Components of the Soy Bean

Abstract

Soy is cultivated worldwide, being the most important protein source and boosting the economy. As nutritionists students will compare the nutritional values of soy in comparison to other food. To do this, students will be given different experiments to work with, which they can further carry out in small groups. Based on the input on the soy plant from previous lessons, as well as general biological knowledge, they are to generate and test hypotheses. Afterwards, they will present their results to the class.

Tags

Discipline: Biology

Target group: Sekundarstufe I

Age range: 11–15

Duration: 1–2 lessons (50 min each)

Inquiry learning dimensions:

- Interpretation and evaluation of results
- Connecting hypotheses and theoretical input
- Planning and carrying out experiments
- Critical analysis of research methods

World of Work Dimensions:

- **Context:** Due to its high protein value, soy is often integrated into various diets as a food supplement and human nutrition in general. Apart from its protein content, soy beans are appreciated for their secondary compounds and unsaturated fatty acids.
- **Roles/Profession:** As nutritionists, students are to detect the components of the soy bean and other plants qualitatively as well as semi-quantitatively and compare the different results. Moreover, they will connect their previous knowledge about human nutrition with these findings.
- **Activities:** The students generate hypotheses, experiment and interpret their results. It is up to the teacher to decide whether the presentation should show the groups' results or the results of the entire class.
- **Product:** Component analysis of food and a recommendation letter regarding human nutrition.

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Task

Soy is cultivated around the world and is stimulating the world market. It has become the most important plant-based protein source. Due to its nutritional components the soy bean has become very popular. Being used as an additive to various food products, soy ends up on people's plates more often than they would know of.

You are working as a nutritionist and therefore need think about which nutritional components you would expect in the selected food samples. Test your hypotheses by carrying out the experiments. The results should be discussed and interpreted within the group before presenting them to the entire class. Connect your results with your knowledge about human nutritional habits and needs.

Material

- Pens, Paper
- Computer and internet access
- Literature
- Plant material (frozen, fresh or dried soy beans, cereal seeds, fruit or vegetables such as apples, pears, potatoes, banana, etc.)
- Microscopes and equipment
- Small glass containers with lids
- Distilled water
- Sudan-IV staining solution
- Ethanol
- Coomassie Brilliant Blue staining solution
- Methyl acetate
- Lugol's iodine
- Knives and cutting boards
- Glass cups
- Juice extractor
- Glass stick
- Coffee grinder
- Scale
- Centrifuge
- Folded filter
- Test tubes
- Merckoquant ascorbic acid test (from pharmacy)
- Combur 4 Test N (urine test strips from pharmacy)

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Teacher Guidelines

Students will analyse different plant material, come up with comparisons, formulate their expectations (hypotheses) and test them. The provided experiments and their instructions serve as guidelines for this. The results should be interpreted based on their relevance for human nutrition.

- Aim: Analysis of the nutritional components of food and their interpretation for human nutrition.
- Topic: Research and experiments.
- Effects: By connecting knowledge about human nutrition and the identified components, students should be encouraged to think critically about human nutrition. The information can be applied to other MASCIL "Soy" tasks.

Methods

- Formulating and testing hypotheses
- Experiments
- Design, presentation, gathering information

Suggestions for Implementation

After reading through the task description the students should come up with a hypothesis on the possible nutritional components of the different food plants. Then, the hypotheses are tested by carrying out the experiments. Finally, they are to build a connection between their results and human nutrition in general in order to draw their conclusions. It is up to the teacher to decide whether the results will be presented per group or as results of the entire class.

(1) Qualitative lipid and protein detection in food plants

Duration: Staining and differentiation takes approximately 30 minutes each. Plant fats and oils in plants are mainly stored in the seeds, but also inside the pulp and serve structural or storage purposes. Lipophilic compounds and lipids are stored as oleosomes (cytoplasm), lipid vacuoles or elaioplasts. Qualitative detection is carried out by staining them with a Sudan-IV solution. Sudan-IV is a highly apolar (lipophilic, hydrophobic) staining substance and thus very liposoluble, which stains the phase red. Thus, Sudan-IV is not soluble in polar (lipophobic, hydrophilic) phases like water and can therefore be used for differentiation. To carry out the detection of lipids with a Sudan-IV solution on cereal seeds (caryopsis), a thin longitudinal slice of the seed is put into the solution. After the differentiation (in ethanol) the red staining of the fatty germ will be visible.

All plant cells contain proteins which are stored colloidally or as crystals. Protein crystals are often visible under the microscope. The qualitative detection is carried out using a Coomassie-Brilliant-Blue Solution, which consists of Coomassie-Brilliant-Blue G250, concentrated acetic acid and concentrated methanol. In this acidic solution protons attach to the amino acids and thus produce a compound with Coomassie-Brilliant-Blue G-250. This stains the proteins blue.

(2) Qualitative starch detection in food plants

Duration: approx. 10 min.

Explanation

During photosynthesis plants produce glucose, which is then stored as starch in storage organs such as fruits and roots, specifically in amyloplasts as starch granules. These starch granules are visible under the microscope. They can be detected qualitatively using iodine solutions. Starch is a macromolecule

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and consists of more than 100 connected glucose units. Some of these units are spirals. Initially some of the interior spaces are filled with red-stained polyiodid ions ($I5-$) from Lugol's iodine. This causes a stronger absorption of orange light and thus strong blue staining. Alternatively other iodine solutions, e.g. Providon-iodine (available as Betaisodona, Mundipharma), could be used.

(3) Semi-quantitative detection of vitamin C, glucose and proteins

Duration: depending on sample preparations approx. 5-20 min.

A calorimetric detection of vitamin C (ascorbic acid), glucose and proteins in solutions is done using prepared test strips. The indicator on the test strips changes its colour when it reacts with the substance to be detected. The detected nutritional values will much likely be far less than values described in literature. However, these values are the results of professional lab methods which are much more selective and exact. Test strips on the other hand only indicate the amounts of free molecules within a solution. In addition, differences between varieties, storage conditions and times as well as other parameters contribute to these results. Therefore a comparison between these values and those from semi-quantitative methods is very difficult and hardly representative.

The ascorbic acid test is carried out using Merck's test strips (Merckoquant ascorbic acid test). The amount of ascorbic acid in the solution is indicated by a colour change caused by the reduction of molybdenum atoms (indicator) by the ascorbic acid. The shift of electrons results in a colour change from yellow (phosphomolybdic acid hydrate) to blue (phosphomolybdenum).

The test „Combur 4 Test N", which was originally developed for clinical urine tests, can also be used for plant material. The experiments with the test strips should be carried out utilising dry cereal seeds, as protein and carbohydrate syntheses start immediately after swelling has begun.

Moreover, stored starch is broken down into sugar molecules. Therefore, a swollen seed contains a much higher amount of proteins and sugar.

Protein detection: The test area contains a buffer and an indicator similar to Bromphenol-blue (3',3",5',5"-Tetrachlorphenol-3,4,5,6-tetrabromsulfophthalein), which in acidic conditions (pH=3,0) is yellow, and in slightly less acidic conditions (pH=3,4) green. The test is based on the „protein error"principle of indicators. Proteins change their structure in acidic conditions. The amino groups (R-NH2) take up protons from the indicator (H-Ind). The produced ions, the amino group (R-NH3+) and the indicator (now green) attract each other. A higher protein concentration, particularly albumin, is thus indicated by a change of colour from yellow to green.

Glucose detection: The test area contains a mix of enzymes and an indicator (3',3",5',5"-Tetramethylbenzidine). Glucose is oxidised by oxygen from the air, but only when the enzyme (oxidase) in the test area catalyses this reaction. This produces gluconolactone and hydrogen peroxide (H2O2). Then, hydrogen peroxide (catalysed by the peroxidase enzyme) oxidises the indicator. This shift of electrons causes a colour change (blue) which stains the test area green.

Challenges

Was the task clear/well-described? Is the provided material up to date? Is it possible to provide the necessary material?

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Didactic Suggestions

By generating hypotheses and testing them, students gain first insights into scientific research methods. Revision and connecting previous knowledge with new contents help the students to internalise what they have learned. The plant material used should be suitable for component detection and at the same time relevant food plants for the students. It is advisable to select a maximum of 7 plant samples.

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Carrying out the task

1. **Considerations and Expectations** Plants play an integral part in human nutrition. Consider which nutritional components and compositions you would expect in the various plant materials. Compare the samples with each other.

2. **Experiments**

   Carry out the experiments as described in the instructions. Document your results. How did you achieve these results? Keep a research log.

3. **Interpretation**

   Describe the results from the experiments and compare them with the hypotheses you generated before. What are your results? Do they match your hypotheses? What could be the reasons for possibly different expectations?

4. **Human nutrition**

   Think about the significance of your results for human nutrition. Write an informative article for a popular magazine.

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Experiment instructions

Source: Brünoth, M. (2008). Nahrungspflanzen und ihre Inhaltsstoffe. Diplomarbeit. Universität Innsbruck

(1) Qualitative starch detection in food plants

Material: Microscope and equipment, tap water, Lugol's iodine, plant material

Aim: A qualitative detection of starch using Lugol's iodine's staining effects.

Safety precaution Be careful when working with Lugol's iodine as it stains skin and fabrics!

Instructions

1. **Preparing the sample**

   Prepare the sample by scraping cells from a freshly cut area of your object using a razor or lancet needle. Add the cells to a drop of water on a microscope slide. Try to indentify granules of starch (amylum) under the microscope.

2. **Staining**

   Add a drop of Lugol's iodine to the edge of the cover glass and create a colour gradient by using filter paper to draw the liquid through.

3. **Observation**

   Observe the stains under the microscope. What do you observe? Can you explain this observation?

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(2) Qualitative lipid and protein detection in food plants

Material: Microscope and equipment, small glass containers with lids, distilled water, plant material, Lipid detection: Sudan"=IV staining solution, ethanol (destaining solution); Protein detection: Coomassie Brilliant Blue staining solution, methyl acetate destaining solution

Aim: The qualitative detection of lipids and proteins in plants by staining them with a Sudan"=IV solution and respectively a Coomassie Brilliant Blue solution.

Safety precaution Be careful when working with methyl acetate (destaining solution) as it contains methanol which is hazardous to one's health!

Instructions

1. **Preparation**

   Prepare thin slices and examine them for lipid drops and proteins crystals under the microscope.

2. **Putting the solutions in containers**

   Lipid detection: Add a small amount of Sudan-IV solution to a small glass container. Add the destaining solution to another glass container. Cover both with lids.

   Protein detection: Add a small amount of Coomassie Brilliant Blue solution to another container. Prepare a fourth container with destaining solution. Cover both containers with lids.

3. **Staining**

   Lipid detection: Add a suitable sample slice to the Sudan-IV solution and transfer it into the destaining solution (96 % ethanol) after approx. 15 min for differentiation.

   Protein detection: Add a suitable sample slice to the Coomassie Brilliant Blue solution and transfer it into the destaining solution (methyl acetate) after approx. 15 min for differentiation.

4. **Destaining**

   After a couple of minutes of differentiation, transfer the samples into distilled water on a microscope slide and observe the decolourisation under the microscope.

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(3) Semi-quantitative detection of vitamin C, glucose and proteins

Material: Knife, cutting board, small cups, juice extractor, glass stick, coffee grinder, balance, centrifuge, folded filter, test strips, colour range, test tubes and holder, measuring pipettes, distilled water, plant material

Aim: The amount of vitamin C, glucose and total protein in food plants is to be tested semi"=quantitatively by using test strips.

Safety precaution Close the test strip container immediately to avoid contamination with other substances and to ensure durability.

Instructions

1. **Sample preparation (fruit and vegetables)**

   Cut the fruit and vegetables and extract their juice using a juice extractor. Put the juice in a glass cup. In case its colour is to strong, dilute it with distilled water. Take notes of the ratio (e. g. 1:1, 1:4)

2. **Sample preparation for cereal grains, seeds and nuts**

   Grind the seeds, grains or nuts with a coffee grinder or any other suitable gadget. Weigh out a defined amount of it (e. g. 3 g) into a glass cup and mix it with distilled water (e. g. 10 ml). Let it sit for 15 minutes.

   Filter the suspension with a folded filter into another glass cup. Instead of filtering it, you could also centrifuge it. Put the filtrate into an empty glass cup.

3. **Vitamin C detection**

   Dip the test strip into the solution for approx. 1 second or press it against a freshly cut part of the fruit or vegetable for approx. 5 seconds. Shake off any excess liquid. After 10 seconds, try to classify the colour shown in the reaction zone of the test strip by comparing it with the vitamin C colour range.

4. **Glucose and protein detection**

   Dip the reaction zones of the test strips into the solution for approx. 1 second or press the test strip against a freshly cut part of the fruit or vegetable for approx. 5 seconds. Shake off any excess liquid. After 60 seconds, classify the colours by comparing them with the glucose and protein colour ranges.

5. **Check and determine**

   Repeat the detection tests three times for each sample and write down the different concentrations. Finally, determine the mean value.

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