Biscuits and Biotechnology

UNIT 3

European Initiative for Biotechnology Education

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EIBE biotechnology

UNIT 3

European Initiative for Biotechnology Education

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World Wide Web

Few areas are developing as rapidly as biotechnology. So that they can be revised and kept up-to-date then distributed at minimum cost, the EIBE Units are published electronically.

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⚠ Safety

In all of the EIBE Units, we have tried to check that all recognized hazards have been identified and that suitable precautions are suggested.

Where possible, the proposed procedures are in accordance with commonly-adopted general risk assessments. If a special risk assessment may be necessary, this has been indicated.

However, users should be aware that errors and omissions can be made, and that different employers and educational authorities adopt different standards. Therefore, before doing any activity, users should *always* carry out their own risk assessment. In particular, any local rules issued by employers or educational authorities MUST be obeyed, whatever is suggested in the EIBE Unit.

Unless the context dictates otherwise, it is assumed that:

- practical work is carried out in a properly equipped and maintained science laboratory;
- any mains-operated equipment is properly maintained;
- care is taken with normal laboratory operations such as heating substances;
- good laboratory practice is observed when chemicals or living organisms are used;
- eye protection is worn whenever there is any recognised risk to the eyes;
- pupils and/or students are taught safe techniques for activities such as handling chemicals and microorganisms.

INTRODUCTION

About this Unit

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Introduction

These materials have been devised by practising teachers and educationalists from several European countries, brought together with financial support and encouragement from DGXII of the European Commission, under the auspices of EIBE, the *European Initiative for Biotechnology Education*.

The EIBE materials have been extensively tested in workshops involving teachers from across Europe.

The views expressed in this Unit and the activities suggested herein are those of the authors and not of the European Commission.

Particular attention should be paid to the general safety guidelines given inthe introduction to this Unit, and to the specific safety guidelines throughout the text.

Contents

The unit includes a series of activities on the use of biotechnology in the food industry. Two specific themes are addressed.

Biscuit production

This deals with the large-scale production of a food generally seen as being very ordinary. We hope to put it back into its historical contexts of the evolution of food and of biotechnology, and to explain scientific, technical, industrial, economic and legal aspects of biscuit production.

Sweeteners

Food additives are introduced as comprising one stage of biscuit production.
As an example of such additives, the unit investigates the use of artificial sweeteners. The production of sweeteners other than sucrose is presented in its historical, economic and technological contexts.

Firstly, there is an **introduction** to the different issues investigated by the unit.

- Sherlock Holmes, magnifying glass in hand : inquiry into a biscuit.
- What will his famous deductive mind uncover ? What kind of questions might he ask?
- What kind of questions might we ask today ?
- What might we discover? This is the point of departure ...

The unit includes five **practical activities**.

1. A game : involving a competition between two teams: each of them has to answer, in turn, preselected questions.
2. Sensory analysis tests: allowing discovery of the different elementary tastes, in particular the sweetness level of different sweeteners.
3. Biscuit-making ('petit beurre' / butter biscuits): giving students first-hand experience of biscuits, ingredients, and of their use on a small scale.

4/5. Experiments into the use of industrial enzymes: showing how one sweetener, fructose syrup, is obtained using industrial enzymes.

Background information is available in the booklet ***The Secret of Biscuits***. It covers:

- the history of biscuits;
- the production of sweeteners from starch;
- fructose syrup;
- improved enzymes;
- legal aspects;
- questions about new products.

Guidelines for the teacher

The different activities can be used independently or to support different educational themes, according to particular teaching goals. There are 3 main areas.

Nutrition

The biscuit is seen as a food. The chemical composition of the ingredients used (flour, sugar, fats), their nutritional roles and their possible modification (use of additives) can all be considered. Taste and its conservation during large scale production must also be taken into account. Finally, biscuit production must be considered within a given economic and legal framework.

Bio-industry

The biscuit can then be seen as the result of a series of complex technical operations. These are considered inside a well defined economic, cultural and legal context. Some examples are given and activities proposed. Nowadays, the production techniques are improved through the use of additives, such as glucose syrup.

Biotechnology

The emphasis will be put on the use of food additives and on the enzymes used to obtain these. Thus information will be given on amyloglucosidase and glucose isomerase, their role in the manufacture of glucose or fructose syrup and on the use of these syrups in an industrial process : biscuit production.

In the following scheme you can see that, even if the specific goals are different, the suggested activities are the same in the different areas, only the order in which they are used will change.

Table 1. Activities grouped according to theme

	Nutrition	Bio-industry	Biotechnology
Main pedagogical aspects to be developed	● Chemical composition of biscuits ● **Nutritive role of the different components** ● Production of the biscuits: - safety: process control - improvement: the use of sweeteners	● Chemical composition of biscuits ● **The biscuit production process** ● Improvement of the process ● The enzymes and their modification (protein engineering)	● Chemical composition of biscuits ● The process of biscuit production (briefly) ● **Improvement of the process: the use of sweeteners** ● The enzymes and their modification (protein engineering)
Different activities suggested	● *Formative evaluation (Game)* ● Sensory analysis of the tastes ● Baking biscuits (practical) ● Use of industrial enzymes (practical) ● *Summative evaluation (Game)*	● *Formative evaluation (Game)* ● Careful study of the booklet ● Sensory analysis of the tastes ● Baking biscuits (practical) ● Use of industrial enzymes (practical) ● *Summative evaluation (Game)*	● *Formative evaluation (Game)* ● Use of industrial enzymes (practical) ● Sensoryl analysis of the tastes ● Baking biscuits (practical) ● *Summative evaluation (Game)*

The secret of biscuits

Some history

Although industrial biscuit manufacture began only in the 19th century, cakes and biscuits have been known for approximately 10 000 years. A 'porridge' of cereals was then cooked in an oven and became the first preserved food.

Cooking in the oven became general practice in the Middle Ages and various fats, sugars and salt were mixed with cereals: thus, with time, different biscuits and cakes originated.

Until the 18th century, these cakes and biscuits were a delicacy, the privilege of favoured classes: the nobles and bourgeois. It was noticed that some biscuits, those with a low fat content, kept well and for this reason they were prepared for naval expeditions and the army. Thus, the biscuit was recognised as both a delicacy and a useful food.

In the 19th century, biscuit production was mechanised. The first industrial manufacture of biscuits was by Carr in Carlisle in the United Kingdom. By 1860, Great Britain exported its dry biscuits to all its colonies as well to all those countries where people drank tea. On the continent, great manufacturing companies were also created: Lazzaroni in Italy, Beukaler and Delacre in Belgium, Kambly in Switzerland, Balsen in Germany, Lefèvre Utile (LU), Biscuiterie Nantaise (BN) and Belin in France.

There was a tendency for small businesses to amalgamate, and after the second world war, biscuit production was carried out by a number of independent companies and large multinational agro-food companies. Diversification of products is necessary to prevent stagnation of the market, to decrease production costs and to incorporate new technological developments. The utilisation of new ingredients that have been produced by biotechnology (e.g. glucose and lactose syrups, flour modified by the action of enzymes) are trends in this direction.

What's in a biscuit?

Flour

There are different qualities of flour, each containing different amounts of proteins. The main flour proteins are glutens, which have elastic and plastic qualities. In biscuit making, flour with a low protein content is preferred; biscuits made with it have a better texture. In addition, fine flour is preferable to coarse flour because it absorbs water when the biscuits are made.

Fat

Fat contributes to the colour, the taste and the texture of biscuits. Fat prevents too much water from being absorbed by the biscuit dough. Consequently the proteins swell up less and the dough is less elastic: it therefore shrinks less after it has been shaped into biscuits. Fat also helps to improve the taste and texture of the biscuit.

Raising agents

Raising agents are often added to biscuit dough. They react chemically, releasing small bubbles of carbon dioxide, which are trapped in the dough, making it rise. Examples of raising agents include ammonium carbonate, ammonium bicarbonate and sodium hydrogen carbonate (sodium bicarbonate).

Milk

Milk may replace water in some biscuit recipes. It moistens and adds protein to the dough.

Eggs

Eggs act as binding agents; they add flavour, colour and protein to biscuits. Air, incorporated into beaten eggs, lightens the dough.

Sugar

In traditional biscuit recipes sucrose ('ordinary' sugar) is used. However, other sweeteners are beginning to replace sucrose. Sugars affect the colour, taste and appearance of the biscuit mixture and finished product. They also help to preserve the biscuits.

Cooking

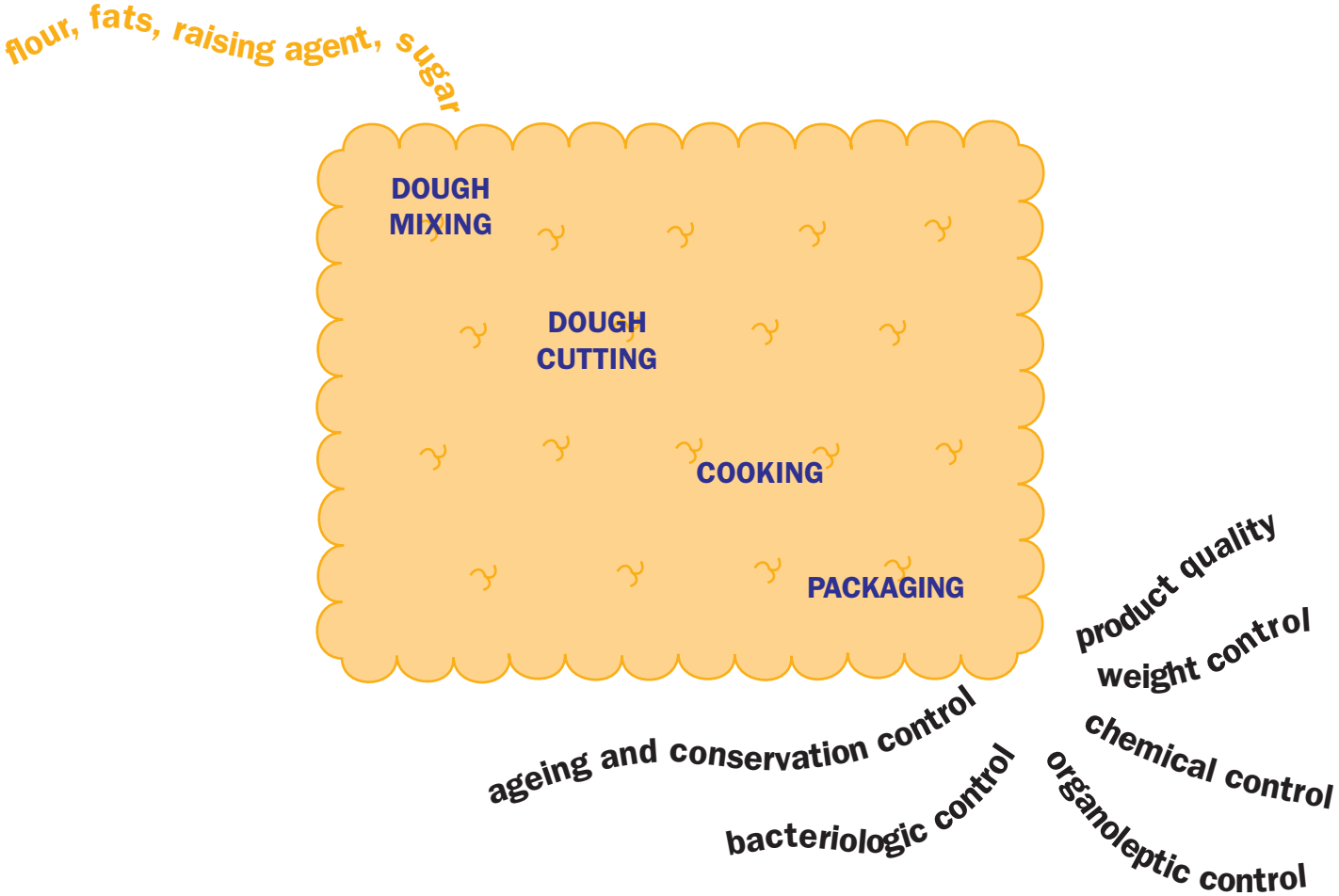
During cooking the dough is heated. Carbon dioxide is released by the raising agents and trapped within the dough. This makes the dough expand. Initially, enzymes catalyse various reactions in the dough, especially the breakdown of starch to sugar. As the temperature rises, proteins in the dough (including enzymes) are partly or wholly decomposed. The structure of starch is changed by heating, making it easier to digest. Any microorganisms in the dough are killed. Sugars and proteins react together, enhancing the flavour and colour of the biscuits.

Recipe for *Petit Beurre*

Flour	100 g
Sugar	30 g
Fat	15 g
Raising agents	1 g
Water	as necessary

Flavouring and colouring

Biscuit Processing



Sweetener production

Sugars from starch

During the Napoleonic wars, Europe was isolated from its major sources of cane sugar in the tropics. In 1811 German chemists succeeded in producing sugar by breaking down starch with acid, this process was adopted in several countries and used until the introduction of sugar beet into European farming. In World War II a milder enzymatic method of converting starch to sugars was developed, this had the advantage that the sugar it yielded lacked the bitter compounds that were characteristic of the product from acid treatment.

Enzyme treatment is now a major way of producing sweeteners, including syrups derived from sucrose or starch containing mixtures of glucose, maltose, fructose and other sugars. High fructose syrup (HFS) from corn (maize) starch has now eclipsed sucrose as the major sweetener used in the US food industry. More than eight million tons of HFS are sold annually, although the production and use of HFS in the countries of the European Union has been limited by quotas intended to protect European sugar beet growers.

High Fructose Syrup

To make HFS, the starch is converted to syrup by several amylase enzymes, which are used in three distinct stages:

Liquefaction

Starch is obtained as a by-product after valuable oil and protein have been extracted from maize. The starch solution is boiled and treated with α-amylase, an enzyme from *Bacillus*. This treatement gelatinises and dissolves the starch and starts to break it down. The partially-degraded starch molecules are known as dextrins.

Saccharification

Depending on the carbohydrate composition required in the finished product a cocktail of various fungal enzymes is then added to the dextrins. For syrups with a high glucose content a mixture of β-amylase or pullulanase with amyloglucosidase is used. Over 1-3 days these enzymes break down the dextrins progressively to glucose. Evaporation of water yields a viscous glucose syrup.

Isomerisation

Glucose shares its chemical composition with fructose but has a different molecular

Enzymes used for the production of sweeteners from starch

Starches are made from glucose units linked to form either a linear polymer called amylose or a branched polymer called amylopectin. Glucose units in both types of polymer are linked by α-1,4 bonds; the side branches in amylopectin are linked by α-1,6 bonds. Both amylose and amylopectin are broken down by extra-cellular amylases that are produced by many kinds of organisms, including bacteria and fungi. These different enzymes act in different ways:

α-amylase hydrolyses α-1,4 bonds in glucose polymers, but only within chains, yielding shorter chains (dextrins). Obtained commercially from bacteria (e.g. *Bacillus spp.*).

β-amylase hydrolyses α-l,4 bonds in glucose polymers, breaking off successive maltose units from the (non-reducing) ends of the chains. Cannot bypass α-l,6 bonds. Obtained commercially from barley and malt.

amyloglucosidase breaks α-1,4 bonds, cleaving glucose units progressively from the (non reducing) ends of the chains. Also hydrolyses α-1,6 bonds, but only slowly. Obtained commercially from the fungi *Aspergillus spp.* and *Rhizopus oryzae*.

pullulanase hydrolyses α-l ,6 bonds. Obtained commercially from the bacteria *Bacillus acidopullulyticus* and *Klebsiella pneumoniae*.

glucose isomerase (more properly known as **xylose isomerase**) transforms glucose into its sweeter-tasting isomer, fructose. Obtained commercially from *Streptomyces murinus*. Usually used in an immobilised form.

structure. This makes glucose about half as sweet as fructose. The enzyme glucose isomerase converts glucose to fructose, thereby increasing the sweetness of the syrup. Immobilised glucose isomerase is packed into a column and glucose syrup, heated to 60 °C, is passed continuously over it. At this temperature, the glucose syrup has a low viscosity, microbial spoilage is prevented and conversion occurs swiftly. Typical HFS has a dissolved sugar composition of 42% fructose and 53% glucose (the remainder being other sugars). Should syrups with a greater fructose content be required, glucose can be separated from the liquid leaving the column. This sugar can be re-cycled over the enzyme column to achieve a greater overall conversion rate.

Improved enzymes

Over the last 25 years much research has been directed towards finding better enzymes for HFS production. In 1974 the Danish company Novo (now Novo Nordisk) introduced a bacterial α-amylase from *Bacillus lichenifomis* that catalysed the breakdown of starch at 100°C or more. This led to significant improvements in the initial liquefaction process. A diverse range of dextrin-degrading enzymes has also become available to satisfy the demand for specialised sugar syrups e.g. for baby food, diabetic confectionery or for use in brewing and winemaking. These developments have resulted from careful selection of microorganisms that produce the enzymes. Finding the ideal production strain takes many years however, and is largely a matter of luck, the chances are that a microbe with one attribute in its favour will lack another equally important characteristic. Modern molecular biology has started to reduce this dependence upon trial-and-error.

Modified microbe

Bacillus stearothermophilus produces an α-amylase which is well-suited to sugar syrup production. Unfortunately, this species makes only small amounts of the desirable enzyme. Several copies of the appropriate gene were transferred into a closely-related species, *Bacillus subtilis*, enabling commercial production of the superior enzyme. After extensive safety tests, this amylase became the second enzyme in the world (after chymosin) from a genetically-modified organism to be approved for food processing in the USA.

A designer enzyme

At the relatively high temperatures (60 °C) used in immobilised enzyme columns, glucose isomerase rapidly becomes inactive. Typically enzyme activity halves every 55 days, so that after several months the expensive enzyme has to be replaced. In 1986, Royal Gist brocades in the Netherlands in co-operation with Plant Genetic Systems of Belgium initiated an ambitious research programme to improve the stability of glucose isomerase. The research team first sought to understand the reasons for the decline in enzyme activity.

Stronger links

Careful examination of crystals of the enzyme revealed its structure. Glucose isomerase was found to consist of four identical sub-units, joined together by rather fragile bonds. At raised temperatures the protein broke apart at these bonds and linked instead to glucose molecules in the syrup as they passed through the column. This explained the inactivation of the enzyme.

Further investigations showed that of the many hundreds of amino acid residues making up glucose isomerase, just two were responsible for the weak links. By substituting these amino acids with others that bound more tightly to their neighbours, the protein 'engineers' were able to produce a more stable enzyme. This was done by altering a small section of the DNA that coded for glucose isomerase, so that one out of the 20 lysine residues in each sub-unit of the protein was replaced by an arginine residue.

The improved glucose isomerase has a half-life that is roughly double that of the original form. This results in a doubled productivity in the enzyme column. In addition, at higher temperatures, a greater proportion of fructose is formed; so the new enzyme may therefore permit one step production of HFS at very high temperatures.

Food biotechnology

Regulation of novel foods and processes

Within the countries of the European Union, regulations have been proposed to cover the use of novel foods or food ingredients. In particular, the regulations will apply to modified or new molecules, to any products that have not previously been eaten by humans to a significant degree, to genetically-modified organisms and their products, and to novel processing methods.

Before it can be placed on the market, the proposed regulations demand that a novel food or ingredient:

- is safe for the consumer when eaten at the foreseeable levels of use;
- is not presented in such a way as to mislead the consumer;
- does not differ from a food or ingredient that it replaces in such a way that its foreseeable consumption is nutritionally disadvantageous to the consumer.

Under the regulations, when a company wishes to place a novel food or ingredient on the market, it must first apply to the responsible authority (e.g the Ministry of Agriculture) in the country where the product is to be marketed for the first time. The application must demonstrate that the three criteria listed above have been complied with. Within 90 days, the authority will either accept or reject the application. This period may be extended if the regulatory authority requests additional information from the applicant.

Points to consider

1. What tests do you think should be carried out to ensure the safety of novel food ingredients (such as sweeteners)?

2. Should novel foods and/or foods in which novel processes have been used be labelled? Is labelling enough, or do consumers need to know more to make informed choices (if so, what should they be told)?

3. Should the process of novel food production be regulated (as is proposed in the European Union) or merely the product of novel processes (as is done in the USA)?

4. Should manufacturers be obliged to demonstrate a need for novel foods and processes, and if so, how could they be asked to demonstrate this need?

5. What consideration, if any, should be made of the effects of novel methods on traditional food production processes?

PRACTICAL ACTIVITIES

The Biscuit Mystery

Is it accurate to assume that Holmes would have had access to biscuits in his day?
Yes, in fact the industrial production of biscuits began in Sherlock Holmes' time (see *The Secret of Biscuits*, page 1).

What might Sherlock Holmes detect while studying this biscuit?
The presence of poison if there is any.... but how? by its taste?
The methods used for its manufacture.

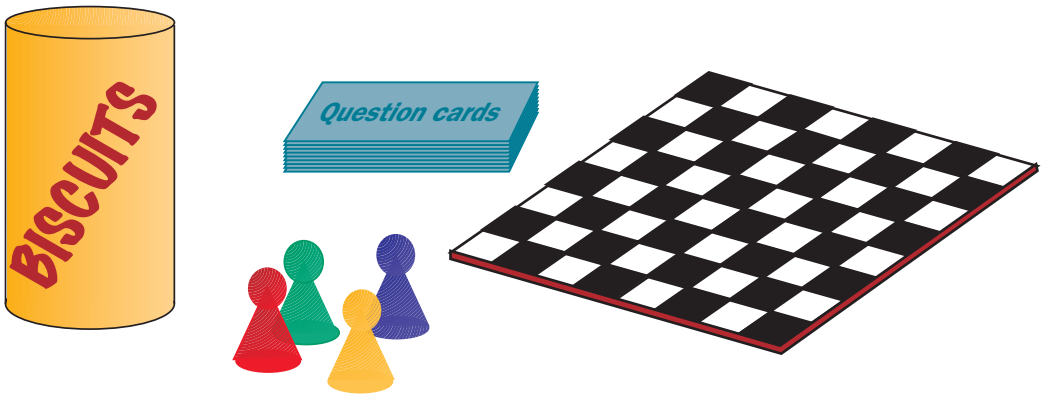
Do these questions correspond to those we might ask today?
Our questions are different... and we have a lot more answers, we know much more.

Why are our questions different today?
We have a better understanding of things.
We have different methods of investigation.
Our society is different : more closely linked to science and technology.

What kind of questions might we ask today? What is the modern version of the 'biscuit secret'?
This will depend on the chosen teaching emphasis; nutritional, bioindustrial or biotechnological.

ACTIVITY 1 *Teacher's notes*

The Biscuit Quiz



Notes for the teacher

The game aims to allow either a formative or summative evaluation of the students' knowledge. The spirit of competition between groups of students will be a useful auxiliary in obtaining the students' interest.

The **formative evaluation**, made at the beginning of some sequences, allows the evaluation of the prerequisite of the students on the chosen theme.

The **summative evaluation**, made at the end, allows an assessment of their knowledge.

Whatever the chosen function of the game, the teacher will have selected the most appropriate questions. In a summative evaluation, this selection may be done at the end of the sequence: one may also specify again in what sense the questions are connected with the chosen pedagogical goals.

Some of the proposed questions are based on knowledge (the answers are in the booklet *The Secrets of Biscuits*), while others are open to debate.

The students are divided into 2 or 4 teams which take turns answering questions drawn at random. The answers are verified by the group under the teacher's guidance. Teams win points for a correct answer, which allows them to put on a pawn on a chess board. Each team begins on a different side of the board. The winning team is the first to reach the opposite side of the board.

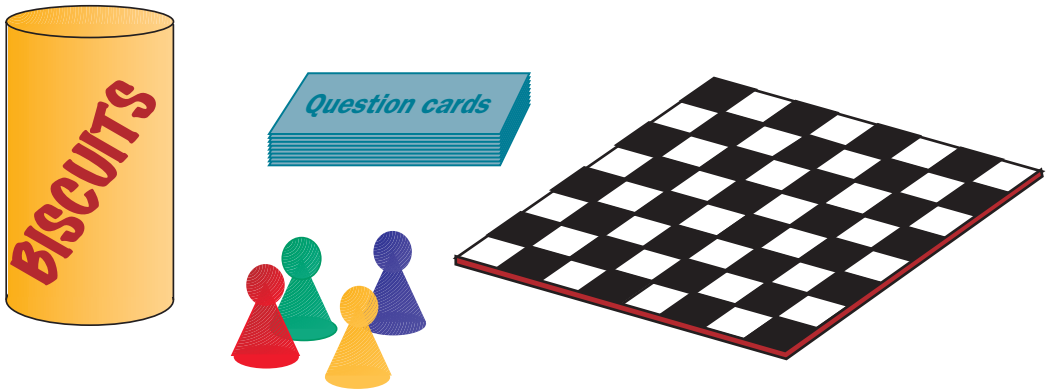
The speed with which the questions are answered and the amount of help needed will be an indication for the teacher of the subjects on which to base future lectures in order to reach the pedagogical goals. The role of the teacher is particularly important both as the game referee and in general present ation.

Materials

The Secrets of Biscuit booklet
Pawns (biscuit shaped)
Question cards (with difficulty levels increasing from 1 to 5)
Chess board (photo)copied on to overhead transparencies
Overhead projector
Pens, non-permanent
Timer
Biscuits for the winners.

WORKSHEET 1

The Biscuit Quiz



Instructions

1. Organise 2 or 4 teams of players.
2. Choose one side of the board and one pawn for each team. The aim is for the pawn to reach the opposite side of the board first.
4. Take turns for each team to draw a card .
5. After a set time for discussion, one person (take turns) should then answer the question to the whole group.
6. As a group, decide whether or not the answer is correct, awards points and move the pawn accordingly.
7. Two pawns may not occupy the same square; one has to go around the other.
8. The team which reaches the other side of the board first wins the game ..... and the biscuits!

ACTIVITY 2 *Teacher's notes*

A Spot of Sensory Analysis - the taste of sugar

Notes for the teacher

To demonstrate pure tastes simple solutions of sucrose, tartaric acid (or citric acid), sodium chloride and quinine sulfate (or another quinine salt, even crystallised caffeine) should be used. For the purpose of this teaching unit a second sweet substance is added to compare its sweetness to that of sucrose at the same concentration.

First prepare the reference solutions at the concentrations indicated in Table 2, then serial dilutions (1 volume of the previous solution plus 1 volume of water) of these. The water used in the preparation of the different solutions should be flavourless, flat, odourless and of comparable purity to distilled water. All concentrations are expressed in grams per litre ($g.dm^{-3}$).

The students should taste each flavour in a series of solutions of increasing concentration. If each solution is designated by a number, the taste tests can be carried out 'blind'.

The recommended numbering and order of taste is :

1 - 6	salty solutions
6 - 12	sweet sucrose solutions
13 - 18	sour solutions
19 - 24	sweet fructose solutions
25 - 30	bitter solutions

For each series, the solutions should be tasted in **increasing** order of concentration, in approximately 15 cm^3 aliquots. The temperature of the solutions tasted should be about 20 °C.

Note : It is better to finish with the series of bitter solutions, as this taste is the most persistent in the mouth.

Table 2. Preparation of stock solutions for sensory analysis.

	Sweet Sucrose or fructose $g.dm^{-3}$	**Sour** Tartaric acid $g.dm^{-3}$	**Salt** Sodium chloride $g.dm^{-3}$	**Bitter** Quinine hydrochloride $g.dm^{-3}$
Reference solution	16	2	6	0.02
Dilution I	8	1	3	0.01
Dilution II	4	0.5	1.5	0.005
Dilution III	2	0.25	0.75	0.0025
Dilution IV	1	0.12	0.037	0.0012
Dilution V	0.5	0.06	0.018	0.0006
Dilution VI	0.25	0.03	0.009	0.0003

ACTIVITY 2 *Students' notes*

A Spot of Sensory Analysis - the taste of sugar

Sensory analysis

Sensory analysis, not to be confused with simple tasting, is a scientific technique which allows the measurement of certain characteristics of food products, including taste and smell. The sense of taste, through receptors (papillae) located on the tongue, makes it possible to detect flavour. The sense of smell, a much richer perception than that of taste, includes two aspects: volatile molecules find their way to a sensitive region of the nasal mucous membrane, either directly through the nose, or by the retronasal passage when the product is in the mouth. When eating it is often difficult to separate gustatory and olfactory perceptions.

Sweet, due to substances known as *sweeteners* is considered to be a pleasant flavour; it is often sought out and chosen over other flavours. Both traditional and modern food products require sweeteners, either in a natural form (a sugar of plant origin), or in the form of an artificial substance (e.g. aspartame). The choice of a sweetener to be used in the manufacture of a food product is the result of a compromise between certain criteria: flavour, degree of sweetness, technology (for example aspartame can not be heated), cost and toxicity.

Perception of taste

Different types of taste papillae can be distinguished according to their shape. Each papilla consists of several hundred taste buds which are made up of about ten cells sensitive to flavoured substances (taste cells), as well as other cell types. Thus we have in all six hundred thousand sensitive cells at our disposition! Their replacement is rapid: the turn over time of the entire taste cell population is four days. They are connected to nerve endings and have fibrils which poke out into a little mucus-filled depression and intercept flavoured molecules.

The taste papillae do not all play an identical role in the perception of flavours. Similarly, any one taste bud is not specialised in a single taste: some of them have the ability to detect simultaneously sweet, sour and bitter, while other perceive only two of these, or even only a single one. Some of them are especially sensitive to bitter. Of a sample of 125 papillae: 79 are sensitive to sweet (including three which detect only sweet), 71 to bitter and 91 to sour (of which twelve detect only sour).

The full explanation of the taste sensation is not entirely known. The membrane containing the taste cells is thought to be equipped with specific receptors for the different flavoured molecules. There are believed, for example, to be several different types of lingual receptors for sweet flavours, such that an individual can be genetically more or less sensitive to different sweeteners.

The information resulting from the excitation of taste cells is transmitted to the brain via three cranial nerves. Inside the brain, the sensory nerve fibres associated with taste end at the receptor centre for taste, where conscious perception occurs: the perception of our senses is not the 'raw data' collected by the appropriate sensors, but the interpretation of these by the brain. Although it may be possible to define a standard individual with respect to vision, the same is not true for the senses of taste and smell, for which large variations exist. The tongue is constantly moistened by saliva, which is necessary for taste: a sub-

stance can only be tasted if it is soluble in the liquid phase of food and saliva. Saliva is secreted continuously and kept at a constant base level. However, eating triggers increased secretion to varying extents depending on the taste of the food: secretion is greater and more fluid for sour and bitter substances than for sweet or more neutral substances. It actually appears that more highly flavoured foods cause greater salivation.

The elementary tastes

Since the end of the XIXth century, the hypothesis has been accepted that the tongue's sensors perceive only four elementary tastes and that these are localised: sweet is perceived by the papillae located at the tip of the tongue, bitter by those at the back of the tongue, salt at the front edges and sour along the edges at the back of the tongue. It is now considered that this 'classical' theory of taste is not sufficiently comprehensive. Nevertheless, until new results, validated by the scientific community, become available, it will remain the foundation on which to base sensory analysis.

All tastes, whether pure or part of a mixture, can be classified into four categories: sweet, sour, salt or bitter, corresponding to the qualities of sweetness, sourness, saltiness or bitterness. A single substance may have just one of these tastes, or may exhibit several different elementary tastes simultaneously, or sometimes successively. All combinations are possible, in varying concentrations, and a good familiarity with the elementary tastes, as well as a degree of analytical effort, are required to define complex mixtures.

When a liquid is tasted containing dissolved substances with the four elementary tastes, they are not perceived simultaneously. The different tastes are said to have different reaction or excitation times. Moreover, they develop differently in the mouth. The sweet taste, for example of a 10 $g.dm^{-3}$ solution of crystallised sugar, occurs immediately, as soon as it comes into contact with the tongue; the reaction is virtually instantaneous. The intensity of the sweet flavour reaches a maximum within two seconds, then decreases progressively to almost disappear after about ten seconds. Salt and sour are also perceived quickly, but these persist longer. The taste 'bitter', on the other hand, develops slowly but increases and remains in the mouth for a long time after the liquid has been removed. The flavours of glutamic acid and succinic acid, simultaneously salt and bitter, persist in the mouth and give a mixture a pleasant character. These differences in perception are thought to be due in part to the fact that the different tastes are perceived by different regions of the tongue, as previously explained. Also, the fact that papillae sensitive to sweet and salt appear at the surface of the tongue while those sensitive to bitter are found further from the surface, may explain bitter aftertastes.

A large part of the surface of the tongue is insensitive: if a drop of a flavoured liquid is carefully placed there, nothing is sensed, until such time as movement of the tongue and diffusion in saliva causes the flavoured substance to come into contact with the part of the tongue where papillae are located. It is important to note that in the mouth true tastes are principally perceived by the tongue. The lips, cheeks, soft palate and epiglottis of an adult have very few papillae and perceive mainly thermal and tactile sensations. One sometimes has the impression that the lips and the back of the throat react to strong sweetness and bitterness. In reality, a flavour on the lips is only perceived when the tongue is brought into contact with them, and it is possible that the considerable stimulation of the pharynx which occurs when eating causes the localisation there of the sensations perceived by the tongue at the time of swallowing.

Acuteness of taste

The sensitivity of different people to different tastes, which has been established using methods which can be compared reliably, appears to be extremely variable.

Although ageusia, a disease characterised by a total inability to detect flavours, is rare, hypogeusia, or diminished sensitivity to taste, is more common, particularly in the case of sweetness.

Attempts have been made to measure taste but are relatively unconvincing since flavours are not additive and disguise each other. However, research into the thresholds of sensitivity to the different elementary tastes in a group of tasters, can be very revealing. The principle is simple: dilution of model substances (e.g. sucrose or tartaric acid) are tasted in order of increasing concentration, and for each person the minimum perceptible doses are recorded. Several protocols can be envisaged, the most interesting of which is where each flavoured solution is compared with pure water.

Acuteness of the tastes sweet and sour can differ by a factor of ten in different individuals. There are reported cases of tasters able to detect 0.5 $g.dm^{-3}$ of sucrose or 0.05 $g.dm^{-3}$ of tartaric acid, while others are not sensitive to 5 $g.dm^{-3}$ of sucrose or 0.5 $g.dm^{-3}$ of tartaric acid. Differences of a factor of two among professional tasters are common. Perceptions of salt are in somewhat better agreement, while trends in ability to detect bitter are the most irregular. The practice of tasting sharpens the perception of taste and the most experienced tasters are generally the most skilled at perceiving substances in the weakest concentrations. The existence of taste thresholds may explain certain eating habits. A person who does not sense bitter to any large extent (and who is not on a diet) will drink coffee without sugar, while another who is unable to taste sweet easily, will take three cubes of sugar.

The following table, established by Emile Peynaud, a world recognised oenologist (wine expert), shows the average results of a large number of similar tests, carried out over several years. The people tested were professionals and people interested in learning about tasting, and therefore represent a particular selection of the population. Other thresholds of perception or recognition have been published, which are clearly higher than those in this table.

Table 3. Sensibility to the different elementary flavours

The threshold is defined as the minimum concentration perceived.
The values given are distribution percentages of the people in the sample according to their perception threshold.

Sweet (sucrose) for 820 people		**Sour** (tartaric acid) for 495 people		**Salt** (sodium chloride) for 100 people		**Bitter** (quininesulphate) for 374 people	
Threshold $g.dm^{-3}$	**%**	**Threshold** $g.dm^{-3}$	**%**	**Threshold** $g.dm^{-3}$	**%**	**Threshold** $g.dm^{-3}$	**%**
>4	4.5	>0.2	11.8	> 1.00	6	>2	23.8
4	12.3	0.2	38.8	0.50	33	2	27.4
2	34.6	0.1	21.2	0.25	40	1	24.5
1	30.6	0.05	28.2	0.10	21	0.5	24.3
0.5	18.0						

WORKSHEET 2

Sensory analysis - Taste

Aims

There are three aims:

- recognition of the flavours;
- determination of your personal perception threshold of the elementary tastes;
- determination of which of the two sweet flavours is the sweetest , i.e. which is tasted at the lowest concentration.

Procedure

1. Each person needs two cups and an evaluation form. One cup is for pure water, the other for the solution being tested.
2. There are thirty samples to be tested consisting of five flavoured substances, representing the elementary tastes sweet (sucrose and fructose), sour (tartaric acid), salt (sodium chloride) and bitter (quinine sulfate), each present in six different concentrations.
3. Compare each solution with pure water and without any discussion, note in column 2 of the form what you tasted. But be careful ! If the concentration is below your own perception threshold, you may very well detect no flavour.
4. When you have filled in your individual results collate the results of the whole group.

Example evaluation form

Test Solution	Perceived taste	Real taste	Concentration $g.dm^{-3}$	Number of people who perceived the taste
18	*salt*	*salt*	*0.25*	*10/15*
26	*none*	*sweet*	*0.5*	*2/15*

Evaluation form

Name: .....

Test Solution	Perceived taste	Real taste	Concentration $g.dm^{-3}$	Number of people who perceived the taste
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
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30				

EIBE *European Initiative for Biotechnology Education* 1997

WORKSHEET 3

Biscuit making - Petit beurre

Equipment

Kitchen scales
Wooden spoon
Bowl
Baking trays
Oven with thermostat
(Biscuit cutters)

Ingredients

Flour	100 g
Sugar	30 g
Fat	15 g
Raising agent	1 g
Salt	0.5 g
Water	as required
(Flavouring)	
(Colouring)	

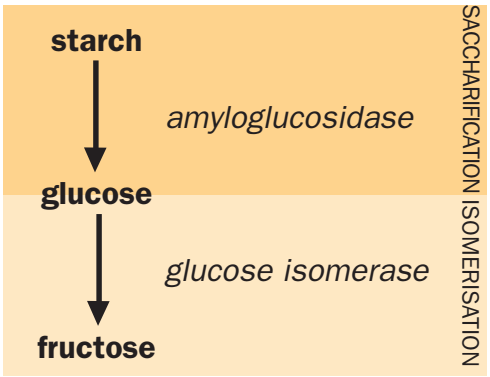
Procedure

1. Grease a baking tray.
2. Preheat the oven to thermostat 4, 150 °C.
3. In a bowl, beat together the butter and sugar until light and fluffy. Little by little, add a mixture of flour, salt and baking powder.
4. Knead the dough, adding water if necessary.
5. Leave in the fridge for at least half an hour.
6. Roll the dough out on a floured surface and cut the dough into different shapes using the biscuit cutters...OR...
   Shape the dough into a cylinder and slice into biscuits.
7. Place the biscuits on the greased tray.
8. Cook in a slow oven (150 °C, thermostat 4).
9. The biscuits are ready when they are a golden colour.

The use of enzymes:
Amyloglucosidase & Sweetzyme®T

Introduction

The enzymes amyloglucosidase (AMG) and Sweetzyme®T (immobilised glucose isomerase) are two enzymes which can be used successively.



In practice, the glucose solution must be concentrated before it can be used as a substrate for the glucose isomerase.

Amyloglucosidase (AMG)

See worksheet 4.

Suggested extension activities:

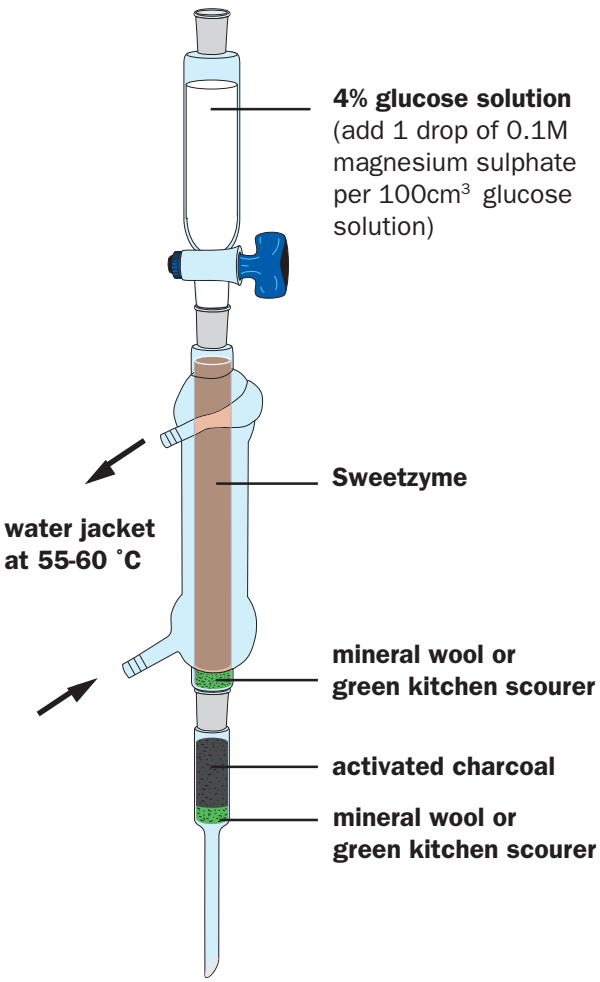
- change the size of the beads;
- change the pH of the conditioning solution;
- change the concentration of the sodium alginate solution;
- change the rate of flow of the starch solution.

Sweetzyme

See worksheet 5.

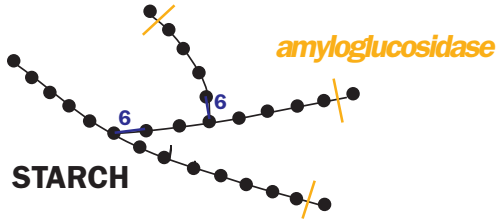
The optimal operating temperature for Sweetzyme is 55-60 ˚C, this is a compromise between optimum activity and enzyme stability. This can be achieved by loosely packing the Sweetzyme granules into a Liebig condenser (see below) and passing hot water through the water jacket, alternatively the method described in the worksheet can be used if solutions are prewarmed in a water bath.

Fig. 1. Apparatus to prepare high fructose syrup.



WORKSHEET 4

The use of enzymes: amyloglucosidase (AMG)



Amyloglucosidase hydrolyses α-1,4 as well as α–1,6 linkages in starch. During hydrolysis, glucose units are removed in a stepwise manner from the non-reducing end of the substrate molecule. The rate of hydrolysis depends on the type of linkage as well as on the chain length: α–1,4 linkages are more readily hydrolysed than α–1,6 linkages, whereas maltotriose, and in particular maltose are hydrolysed at a slower rate than higher oligosaccharides.

Saccharification is the process by which starch is made sweeter by treatment with AMG. The AMG can theoretically hydrolyse starch completely to glucose. In practice, a little maltose and isomaltose are produced too.

Materials

Amyloglucosidase, 1 cm^3 (AMG, Novo Nordisk, available from the NCBE)
5% sodium alginate solution, 10 cm^3
1.4% calcium chloride soln., 100 cm^3
0.05M sodium acetate pH 5.0, 20 cm^3 (adjust with acetic acid)
1% buffered starch solution, 50 cm^3 (1% soluble starch in the above buffer)
Semi-quantitative glucose test strips e.g. Boehringer Mannheim Diabur-5000
10 cm^3 plastic syringe
Glass rod
Small beakers (e.g. 100 cm^3), 2
Small sieve
Small square (about 1 cm^3) of nylon gauze
Syringe barrel (10 cm^3) with small tube attached to the nozzle
Tubing clip (Hoffman clip)
Clamp stand

Procedure

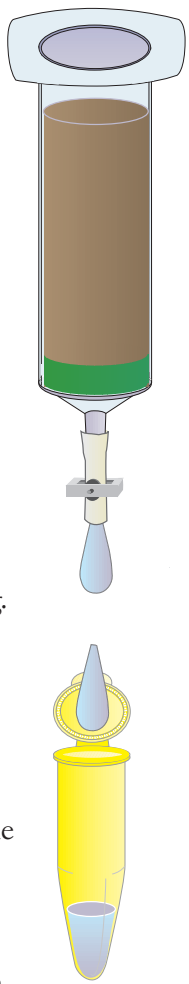
1. Add 1 cm^3 of the enzyme to 10 cm^3 of 5% sodium alginate solution.
2. Carefully stir the enzyme into the alginate solution to ensure a thorough mix. Try not to stir bubbles into the mixture.
3. Using a 10 cm^3 syringe draw up the enzyme alginate mix, again avoiding the formation of air bubbles.
4. Drop the enzyme-alginate mix drop by drop, into the calcium chloride solution so that it forms small regular beads.
5. To ensure the beads set fully leave them in the calcium chloride solution for about ten minutes.
6. Separate the beads from the calcium chloride using a small sieve.
7. Cut a disc of nylon gauze and fit it into the bottom of the syringe barrel. This will prevent the column becoming blocked by one of the beads. Pour the beads into the syringe barrel to form a column.
8. Wash the column with about 20 cm^3 of 0.05M sodium acetate (pH 5.0) solution.
9. Test the starch solution for the presence of glucose with a glucose test strip (Diabur 5000).
10. Pour the buffered starch solution into the column so that it runs slowly over the beads, controlling the rate of flow with the tubing clip.
11. Test a few drops of the processed starch for the presence of glucose with a glucose test strip. The Diabur 5000 test strips are semi-quantitative and can be used as a measure of the amount of glucose formed.

WORKSHEET 5

The use of enzymes:

Sweetzyme® (glucose isomerase)

Sweetzyme ® is an immobilised glucose isomerase that catalyses the conversion of glucose into fructose. It has been developed for the conversion of high dextrose syrup (obtained by enzyme hydrolysis of starch) into a sweeter tasting high fructose syrup in a continuous fixed bed process. Immobilising the enzyme gives higher yields with fewer by-products and enables the enzyme to be used continuously for several months. Syrups with a fructose content of 42 % can be obtained in this way. As fructose is about twice as sweet as glucose this high fructose syrup is as sweet as ordinary cane or beet sugar.

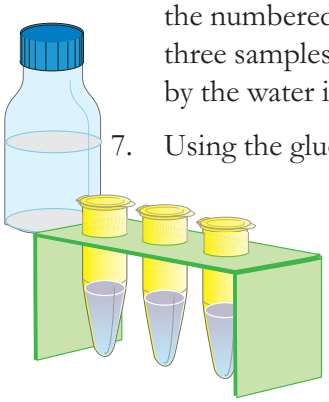


Materials

- Sweetzyme, 5 g (Novo Nordisk, available from the NCBE)
- 4 % glucose solution, 50 cm^3
- Semi-quantitative glucose test strips e.g. Boehringer Mannheim Diabur-5000
- Syringe barrel (20 cm^3) with small tube attached to the nozzle
- Tubing clip (Hoffman clip)
- Clamp stand
- Mineral wool (or green kitchen scourer)
- Small beakers, 3
- Minitubes (1.5 cm^3), 10
- Water bath at 60 °C

Procedure

1. Fix the syringe barrel, tubing and tubing clip vertically in the stand as shown and place a wad of mineral wool (or a circle of scouring pad) in the base of the syringe. Open the tubing clip.
2. Mix 5 g Sweetzyme with 20 cm^3 distilled water to form a slurry and pour into the syringe.
3. Rinse with 20 cm^3 distilled water at 55-60 °C and adjust the tubing clip to allow a flow of about 1 drop every 5 seconds. Allow all the water to pass through the column.
4. Prepare at least ten numbered minitubes to collect the column effluent.
5. Slowly add 10 cm^3 warm glucose solution to the column.
6. Collect 1 cm^3 fractions of effluent into the numbered tubes, discard the first three samples (as these will be diluted by the water in the column at the start).
7. Using the glucose test strips test the starting glucose solution and fraction number 4 onwards until there is a steady reading (3 the same) of the glucose concentration.



8. Compare the glucose concentration in the starting solution with the final sample tested and from this estimate the fructose concentration of the final sample.
9. If there is time investigate the effect of altering:
   - the temperature;
   - the initial glucose concentration;
   - the flow rate in the column.

QUESTION CARD 1

Name four of the main ingredients of biscuits. 1.1	What disadvantages caused by public concern could manufacturers expect from using ingredients derived from biotechnology? 1.8
Describe the roles of the ingredients of biscuits in the production process. 1.2	Which is sweeter, glucose or fructose? 1.9
Describe the nutritional function of one of the ingredients of biscuits. 1.3	Should biscuits made with sweeteners derived from modern biotechnology be specifically labelled? (Justify your answer) 1.10
Name the different types of sweeteners and their origin. 1.4	What disadvantages relating to regulations may the use of ingredients derived from biotechnology bring to biscuit manufacturers? 1.11
What economic advantages may the use of ingredients derived from biotechnology bring to biscuit manufacturers? 1.5	What nutritional disadvantages may the use of ingredients derived from biotechnology have for consumers ? 1.12
What advantages (other than economic) may the use of ingredients derived from biotechnology have for biscuit manufacturers? 1.6	What nutritional advantages may the use of ingredients derived from biotechnology have for consumers? 1.13
What advantages may the use of ingredients derived from biotechnology have for consumers ? 1.7	Will the use of food ingredients derived from biotechnology provide consumers with greater choice? (Justify your answer) 1.14

QUESTION CARD 2

Name three different sugars that may be used in biscuit recipes. 2.1	Argue against the European Union quota on the production of high fructose syrup. 2.8
Argue for the use of sugar (sucrose) instead of novel sweeteners in biscuits. 2.2	Argue in favour of the European Union quota on the production of high fructose syrup. 2.9
Do novel sweeteners pose greater safety risks to consumers than traditional ingredients? (If so, how?) 2.3	What are the advantages of high fructose syrup compared with glucose as a sweetner? 2.10
Explain (briefly) how genetic modification is done. 2.4	What is the function of α-amylase in the production of high fructose syrup? 2.11
Describe the difference between genetic modification and protein engineering. 2.5	What is the function of glucose isomerase in the production of high-fructose syrup? 2.12
What disadvantages could the use of novel sweeteners bring to farmers in Europe? 2.6	What is the function of amyloglucosidase in the production of high fructose syrup? 2.13
What disadvantages could the use of novel sweeteners bring to farmers in countries where sugar cane is produced? 2.7	At approximatively what temperature are commercial glucose isomerase enzymes used? 2.14

QUESTION CARD 3

What advantages do biscuits have as a food, compared with bread? 3.1	Should foods produced with the help of enzymes from genetically-modified organisms be labelled, even when there is no enzyme in the finished product? (Justify your answer). 3.8
The first genetically-modified enzyme used in the manufacture of a sweetner was a type of α-amylase. What advantage did this enzyme bring? 3.2	Name two potential benefits that come from 'protein engineered' glucose isomerase. 3.9
Why are enzymes used instead of acid treatment when producing sweeteners from starch? 3.3	What are the advantages of using immobilised (rather than free) glucose isomerase? 3.10
Name four factors that contribute to the popularity of biscuits as food. 3.4	What can be done to make glucose isomerase more stable at higher temperatures? 3.11
Argue in favour of the use of novel sweeteners (rather than sucrose) in food. 3.5	Describe the three main steps in the production of high-fructose syrups from starch. 3.12
What advantages could the use of novel sweeteners bring to farmers in Europe? 3.6	What is the 'active site' of an enzyme? 3.13
Why are peptide or protein sweeteners (e.g.aspartame) unsuitable for use in biscuits? 3.7	Name ten different types of biscuits. 3.14

QUESTION CARD 4

Do you think it is sufficient merely label food as *'a product of gene technology'* ? What additional information do the consumers need to make informed choices? 4.1	Argue in favour of enzyme producers being allowed to patent new enzymes they create by 'protein engineering'. 4.8
Why is high-fructose syrup the main sweetener used in the USA, whereas it is relatively uncommon in Europe? 4.2	Are enzymes alive ? 4.9
What are the main traditional sweeteners used in food? 4.3	Argue against enzyme producers being allowed to patent new enzymes they create by 'protein engineering'. 4.10
Describe the specific action of α-amylase on amylose and amylopectine molecules. 4.4	What happens to the structure (and consequently the function) of enzymes at very high temperatures? 4.11
Argue for the involvement of consumers in drafting regulations on novel foods. 4.5	The use of novel sweeteners could adversely affect traditional agriculture. To what extent should scientists be held responsible for this? 4.12
In the USA, regulations are applied to the products of genetic modification (in the food industry). In Europe, we regulate the process. Which is the better approach, and why ? 4.6	Who should oversee the safety of novel food? (Give reasons for your answer). 4.13
What sort of tests do you think should be done to ensure the safety of novel food? 4.7	From what source are commercial α-amylases obtained? 4.14

QUESTION CARD 5

Apart from safety, what factors should be considered when licensing novel food? 5.1	Would you eat biscuits that carried a 'gene technology' label? Explain your reason(s). 5.8
Give three reasons why consumers may want to know whether they are eating 'a product of gene technology'. 5.2	Describe (briefly) the structure of proteins. 5.9
What ingredients of biscuits, apart from sweeteners, could be produced with the help of gene technology? 5.3	Argue in favour of laws to prevent the import of foods derived from gene technology into Europe, when those foods are not approved here. 5.10
What factors have contributed towards the development of alternatives to traditional sweeteners? 5.4	Argue against laws to prevent the import of foods derived from gene technology into Europe, when those foods are not approved here. 5.11
From what plant is starch for high syrup production obtained, and in which countries does this plant grow? 5.5	Argue for international regulation of gene technology. 5.12
Do you think that it is morally wrong to alter naturally-occurring enzymes? (And how do you define 'moral' and 'natural'?) 5.6	Should manufacturers be called upon to demonstrate a need for the products of gene technology before they are approved? Justify your answer. 5.13
The production of high-fructose syrup in the USA increased dramatically after the take-over by the communist regime in Cuba. How could the two events be related? 5.7	Regulations in Europe may have hindered the commercial development of gene technology here. Should we be concerned about this ? Justify your answer. 5.14