Looking for antioxidant food

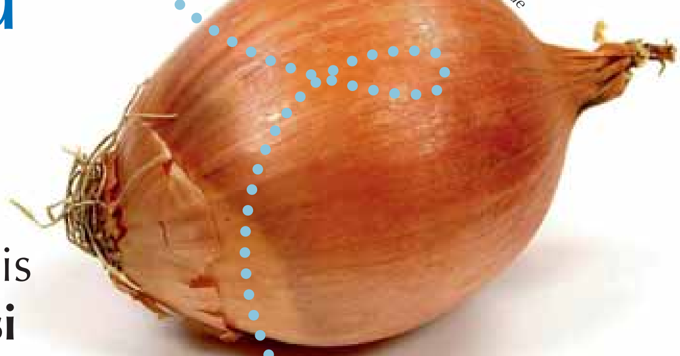


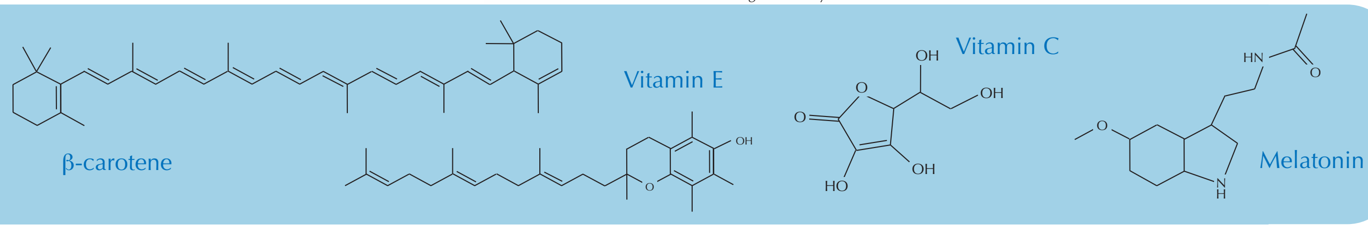
Image courtesy of wrw / pixelio.de

The author, Gianluca Farusi

Image courtesy of Gianluca Farusi

We've all heard that an antioxidant-rich diet is healthy. Together with his students, **Gianluca Farusi** compared the antioxidant levels in a range of foods and drinks.

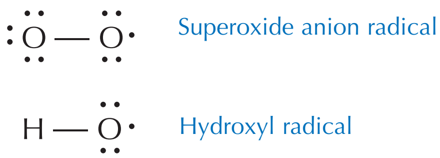
Image courtesy of Nicola Graf



Many health problems, including atherosclerosis, heart attack, Alzheimer's disease, some tumours and senile cataracts, are associated with very reactive molecules called *free radicals*. These molecules are produced normally during aerobic respiration and are used by the body, for example to defend it against micro-organisms. If, however, there is an imbalance between radicals (oxidants) and antioxidants, this can lead to disease.

Free radicals are so reactive because they possess one or more unpaired electrons. They are produced and found in many cells and cell organelles; the superoxide anion radical (O_2^-), for example, is the most common radical in the body, being used by white blood cells to attack viruses and bacteria. By far the most reactive radical, however, is the hydroxyl radical (HO·), found in the peroxisome (where fatty acids are

Images courtesy of Gianluca Farusi



broken down) and the endoplasmic reticulum. External factors influence the production of radicals too; for example, ultraviolet (UV) light shining on our skin causes singlet state oxygen radicals ($^1\Delta O_2\cdot$) to be formed.

Within the body, free radicals can lead to a variety of problems. In particular, they react with – and damage – lipids, proteins and nucleic acids, including DNA (Arking, 2006). To protect itself from the continuous radical attack, our body has two basic forms of protection: enzymatic and non-enzymatic. The most important enzymes used to defend our bodies from free radical attack are the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. The principal non-enzymatic antioxidants are melatonin, α-tocopherol (vitamin E), ascorbic acid (vitamin C) and β-carotene (the vitamin A precursor).

All four non-enzymatic antioxidants are essential in the diet and are found in a range of foods. Cancer in particular is less common among people who eat plenty of fruit and vegetables, and it has been suggested that the health benefits are due to the antioxidants they contain (Polidori et al., 2009; Swirsky Gold et al., 1997), which counteract the damaging effects of free radicals. Currently, there is little evidence that antioxidant supplements (e.g. tablets) have any health benefits.

The experiment below compares the levels of antioxidants in various types of food and drink, i.e. the effectiveness of the different types of food and drink as radical scavengers.

The experiment: searching for antioxidants in food and drink

Image courtesy of Valentyn Volkov/ iStockphoto

To educate my 17-year-old students about a responsible diet, in the hope of reducing their risk of developing the diseases mentioned above, I designed an activity based on the Briggs-Rauscher reaction: an oscillating reaction in which amber radical and blue non-radical steps alternate. By adding samples of different types of food and drink to the reaction and measuring the time intervals between colours, the students could compare the effectiveness of the samples as radical scavengers. Of course it is a comparative and not an absolute evaluation. But one thing at a time….

Materials and equipment

- 4 M hydrogen peroxide (H_2O_2) aqueous solution
- 0.20 M potassium iodate (KIO_3) and 0.077 M sulphuric acid (H_2SO_4) aqueous solution
- 0.15 M malonic acid ($CH_2(COOH)_2$) and 0.20 M manganese sulphate ($MnSO_4$) aqueous solution
- Distilled water
- Food and drink, e.g. samples of wine, teas, infusions; samples of food as aqueous extracts (see Table 1[w1])
- Magnetic stirring plate with magnetic stirrer
- 100 ml and 400 ml beakers
- 2 ml and 10 ml pipettes
- Wash bottle
- Spatula
- Glass rod
- Test tubes
- 1 l flask
- Bunsen burner

Preparing the solutions

4 M hydrogen peroxide solution: pour 400 ml distilled water into a 1 l flask. Wearing gloves, add 410 ml 30% hydrogen peroxide. Using distilled water, dilute the solution to 1.0 l.

0.20 M potassium iodate and 0.077 M sulphuric acid solution: place 43 g potassium iodate and approximately 800 ml distilled water into a 1 l flask. Add 4.3 ml concentrated sulphuric acid. Warm and stir the mixture until the potassium iodate dissolves. Dilute the solution to 1.0 l with distilled water.

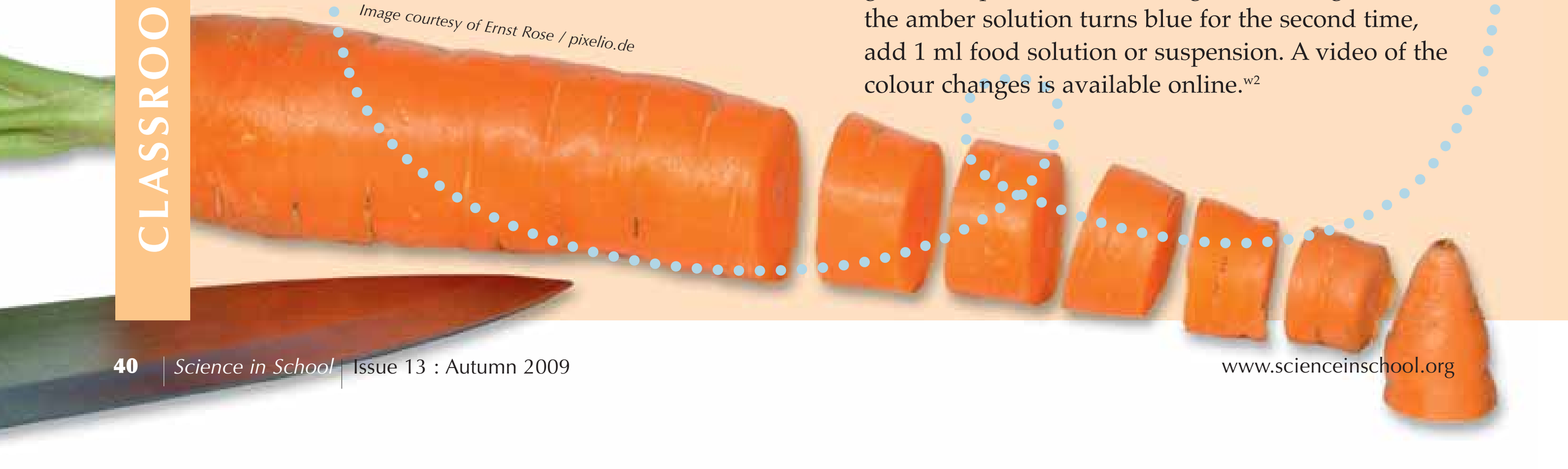
0.15 M malonic acid and 0.20 M manganese sulphate solution: dissolve 16 g malonic acid and 3.4 g manganese (II) sulphate monohydrate in approximately 500 ml distilled water in a 1 l flask. In a 100 ml beaker, heat 50 ml distilled water to boiling. In a 50 ml beaker, mix 0.30 g soluble starch with about 5 ml distilled water and stir the mixture to form a slurry. Pour the slurry into the boiling water and continue heating and stirring the mixture until the starch has dissolved. Pour the starch solution into the solution of malonic acid and manganese (II) sulphate. Dilute the mixture to 1.0 l with distilled water.

Food samples: to prepare the food samples as aqueous solutions or suspensions, put 2.0 g into a 400 ml beaker. Add 100 ml distilled water and stir with a glass rod. Decant, pour a portion into a test tube and centrifuge. For drinks, such as coffee or wine, take 2.0 ml, add 100 ml distilled water and stir.

Method

Into a 100 ml beaker containing a magnetic stirrer, pipette: 10 ml 4 M hydrogen peroxide aqueous solution, 10 ml aqueous solution of 0.20 M potassium iodate and 0.077 M sulphuric acid, and 10 ml aqueous solution of 0.15 M malonic acid and 0.20 M manganese sulphate. Start the magnetic stirring. When the amber solution turns blue for the second time, add 1 ml food solution or suspension. A video of the colour changes is available online.[w2]

Image courtesy of Ernst Rose / pixelio.de



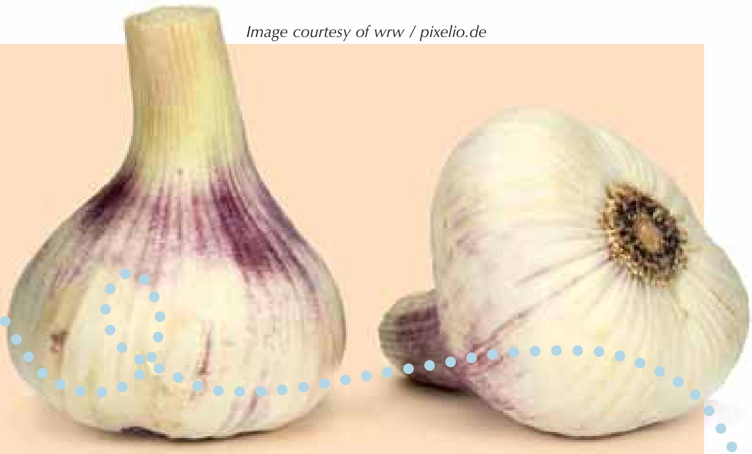


Image courtesy of wrw / pixelio.de

The Briggs-Rauscher reaction is an oscillating reaction – that is, a mixture of chemicals goes through a sequence of colour changes which repeats periodically. The exact mechanism of the reaction is still under investigation, but the nature of the oscillations is sufficiently clear. For the purposes of this article, it is enough to know that as long as the **radical** process maintains the concentration of the intermediate HOI higher than the concentration of the intermediate I^-, the solution remains **amber**; when the **non-radical** process takes place, $[I^-]$ is greater than [HIO] and the iodide ion combines with I_2 to form a **blue** complex with starch. A more detailed description of the reaction can be downloaded from the *Science in School* website[w1].

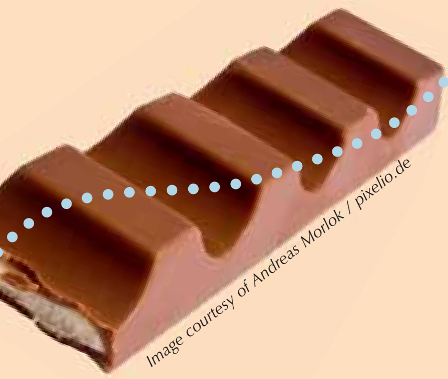


Image courtesy of Andreas Morlok / pixelio.de

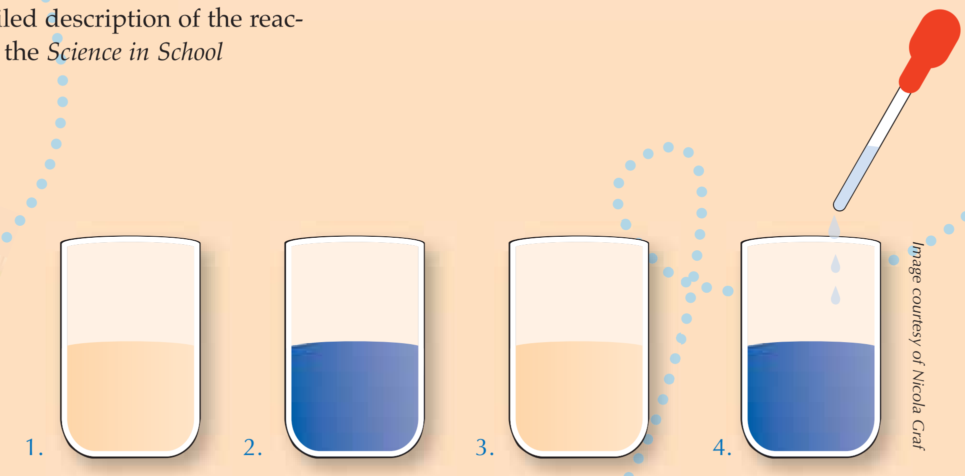


Image courtesy of Nicola Graf

Since we add the food solution or suspension after the second blue phase, when the non-radical phase is ending and the radical phase is about to start, the longer the time interval between the second and the third blue phases, the greater the antioxidant capacity of the food. In other words, the food has reacted with the radicals produced and the reaction takes longer to produce enough radicals to allow the oscillating reaction to continue.

We dedicated much time to choosing the best food or drink concentration to use, since too-dilute solutions lowered the antioxidant capacity and too-concentrated solutions increased the reaction time so much that it was not practical to make a statistically significant number of trials during the course of the lesson.

Safety notes:

Both malonic acid and iodine (produced during the reaction) can irritate the skin, eyes and mucous membranes; for this reason the reaction must be carried out in a fume cupboard.

Because 30% hydrogen peroxide is a very strong oxidising agent, eye protection, lab coats and gloves must be worn. Any contact between hydrogen peroxide and combustible materials must be avoided.

Sulphuric acid is a strong dehydrating agent; eye protection, lab coats and gloves must be worn.

To safely dispose of the mixture at the end of the experiment, slowly add sodium thiosulphate ($Na_2S_2O_3$) to the reaction products, until the excess iodine turns into colourless iodide ions (the reaction is quite exothermic).

Image courtesy of Gianluca Farusi

Sample results

When I performed the activity with my students, we found the greatest antioxidant activity in espresso coffee: 6970 seconds. See the graph below.

More details of our results can be downloaded from the *Science in School* website[w1]. Table 1 shows the foods we tested, the antioxidant activity (time interval) and the main substance presumed to be responsible for the activity.

Discussion

What, then, should we conclude from the results? Clearly, a diet consisting purely of espresso may contain high levels of antioxidants but would be far from healthy. When I did this activity with my students, it led to detailed discussion of radical reactions. Below are some questions that could be used to start a discussion.

1. Which groups of foods tested tend to contain the highest levels of antioxidants? Is that what you expected? Why/why not?
2. Table 1[w1] lists the antioxidant molecules presumed to be responsible for the antioxidant activity of the foods tested. Choose five antioxidants from the table, find their chemical formulae and discuss which functional groups are responsible for their antioxidant (free-radical scavenging) activity.
3. Some of the foods tested have been heat-treated (e.g. jams, chocolate). Does that appear to affect their antioxidant abilities? Table 2, which can be downloaded from the *Science in School* website[w1], shows the molecular structures of several antioxidant molecules, and gives examples of the foods they are found in. Looking at the molecular structures, would you expect these antioxidants to be thermolabile?
4. Apart from antioxidant activity, why might a diet rich in fruit and vegetables reduce your chance of developing the diseases mentioned at the beginning of the article? What other health benefits could it offer?

Antioxidant activity of the types of food and drink tested. The values in the graph were obtained from about 0.02 g samples (100 ml distilled water added to 2.0 g food or drink sample). Thus 0.02 g blackberry jam, for instance, has an antioxidant activity 50 times greater than that of distilled water!

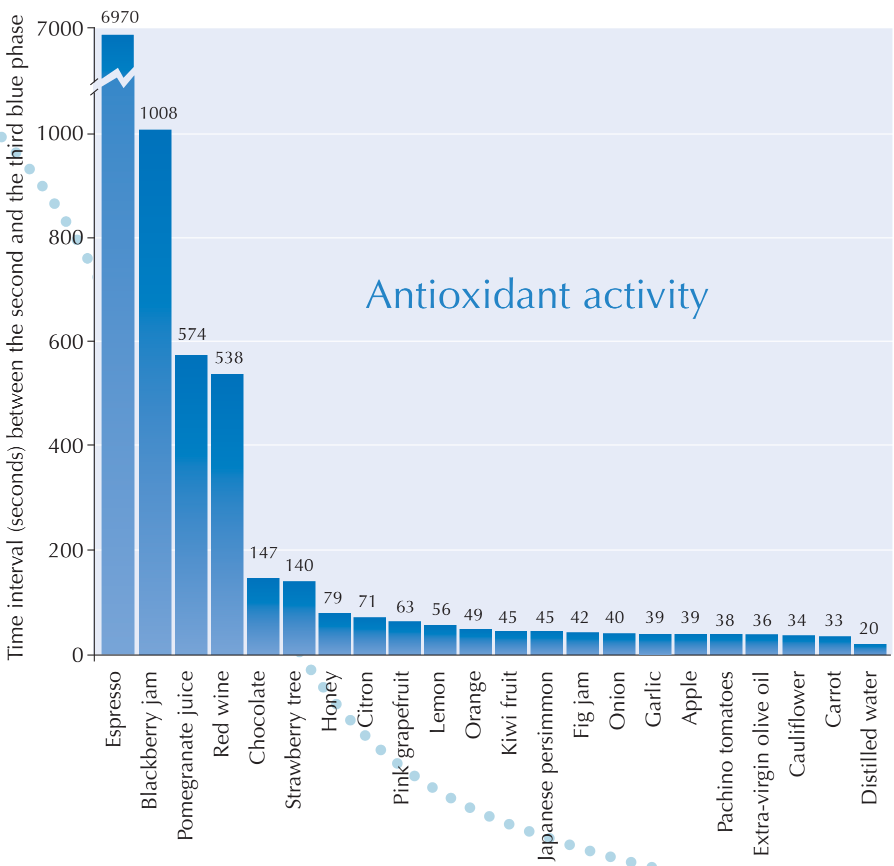


Image courtesy of Nicola Graf

References

Arking R (2006) *The Biology of Aging: Observations and Principles* 3rd edition. Oxford, UK: Oxford University Press. ISBN: 9780195167399

Polidori CM et al. (2009) High fruit and vegetable intake is positively correlated with antioxidant status and cognitive performance in healthy subjects. *Journal of Alzheimer's Disease* **17**: 921-927. Brief coverage of the article is available on the *Science Daily* website: www.sciencedaily.com/releases/2009/09/090909064910.htm

Swirsky Gold L, Slone TH, Ames BN (1997) Prioritization of possible carcinogenic hazards in food. In Tennant DR (ed) *Food Chemical Risk Analysis*, pp 267-295. New York, NY, USA: Chapman and Hall. This chapter is freely available online: http://potency.berkeley.edu/text/maff.html

Web references

w1 – Tables 1 and 2 and a detailed description of the Briggs-Rauscher reaction can be downloaded from the *Science in School* website: www.scienceinschool.org/2009/issue13/antioxidants

w2 – A video of the colour changes can be viewed here: www.youtube.com/watch?v=WXf6-bRwPfM

Resources

For descriptions of experimental methods similar to the one used in this article, see:

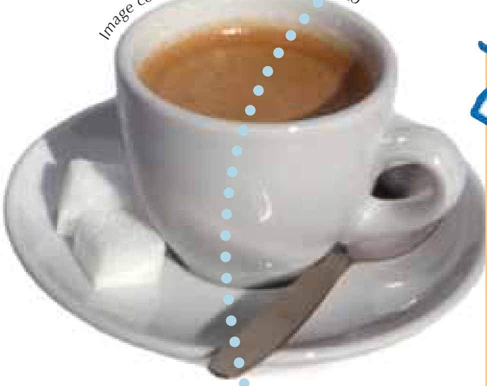


Image courtesy of colosal / iStockphoto

Höner K, Cervellati R (2002) Attività antiossidante di bevande. Esperimenti per le scuole secondarie superiori. *La Chimica nella Scuola* **24**: 30-38

Shakhashiri BZ (1985) *Chemical Demonstrations* Volume 2. Madison, WI, USA: University of Wisconsin Press. ISBN: 9780299101305

For a good (freely available) article about the effects of free radicals, see:

Sies H (1997) Oxidative stress: oxidants and antioxidants. *Experimental Physiology* **82**: 291-295. This article and all other *Experimental Physiology* articles older than 12 months can be downloaded free of charge from the journal website: http://ep.physoc.org

To learn about haemochromatosis, a disease in which free radicals play a role, see:

Patterson L (2009) Getting a grip on genetic diseases. *Science in School* **13**: 53-57. www.scienceinschool.org/2009/issue12/insight

For more information about free radicals and their activities in the body, see:

Dansen TB, Wirtz KWA (2001) The peroxisome in oxidative stress. *IUBMB Life* **51**: 223-230. doi:10.1080/152165401753311762

Rosen GM, Rauckman EJ (1981) Spin trapping of free radicals during hepatic microsomal lipid peroxidation. *Proceedings of the National Academy of Sciences USA* **78**: 7346-7349

See also Gianluca Farusi`s previous articles in *Science in School*:

Farusi G (2006) Teaching science and humanities: an interdisciplinary approach. *Science in School* **1**: 30-33. wwwscienceinschool.org/2006/issue1/francesca

Farusi G (2007) Monastic ink: linking chemistry and history. *Science in School* **6**: 30-40. www.scienceinschool.org/2007/issue6/galls

A complete list of all chemistry-related articles published in *Science in School* is available here: www.scienceinschool.org/chemistry

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Gianluca Farusi teaches chemistry at the technical school (Istituto Tecnico Industriale) Galileo Galilei in Avenza-Carrara, Italy, and stoichiometry at the University of Pisa, Italy. He has been teaching for 12 years and nothing gratifies him more than the delight on his students' faces when they grasp a difficult chemical concept.

This activity, which was carried out with his school students, won Gianluca an EIROforum Science Teaching Award: the ILL Prize (Science on Stage 2, 2007). He has also been awarded the ESRF Prize (Science on Stage 1, 2005) and the Italian Chemical Society's Illuminati Prize for Chemistry Didactics (2006).


Image courtesy of Gianluca Farusi

REVIEW

This is a superb article which underlines the importance of chemistry in the behaviour of biological systems. It is so important to instil an appreciation of the way in which scientific knowledge is multidisciplinary. Teachers and students could use the article for a practical activity in chemistry, biochemistry, food-science or health-science lessons. It could also form the basis of science-fair projects.

If the experimental part were left out, the introduction, discussion and results could be a sound basis for a comprehension exercise. Suitable questions, which could be used to spark off a discussion about food and health, or chemistry in everyday life, could include:

1. Explain the term radical.
2. What do you understand by the term 'antioxidant'? Explain how this type of substance can be important for good health.
3. What types of food are the best sources of antioxidant molecules?
4. How could an average person ensure that their diet maximises the levels of antioxidants?

Marie Walsh, Republic of Ireland