The antioxidant activity of honey

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What are antioxidants?

Antioxidants are substances which protect the body from damage caused by free radicals. Free radicals are very reactive chemicals which react with and change other molecules next to them. The changed molecules no longer function normally.

If they are protein molecules that get changed, these can cause the alterations in the body that cumulatively give the deterioration seen in ageing, such as hardening of the skin and the arteries., and the development of cataracts in the eyes. If the changed molecules are the fatty acids in the membranes around the outside of cells, these membranes break and the cells die.

Free radicals can also activate inflammation and can activate very destructive enzymes which damage the structure of the body's tissues.

If the molecules changed by free radicals are the DNA in the cell, then mutations occur in the genes. This can cause cancer. If it occurs in the sperm or egg cells it can be passed on to the next generation as abnormalities.

Free radicals set off chain reactions. Each time they react with another molecule a new free radical is formed, which reacts with another molecule, and so on. This leaves a trail of damage behind.

Where do free radicals come from?

Free radicals can arise from chemicals (both synthetic and natural ones) that enter the body from food and drinks (*e.g.* alcohol) and polluted air. They also arise from exposure of the body to ionising radiation such as gamma rays from the sun and from X-rays. Mostly the free radicals formed in the body arise from the body's own metabolism. Most of all the production of free radicals in the body comes from the breakdown of hydrogen peroxide that is produced in metabolism.

The formation of free radicals from hydrogen peroxide involves catalysis by free iron atoms. Iron is mostly kept safely in a non-catalytic form by being bound up by proteins. However, in many situations (*e.g.* sites of infection, injury or inflammation, in extreme aerobic exercise, and in alcoholics) iron gets released from the proteins which keep it safely bound up. Unbound iron causing free radicals to be formed is the cause of most of the degenerative diseases associated with ageing. A comprehensive review of the very large number of scientific papers on this has been published by D. Kell: "Iron behaving badly" http://www.biomedcentral.com/content/pdf/1755-8794-2-2.pdf .

How do antioxidants work?

Antioxidants work in one (or sometimes both) of two ways. They can stop the chain reaction by getting involved in it, or they can stop free radicals being formed in the first place.

Because free radicals react so quickly, if an antioxidant is to be effective in stopping a damaging chain reaction, the antioxidant molecule has to be right next to the free radical formed, so that it is the next thing that the free radical reacts with. The chance of there being an antioxidant molecule next to each free radical as it is formed is low unless the quantity of antioxidants taken into the body is massive, so some damage is inevitable.

Antioxidants which prevent free radicals being formed in the first place give much better protection. These work by binding up free iron. There is time for them to diffuse around and 'find" the iron atoms. If there is sufficient antioxidant for all the iron to be safely bound up, then there is little chance of free radicals being formed to cause damage.

Some antioxidants which break free radical chain reactions can actually increase damage from free radicals. This is because they react with iron to convert ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). When iron catalyses the formation of free radicals from hydrogen peroxide it is only the ferrous (Fe^{2+}) form of iron that works. In the reaction this is converted to the inactive ferric (Fe^{3+}) form. Antioxidants such as Vitamin C and some of the polyphenols from plants recycle the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) to give a lot more reaction forming free radicals. Only when they are in the body at very high levels do their protective action against free radicals outweigh their simulation of production of free radicals.

Otherwise, are all antioxidants equally effective?

There are many foods and drinks claimed to have a high antioxidant content, but some methods used to measure antioxidant activity in foods and drinks are not all that relevant to protection of health, and some antioxidants are not very "bioavailable" (*i.e.* are poorly absorbed into the circulation from the gut).

Various methods are used to measure antioxidant activity. These measure activity in different ways, and can give different results for the same food or drink. The most commonly used method is the ORAC assay, but this picks up antioxidant molecules with slow reaction inactivating free radicals as well as those with rapid inactivation. The slowly reacting ones are not of benefit if one is considering protection of health rather than prevention of oxidative spoilage of foodstuff. Any antioxidant that does not give an immediate reaction will not stop the damaging free radicals (the very reactive ones) from damaging essential cell components – reactive free radicals react within a millisecond with whatever molecule is next to them.

Another commonly used method for measuring antioxidant activity is the FRAP assay, which measures the conversion by antioxidants of the oxidised form of iron (Fe^{3+}) to the reduced form (Fe^{2+}). But as described above, antioxidants which do this recycle any unbound iron in the body to give a lot more reaction forming free radicals. Antioxidants which do not do this will not be measured by the FRAP assay.

There are also assay methods which measure inactivation of relatively stable free radicals (ABTS or DPPH). These can be used to measure instantaneous inactivation of free radicals.

None of these methods distinguish bioavailable antioxidants from ones which are not bioavailable. Many of the of the polyphenols from plants are poorly absorbed from the gut into the bloodstream. A good example of this are the anthocyanins in red wine, which are famous antioxidants but are poorly absorbed from the gut. (It is probably the antioxidants that come from the grape seeds that give red wine its health-protecting action.) At the University of Waikato we have been developing a test to find if antioxidants can cross the membranes of cells cultured in the laboratory. That will allow us to be able to easily test if the antioxidants in different types of honey are bioavailable.

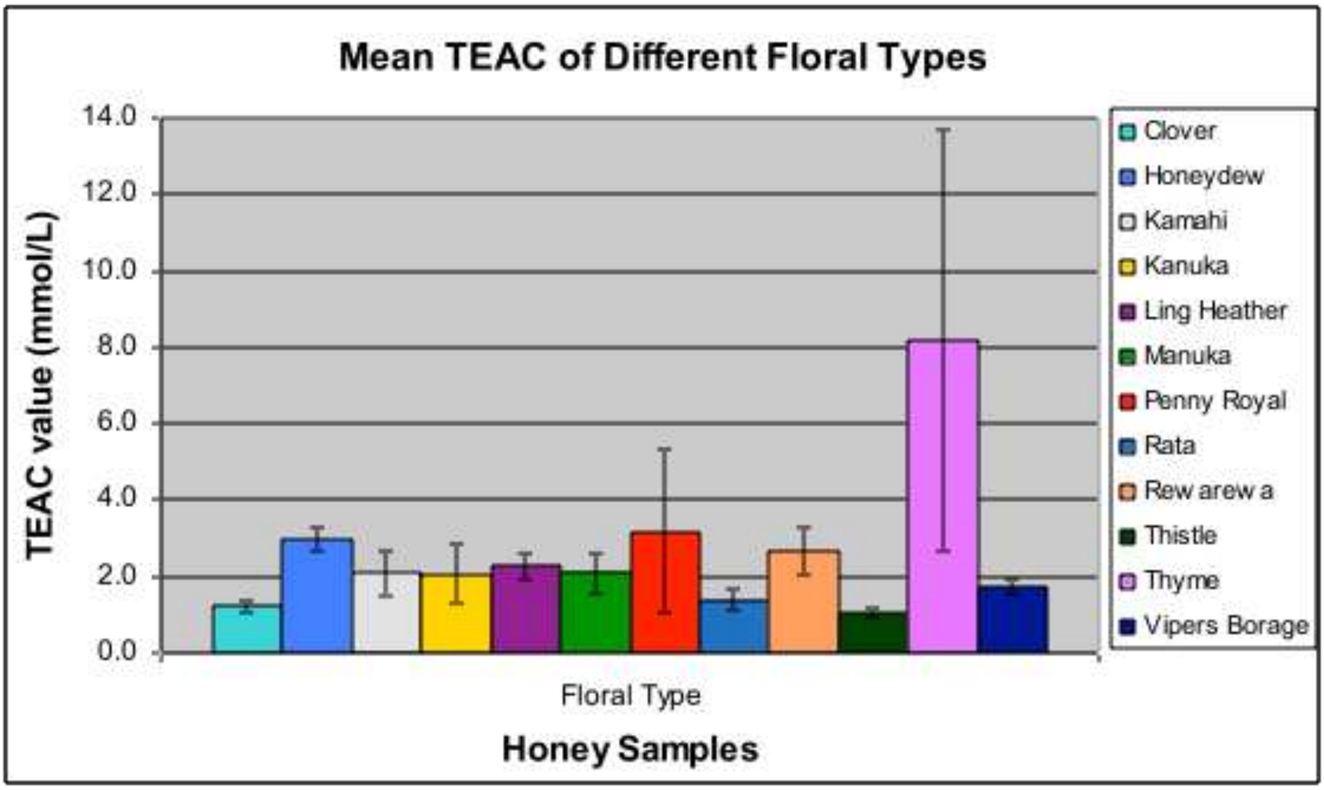
How good is honey as a source of antioxidants in the diet?

A comprehensive review, by Erejuwa *et al.*, of the papers published on antioxidants in honey can be downloaded free of charge from http://www.mdpi.com/1420-3049/17/4/4400.

Relative to other foods the antioxidant activity of honey is quite high. However, the quantity of honey consumed in the diet when it is used as a spread on bread is low compared with the quantity of many other food sources of antioxidants. But if honey is used instead of refined sugar as a sweetener for food and drinks it can make a substantial difference to the quantity of antioxidants consumed in the diet, because the typical diet contains a lot of refined sugar and this contains no antioxidants.

It has been shown that consumption of honey increases the level of antioxidants in the bloodstream. However, it was only buckwheat honey that was used, so it is not known if the antioxidants in other types of honey are bioavailable. But there have been some other reports published, from studies with non-specified types of honey, where effects have been seen which indicate that antioxidants are being absorbed into the body from the honey.

There has been a lot of research published on studies measuring the antioxidant activity in honeys in various countries, and differences have been found between honeys from different species of plants. In the USA buckwheat honey has been found to have the highest antioxidant activity. At the University of Waikato we have surveyed the main commercial types of honey to compare their antioxidant activity. We measured the inactivation of the ABTS free radical, with the method modified so that we measured only instantaneous reaction. We collected at least ten samples of each type of honey to ensure that the results were representative. The results are shown in the following graph:



The 'T' lines on each bar on the graph show the standard deviation from the average values that the bars show. It can be seen that there is a large variation from sample to sample within a single type of honey. For this reason consumers seeking honey with high antioxidant activity cannot rely on purchasing a honey of a type which on average has a high activity. As an illustration of this, in our survey thyme honey was found to be (going on average values) the type of honey with by far the best antioxidant activity, but of the total of 215 samples tested in the survey one of the samples of thyme honey had the lowest activity of all. Marketers of honey are now starting to sell honey with the level of antioxidant activity of tested batches given on the label.

"Pre-emptive antioxidant activity" in honey

As discussed above, antioxidants which bind up iron and prevent free radicals from being formed in the first place give superior protection compared with other antioxidants where you are relying on them maybe intercepting free radicals before they do any damage to the body. This type of antioxidant activity has been termed "pre-emptive antioxidant activity". At the University of Waikato we have devised a method for measuring this. It is based on a model of the damage that is done by free radicals in the body to cell membranes and to the lining of arteries. A small amount of iron is added to make the damage occur very rapidly. (This level of iron is as occurs in some of the conditions under which free iron causes damage in the body.) Honeys are tested in this model to find how much protection from damage they give.

In this testing thyme honey, which is the type that had by far the highest antioxidant activity in the survey of honeys for their ability to inactivate free radicals, showed no protection at all from damage caused by unbound iron. New Zealand beech honeydew honey was found to have a very high level of "pre-emptive antioxidant activity". It was found to be more protective than massively high levels of vitamin C which is a well known for its ability to mop up free radicals. (The text of the paper published on this work can be downloaded free of charge from the University of Waikato 'Research Commons': http://researchcommons.waikato.ac.nz/handle/10289/4687 .) As with all features of honey, this activity varies from batch to batch, so each batch needs to be tested for consumers to know that they are purchasing a batch with a high level of activity. There is New Zealand beech honeydew honey on sale with its level of "pre-emptive antioxidant activity" marked on the label, as a 'P−AXC' rating.

