Co-funded by the European Union

Learning Unit:

Learning about the 'antioxidant' compounds in our food.

Theme:
Nutritious and Healthy Food Consumption.

Main School subjects involved: Biology, English, Chemistry, Mathematics, Art.

Worksheets and relevant digital tools are available at the end of this unit and within the GOODFOOD Resources Library (website: https://goodfoodeplus.cebas.csic.es/)

Total duration: 8-11 h (complete activity).
1-3 h (separate activities).

Core concept:

The health benefits of consuming certain foods have been known since ancient times. Nowadays, consumers increasingly look for foods with health benefits and the ability to reduce the occurrence of disease. In this sense, many foods, especially fruits and vegetables, are excellent sources of healthy compounds, commonly known as 'antioxidants'. Learning about these compounds, their properties, and their presence in "healthy foods" will help the students to improve their food choices. Knowledge about their weekly intake of these compounds could encourage them to increase their intake.

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Learning Objectives:

Students will learn to:

- Plan and develop a research project following a number of steps based on the scientific method.
- Understand the concepts of 'antioxidants', and 'polyphenols'.
- Measure and compare the content of 'antioxidants' and of 'polyphenols' in foods by means of an experimental method.
- Explore the nature and quantity of 'antioxidants' and of 'polyphenols' present in different food products using specific tools (databases, websites).
- Estimate the weekly intake of polyphenols and identify ways to improve this value (Excel app).

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Steps of the Learning Unit

Orientation

Duration: ≈1.5 h.
School subjects involved: Biology, English, Chemistry, Art.
Where the activity takes place: classroom.
Method (how the students have to work): brainstorming as a class or in groups.
Equipment / materials: Notebooks, pens, board, cardboard, scissors, etc.

Description:

What do the students know about bioactive compounds and "antioxidants"?

Phase 1. – Brainstorming exercise: How much do they know/understand about the concept of 'antioxidants' in foods? Teachers will encourage students to answer a list of questions to create a brainstorming in class about the concept of 'antioxidants'. Some examples could be:

1. Do you know why the plants (flowers, fruits) have those beautiful colors?
2. Have you ever heard about the term 'antioxidants'?
3. Have you ever heard about other terms like 'polyphenols'? Did you know that 'polyphenols' are a type of 'antioxidants'?
4. Do you understand what all these compounds are?
5. Can you mention any market products that you have heard contain 'antioxidants' or 'polyphenols'?
6. Do you know what 'oxidative stress' is and what consequences can have for our health?
7. Did you know that the 'antioxidants or polyphenols' that we may eat with our food can have some beneficial effects on our health?
8. Can you mention /think of some the health benefits of these type of compounds?
9. Can you think of any examples of foods that you might have seen in the supermarket with a message of a beneficial effect for our health? Can you remember if the food with the health message did also indicate to contain or be rich in any specific compounds?
10. Have you ever heard about the terms 'functional foods' or 'nutraceuticals'? If so, could you propose some examples of this type of products? Prepare a list with the examples provided by everyone. (≈30 minutes).

Phase 2.- Which foods contain 'antioxidants' or 'polyphenols'?

Each student (or in groups of different students) will write in a paper three foods that he/she considers contain antioxidants. Then, each one will explain to the rest of the class the three chosen foods and the teacher will write them on the blackboard. They will check what foods are recognized by most of the students as rich in antioxidants and the knowledge gaps about these compounds.

A second option is that considering the list of foods and ingredients used in their initial recipe, the students will select those that according to their opinion could contain antioxidants. They can cut out those foods from a supermarket brochure and paste on a cardboard. This activity constitutes also a good opportunity for creative Artwork since the students, alternatively, can draw/paint nice illustrations of the different fruits and vegetables with indication of one major bioactive compound typical of each food. Then, they can put the drawings in a poster of antioxidant foods (≈60 minutes).

Conceptualisation

Duration: ≈30 min.
School subjects involved: English, Biology.
Where the activity takes place: classroom.
Method (how the students have to work): as a class or in groups.
Equipment / materials: notebooks, pens, board

Description:
This step aims at formulating a hypothesis or questions on the specific recipes of the food (dish or meal) students have chosen and that will be explored through the investigation.

Hypotheses (examples).

- How can we estimate the amount of the antioxidants/polyphenols in the different foods/ingredients examined?

The hypotheses to investigate within this Learning Unit will be formulated/discussed in class by the students. Based on the Orientation phase, the questions will focus on finding out more about the content of antioxidants or polyphenols in foods. The students will include the foods/ingredients present in the recipe they have initially prepared/selected. They may also include additional foods examined during the orientation phase and (or) other foods suggested by the teachers/other students.

Investigation

Core concept: Higher intakes of antioxidant- or polyphenol-rich foods like fruits, vegetables, and legumes are associated with a lower risk of chronic oxidative stress-related diseases like cardiovascular diseases, cancer, and deaths from all causes. It is important to know what foods contain these antioxidants, in what quantity and how they exert their effects. During this investigation, the groups of students will carry out a specific activity to learn:

How can we measure the antioxidant activity with a simple method in the lab? – Activity 1

How can we experimentally estimate the content of polyphenols in different foods? – Activity 2

How can we know the polyphenols/antioxidant content in foods using tools from the web? – Activity 3

How can we estimate the amount of dietary polyphenols we eat weekly? – Activity 4

Activity 1. – Simple experimental measurement of the antioxidant activity (example with lemon juice).

Description:
Antioxidants work by preventing oxidation reactions that produce free-radicals which can cause harm to the body. A simple way to visualize the antioxidant activity is using a reduction-oxidation indicator solution (redox) of iodine-amylose which will change color (blue to colorless) due to the reaction with the antioxidant.

Duration: ≈1-2 h.
School subjects involved: Chemistry.
Where the activity takes place: In the classroom, laboratory.
Method (how the students have to work): in groups of several students with the help of the teachers.

- *1. Planning the investigation activities to be implemented.*

  - In this example, we will visualize the antioxidant capacity of lemon juice (other juices or extracts from other foods/ingredients can also be tested).
  - Prepare the lab material for the experiment: cornstarch, iodine solution (Betadine), glasses, syringe or dropper, lemons.

- *2. Performing the investigation activities.*

  - Mix 5 grams of cornstarch with 100 mL of water.

- Add the iodine solution (Betadine) until a blue color is achieved (≈2 drops) (blue indicator).

- Pour 10-20 mL of the blue indicator solution into a beaker.
- Extract the lemon juice (or the juice from the different juices or ingredients that you want to test) (antioxidant).
- Add drop-by-drop of the lemon juice to the indicator mixture until the blue color disappears (note down the number of drops).

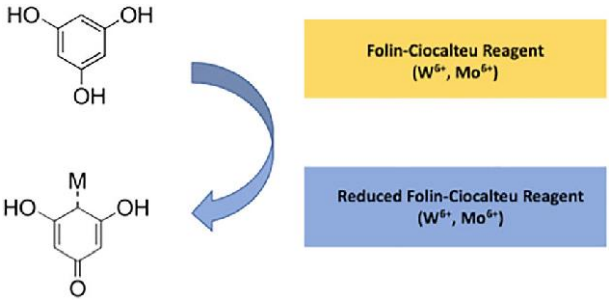
➢ *3. Analysis of results and main findings*.

Cornstarch contains a macromolecule called amylose. This molecule has a helical shape and is capable of forming with iodine a iodine-amylose compound with a deep blue color. When a solution containing antioxidants is added to this blue solution, a reaction occurs between the antioxidant and the iodine-amylose compound. The antioxidant is oxidized and the iodine present in the iodine-amylose compound is reduced to iodide causing the solution to lose its intense blue color. The number of drops that you need of each product to change the color can be used to estimate and compare the antioxidant content of each product. The fewer drops you need to change the color, the more antioxidants the product contains.

Activity 2. – Experimental approach to estimate the total amount of polyphenols in different foods/ingredients.

Description:

The students will learn a basic experimental protocol based on the Folin-Cioalteu method to estimate the total amount of polyphenols present in foods. The Folin-Ciocalteu test is based on the fact that the polyphenols can react with the Folin-Ciocaltu reagent. At basic pH, the polyphenols are oxidized by the Folin-Ciocalteu´s reagent yielding a blue color which is proportional to the polyphenol content, i.e. more color, higher amount of polyphenols. Using the pure polyphenol 'gallic acid', it is possible to construct a standard curve and quantify the amount of polyphenols by directly relating color and quantities. See the process below:



Duration: ≈2-3 hours.
School subjects involved: Chemistry, Mathematics.
Where the activity takes place: Laboratory hands-on experience.
Method (how the students have to work): in groups of several students with the help of the teachers.

- *1. Planning the investigation activities to be implemented.*

  - Select and buy the foods that will be used in this experiment, including some of the foods/ingredients used in the initial recipe and some alternative foods. They should try with, at least, three or four very different ones.

  - Prepare the lab material for the experiment: Flasks (20 or 50 mL) or falcon tubes (50 mL) and measuring cylinders (20 or 50 mL), markers, timer, safety goggles, grinder or similar, analytical balance, pipettes, centrifuge, agitator, test tubes. Chemical reagents: gallic acid, methanol, distilled water, Folin-Ciocalteu reagent, sodium carbonate.

➢ *2. Performing the investigation activities.*

The students shall follow the next protocol:

- Prepare a standard curve using the standard compound gallic acid (SIGMA). For this, we first prepare a solution of 1000 mg/L gallic acid: weigh 10 mg of gallic acid in a measuring cylinder and dissolve in 10 mL of water. From this starting solution we shall prepare increasing concentrations of gallic acid between 0 and 1000 ppm (or mg/L) as detailed in the next table and using test tubes:

	Concentration (mg/L) of the calibration curve of gallic acid						
Reactives	0	50	100	250	500	750	1000
Gallic acid (uL)	0	50	100	250	500	750	1000
Water (uL)	1000	950	900	750	500	250	0

- Prepare the samples containing the polyphenols from the selected food samples: First of all, solid samples must be grinded to the smallest particle size possible to get a most homogenous mixture. This can be done, for example, using a grinder or a plastic bag and a hammer.
- Using the digital scale, weight out a certain amount of the grinded sample (e.g. 5-10 g) into a glass laboratory flask and add methanol/water (50:50, v/v) (25 mL) (make sure you work in a properly ventilated area, outdoors or in an extraction hood if available; IMPORTANT! wear safety goggles and gloves). Agitate thoroughly the sample in the solvent during a few minutes (e.g. 5 min) to extract the polyphenols. Let the solution settle for a while. Carefully pour the solvent into a new empty clean flask covered with a strainer, filter or mesh so that it retains any solid particles. Only the solvent with the dissolved polyphenols will go through into the flasks. Liquid food samples (i.e. juice) can be filtered and tested directly.
- Take 250 µL of each standard gallic acid solution and of the extracted sample containing the polyphenols. Place them in Falcon tubes (50 mL). Add 15 mL of distilled water and 1.25 mL of Folin-Ciocalteu reagent. Homogenize the contents of the tube and leave to stand for 8 minutes in the darkness. After this time, add to each flask 3.75 mL of the 10% sodium carbonate solution and bring to a volume of 25 mL with distilled water. Homogenize the flasks and keep in the dark at room temperature for ≈2 hours. Then, put a small quantity in test tube to evaluate visually the results.
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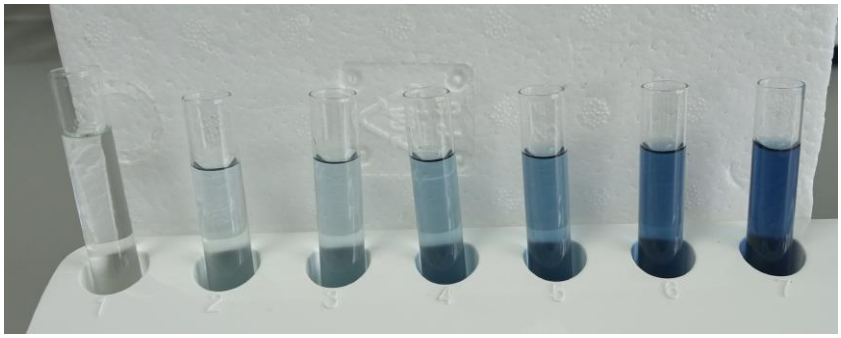
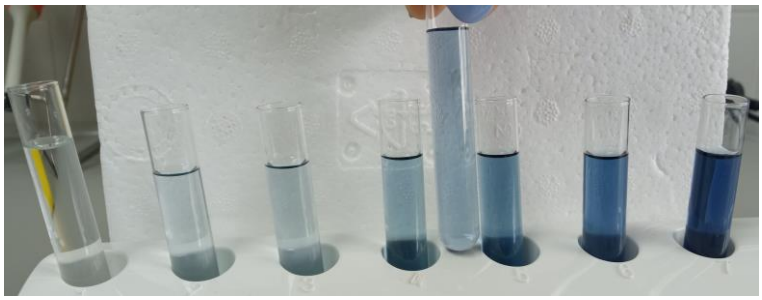


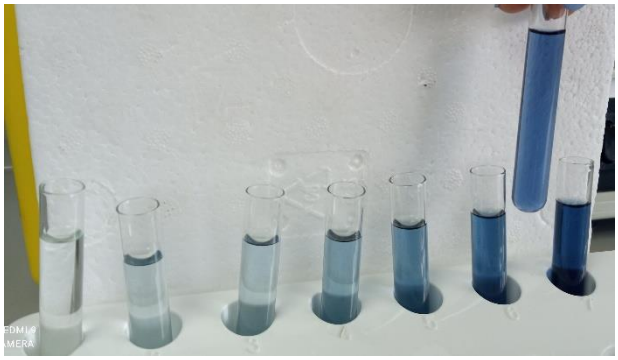
Standard curve constructed with the different gallic acid concentrations. Higher concentration, more intense blue color.

➢ *3. Analysis of results and main findings*

- Compare the color attained from the food extract sample with the color of the different points of the standard curve and select the concentration of the gallic acid with the color most similar to your sample.



Example: Apple sample compared with the standard curve.



Example: Juice sample compared with the standard curve.

Activity 3. – Applying current information available from the web to learn about the polyphenol /antioxidant content in food.

Description:

In this activity, the students will learn to use some digital tools to know more about the nature and amount of polyphenols/antioxidants present in different foods.

Duration: ≈1-2 h.
School subjects involved: Biology, Chemistry, Mathematics, English.
Where the activity takes place: In the classroom and (or) as homework.
Method (how the students have to work): in groups of several students with the help of the teachers.

- *1. Planning the investigation activities to be implemented.*

  - Selection of the foods/ingredients that will be investigated including the initial recipe ingredients and some alternative ones
  - Computers/Mobile phones with Internet connection and Excel program.
  - Access to databases where you can search for the polyphenol/antioxidant composition of foods (List with recommended tools included at the end of the Learning Unit).

- *2. Performing the investigation activities.*

For the analysis and comparison of the bioactive compound composition:

  - Students will study the labels to identify the different ingredients in a product.
  - Use digital tools such as Phenol-Explorer database (http://phenol-explorer.eu/) to investigate the polyphenol content of the foods/ingredients selected.
  - Use the table included at the end of the learning unit The Antioxidant Food Table (https://drive.google.com/drive/u/0/folders/17RQnwvMmgIPBkf1xpGvzNw21vkEdrmGL) to compare the total antioxidant content of different foods and ingredients.
  - Using this information and the amount of each ingredient or food used in the recipe, they can then calculate the amount of polyphenols and (or) of total antioxidant content that they are consuming in a serving of a recipe.

In the report of this activity, the students should prepare a list of inferences about the use of these tools (problems encountered, best informative source, etc). These issues will be later presented and discussed during the **CONCLUSION** and **DISCUSSION** sections of the project.

Activity 4. – Estimation of the amount of dietary polyphenols we eat weekly.

Description:

The students can estimate the amount of dietary polyphenols they may eat weekly to know if they take sufficient amount of these compounds and compare between students. The students will use a tool to

estimate the intake of dietary total polyphenols developed by a group of researchers under the framework of the European project Stance4Health.

Duration: ≈1 h.
School subjects involved: Biology, Chemistry, Mathematics, English.
Where the activity takes place: In the classroom and (or) as homework.
Method (how the students have to work): in groups of several students with the help of the teachers.

1. Planning the investigation activities to be implemented.

- Excel file provided with this learning unit Intake of total dietary polyphenols (https://docs.google.com/spreadsheets/d/1BgW5lMUMqTZcUqdaN2hpLOhu_DinSP9Q/edit?gid=664609864#gid=664609864) .
- Instruction manual for the use of the tool.

2. Performing the investigation activities.
To find out how much polyphenols they take weekly, the students shall:

- The students will fill the Excel file indicating the foods consumed in the different meals along the day for one week. Only those containing polyphenols can be chosen from the available dropdown table.
- They will determine and compare the weekly intake of total dietary polyphenols in different people (e.g. some of the participant students, friends, relatives, teachers, etc) using the specific recommended tool. Try to include very different people in terms of age, sex, body weight and level of activity to search for potential differences.

3. Analysis of results and main findings.

- Inspect and note down the results found using the Excel file. The tool will show total polyphenol intake provided by the specific food, by the complete meal, per day and per group of food. It also calculates the total polyphenol intake by week. The students will identify the amount of polyphenols ingested compared with their colleges and they will learn how they can improve the intake, for example with the new recipe.

Conclusion

Duration: ≈1 h.
School subjects involved: Maths, English, Biology.
Where the activity takes place: classroom.
Method (how the students have to work): in groups of several students with the help and collaboration of teachers of different subjects.
Equipment / materials: Notebooks, pens, foods (or photographs of foods/nutrition labels), computers, mobile phones

Description:

With the results acquired:

- Reporting: the different groups of students will elaborate a report with their main findings (Tables of results) and present/explain their conclusions to their colleagues. For this, the students with the help of the Maths teachers can produce graphs and (or) a poster. Then, in the English class, they will prepare a presentation of their findings (they can use PowerPoint or alternative tools that they like).
- Brainstorm: the different groups of students will try to come up with some general common ideas with regards to:

  - ✓ Which type of foods contain higher amount of antioxidants or polyphenols?
  - ✓ How these compounds exert their antioxidant capacity?
  - ✓ How to include food in our diet rich in these types of compounds.
  - ✓ How/Which Apps and Web-based tools use to investigate the antioxidants and polyphenols of foods.
  - ✓ How to improve their recipe or prepare a healthier one incorporating these type of compounds.
  - ✓ Propose a video with some recommendations to have a healthier diet including antioxidnats or polyphenols.

Discussion

Duration: ≈1 h.
School subjects involved:
Where the activity takes place: classroom
Method (how the students have to work): in groups of several students with the help and collaboration of teachers of different subjects.
Equipment / materials: Notebooks, pens, foods (or photographs of foods/nutrition labels), computers, mobile phones.

Description:

In this phase, the students will discuss their own findings by talking about the whole experience and indicating aspects like: what were the main difficulties they found, what things they did not understand, what phases they found more difficult to perform, what they liked the most, what they did not like, what they have learned about antioxidants and polyphenols, etc.

Recommended Digital Tools

Phenolexplorer:

http://phenol-explorer.eu/

It is the first comprehensive database on polyphenol content in foods. The database contains more than 35000 content values for 500 different polyphenols in over 400 foods. English

The Antioxidant Food Table:

https://drive.google.com/drive/u/0/folders/17RQnwvMmgIPBkf1xpGvzNw21vkEdrmGL

In this table, you can find the total antioxidant content of more than 3,100 foods, beverages, spices, herbs and supplements used worldwide.

Intake of total dietary polyphenols:

https://docs.google.com/spreadsheets/d/1BgW5lMUMqTZcUqdaN2hpLOhu_DinSP9Q/edit?gid=664609864#gid=664609864

In this table, you can calculate the amount of polyphenols you eat in a day or a week by filling in the data in the Excel sheets. You can select the food items (dropdown menu) and include the estimated quantity (grams) you eat.