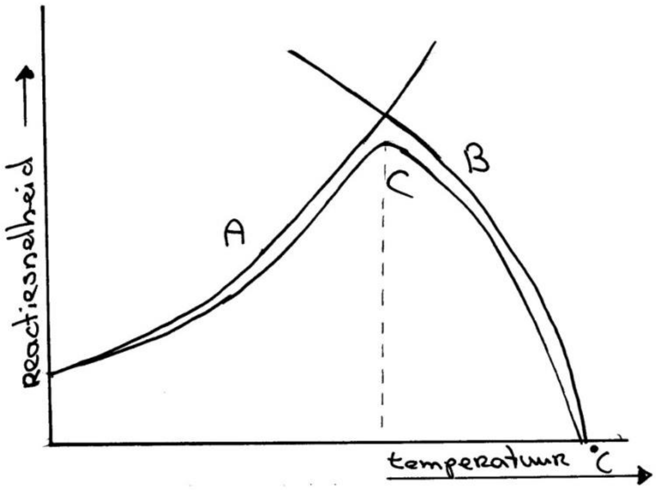

Figure 3.35 Optimum curve of enzymes

In addition to temperature, the pH also plays a role in the functioning of an enzyme. When the pH value deviates too strongly from the normal circumstances for an enzyme, it leads to denaturation. This sensitivity to pH and the accompanying optimum differs per enzyme (see figure 3.35).

The substrate can only attach to the protein/enzyme in one specific spot. This specific location is called the active center of the enzyme. During attachment, various interactions play a role depending on the residual groups of amino acids in the protein in this location.

Now complete assignments 12 and 13.

From cow's milk to formula

At the end of the 19th century, two-thirds of mothers breastfed their babies. Yet this led to an infant mortality percentage of 17%. This percentage was especially due to the social circumstances; malnutrition, infections, dehydration, and nutrition disorders often occurred in the lower classes. As a reaction to this, child welfare centers (in Dutch: consultatiebureaus) were founded in 1901. These centers distributed free milk for babies. This was cow's milk from cows that were fed according to a certain procedure. In the summer, the cows grazed in special meadows and in the winter they only ate hay, linseed cakes, and brans. However, this had an adverse effect. The infant mortality rate increased. Consequently, the nursing premium was reinstated in 1935 to stimulate breastfeeding.

In the middle of the twentieth century, breast milk substitutes for babies were being adjusted to better varieties. They mixed ⅔ part cow's milk with ⅓ part water and subsequently added additional sugar and flour. This "humanized" the milk and made it resemble breast milk more closely.

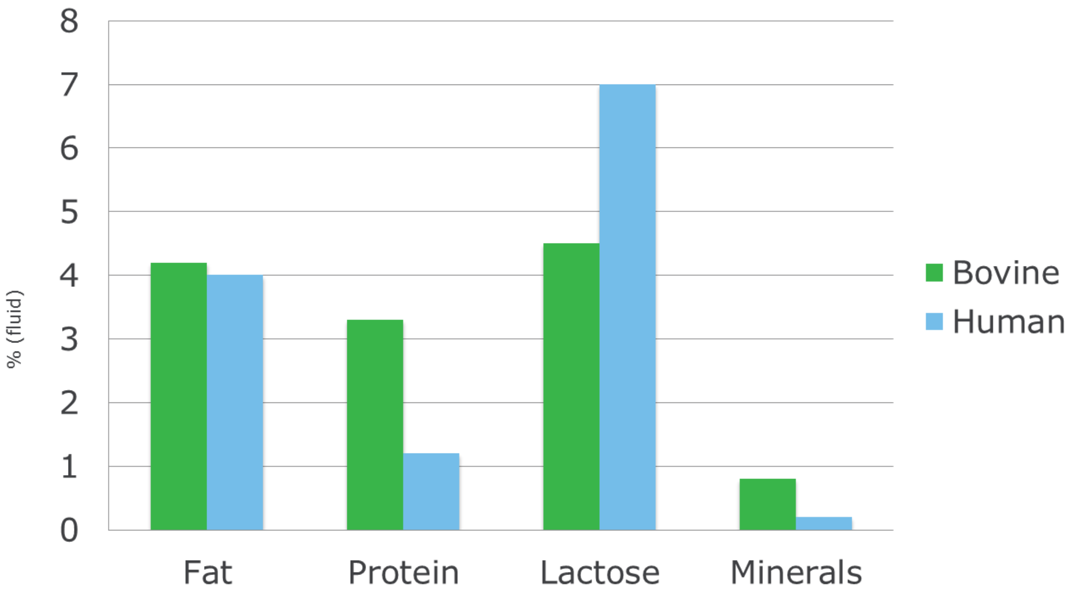

Figure 3.36: The figure above shows the composition of cow's milk and breast milk. Breast milk contains fewer proteins and minerals and more sugars. By diluting and adding sugars to cow's milk, the difference in composition has been reduced.

In the fifties, the firm Nutricia marketed the first real formula. Nutricia called this product "humanized formula". By giving it a name, people assumed it was an improved version of the diluted cow's milk.

In the sixties and seventies, feminism arose. Women became more independent and took it upon themselves to decide whether they wanted to breastfeed or bottle feed their children. The increase in bottle feeding also occurred because the time schedule made women insecure, feeding every three hours as the experts advised for breastfeeding. Also, more women gave birth in the hospital. In the sixties and seventies, babies were immediately separated from the mother due to which they mainly received formula. Around the seventies, only one in ten children were breastfed.
Around the turn of the century, increasingly more became known about the composition of breast milk and its positive effects on the baby's health. Consequently, the advice increasingly became to breastfeed as long as possible. The last ten years, a lot of research has been conducted into a possibility to develop a breast milk substitute that is comparable to breast milk. One of these developments is converting lactose from cow's milk into galacto-oligosaccharides and adding these to formula.

Now complete assignment 14

From lactose to GOS

Scientists have succeeded in producing galactose-oligosaccharides from lactose from cow's milk. Isoforms of β-galactosidase play a large role in this. In a biological cell, a hydrolysis reaction will occur in the presence of water. When you take an extremely high concentration of lactose in a reactor vessel, synthesis of GOS will occur.

This latter process occurs in three steps:

1. First, a lactose molecule will attach itself to the active center of the enzyme.
2. The enzyme catalyzes the hydrolysis of lactose. The glucose molecule detaches and a covalent bond arises, between the enzyme and residual part galactose. An enzyme-galactose complex is formed.
3. In the next step, the galactose-enzyme complex reacts with a hydroxyl group of an acceptor molecule. This can be a water molecule. In that case, we speak of hydrolysis. It can be a lactose molecule. In that case, a trisaccharide develops, a GOS. In the case of a trisaccharide, a tetrasaccharide develops. This can occur in up to 10 units.

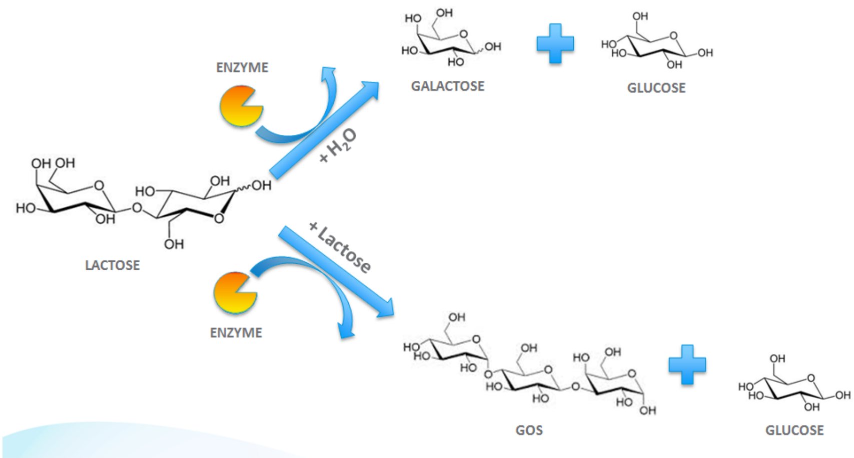

Figure 3.37 Synthesis of GOS

It seems strange that an enzyme that cuts sugars "in life" pastes galactose units together with a glucose unit somewhere in the chain. The body creates controlled circumstances under which the enzyme cuts. This is not strange at all when you look at the reactions as equilibrium reactions and get insight into the equilibrium conditions (reaction speed constants).

In order to improve the stability of an enzyme (and in doing so extend its lifespan), it is immobilized in the bio-industry. Immobilizing means fixing on a carrier or enclosing in a matrix. The advantage of this is that the unfolding of the chains is no longer possible, due to which the enzyme is less sensitive to pH and temperature changes. Additional advantages are that an immobilized enzyme can more easily be isolated from a reaction mixture than a non-immobilized enzyme and that this means that the production process can be better controlled.

The enzyme functioning can also be inhibited by certain substances. The blocking of enzymes can be understood when you depict them with spatial structures. There are two types of inhibitors: irreversible and reversible inhibitors. Irreversible inhibitors destroy enzymes by binding with the specific amino acids in a protein due to which the structure is irreversibly damaged. Reversible inhibitors temporarily bind with an enzyme and such a bond is reversible.

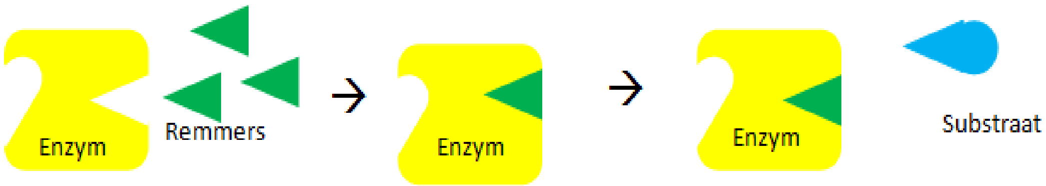

Figure 3.38 Enzyme inhibitor blocks the reaction location

When the inhibitor strongly resembles the substrate in terms of stereo-chemistry, it can bind in the active location; in that case, the active location is 'occupied' (see figure 3.4.26).

When both the inhibitor and substrate are present, a competition takes place between these two substances. When a lot of substrate is present, most enzymes will bind the substrate due to which the reaction is a bit slower. This form of inhibition is called competitive inhibition: competition exists between the inhibitor and substrate to bind with the enzyme.

There are also reversible inhibitors that bind in a different location than the active location. Consequently, the spatial structure of the enzyme changes due to which no substrate molecules can be converted.

Experiment

Immobilization of an enzyme. Follow your teacher's instructions when doing this experiment. See appendix Experiments

Box 3.6: Catalysis

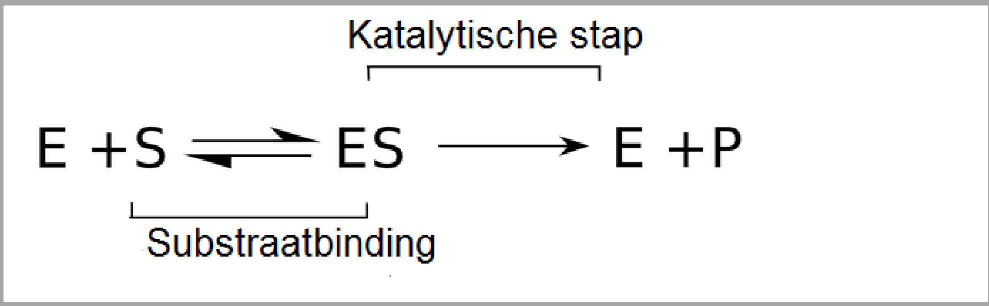

Figure 3.39 Catalysis

Adaptation from http://bcrc.bio.umass.edu/intro/content/enzyme-kinetics-lab-protocol

The equilibrium condition of the substrate bond can be defined by the ratio of the reaction speed constants. The reaction speed constant is defined by s1 = k1x [E] x [S], in which s1 is the reaction speed of the forward reaction and k1 is the accompanying reaction speed constant. Consider that, in case of equilibrium, the following applies: $K = \frac{[ES]}{[E].[S]}$ = $k1k2$.

The catalytic step is not an equilibrium reaction. The reaction speed of this step is dependent upon the speed with which the enzyme-substrate complex is formed. Unfortunately, the process of the GOS formation is a little more complicated than the abovementioned process.

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Assignments 3.2 –

(Bio)Chemistry of milk

1. Make a 3D model of a saturated and unsaturated fatty acid.
2. What is the systematic name of glycerol? Draw the structural formula.
3. Draw the structural formula of a triglyceride consisting of two caprine acids and a palmitoleic acid.
4. Give the hydrolysis reaction of the fat from assignment 3.
5. Draw the piece of protein that has the primary structure Val-Al-Thr.
6. Build a 3D model of a chain with at least 20 amino acids, simplify the side chains. Construct hydrogen bonds between the oxygen atom of the carbonyl group and the nitrogen atom of the amide group in such a way that you get an alpha-helix structure. Carefully consider the materials you will use for this.
7. Which amino acids will occur most in the k-casein? Explain your answer by means of information from the text.
8. Explain why van der Waals forces in proteins result in such a strong binding.
9. Make a 3D model of the alpha and beta variety of glucose.
10. Make a 3D model of the alpha and beta variety of glucose.
11. Build the D-galactose, clearly show that it is an epimer of D-glucose.
12. In table 67 H of Binas, find the various amino acids. Which types of interactions are possible between the substrate and the active center of the enzyme? Give at least one amino acid that plays a role in every type of interaction.
13. Which interactions will especially play a role between lactose and the active center of lactase? Clarify your answer.
14. Explain why a newborn baby cannot digest the amount of proteins and minerals yet.

3.3 Process chemistry

Milk – why process?

Milk contains a lot of high-quality nutrients, such as proteins, fats, carbohydrates, and minerals. The main disadvantage of fresh milk is that it is highly perishable. In order to maintain the nutritional value of these substances, food with a longer expiration date is made from fresh milk.

This section is about how fresh milk is processed in the milk factory. The emphasis is on the production of formula: a milk product suitable for feeding babies. To be clear: breast milk is always better for babies than formula made of cow's milk. However, if formula is necessary because of certain circumstances, it is a suitable alternative.

The composition of formula has clearly improved over the years. More is still being discovered about the influence of the components in milk on the health of growing babies. In factories, it is pursued to add beneficial substances to formula as much as possible. However, it is still true that the composition of breast milk is best for babies.

Fresh milk

A cow that's given birth to a calf produces approximately 8000 liters of fresh milk during 300 days in the Netherlands. About three to four times a week a tank truck from the milk factory picks up the milk at the farm.

Before the driver pumps the fresh milk into the truck's tank, he takes a sample to determine the quality. Depending on the quality the farmer will receive less or more money per liter of milk. The milk price is partly dependent upon the mass percentages of protein, fat, and lactose. Furthermore, it is checked whether the farmer has processed the milk hygienically.

Figure 3.40. The FrieslandCampina/Domo plant in Beilen, Netherlands

During the drive to the milk factory, the driver uses a device to check whether the milk has no antibiotics. Milk cows sometimes get udder infections that must be treated with antibiotics. The farmer is not allowed to deliver the milk of these cows to the factory and the Dutch dairy factories check this very strictly. Contrary to the United States, no antibiotics may be present in milk in the Netherlands.

If a farmer delivers milk with antibiotics anyhow, he will have to pay for the destruction of both the milk containing antibiotics and the possibly contaminated milk that was already present in the tank truck. Additionally, the farmer will receive a lower price for his milk for the duration of one year, even if this milk were to be of the highest quality. It is clear that farmers have to be very careful during milking. When the milk is ok, it is pumped from the tank truck to a temporary storage tank at the milk factory.

Three groups of food can be prepared from milk:

1. Day-fresh and sterilized milk (low-fat, skimmed, and whole milk, but also whipped cream)
2. Fermented products (cheese, yoghurt, buttermilk, butter)
3. Raw materials for the food industry (e.g., powdered milk for ice cream, whey powder)

Figure 3.41 – Dutch cows

The raw materials for formula

The composition of fresh cow's milk differs greatly from breast milk. To make formula from cow's milk that can be digested by babies, the composition of cow's milk must be adjusted. In the milk factory, the various components of fresh cow's milk are separated. These components will later be mixed in the right ratios to produce nutritious and well-digestible formula.
As you know, cow's milk mainly contains water. The other components are fat, protein, carbohydrates, and minerals. There are two types of milk proteins: casein proteins that are solid, and dissolved proteins, the so-called whey proteins.

The main differences in the composition of cow's milk compared to breast milk are not just the amount of fat and protein, but also the type of protein, see table 3.4.2. The composition of the milk must therefore be adjusted to make it suitable as formula for babies. The process for the separation of the milk components and the subsequent production of formula is described below.

Skimmed milk

In the milk factory, the milk is skimmed by means of centrifuges, whereby the fat is removed from the fresh milk in a few steps. This creates multiple milk flows with an increasingly lower fat percentage. Part of the cream is used to make butter, another part is later added to some products. The milk has now become skimmed (low-fat) due to the skimming, and skimmed milk is one of the ingredients of formula.

Whey

Cheese is made from cow's milk. Fresh milk that is partly skimmed is used for this purpose. A mixture of enzymes is added to the milk causing the casein proteins from the milk to denature and therefore clot. The clotted proteins, containing a large portion of the milk fat, are filtered and pressed into fresh cheese. The filtrate from the cheese production, that is, the liquid that is squeezed from the clotted casein proteins, is called whey. Whey contains approximately 1 mass percent of protein, the so-called whey proteins. These water-soluble whey proteins are the most important proteins for the production of formula. Additionally, the whey still contains a lot of lactose and dissolved salts that are called minerals.

Cow's milk has a much higher mass percentage in terms of dissolved salts than breast milk does. During the production of cheese, these salts largely remain in the whey, which means that they have to be removed in order to make the whey suitable for formula. This is done in so-called ion exchangers. As you can read in box 3.7, the whey proteins undergo an enormous pH change. Most proteins hydrolyze when the pH is low, but whey proteins are virtually insensitive to this. A very small part is hydrolyzed into shorter protein chains in ion exchangers, but this has no effect on the flavor or nutritional value of the proteins.

The whey is concentrated in a large vacuum tank after demineralization. A large part of the water evaporates and the percentages of protein and lactose quickly increase. The solubility of lactose in watery solutions is low, due to which it soon crystalizes. After filtration, whey concentrate is left that is demineralized and lactose-free.

Lactose and GOS

Breast milk contains lactose and the right dosage is again added to formula in a later stage of production. Part of the lactose is converted into GOS in a separate factory and is also added to formula. The chemistry behind the GOS production has already been discussed in section 3.4.

Production of formula

At most factories, formula is produced as follows:

- Demineralized whey and skimmed milk are mixed in the right proportions to obtain the correct ratio of casein and whey proteins.
- This mixture is concentrated under low pressure by evaporating water.
- The mixture could also be boiled for a long time in order to evaporate the water, but this would cause a Maillard reaction which strongly changes the composition and digestibility of the proteins.
- The mixture is heated briefly to kill bacteria.

Both vegetable fats and part of the previously removed cream are added to the protein concentrate. Vegetable fats are unsaturated, which means they can oxidize with oxygen. Therefore, the fats are added to the protein concentrate in a nitrogen atmosphere.

BOX 3.7 Demineralization

The whey is demineralized in two ion exchangers that first remove the positive ions and subsequently remove the negative ions. The first ion exchanger contains a resin (= grains of a synthetic material) to which H^+-ions are bound. When the whey comes into contact with the resin, positive ions from the whey bind with the resin, while at the same time the H^+-ions dissolve while oxonium ions are being formed. The electric charge of the whey does not change, but the pH decreases strongly, to approximately 1.5.

The second ion charger contains grains of resin to which hydroxide ions are bound. This exchanger removes negative ions such as phosphate and carbonate, while at the same time hydroxide ions dissolve. The pH of the whey increases to approximately 7.

After some time, the ion exchangers become saturated with positive and negative ions respectively. The positive ion exchanger can be regenerated by leading concentrated hydrochloric acid through it. The binding of positive ions from the whey is an equilibrium process; due to the high $[H^+]$, the positive ions dissolve again, while the H^+-ions bind with the resin.
Similarly, the second ion exchanger can be regenerated with lye. Finally, the ion exchangers are flushed with water several times after which they can be demineralized again.

After adding the right amount of fat, the percentages of protein and fat match those in breast milk. Earlier in the process, sodium, potassium, and calcium ions were removed from the whey. Now, the correct amount of each type of ion is added again. Moreover, vitamins (A, D, E) and several essential nutrients are added, such as choline, taurine, and inositol; substances that are also present in breast milk.

The mixture is subsequently pasteurized to kill possible bacteria. This step must be brief to prevent both denaturation of proteins and the Maillard reaction. Immediately after pasteurization, the already heated mixture is dried into a powder with a moisture percentage of <2%. This is done by means of spray drying. The dry powder is not yet completely ready to be packaged as formula.

Part of the metal ions removed during demineralization is multivalent. These metal ions have multiple electrovalences, which means that they can take part in redox reactions. In milk,

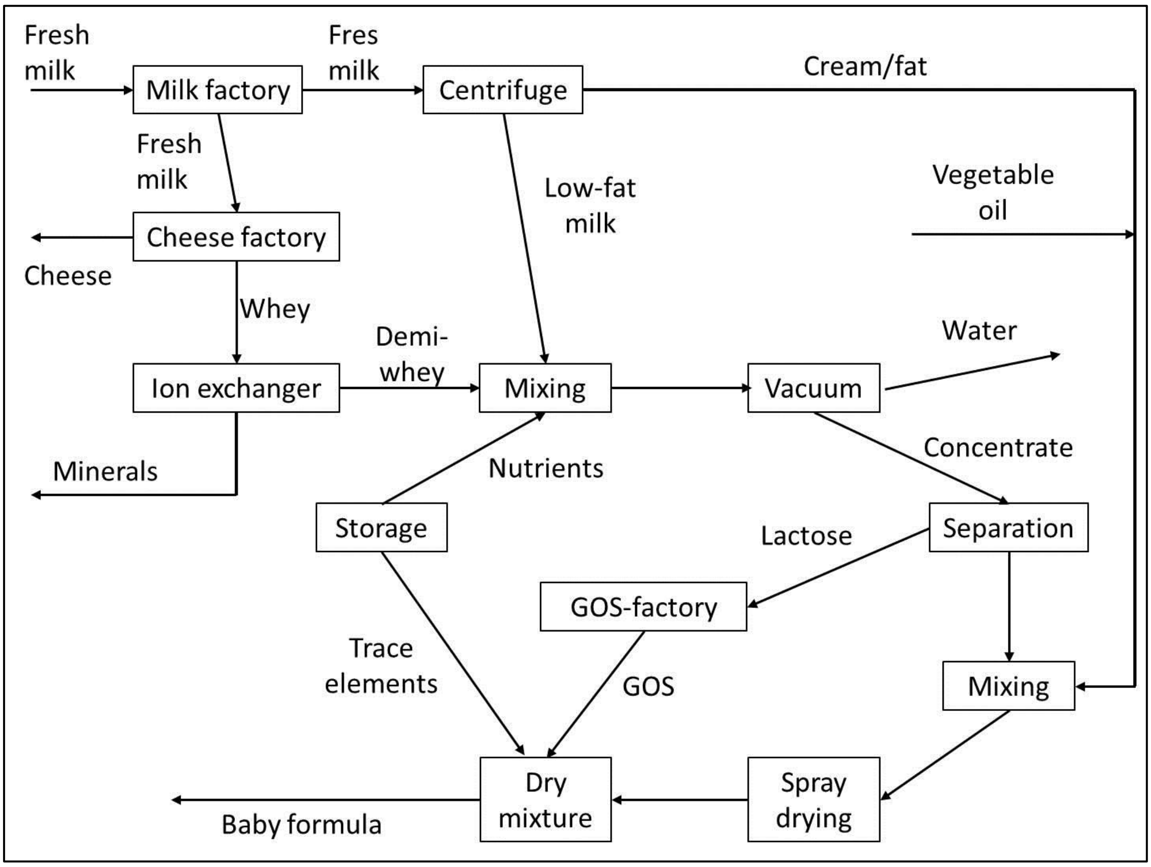


Figure 3.42 Production process of formula

unsaturated fatty acids can oxidize: a process that is sped up in the presence of multivalent metal ions, such as Fe^{2+}, Cu^{+}, Mn^{2+}en Co^{2+}.

In case of oxidation, the double bindings in the unsaturated fats react with oxygen into aldehydes and carbon acids, substances that are not necessarily healthy and often have an unpleasant smell and flavor.

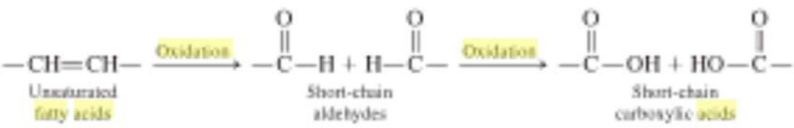


Figure 3.43 Oxidation

The multivalent metal ions are so-called trace elements that are crucial for babies as cofactor in various enzymes. Of course, the trace elements are added again after spray drying, but now in powdered form. As of this point in the production of the formula, even more attention is paid to microbiological hygiene. Liquid products can easily be decontaminated by means of heating, but if the milk powder becomes contaminated now, there is no suitable way to kill bacteria, viruses, and fungi. The powder is stored in large, reinforced plastic bags, or immediately put in cans or sachets – the formula is now ready to be sold.

Assignments 3.3 –

Process chemistry

1. Explain why milk in the Netherlands must be free of antibiotics.
2. What is the normal fat percentage of cow's milk? And explain why the fresh milk is skimmed.
3. Find out how fresh cow's milk is skimmed in milk factories.
4. Find out what the disadvantage is for babies and toddlers of a too high level of sodium, potassium, and calcium ions in formula.

4

Elaborate – RRI in research about breast milk and formula

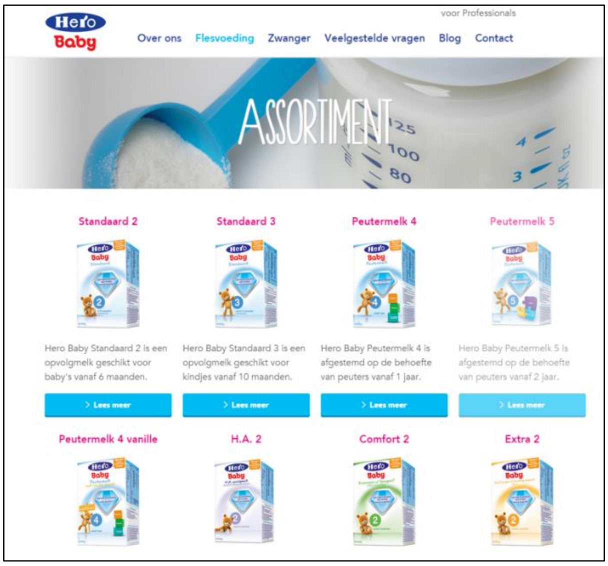
Responsible research and Innovation

In the first chapter, you read about *Responsible Research and Innovation (RRI).* In this chapter, you will learn how RRI is important in the research about the effects of breast milk and the production of better formula.

Advertising for formula:

The EU has strong rules for advertising of and marketing for formula. First, manufacturers of formula must always state that breast milk is the best choice for children in all their advertisements and commercials.

Take a look at the websites of the two most important manufacturers of formula in the Netherlands, Nutrilon and Hero Baby. What is another important rule? What do you notice? Take a look at the websites of the two most important manufacturers of formula in the Netherlands, Nutrilon and Hero Baby. What is another important rule? What do you notice?



Figuur 4.1 Website Hero Baby

Formula in developing countries:

In the Netherlands, mothers who give formula to their babies have money to buy sufficient food, have access to clean water, and the possibility to clean bottles and nipples well. In developing countries, this is often not the case. Women who give their babies formula in developing countries sometimes give too little food because they don't have enough money, or give contaminated food because the water or bottles are not clean. Lots of babies die every year as a result; something that could be prevented if these mothers would breastfeed their children.

In the previous century, manufacturers of formula viewed third world countries especially as a new market that could and had to be supplied and they sometimes aggressively advertised their formula. Their products were advertised by means of pictures of healthy Caucasian babies. To women in developing countries, formula became synonymous with Western society and was therefore seen as better than breast milk. In 1977, manufacturer Nestlé was reprimanded because it tried to persuade women in developing countries to buy formula using too aggressive marketing techniques, while they were aware of the harmful effects on babies.

You can read about this in the following articles about the Nestle boycott and The Baby food Tragedy.

Unfortunately, despite the Nestlé boycott and the *International Code on the Marketing of Breast-Milk Substitutes (Link)* from 1981 of the WHO, aggressive marketing in developing countries still occurs. For example in Bangladesh, as you can read here: Milking It .

Formula scandals:

There have also been a recent scandal in the production of formala in China in 2008, where formula was contaminated with the toxic substance melamine to make the protein level of the milk seem higher. Three hundred thousand children became ill and six babies died. Ever since, the Chinese no longer trust their own formula and they import formula from the Netherlands (both legally and illegally), leading to scarcity in the Dutch supermarkets. Dutch mothers can no longer rely on the stocks in stores and find themselves standing for empty shelves. Shops have put restrictions on how many containers of milk to buy (only one at a time), but still after opening hours, stocks run out quickly by Chinese traders working in teams. Stores that prohibit Chinese-looking people from buying formula, have been accused of racism, as you never know if they might be ´real´ fathers or mothers.

Read the following articles for more information on this topic: Poeder van de wereld: Trouw, 27-11-2014 and Hoe melkpoeder in Hong Kong lucratiever werd dan de handel in drugs, Volkskrant, 8 mei 2013.

Women, Emancipation and Breastfeeding

After the first ‘formula’ made out of diluted cow’s milk around 1880, only after the Second World War real formula became available for everybody. In the ‘60s, many women saw formula as the way to free them from their role as mother and housewife. Through formula, they were no longer home-bound and the baby was not necessarily bound to the mother. Women were very happy with their freedom and started bottle-feeding en masse. In 1975, only 10% of women (in the Netherlands) breastfed. Also hospital-guidelines, separating the baby from the mother directly after birth and putting them in a different room, didn’t help stimulating breastfeeding.

Renewed insights in the superior quality of mother’s milk in the next decennia lead to a stimulation of breastfeeding by caregivers. Also, many more hospitals let women keep their babies with them after birth. Many more women started to breastfed and now, about 80% of women breastfeed their children one week after birth. Over the past five years, the number of women that breastfeed their children longer, after six months, has doubled. Breastfeeding is in fashion again!

Figure 4.2 Advertisement for Nestlé, source: https://nl.pinterest.com/hess0815/infant-formula-company-ads-then/

But now the problem is that many women start working again, shortly after the baby is born (in the Netherlands, after three months). Because of the increased quality of formula, many women decide to quit breastfeeding upon returning to work. The government has made regulations to make it easier for breastfeeding women to breastfeed – for example, in the first 9 months after the baby is born, a woman can use ¼ of her work time to pomp milk, and the employer has to make sure there is a suitable room to do so. However, in practice this might not always be satisfactory: it is difficult to free time when you are a teacher, a surgeon or always have a lot of meetings… In addition, breastfeeding in public, however increasingly accepted, is not always appreciated, whereas bottle feeding is always ok.

In addition, there are many women who experience start-up problems with breastfeeding: inflammations, painful nipples, too little or too much milk. Such problems usually solve themselves over time, but because there is a decent alternative (formula), these can be reasons to quit breastfeeding. Women with these problems can be supported by lactation specialists, but not all women are aware of their presence, keep on struggling and give up.

Breastfeeding and bottle-feeding women can get into rather sharp discussions about this. One part is sometimes called the 'breastfeeding police' of 'maffia' because they are very strict in advising breastfeeding and disapproving formula, putting it down as poison for babies. On the other hand, with more help, some women would persist when experiencing start-up problems and breastfeed longer. But, most of all, bottle-feeding or breastfeeding is an individual choice, that only the mother can make, and is usually the best decision for that specific moment. So don't judge too fast!
Watch this movie for a view on this topic: https://www.youtube.com/watch?v=Kz4BUwaxj5c

Assignments:

In class, you will further deal with these questions by a debate, and by using the RRI-topics in the exhibition that you will be making in the following chapter.

For the debate, you will use the following statements to debate. Use the material that is provided to you (newspaper clippings, movies) to make arguments.

- Nestlé has the right to sell their formula all over the world
    - Team 1: Nestlé
    - Team 2: OxfamNovib
- Chinese are no longer allowed to buy formula in Dutch stores
    - Team 1: Dutch mother who finds herself at empty shelves all the time
    - Team 2: Chinese mother who obtains formula through a friend who lives in Holland.
- Women that do no breastfeed or quit quickly are lazy
    - Team 1: Struggling mother that changed to formula and is quite happy
    - Team 2: Breastfeeding-police-mum who thinks that she had to persist longer

Assignments 4 – Elaborate

1. Which aspects of RRI match the ethical issues regarding breast feeding and the production of breast-milk substitutes?
2. What could manufacturers do differently?
3. How can the government intervene in this matter?

These questions will be further discussed in class by means of role playing, a (parliamentary) debate, the topic of a scientific exhibition, and or with ‘RRI-dices’. Follow your teacher’s instructions.

5

Exchange – Making an Exhibition

Making an exhibition

In this chapter, you will start making an exhibition about the studied materials. In this manual, we will give you guidelines for doing this successfully and to make a nice exhibition.

The process for making an exhibition has three phases that are all equally important: pre-production (design), production (making the exhibition), and post-production (evaluation). Furthermore, it is important to think about how you can make your exhibition interactive, meaning that visitors have to be involved with the exhibition and not just watch. Finally, exhibition texts must be written that are clear and legible and which are especially not too long. Also, we will show you how you can make a great exhibition with a simple IKEA bookcase and give examples of how you can design it.

Interactivity

The word 'interactivity' often evokes associations with a computer screen that you can do something with. However, nothing could be further from the truth: an exhibition without a computer can be interactive, while some set-ups of computer screens are anything but interactive. It's all about the way the information is presented. A set-up is interactive when it '*changes the form of presentation as a function of the visitor's response*'.

What is interactivity?

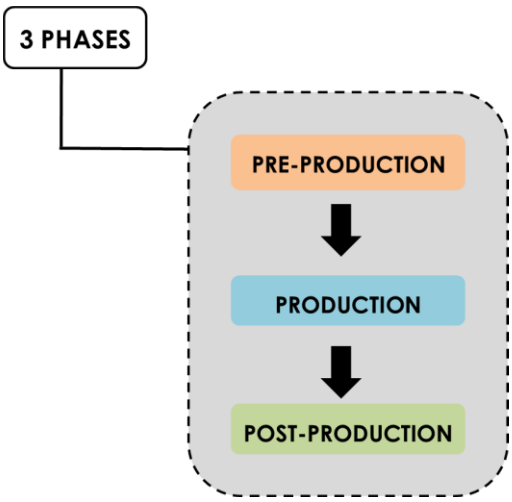
Interactivity does not always go hand in hand with technology and ICT. There are three types of interactivity: hands-on interactivity (doing something), mental interactivity (thinking about something), and cultural interactivity (feeling engaged with something). Asking questions and promoting discussion can attract the visitor's attention, and a conversation may arise. This is also interactivity.

The three phases in the production process:

There are three distinctive phases in the production process of an exhibition, that all require equal attention: pre-production, production, and post-production.

Pre-production:

Don't immediately start building, no matter how enthusiastic you are. The preparation is just as important as the real work. In the pre-production phase, you conduct research: clarifying research questions, summarizing information, and taking from this what you wish to show. Additionally, you come up with the design: what are you going to use to tell the story, what will the exhibition look like? You discuss the design with the entire class (or the group that makes the exhibition) to make sure that all separate components fit together.



Production:

In the production phase, the real work begins. The plans should be ready now, it is clear what needs to be done, what it will look like, and which materials are needed. Make a clear construction plan that lists **who** does what and **when per activity**. It is very important to plan this well; most processes needed to build an exhibition are dependent upon things that must be done first. If one process is late, the rest will also be delayed.

Post-production:

The exhibition is finished and it may be tempting to immediately return to your everyday activities after breaking down the exhibition. But it is very important to evaluate an exhibition.

Because, what did the visitors think of the exhibition? Did they understand it? Did they use it as you had intended? Did you learn enough?

The visitor's experiences must be collected to properly evaluate the exhibition. You can do this in various ways: by observing visitors (what do they do at the different exhibits, how much time do they spend on the various components), by interviewing the visitors afterwards (what did you think of it, what have you learned), or by asking visitors to fill in a questionnaire. A proper evaluation takes time, so prepare this beforehand by thinking about the evaluation method and by formulating the questions you wish to ask.

Writing exhibition texts

A good exhibition has texts. Writing these texts requires particular skills. The most important key points are that the text must be short, clear, and have a good layout. Texts in an exhibition can have a certain hierarchy, where the various components can have different lengths. The text of the entire exhibition can only be approximately 1000 characters long, for instance, but the text of a single exhibit should be limited to approximately 350 characters.

table 5.1

Writing exhibition texts

Textual aspects	Readability
Use simple, clear sentences,	Don't use too many fonts, text heights etc. all at once
Use common words, don't use many words from other languages or technical terms	Avoid writing in capitals only, don't highlight words and make sure type size and contrast are suitable for reading.
Avoid filler words, iterations, adjectives if not really needed	Make sure the structure of the text is appropriate (position of line breaks , paragraphs, etc)
Use concrete and figurative phrasing	Make sure you put op the tekst in the right place (not too high or too low, enough light, close the the exhibit)
Use an active, verbal writing style	Make sure you place the text in a suitable position (not too high or low, close to the exhibit)

To clarify, see a good and bad example of an exhibition text below:

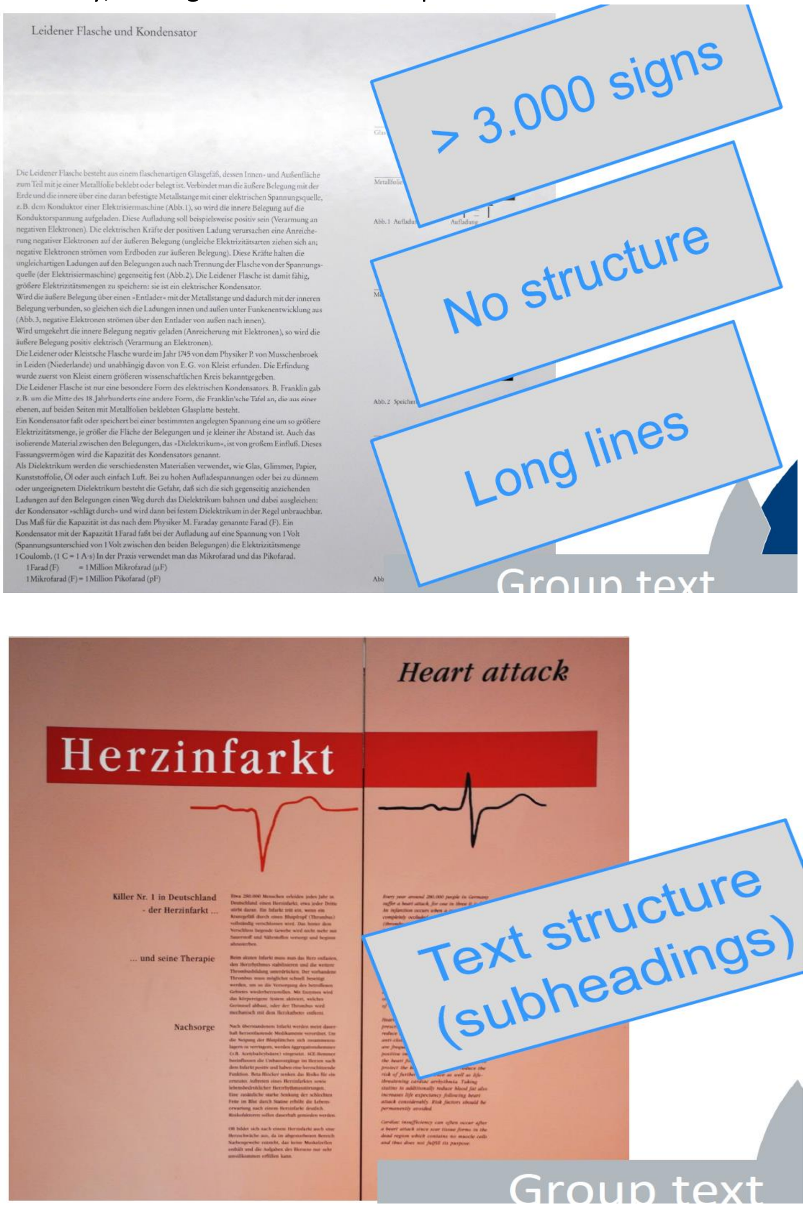


Figure 5.2 – a good and bad example of an exhibition text

EXPONEER: an exhibition with an IKEA closet

You can make great exhibitions with the famous IKEA closet with the various compartments (previously called Expedit – now Kallax)! The closet of 4 x 4 compartments has 16 possibilities for text panels, objects, experiences, screens, and other exhibition options. This idea has been referred to as EXPONEER.

Figure 5.3 – an example of an EXPONEER exhibition

For this project, you can fill an entire closet about this module with your class, where every group takes care of one compartment.

Discuss the contents of the exhibition with the entire class, so all the different topics of the module will be included and make a clear plan. Subsequently make an exhibition object that fills one of these compartments in groups of approximately 3 students.

Assignments 5 –

Exchange

Make an exhibition, poster, a 3D-object or something else about what you have learned in this module, including the RRI-aspects.

Follow the instructions of your teachers.

6

Evaluate

Test and/or evaluation

Evaluate

Your teacher will give you a test and/or will grade your exhibition. This will be discussed in class.