PRACTICAL EXERCISES FOR THE COURSE OF FUNCTIONAL FOODS

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Exercise 1.

1. Different definitions of the functional foods

Functional food is a food that contains components, which have a positive effect on one or more life functions, contribute to the state of mental well-being, and their regular consumption can reduce the risk of nutritional diseases. Besides the energy and nutrition value of foods, they also have a health-protective effect.

2. What type of functional foods exist

A functional food is a food that contains more of one or more component that has a positive effect on the health of the human body. Those foods are also functional in which one or more component is less than in usual foods of the same category (reduced fat, reduced carbohydrate content with reduced protein content), or more than usual due to enrichment of some substances.

3. Nutraceuticals, prebiotics, probiotics

Nutraceuticals are foods that are at the margins of medicines and foods. Their exact names are dietary supplements, medicinal products, which are not considered as medicines. Prebiotics penetrate into the guts, promote proliferation of beneficial microorganisms, suppress harmful microorganisms, and promote the formation of the most favourable microflora. Probiotics are living microorganism cultures that are either present or enriched in food, proliferating in people's digestive tract, suppressing the life cycle of harmful microorganisms.

4. What makes a food product functional?

There are three topics to be discussed about functional foods: what ingredients should be added to the food or what ingredients should be found in it, which is present in a larger quantity and makes it functional? What effect can be achieved by dosing this component? What will be the product, what do we manufacture? What is the specific component, which in greater proportion can make a food functional?

5. Which are the constituents of the functional foods?

Such constituents are for example dietary fibres and natural antioxidants, which may be effective against cancers, and microelements that are essential to the function of enzymes as cofactors. Polyunsaturated fatty acids, which are heat-sensitive, and special proteins may also be components of functional foods. Baking products can be enriched with milk, whey, and casein, but also enrichment with certain peptides can occur. Many health-protection components are produced from colostrum or milk and used to prevent or cure certain diseases.

6. May oligosaccharides, vitamins, phytochemicals and antioxidants are prebiotics?

Oligosaccharides may be prebiotics because they favourably influence the development of intestinal microorganisms. Supplementation with vitamins is very important, but the overdose of certain vitamins can be dangerous. Phytochemicals are chemical compounds, many of which utilized by the pharmaceutical industry. They contain natural antioxidants and microelements. Natural sweeteners are used to replace energy-rich sugars.

Exercise 2.

7. What is the physiological effect of functional foods? What physiological effects can be expected from functional foods and which diseases might be prevented by the consumption of these food products

The effects may include inhibition of oxidative damage, antimutagenic activity, inhibition of microbial infection, dietary fibre activity, immunomodulatory effect, neuro-regulative (nervous system stimulating) effect, estrogenic effect, antihypertensive effect, cholesterol-lowering effect and anti-allergen effect. Heart-friendly foods have been developed for the prevention of heart and circulatory diseases and reducing the cholesterol level. Functional foods have also been developed to prevent obesity, and salt-reduced foods have also been marketed.

8. How to make functional foods? What to produce, how to make it to become functional food?

The concentration of a typical beneficial component of a given food should be increased, during the enrichment process a useful but not necessarily food-typical component should be added, the existing useful component should be modified, the harmful components, such as the allergen protein should be removed, or the harmful components replaced with something else. Thus, the increase of dietary fibre content, the addition of vitamins, microelements, the production of gluten-free products, and the enrichment of protein are used when most milk and milk-related products are produced and, due to its high protein content, soy is used. The applications of fruit juice concentrates, extracts, herbal concentrates and extracts are spreading and functional beverages are also used to enrich normal fruit drinks with multivitamins, calcium, magnesium and carotenoids.

9. Consumer expectations for functional foods

Be delicious, tasty, practical, varied, "like the rest", but have a positive effect on your health! The trust in the buyer for special foods shall be developed, buyers should believe that they need these products which shall increase their well-being.

10. Food safety and functional foods

From the point of view of food safety, functional foods are subject to the same laws and rules as other foodstuffs. Food quality is the sum of all the properties of the food that make it suitable to satisfy the requirements set out in the regulations and the consumer's expectations. Food is safe, which does not constitute any health risk for the consumer. Absolutely risk-free food consumption does not exist, so we are always exposed to a certain likelihood of health damage.

11. What are the typical dangers of functional foods?

It is a relatively new food group, so for the first time it is not known whether it is good, does it cause other problems with over-consumption, whether there is no adverse cross-reaction with commonly consumed foods, medicines, novel foods that are not toxic, whether there are sufficient amount of nutrients in it suitable for human consumption.

12. Functional foods and legislation in Europe

In Europe, regulation is quite heterogeneous, and different countries are using their own directives for the time being. Integration of functional foods is ongoing and there is no uniform definition of what we call food. There is regulation for pure, isolated additives and supplements.

Exercise 3.

13. Production of functional foods with food supplements

Supplementation of foods with various micro-nutrients may have a history of centuries: iron nails put in apple, maize soaked in calcareous water, salt supplemented with iodine to prevent gout, vitamin A deficiency was suppressed by addition of vitamin A to margarine, wheat flour supplemented with thiamine, niacin and iron, and even flour supplemented with calcium was produced.

14. Fortification, restoration, enrichment, supplementation, standardization, substitution

There are several notions about supplementing foods with nutrients. Fortification is the process by which a nutrient is added to a food that was originally not present or the concentration remained below the limit of detection. A good example of this is to strengthen margarine with vitamin A and later with vitamin D. During restoration, they replace the nutrients that have been lost or transformed, for example during the technological processes, such as supplement of flour with iron and vitamin B_1 or supplement of potato products with vitamin C. The production of fruit juice reduces vitamin C content, which should be compensated.

In enrichment, the food is supplemented with a substance that contains more than one ingredient than the basic food. In practice it is also used as a synonym for reinforcement and restoration. During standardization, the different compositions of a foodstuff of the same category is approximated to a standard in some way. Substitution is the process of adding nutrients to foods to reach the same levels as in the original non-substituted food. During the supplementation, a micro-component is added, alone or in combination, to the food to increase its nutritional value.

15. Increasing the functional component content of foods

In addition to the well-known supplements (iodized salt, margarine supplementation with vitamin A and vitamin D or flour restoration and strengthening), methods have been developed to prevent rickets by increasing the vitamin D content of milk, niacin, thiamine and folic acid have been added to the flour to prevent beriberi and pellagra and iron for the prevention of anaemia. The addition (fortification) does not affect the organoleptic properties of the food. In developing countries, the complementation of the most important basic alimentary products with vitamins and minerals has become a daily routine such as the supplementation of cereals with micro nutrients or the enrichment of juices or potato-based foods with vitamin C.

16. Supplementation of the food with micronutrients

Most of the micronutrients studied the effect of folic acid supplementation on health. As folic acid deficiency increased the proportion of infants born with open spine, folic acid was added to foods made from grain in the United States and the consumption of such foods with high levels of folic acid is recommended for pregnant mothers. Folic acid supplementation, however, has led to a deficiency of vitamin B_{12} in older people, so if you want to supplement our foods with a micronutrient, you should be careful with it because this addition may have potentially damaging consequences beyond beneficial effects in certain physiological conditions.

17. Substitution of food components

A problem is the substitution of fatty milk and milk products with other food ingredients because fatty milk contains significant amounts of fat-soluble vitamins. When meat was replaced with soy, vitamins, minerals and essential amino acids were added as supplements. Producing fat replacement margarines, it was necessary to supplement them with vitamin A and vitamin D as well as with carotene.

18. Enrichment of the food with one or more food components

Enrichment results in a special product that contains a significant amount of one or more food component and is aimed to fast modify a small target group of the population in a good direction. The best-known recipes are multivitamins, vitamins mixed with minerals, and recently very popular capsules containing different amounts of vitamin C. Supplements for safe food and nutrition are used for therapeutic purposes, and sometimes we expect a specific therapeutic effect. Elderly people could effectively fight against osteoporosis with preparations containing mainly calcium and vitamin D at different concentrations.

Exercise 4.

19. Supplementation of foods with vitamins and minerals. The necessity of supplementation

Especially the widely consumed foods and their raw materials, such as cereal flour, salt, sugar or soy sauce, are complemented with micro components. Mineral deficiency mainly includes low levels of zinc, selenium, iron, iodine and calcium, while the deficiency among vitamins occur with A and D vitamins and folic acid, frequent niacin deficiency has been observed in the corn-consuming societies, thiamine deficiency occurs in rice-consuming populations, and scurvy (vitamin C deficiency) in those groups who consume little fresh fruit or vegetables. Today, two billion people suffer from iron deficiency, 1.9 billion in diabetes, and in the absence of vitamin A 250 million school-age children are exposed to the risk of blindness. Deficiency diseases occur mainly in those regions where nutrition is based on grain and legumes and the population does not consume sufficient quantities of animal food, especially meat, fresh fruit and vegetables. Low selenium and yeast deficiencies in food are due to the low concentrations of these elements in the soil, which are reflected in both plant and animal foods.

20. Iron deficiency and its consequences

As a result of iron deficiency, 30% of the world's population is anaemic and one billion suffer from iron deficiency anaemia (IDA), another one billion in iron deficiency, without anaemia. In the human body, 95% of the total iron content is found in haemoglobin and myoglobin and even cytochromes and NADH dehydrogenase contain iron. Some iron-containing enzymes are a means of immunization; iron deficiency causes fatigue, weakness, resistance to infections decreases, work capacity decreases, mortality increases, babies are born with lower body mass, children's learning and learning ability decreases. Very good iron sources are meat and meat products because they contain iron in hem form. The absorption of hem iron is not affected significantly by the condition of the food or its iron, but the absorption of non-hem iron is significantly influenced by the condition of the iron and other nutritional components. In recent years iron supplemented flour, rice, fish, soy sauce, corn, milk and dairy products have been produced.

21. Lack of iodine and its consequences

Iodine deficiency occurs throughout the world. In Europe, 60% of children suffered from iodine deficiency until the use of iodized sodium salts has spread in practice. Iodine is an essential component of thyroid hormones that are necessary for the development of nerve tissue and brain in intrauterine life and postnatal period. Inadequate iodine intake results in functional abnormalities, which are commonly called iodine deficiency disorders. Illness due to low iodine intake is exacerbated by the lack of selenium and iron because both microelements are necessary for the synthesis of thyroid hormones. The best solution to eliminate iodine deficiency is the use of iodized salt. In addition to iodized salt, iodized water, various iodized sauces and iodinated wheat flour can be a good iodine source for humans.

22. Vitamin A deficiency and its consequences

The vitamin A deficiency is responsible for the development of childhood blindness. About 500,000 children acquire blindness every year due to vitamin A deficiency and 50% of them die within one year. Vitamin A is needed for rhodopsin, the formation of the visual system, and retinoic acid, a metabolite of vitamin A, is required for growth and development, for the development of immune functions and for reproduction. In case of intake of large amounts of vitamin A, the excess amount, which is released from the diet is stored in the liver, and will be available to the body when needed. Milk and dairy products as well as the liver are the most important vitamin sources. The vitamin A content of cereals and legumes is low, so vitamin A deficiency is very common in those individuals who rely on grain and legumes in their diet. To satisfy vitamin A requirements, vitamin A supplementation of margarine, vegetable oils and cooking oils has been used for a long time.

23. Zinc deficiency and its consequences

Zinc is required for the function of about 100 enzymes that are involved in metabolism, growth, immune system, reproduction and the development of the nervous system. Zinc deficiency occurs primarily in cereal and leguminous nutrition, coupled with low meat, milk and dairy consumption. Supplementation of foods with zinc has not spread in practice.

24. Calcium deficiency and its consequences

Calcium deficiency occurs in populations worldwide where there are no traditions of milk and dairy consumption. The calcium concentration is well controlled in the body, and in case of deficiency, the body replaces the required amount from the bones. In children, inadequate calcium and vitamin D supply does not allow the development of strong bones, and later on this organism will be more prone to osteoporosis. In industrial countries 60-70% of the calcium requirement comes from milk and dairy products. Where the consumption of milk and dairy is minimal, calcium deficiency can be expected. Calcium absorption is closely related to optimal vitamin D intake, because where the vitamin D content of foods is low, disruption of calcium absorption can be expected. Foods enriched with calcium and vitamin D help the young organism reach the genetically determined maximum calcium content in the bone, which subsequently reduces the risk of osteoporosis.

Exercise 5.

25. Folic acid deficiency and its consequences

Folic acid deficiency can develop where foods are consumed from refined feedstocks or people do not consume enough leafy vegetables. Folates contribute to the synthesis of coenzyme A in the body as part of Vitamin folic acid in B complex, in the absence of which many biochemical processes stop in the body. Inadequate folic acid supply or disturbance of folic acid metabolism may result in increased number of infants born with open vertebrate column, megaloblastic anaemia, neurological degeneration, cancer and cardiovascular complaints. Supplementation of foodstuffs, especially flour, with folic acid, significantly reduced the occurrence of diseases previously attributed folic acid deficiency.

26. Supplementation of foods with vitamins I.

Vitamins are among the less stable food components. Stability changes from the type of vitamin; there are more stable (niacin) and are less stable ones (vitamin B_{12}). The stability of the vitamins is mostly influenced by temperature, moisture, oxygen, light, pH, oxidation or reduction components, heavy metal ions (copper, iron), sulphur dioxide, other vitamins or the combination of the aforementioned effects. Of these factors, most important are temperature, humidity, oxygen, pH and light. Particularly high vitamin losses can be expected, where substantial heat treatment is applied. Vitamin content may vary from time to time. Inspection authorities are always testing the component that is most sensitive to technology, warehousing and transport conditions. Adhering to the declared vitamin content is rather difficult because every vitamin is broken down in different ways and it is very difficult to declare how the vitamin composition of a product will change during its lifetime.

27. Supplementation of foods with vitamins II.

Vitamins may interact with one another and help each other to break down. Studies in fluid multivitamin preparations have shown that 13 vitamins have been identified to facilitate the breakdown of other vitamins by their interaction. The most important of these are ascorbic acid, thiamine, riboflavin, and cyano-cobalamin. Ascorbic acid increases the instability of folic acid and cyanocobalamin, thiamine has a similar effect on folic acid and cyanocobalamin and riboflavin increases the instability of thiamine, folic acid and ascorbic acid. Vitamins can reduce or increase the solubility of the other vitamins. Irradiation decreases the vitamin content of foods, the degree of this decrease is clearly related to the intensity of irradiation. Already at 3-10 kGy irradiation, in the presence of air, vitamin loss can occur, which increases with storage. The fat-soluble vitamins A, E, and K are sensitive to irradiation, of the water-soluble ones thiamine is the most sensitive, while niacin, riboflavin and the fat-soluble vitamin D are not sensitive to irradiation.

28. Supplementation of foods with polyphenols. Properties of polyphenols

Polyphenols, also known as flavonoids, are secondary metabolism products of plants, of which over six thousand have been identified to date. The hydroxyl groups attached to the phenolic ring make them an excellent antioxidant. The flavonoids can be divided into six groups according to their structure: flavonols, flavones, catechins, flavonones, anthocyanidins and isoflavones. They are antioxidants and can modify the activity of key enzymes, they have vasodilatatory, anti-cancerogenic, anti-inflammatory and immune-enhancing effects. Most important flavonoid sources include fruit juices, coffee, tea, red wine, onions, apples and berries, black currants and blueberries. The main flavonoids that occur in foods are catechin and catechin gallates, as well as quercetin and campherol, and their glycosides.

29. Technological aspects of making plant extracts

The possibility to use the extracts for production of functional foods depends on whether the plant has been used as a food traditionally and on the physiological and pharmacological properties of the active ingredient. Aqueous solvents that don’t alter the composition of the desired material are suitable for extraction. Plant extracts may contain hundreds of substances that may have synergistic effects. The added extract might change the colour and taste of the supplemented food, turn it brown or cause a bitter taste. For these reasons, the extract should always be checked before being added to food. The most popular vegetable extract is green tea followed by rooibos tea extract, and the cactus extract closes the line.

30. Supplementation of foods with carotenoids

Carotenoids form a large group of natural plant pigments. Their colour may vary from yellow to red in nature. Foods contain approximately 50 to 60 different carotenoids. β-Carotene is provitamin of vitamin A, of which the organism can synthesize two molecules of vitamin A with the carotinase enzyme. Supplementation of foods with β-carotene has a long history, for example fruit juices have long been complemented by β-carotene. Earlier carotenoids were used as natural food dye. Among the many carotenoids, β-carotene, α-carotene, β-cryptoxanthine, lutein and, which are not A-provitamins, zeaxanthin and lycopene are most important. Their health-protective effect is attributed to their outstanding antioxidant effect. β-Carotene, which can transform into two vitamin A molecules, lutein and zeaxanthin contribute to the healthy functioning of the eye, and lycopene helps prevent prostate cancer. The amount of added carotene is primarily determined by the achievement of the desired colour and health-related efficacy. Today, β-carotene and lycopene are widely used in the food industry to colour food. β-carotene is used in large quantities for margarines, butter, cheese, yoghurt and ice cream, in bakery products, soups, sauces, salads and sweets, and in particularly large quantities in the production of multivitamin drinks.

Exercise 6.

31. Supplementation of foods with essential fatty acids

Some lipids have been identified to have a health-promoting effect and sometimes are essential to the human body. The best known of these beneficial effects are plant steroids that have cholesterol-lowering effects or fatty acids that have anti-inflammatory effects. Plant steroids are highly cholesterol-related in terms of their chemical structure. Their most important representatives are sitosterol, campesterol and stigmasterol. Plant stanols are saturated plant sterols, as there is no double bond in the steroid ring. Plant steroids have no effect on HDL cholesterol, but as they improve LDL/HDL ratio, they have a health-protective effect.

32. Supplementation of foods with polyunsaturated fatty acids

The term polyunsaturated fatty acid (PUFA) is used for all fatty acids containing at least two unsaturated bonds. For humans, linoleic acid (C18:2) and linolenic acid (C18:3) are also essential because our body cannot produce them. The body produces substances from both fatty acids such as eicosanoids, which are hormone-like compounds and modulate the cardiovascular system, respiratory system, immune system and reproductive functions and play a key role in preventing inflammation. The synthesis of eicosanoids in the human body depends on the type of fatty acids available, so the ratio of the food ω-3/ω-6 determines the amounts of eicosanoids that can be produced from them. It is believed that the ratio of 4:1 ω-3/ω-6 is optimal for the human body, but in many countries, ratios between 7:1 and 14:1 were measured, which is far from the optimum values.

33. Supplementation of foods with essential fatty acids. Sources of polyunsaturated fatty acids

The optimal raw materials for supplementing with PUFA are the various vegetable oils such as, for example, the primrose-oil and flaxseed oil, characterized by a typical ω-3 / ω-6 ratio, and in particular the fish oil, which contains high amounts of eicosapentaene (C20: 5 n-3) and docosahexane (C22:6 n-3) fatty acids. Generally, vegetable oils contain many n-3 PUFA fatty acids in linoleic acid form. Since the same enzyme system further transforms linoleic acid and linolenic acid into longer chain unsaturated fatty acids, the two fatty acids are competitive inhibitors of each other, therefore only a small portion of linolenic acid is converted to eicosapentanoic acid (EPA) and arachidonic acid. The main sources of EPA and DHA (docosahexaenoic acid) are fish oils, which are "by-products" of fishmeal production. The fatty acid composition of fish oil depends on the composition of the feed consumed, and therefore the differences in the fatty acid composition of fish oils from different sites may also be present. EPA content of fish oils range from 5 to 18%, DHA content ranges from 6 to 13%. Sources of the n-3 PUFAs can also be various microorganisms, because these microbes are capable of synthesizing n-3 fatty acids having 20 or more than 20 carbon atoms. Sea microalga appears to be the best n-3 fatty acid source because they are able to accumulate long chain n-3 fatty acids in their bodies.

34. Supplementation of foods with essential fatty acids. Conjugated linoleic acids

Conjugated linoleic acids (CLA) also contain two double bonds, but they are in a conjugated position in the molecule. CLA reduces fat storage after meals, reduces the total amount of fat cells and increases fats' involvement in energy production processes. CLA also has an immunomodulatory effect that influences the immune response of cells to vaccines, influences the body's cytokine levels and thus may also play a role in treating inflammation. Among the many CLA isomers, cis-9, trans-11, and trans-10, cis-12 isomers have biological activity. Commercially available CLAs are made from safflower oil, containing both isomers at 50:50 percent.

35. Supplementation of foods with essential fatty acids I. Technological aspects of lipids

In CLA production, it is essential that isomers with biological activity be produced at the highest concentrations. Products containing many unsaturated fatty acids are very sensitive to oxidation, so often antioxidants are used in such compositions such as tocopherol blends, ascorbyl palmitate, rosemary extract or citric acid. Basic compounds of lipid oxidation are double bonded fatty acids. The more double bonds are in the molecule, the higher is its susceptibility to oxidation. Thus, DHA is five times more susceptible to oxidation than linoleic acid. Autooxidation is initiated by initiators, resulting in free radicals localized on carbon atoms from unsaturated fatty acids. These, after further degradation, form volatile or non-volatile secondary decomposition products.

36. Supplementation of foods with essential fatty acids I. Volatile components, light sensitive compounds, artificial and natural antioxidants

Volatile components include aldehydes, ketones and alcohols, hydrocarbons and those alcohols that are responsible for the formation of odour and taste. Photooxidation is a non-radical reaction that leads to the formation of hydroperoxides and volatile components, such as those produced by radical reactions. Light sensitizing compounds in foods are chlorophyll, riboflavin and hem-proteins. Lipid oxidation may in some cases be prevented by the administration of antioxidants. Primary antioxidants are also called free root binders because they neutralize free radicals to stop radical reactions. Phenolic compounds are for example, BHA (butylhydroxyanisole), BHT (butylhydroxytoluene) and propyl gallate, and natural antioxidants include tocopherols and vegetable polyphenols.

Exercise 7.

37. Supplementation of foods with biologically active lipids

Long chain PUFA, CLA, is usually formulated in esterified form after deodorization. It is always advisable to add emulsifiers to the oils, which increase dispersion, provide stability to the food, preventing the separation of the various phases. Generally, homogenization is also interspersed due to the degree of dispersion and stability required. To facilitate the addition of functional lipid components, various spray-dried products were produced which can easily and homogeneously be mixed in an aqueous medium. Sterols of the original state are difficult to disperse evenly in the aqueous medium because these compounds are highly hydrophobic and immediately separate from the aqueous medium after simple mixing.

38. Examples of increasing sterols, CLAs and PUFAs in foods

In the United States, drinks and cereals complemented with sterols are currently the most popular. The one-cup milk-based drinks are popular with a plant sterol content of 2-3 grams in 100 grams of liquid and people love yogurt, milk, milk powder and spreadable cheese with herbal sterol. Recently, infant formulas and food products for older people have been supplemented with PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition to milk and milk products, many so called Omega-breads are commercially available, with PUFA concentrations averaging 80 mg/100 g.

39. Flavonoids as functional food components

The basic structure of the flavonoids, aglycone, is linked to a sugar molecule, so flavonoids are in fact glycosides. Flavonoids are derivatives of 1,3-diphenylpropane, isoflavonoids are derivatives of 1,2-diphenylpropane and neoflavonoids are 1,1-diphenylpropane derivatives, which include anthocyanide, cyanidine, anthocyanin and cyanine. Secondary metabolic products of plant metabolism are found mainly in the skins, seeds and stems of the fruits. As pigment-forming substances, they play a role in protecting against UV light and microorganisms and other plant pests, regulating enzyme reactions, and they have also signalling functions for nitrogen-binding bacteria. As a food ingredient in plant materials, they are colouring agents, flavour components and antioxidants.

40. Flavonoids as functional food components. Classification of flavonoids

Typical representatives of the flavonol group are the catechins, the proanthocyanidins include oligomeric catechins, flavones are the quercetin and campherols, the biflavones are represented by the amentoflavone and bilobetine, the flavonones include the hersperidine and naringin, typical flavononol is the taxifolin, anthocyanins include the cyanidine, delphidine, malvidin and petunidin, the silmarin is a flavonolignane and isoflavones are the genistein and diadene.

41. Flavonoids as functional food components. Intake of flavonoids with food

In 2001 adults in Hungary consumed 18.8 mg/person/day (0.5-309.7 mg) and children consumed 19.5 mg/person day (0-179.3 mg) of flavonoids while the recommended total dose is 1000 mg/person/day. It can be concluded from the above data that the consumption of vegetables and fruit in Hungary is far from below the desirable level.

42. Absorption and metabolism of flavonoids

The absorption of flavonoids depends on the chemical structure, molecular size, polymerization, glycosidation and solubility. Badly absorbed, only 0.2-0.5% of all consumed quantities are utilized in the body. They are easily adsorbed, transformed by decarboxylation, demethylation, by the saturation of double bonds, and the aglycons are absorbed through the small intestine. Glycosides have to be hydrolysed prior to absorption, but the human body lacks the β-glucosidase enzyme, therefore the microflora of the colon hydrolyzes the glycosides, after which the metabolites pass through the blood into the liver where methylation and sulphonation occur, and the derivatives reach the kidneys with the blood where they are excreted in the urine.

Exercise 8.

43. Biochemical properties of flavonoids

The most important biochemical properties of flavonoids include antioxidant activity with free radical capture, anti-inflammatory, anti-asthma and antiallergic activity, modification of enzyme activation, usually inhibition, antiviral, antibacterial effect, estrogen activity, influencing mutagenetic and carcinogenetic effects, hepatoprotective effect and they affect even the function of the blood vessel system. According to our present knowledge, cancer cannot be prevented by any nutrition or nutritional supplements, but the risk of cancer is lower among those who consume a lot of vegetables and fruits.

44. Lower occurrence of cardiovascular diseases and the flavonoid content of red wine consumed

In Mediterranean countries, the lower occurrence of cardiovascular diseases are believed to be due to the flavonoid content of red wine consumed. Flavonoids reduce fibrinogen and elevate plasminogen concentrations, increase protective HDL, while simultaneously reducing harmful LDL levels. Red wine is a functional food because the flavonoids in the aqueous, alcoholic liquids are absorbed more efficiently, but most likely not only the red wine but also the lifestyle and genetic differences cause the absence of the disease.

45. Connection between the flavonoids consumption and osteoporosis and osteogenic diseases

Other beneficial effects of flavonoids have also been established: in case of osteoporosis and osteogenic diseases they restore the physiological metabolism of bones, increase insulin production in people with diabetes, affect oestrogen production in gynecological problems, play a role in the prevention of Alzheimer's disease, promote absorption of drugs (medicines), and quercetin inhibits the xanthinoxidase enzyme required for uric acid formation.

46. Medicinal products with high flavonoid content

Functional foods may have high flavonoid content, and there are medicines and preparations such as Rutascorbin, which strengthens the walls of the capillary blood wessels, the liver protector Legalon, medicines containing visual enhancement anthocyanins, and a large number of therapeutic and functional preparations, cosmetics and dietary supplements, contain flavonoids.

47. Summary of the functional foods I.

In terms of functional foods, food production was about to create a new area of knowledge, which is now being developed and accepted by consumers, and producers and consumers will jointly guide the processes that, in a few years, will become part of the traditional food production. In a brief statement, of course, we could only touch the most important knowledge concerning functional foods, including the definitions of what kind of food would be functional, what are the physiological effects of functional foods, how to produce functional foods, what consumer expectations are for functional foods, and we were briefly dealing with food safety and legal regulation.

48. Summary of the functional foods II.

Increasing the proportion of functional components of food includes substitution, enrichment and supplementation. More specifically, it is about supplementing foods with vitamins and minerals, enriching them with polyphenols and essential and health-promoting fatty acids. The scope of this study does not allow including the bioactive components of the milk, their recovery, enrichment and production, bioactive proteins, lipids and carbohydrates, prebiotics, probiotics and synbiotics, and functional or potentially functional foods such as meat, eggs, soy, various cereals, vegetables and fruits, nutritional germs, and red wine. The main purpose of this study was to make readers aware of the importance of functional foods and their role in healthy nutrition.

Exercise 9.

49. Casework for production of prebiotics via reactions involving lactose as well as malic acid and citric acid

Prebiotics are non-digestible polysaccharides and oligosaccharides, which, upon reaching the colon, inhibit the harmful bacteria and promote the growth of bifidobacteria and lactic acid bacteria. In 2004 outlined the conditions required for a nutrient to have prebiotic qualities. These are as follows: they should resist gastric acid and the pepsin found therein, mammalian enzymes should not break them down in the gastrointestinal tract, and they should serve as nutrients for those bacteria in the gastrointestinal tract that contribute with their metabolites to humans' well-being and health. A great number of food ingredients meet these criteria. They are inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), lactulose, and polydextrose, whereas isomalto-oligosaccharides, xylo-oligosaccharides (XOS), and lactitol were grouped in the category of emerging, potential prebiotics.

Solution of the problem

Objectives and used materials. Relying on literature results we aimed at producing prebiotics, during which we established such linkages between lactose and malic acid as well as between lactose and citric acid that can resist acidic medium and the attack of carbohydrate-degrading enzymes in the human stomach and the forward section of the gastrointestinal tract and that get into the colon, where they function as nutrients for the probiotic microorganisms settled therein.

Our experiments were performed with pharmaceutical-grade lactose, citric acid, and malic acid. The malic acid we used had a purity of 95%, containing less than 1% of fumaric acid and less than 0.05% of malonic acid. The citric acid used for our experiments was also food-grade, or, even better, pharmaceutical-grade citric acid monohydrate. Its citric acid monohydrate content is nearly 100%, it has a maximum water content of 8.8% and an oxalic acid content of less than 100 mg/kg.

The lactose used in our experiments was a 95% pure, food-grade, finely pulverized D(+)-lactose 1-hydrate, isolated from bovine milk and spray-dried produced.

Engineering the reactions between malic acid and citric acid as well as lactose. In the first step, we added 20% of citric acid, while in the second step the same percentage of malic acid to the pharmacy-quality lactose. Following a thorough examination of the literature and the patents at our disposal, we ascertained that most reactions were performed at a temperature between 130 and

180 °C – therefore, we opted for 170 °C. The samples were blended in a mortar, ensuring their maximum homogeneity. Following this, the samples were distributed in quantities of approx. 10 g into glass vessels and heat treated for 5-10-20-30-40-50-60 minutes; after cooling down, the lactose content of the samples was determined.

The following experiment investigated into the effect of temperature on the reaction between citric acid and malic acid as well as lactose. In the first stage, the samples containing 20% citric acid, 20% malic acid, and 80% lactose were treated at 130 °C for 30 minutes, in the second stage at 140 °C, in the third stage at 150 °C, in the fourth stage at 160 °C, while in the fifth stage at 170 °C. After cooling down, the lactose content of the samples was determined.

Results and conclusions. By adding 20% of citric acid to the lactose and performing a heat treatment of 170 °C for various times, we established that upon heat treatment the initial white mixture changed to yellow in 5 minutes and turned to a brownish colour in 10 minutes, after which only its colour became increasingly darker, but its volume remained practically unaltered.

By adding 20% of malic acid to the lactose and performing heat treatment for various times, similarly to the case of citric acid, we found sample colour almost unchanged in 5 minutes' time, within ten minutes, it took on a somewhat yellowish colour, in 20 minutes, it turned yellowish brown, and then it became increasingly swollen, while the last sample changed to dark brown.

Upon a 30-minute heat treatment at 130 °C, the samples practically maintained their white colour; at 140 °C, both the samples containing citric acid and those containing malic acid changed to yellow; at 150 °C, this yellowing further intensified in both carboxyl acids; at 160 °C, the sample with citric acid as well as the one with malic acid took on a deeply brown colour; at 170 °C, the sample with citric acid formed a brown-coloured mass, just as the sample containing malic acid, which, however showed a less brownish discolouration.

Time dependence, temperature dependence. The determination of lactose content performed with the use of 24 samples yielded the following results. In the first experiment, 20 g of citric acid was added to 80 g of lactose, while in the second experiment 20 g of malic acid was admixed with 80 g of lactose, thereafter heat treatment was carried out at 170 °C for a duration of 5, 10, 20, 30, 40, 50, and 60 minutes. A subsequent experiment investigated heat dependency, treating the above listed samples (citric acid, malic acid) at 130, 140, 150, 160, and 170 °C for 30 minutes. This time, we tried to identify the optimal temperature at which an adequate reaction can be produced between lactose and the various added carboxylic acids.

In the first case, we measured 79.1% from the control sample (a heat-treated mixture of erythrite and lactose), which in fact gives us the theoretical value. Exposing the lactose with citric acid to heat treatment for 5 minutes, its quantity decreased to 73.6%, while upon a 60-minute heat treatment it decreased to 7.1%. Therefore, it seems that with the application of citric acid 93% of the lactose transformed into some kind of oligomeric or polymeric compound.

In the second experiment, we measured a 70.6% lactose content for the sample containing malic acid and heat treated for 5 minutes, whereas the sample heat-treated for 60 minutes had a lactose content of 16.4%, meaning that 83–84% of the lactose transformed into some sort of product during those 60 minutes of heat treatment. Therefore, we may conclude that both malic acid and citric acid are suitable for forming oligomers or polymers with lactose.

What conclusions can be drawn from these investigations? First of all, we can point out that most of the lactose did neither disappear nor degrade nor get damaged but got transformed into an oligomer or polymer that manifests Fehling's reaction to a minimum extent. However, when we used hydrochloric acid to transform the oligomers and polymers into mono- or disaccharides, the resulting sugar-like substances (most probably, glucose and galactose for the most part and lactose to a lesser extent) did manifest Fehling's reaction and could be determined as total sugar.

Enzymatic treatment of the 'obtained' prebiotic with *amylase.* The same samples were hydrolysed with *amylase* as well, thus modelling the reactions taking place in the forward section of the gastrointestinal tract. Following hydrolysis with *amylase*, total sugar content remained practically unchanged, meaning that *amylase* did not react naturally with disaccharide lactose and was not able to split the oligo- and polysaccharide derivatives. This way, the not yet identified, presumably oligomer or polymer product has all the conditions to become a prebiotic, that is, it is not degraded in the forward section of the gastrointestinal tract and most probably gets into the colon, where it can serve as a nutrient for the probiotics found there.

Exercise 10.

50. Casework for production of high-lysine-content biscuit and examination of the absorption of lysine in humans

Lysine (also known as α,ε-diamino caproic acid) is one of the twenty protein-building amino acids, perhaps the most important essential amino acid, which also plays the role of limiting amino acid in most plant foods (except for leguminous plants), since compared to human needs it can be found in the smallest proportion. It is essential for humans and all farmed animals; the daily human need is 1.0–1.5 g, which can be satisfied only with the combined consumption of appropriate animal and plant proteins.

Besides its many biological functions, we must highlight that lysine takes an active share in treating the symptoms of the herpes simplex virus (HSV). To make use of this functionality, our intention was to introduce a significant amount of lysine into the human organism to monitor through our experiment how it becomes effective and gets absorbed, whether it has therapeutic benefits, and if so, then to have a look at its efficiency in preventing the development of herpes as well as in the treatment of the already developed disease.

Solution of the problem

Objectives and used materials. Lysine supplementation can be extremely useful in the prevention of many diseases as well as in their treatment in case they have already developed. The question arises, then, as to in what form lysine should be introduced into the organism. We have decided to deliver lysine into the organism in the form of biscuit and examine how lysine is absorbed in the human body and how it increases the free lysine content of the blood serum. Our secondary aim was to look into how the duration and temperature of baking influences the utilization of lysine in the body.

The product under study. For our study, we made use of the *"X"* biscuit, previously prepared with lysine supplementation, having the following major properties: lemon-flavoured calcium source, increased lysine content, and tested antioxidant activity – in the production of the *"YYY Ltd"*. The product contained the following ingredients: 63.4% wheat flour, vegetable fats, sugar, isosugar, whey powder, 3.8% L-lysine hydrochloride, gluten, 0.8% calcium carbonate, aromas, raising agents, antioxidants, tartaric acid, soya lecithin as emulsifier, salt, and it may contain traces of nuts, peanuts, and egg-powder. Biscuit ingredients per 100 g of product were: caloric value:

1,955 kJ, protein: 8.9 g, carbohydrate: 67.6 g – containing sugar: 21.1 g, fat: 17.5 g – containing saturated fatty acids: 8.2 g, dietary fibre: 0.2 g, sodium: 17 mg, calcium: 400 mg, L-lysine: 3,000 mg – containing free L-lysine: 2,300 mg, antioxidant activity: 76.5 mg vitamin C equivalent determined with FRAP method.

Baking time and temperature combinations in the production of biscuit.

Since the baking process of biscuit takes up 30 minutes on average, we did not change the baking time, only the temperature in the production of the biscuit. Baking was performed at 120, 130, 140, 150, 160, 170, and 180 ºC, each time followed by an examination of the biscuit composition – more specifically its free amino acid and total amino acid content, paying particular attention to free lysine – and its antioxidant activity, determined with FRAP method and expressed in vitamin C units (mg/kg).

The study protocol of lysine absorption. Six participants consumed six 100-g biscuits per head, each portion containing 333 mg of lysine hydrochloride; thus, the total lysine consumption amounted to 2,000 mg at the beginning of the experiment. At the same time, two further participants consumed six biscuits likewise, but without lysine supplementation – they formed the control group. Blood was drawn and the composition of these blood samples was determined in the members of both groups immediately before consuming the biscuits and then after 15, 30, 60, 120, 180, and 240 minutes. Following centrifugation, blood plasma was divided into three parts, and its antioxidant, lysine, and calcium content was determined.

Results. During the tests we examined the changes in the free amino acid content of the blood serum of control and experimental individuals after consumption of high-lysine-content biscuits. We opted for a baking temperature of the biscuit (130 ºC) with a view to leaving the greater part of lysine in free form and converting only a smaller part (20–25%) through the Maillard reaction. By changing baking temperature between 120 and 180 ºC, we were able to change the concentration and ratio of free lysine and of the Maillard reaction products, but since our aim was to increase the lysine level in the blood serum we kept to a temperature of 130 ºC. We set the free lysine content of the biscuit to 3% so that the 5-g biscuit would contain 100 mg of lysine: this way, we could work out that a daily consumption of 10–30 biscuits would secure the necessary amount of lysine intake in order to achieve the desired therapeutic effect. 30–60 minutes after the consumption of 2,000 mg of free lysine, the free lysine content of the experimental group's blood serum showed a significant increase of 41–46% in comparison with the initial value, yielding a 20% higher concentration in relation to the initial measurement even after three hours have passed following consumption. After the consumption of control biscuits, the free lysine concentration increased in the control subjects' serum, but this was not a significant change.

The free arginine content of the blood serum did not change either in control or in experimental subjects following the consumption of the control and the high-lysine-content biscuit respectively. As a consequence, the free lysine/free arginine ratio in the blood serum of the individuals consuming lysine increased significantly compared to the initial value and to the control group alike.

The antioxidant level of the control subjects' blood serum has remained virtually unaffected by the consumption of control biscuits, whereas in the case of experimental individuals consuming high-lysine-content biscuits this level has increased by 40–45% in relation to the initial value.

In summary, we may conclude that after the consumption of biscuits with 2,000 mg of free lysine the concentration of free lysine in the blood serum, its free lysine/free arginine ratio and antioxidant level increased significantly. Our researches have clearly demonstrated that the active substances of the biscuit got into the blood serum, so the investigation of the active substance and the evaluation of the physiological effects are definitely recommended in the long run.

Exercise 11.

51. Casework for production of highly nutritious functional food with the supplementation of wheat flour with lysine

Nowadays, there is a growing interest in functional foods with therapeutic effects. In several countries of the developed world, protein deficiency is a prevailing factor due to the predominance of low-protein plant-based diet. Protein deficiency may lead to growth retardation/delayed growth, oedema formation, and anaemia, whereas in case it is combined with energy deficiency the resulting malnutrition may cause death in many infants and young children. As a result of researches carried out in the past decades, today's subject matter is not the protein needs in general but the specific quantitative requirements in terms of the essential amino acids. It has also been revealed that we must not only cover the deficiencies in limiting amino acids but we must also aim at achieving a balanced ratio of essential amino acids. Moreover, we must also pay close attention to obtaining an optimal ratio of essential/non-essential amino acids in our foods. Once the industry-like production of amino acids has started, green light is given for amino acid supplementation, during which growth will approximate the optimal value.

Enhancing the nutritional value of wheat flour and wheat-flour-based products. The protein needs of an adult person is 80–110 g/day depending on age and the exerted physical effort. In the case of a mixed diet, this amount of protein contains sufficient essential amino acids, but a one-sided diet may result in essential amino acid deficiency even if combined with adequate protein consumption. Plant-based proteins, however, lack lysine, methionine, threonine, and tryptophan to varying degrees. Primarily, they used to fortify wheat-flour-based products – the efficiency of this protein supplementation is examined with the help of biological or chemical verification methods.

Solution of the problem

Objectives and used materials. The use of lysine in flour enrichment. The amount and ratio of amino acids in wheat is far from the optimal values necessary for the human organism, giving the nutritional value of wheat protein a score of approx. 53 on a scale of 100. Since there is a low proportion of lysine first of all, the high-lysine-content materials are the most suitable for the amino acid enrichment of wheat flour (potato (biological value: 73%) and soy (biological value: 74–78%)).

Aims of the experiments is to produce a lysine-fortified bread as a functional food which helps in treating the symptomatic herpes simplex virus and has beneficial effects in the treatment of osteoporosis and other diseases of the circulatory system. We expect that adding a right dosage of lysine – according to our previous researches and preliminary estimates: 0.5–2% – to wheat flour will increase the amount of essential (and, in the case of wheat flour, limiting) amino acids and that adding an amount higher than necessary for increasing the biological value will result in such a functional, health-protective and health-preserving product that will help eliminate the damages caused by the herpes virus in humans.

Combination of the basic ingredients for bread and the baking process. Using any amount of lysine under 0.5% should be avoided in order to achieve the desired therapeutic effect, while an amount of lysine exceeding 2% may significantly spoil the product's palatability traits, as according to our pre-experiments, since the large amount of Maillard reaction products may come with unwanted flavour and colour effects.

L-lysine was added to the flour in the form of L-lysine-hydrochloride, in a solid, powdery state, and then mixed with the other ingredients and water, in accordance with bread baking technology requirements. To determine the optimal amount of lysine, it was added to the wheat flour in 0.5, 1, 1.5, and 2% concentrations, followed by the examination of all properties of the obtained breads, in compliance with the standard practical procedures used in the qualification of bread.

Total protein content of the bread. We measured the following amounts of protein content: control bread – 8.86±0.18%, bread made from 0.5% lysine-enriched flour – 9.20±0.065%, bread made from 1% lysine-enriched flour – 9.24±0.038%, bread made from 1.5% lysine-enriched flour – 9.67±0.037, and bread made from 2% lysine-enriched flour – 10.14±0.023%.

Lysine content of the bread. We measured the following amounts of lysine content: flour – 0.32±0.05, control bread – 0.38±0.06, bread made from 0.5% lysine-enriched wheat flour – 0.48±0.12, bread made from 1% lysine-enriched wheat flour – 0.67±0.14, bread made from 1.5% lysine-enriched wheat flour – 0.95±0.13, and bread made from 2% lysine-enriched wheat flour – 1.11±0.13%.

Lysine loss can be explained by the lysine transformation (Maillard reaction) that took place in the breadcrumb, but mostly in the crust during baking. This loss is most striking in the case of the bread made from 2% lysine-enriched wheat flour, where we measured an amount much less than expected.

Sensory analysis results. According to sensory analysis results, 1.5% lysine supplementation enhances the palatability traits of the bread, namely its taste and colour, but it also affects the development of its internal content in a positive way. Performing the sensory analysis, we concluded that the enrichment of wheat flour with 0.5–1.5% lysine either improved sensory properties or did not change them at all as compared to control bread. The breads cannot be enriched with a proportion exceeding 1.5% since that would increase the concentration of the Maillard reaction products, which has a negative influence on the taste factor.

Conclusions. Besides taste, bread-crust gains a much darker shade, which is undesirable for customers. The hydroxyl-methyl-furfural content of the bread made from 2% lysine-enriched flour is much higher than that of the breads with a lower amount of lysine supplementation – therefore, we put down the appearance of the strong bitter taste to this fact.

Exercise 12.

52. Casework for production of a high-nutritional-value functional food, with the supplementation of the wheat flour with high-protein-content raw food materials

In many countries of the developing world, the population is often grappling with protein deficiency due to the prevalence of low-protein-content plant foods. Protein deficiency can lead to growth retardation, oedema formation, and anaemia; if protein deficiency is coupled with lack of energy, malnutrition may cause the death of many infants and young children.

Once the industry-like production of amino acids had started, green light was given for amino acid supplementation, as a consequence of which a nearly optimal amino acid composition can be obtained, one of the methods leading up to producing protein of high biological value. Due to the widespread application of amino acid analysis, the amino acid composition of many food ingredients has become common knowledge, and by combining them we can again produce foods, such as bread, with optimal ingredients – yet another way of producing foods of high biological value.

Enhancing the nutritional value of wheat flour and wheat-flour-based products. Proteins that contain all essential amino acids in a sufficient amount and in an adequate proportion are called complete proteins. Such are the proteins of meat, egg, and milk. Plant-based proteins, however – compared to the needs –, lack lysine, methionine, threonine, and tryptophan to varying degrees. The supplementation would be most needed in the case of cereal-based foodstuffs because the proteins of wheat and rye contain only small amounts of lysine, methionine, and threonine.

In Europe, people usually tend to opt for a supplementation with natural protein resources, for which the various soy-based products are the most suitable as soy protein contains a large amount of lysine and is stocked up with threonine above the average level. Its disadvantage is, however, the relatively low methionine and cystine content. Primarily, they used to fortify wheat-flour-based products. The high lysine and threonine content of soya bean is an excellent supplement to the amino acid composition of the wheat flour, but an even better result can be obtained with a reasonably assorted protein supplementation, just as it happened in the case of our bread, where, besides soy protein, egg-white powder, gluten isolate, and dried yeast supplementation also took place. Due to protein supplementation, the carbohydrate content of wheat flour decreased by half, making this type of bread perfectly suited for the diet of diabetics and those who wish to lose weight.

Solution of the problem

Objectives and used materials. The aim of our research is to produce a type of bread as a functional food, fortified with lysine and other essential amino acids, which helps in satisfying the organism's optimal essential amino acid needs and has beneficial effects in the treatment of osteoporosis and other diseases of the circulatory system. We expect that adding a right dosage of extracted soya bean meal, egg-white powder, gluten, and dried yeast to wheat flour will increase the amount of essential (and, in the case of wheat flour, limiting) lysine as well as the biological value of wheat protein, whereas that adding an amount higher than necessary for increasing the biological value will result in such a functional, health-protective, and health-preserving product that will help prevent diseases caused by amino acid deficiency, such as weakness of the bones or osteoporosis.

Composition of the basic ingredients for bread and the baking process

The raw mixture was composed of wheat flour of 12–13% protein content, egg-white powder of 83% protein content, gluten of 79.4% protein content, extracted soya bean flour of 49.3% protein content, dried yeast of 16% protein content, and the practically protein-free bamboo fibre.

Results and conclusions

Protein content and amino acid composition of bread ingredients. Based on measurement data, we can establish that the flour is a flour mixture of high protein and fibre content and reduced carbohydrate content, produced from natural ingredients, being sufficient of itself to be used in the preparation of various products with reduced carbohydrate content (e.g. breads, bakery products, desserts, pastries, muffins, etc.). It might be a convenient solution for those who do not like to experiment with the proportions. It contains fine-ground wheat flour, defatted soya bean flour, wheat gluten, dried yeast, bamboo fibre, and soya protein isolate. According to official data, its caloric value is 1364 kJ/100 g, its fat content 1.4%, of which the saturated fatty acid less than 0.5%, its carbohydrate content 35.4%, of which sugar 2.8%, starch 32.6%, fibre 14.4%, protein 35.0%, and salt 0.125%. Overall, it can be said this is a natural food ingredient with a protein content almost three times the fine-ground flour and a carbohydrate content decreased by half.

Total protein and essential amino acid composition content of the breads

We measured the following amounts of protein content: control bread – 12.4±0.18%, fortified bread – 35,2±0,26%. Results immediately make it clear that due to the added extracted soya bean flour, egg-white powder, and gluten, the protein content of the fortified bread has increased 2.86 times compared to the control bread. This increase was an expected outcome as all of the additives had a higher protein content than the wheat flour.

Wheat flour and gluten contained the smallest amounts of essential amino acids among the applied ingredients, yielding therefore the lowest biological value for the protein of wheat flour (53) and gluten (47). The high lysine and threonine content of the extracted soya bean meal is in line with expectations, but its sulphur amino acid content is rather moderate, and so the biological value of the protein is 75. Due to its outstanding essential amino acid content, egg white disposes of the highest biological value (98), and thanks to its high methionine and cystine content it serves as an excellent supplement to the low levels of sulphur amino acids in the soya and wheat protein as well as in gluten. The high lysine and, especially, threonine content of egg white also contributes to the high biological value of the fortified bread for which it serves as a supplement. Owing to its low lysine, threonine, and sulphur amino acid content, gluten has the lowest biological value.

The biological values are as follows: gluten – 47, wheat flour – 53, soya bean meal – 75, and egg-white powder – 98. The biological value of the control bread was measured at 61 and that of the fortified bread at 75. Overall, the wheat protein's biological value of 53 could be raised to 75 with the help of extracted soya bean meal, egg-white powder, and gluten supplementation, which is a more than 40% increase. (In all cases discussed, data refer to the protein.)

Comparing the protein content of the LysBread and the amino acid composition of the protein with data, we may state that 3–4 slices of LysBread can cover most part (60–70%) of an adult's daily essential amino acid requirements.

Exercise 13.

53. Casework for calculating the biological value of the protein of a functional food

Determination of protein quality starts with determining protein content, followed by determining the amino acid composition of the protein. Data on amino acid composition let us determine the limiting amino acid of the protein as compared to the amino acid composition of the reference protein made according to the FAO/WHO recommendations. The amino acid occurring in the lowest percentage is the limiting amino acid, whose numerical value is the chemical index (CS). Besides CS, other indices can also be calculated from the amino acid composition if we consider the rest of the essential and non-essential amino acids as well.

Nowadays, during the determination of biological value in human terms, many tend to use the Morup and Olesen biological value calculation method, who, in 1976, based on their experiments carried out with young male university students, experienced the highest rate of nitrogen retention when the experimental subjects were consuming a mixture made up of 66% potato and 34% whole egg, which in our country is mostly similar to layered potato casserole of a very good quality, prepared without sausages. They elaborated the following equation for the calculation of biological value:

$$\text{Biological value} = 10^{2.15} \cdot q_{Lys}^{0.41} \cdot q_{Arom}^{0.60} \cdot q_{Sulf}^{0.77} \cdot q_{Thr}^{2.41} \cdot q_{Trp}^{0.21},$$

where: $q = a/a_{ref}$,

a = a particular essential amino acid/all essential amino acids in the protein under examination,

a_{ref} = a particular essential amino acid/all essential amino acids in the reference protein,

Arom = aromatic amino acids (Tyr, Phe),

Sulf = sulphur-containing amino acids (Cys, Met).

According to this, the biological value of the reference protein is $10^{2.15} \cdot 1 \cdot 1 \cdot 1 \cdot 1 \cdot 1$, that is = 141.25. When calculating the biological value, aromatic amino acids (phenylalanine + tyrosine) and sulphur-containing amino acids (methionine + cystine) were evaluated together besides lysine, threonine, and tryptophan. Among amino acids, tryptophan figures on the 0.21 power, and thus it has the lowest influence on biological values, while threonine figures on the 2.41 power, having the highest influence on biological value. As for the rest of the amino acids, this index varies between 0.41 and 0.77, thus exerting by and large a similar degree of influence on biological value.

Exercise 14.

54. Casework for determination of essential amino acid composition and biological value of the control and the LysBread

Prior to determining the essential amino acids, the proteins of the breads were hydrolysed with 6M HCl at 110 ºC for 24 hrs, and then, after proper dilution and derivatization, 20 µl of hydrolysate was injected into the high-performance liquid chromatography. In the determination of tryptophan, protein was hydrolysed with 4M sodium hydroxide at 110 ºC for 24 hrs, and then we determined tryptophan content using once again the high-performance liquid chromatography method. The next table includes the essential amino acid content of wheat flour, extracted soya bean meal, egg-white powder, gluten, control bread, and the bread fortified with lysine (LysBread) as well as the biological value of the protein.

Essential amino acid content of wheat flour, extracted soya bean meal, egg-white powder, gluten, control bread, and the LysBread bread as well as the biological value of the protein

Amino acid	Amino acid, mg/g protein						
	Wheat flour	**Extracted soya bean meal**	**Egg-white powder**	**Gluten**	**Control bread**	**LysBread 1**	**LysBread 2**
Histidine	23	32	22	18	21	27	26
Isoleucine	40	41	54	41	40	44	43
Leucine	71	77	86	72	71	78	79
Lysine	30	61	70	23	26	46	47
Methionine + cystine	41	31	57	35	38	41	41
Phenylalanine + tyrosine	78	88	93	78	77	87	86
Threonine	32	40	47	28	30	38	39
Tryptophan	12	16	17	11	11	15	14
Valine	48	47	66	46	49	52	51
Total							
with histidine	375	433	512	352	363	428	426
without histidine	352	401	490	334	342	401	400
Biological value*	53	75	98	47	51	74.8	74.7

* Calculated according to Morup and Olesen's (1976) method.

As it can be seen from the table, wheat flour and gluten contain the smallest amounts of essential amino acids among the applied ingredients, yielding therefore the lowest biological value for the protein of wheat flour (53) and gluten (47). The high lysine and threonine content of the extracted soya bean meal is in line with expectations, but its sulphur amino acid content is rather moderate, and so the biological value of the protein is 75. Due to its outstanding essential amino acid content, egg white disposes of the highest biological value (98), and thanks to its high methionine and cystine content it serves as an excellent supplement to the low levels of sulphur amino acids in the soya and wheat protein as well as in gluten. The high lysine and, especially, threonine content of egg white also contributes to the high biological value of the LysBread for which it serves as a supplement. Owing to its low lysine, threonine, and sulphur amino acid content, gluten has the lowest biological value.

As a consequence of the amino acid composition of the additives used during dough preparation and due to the baking processes, the control bread has practically the same level of amino acid content as wheat flour, but because of a minimal loss of lysine, methionine, and cystine, its biological value is slightly lower.

The table shows data on the LysBread from the two different batches. Since data practically coincide, the two measurement results can be evaluated together. Due to the high histidine content of the extracted soya bean meal, the histidine content of the protein in the LysBread is slightly higher (27 mg/g) than that of the control bread. It has a slightly higher isoleucine content, which is a consequence of the egg white's higher isoleucine content. The leucine content is again a higher one as both soya protein and egg white have a higher leucine content than wheat protein. It has an approx. 80% higher lysine content than the control bread owing to the higher lysine content of the soya bean and egg white. Despite supplementation, its sulphur amino acid content (methionine, cystine) is not higher than that of the control bread due to the low sulphur amino acid content of the soya bean, which is, however, compensated by the sulphur amino acid content of the egg white, being well above the required level. On the whole, the sulphur amino acid content of the protein in the control bread and the LysBread may be considered identical.

The protein of the LysBread has a more than 10% higher aromatic amino acid (tyrosine, phenylalanine) content, while there is an even bigger difference, of almost 30%, in the threonine content. This 30% difference can also be observed in the tryptophan content, but the valine content of the two proteins shows only a slight difference in favour of the LysBread protein. (Regarding all amino acids, evaluation refers to the protein and the amount of amino acids. As the LysBread contains protein in an amount of almost three times the control bread, the sulphur amino acids are also present in the LysBread in a concentration almost three times higher, while in regard to the rest of the amino acids this difference can be even four- of fivefold.)

Using Morup and Olesen's (1976) method in our calculation, the biological values are as follows: gluten – 47, wheat flour – 53, soya bean meal – 75, and egg-white powder – 98. The biological value of the control bread was measured at 61 and that of the LysBread at 75. Overall, the wheat protein's biological value of 53 could be raised to 75 with the help of extracted soya bean meal, egg-white powder, and gluten supplementation, which is a more than 40% increase. (In all cases discussed, data refer to the protein.)

Comparing the protein content of the LysBread bread and the amino acid composition of the protein with data shown in tables 1–3, we may state that 3–4 slices of LysBread can cover most part (60–70%) of an adult's daily essential amino acid requirements.