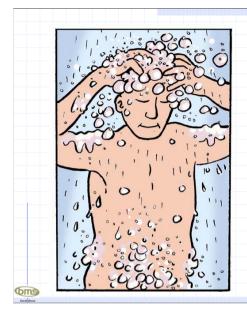


Fungi are used every day in a very wide variety of ways. Fungi are an ideal food because they have a fairly high content of protein (typically 20 to 30% crude protein as a percentage of dry weight) and contain all the amino acids which are essential to human health - and several of the vitamins, too. Fungi are easily digested and also provide a good source of dietary fibre. Possibly most important of all, though, is that they are virtually free of cholesterol. Fungi just don't use cholesterol to the extent that animals do (fungi use a different sterol which doesn't accumulate in humans). Consequently, fungal foods compare very favourably with meats on health grounds as well as on grounds of nutritional value.

But our dependence on fungi goes way beyond foods. We use products of their metabolism in industrial processes, in housekeeping, in our medicines and in our social life. Every hour of every day!



Dave has a quick shower, sharing his bathroom with a few fungi.

Fungi are all around us wherever we live, work or play.

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Houses are just extensions of the natural environment. The timber and fabrics (carpets, curtains, even the wallpaper) of an average house are just like the plant litter – the leaves, twigs and branches – of the forest floor as far as fungi are concerned.

Ever wondered what happens to all the leaves that fall from the trees, or the branches that fall to the ground? Somehow there's never a build up of all this organic matter, but what happens to it? Well, it's broken down and recycled by many small organisms like bacteria, insects, worms and many types of fungi. The resultant humus is full of nutrients for plant growth.

The cell walls of plants are very strong. The polymers cellulose and lignin provide the strength. Fungi are the only organisms capable of breaking down both cellulose and lignin. Brown rot fungi breakdown cellulose best and they're called brown rots because a lot of the lignin remains intact so the wood keeps its brown colour. The enzymes released by brown rot fungi break the cellulose into molecules of glucose sugar to feed the fungus.

Lignin is the other strong polymer. Fungi that break down lignin are called white rot fungi, because as the content of lignin is decreased, the wood becomes lighter in colour. White rot fungi degrade lignin by releasing oxidising enzymes from their hyphae – they 'burn' the wood in an enzyme-controlled way. Lignin contains phenols and the white rot fungi are the only organisms that can turn these chemicals into nutrients.

These two types of fungi have important roles in recycling nutrients. Without them, dead plant material would not decay and soil nutrients would be locked into a mass of undegradable biomass. The downside is that the fungi that can degrade wood in the forest, can become the wood-decaying pests in our homes – dry rots, wet rots, moulds and mildews. You're never far from a fungus!



We use fungal enzymes as 'fabric conditioners' to restore fabrics in the wash. The enzyme removes broken fibre ends and makes the fabric 'look like new'.

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USES OF MICROBIAL ENZYMES (* indicates a process in which bacterial enzymes may be used)

- a. Textiles. Cellulolytic enzymes denim treatment (stone washed finish);
 biofinishing of fabrics (removes loose fibres).
- Biological detergents Have to be active under alkaline conditions: proteases*, lipases, amylases and cellulases (for colour brightening and restoration).
- Starch hydrolysis in production of high fructose syrup. Second largest use of enzymes after use in detergents.
- d. Cheese making Rennilase® from a fungus called *Rhizomucor miehei* used instead of rennin (chymosin).
- e. Brewing amylase, glucanases and proteases used to 'mash' barley in beer production.
- f. Wine and fruit juice Pectinases used to remove pectin to pulp fruit.
- g. Proteases used to modify protein from a wide variety of sources, e.g. milk (whey protein), soya bean, wheat, fish, meat.
- h. Baking. Many flours are deficient in amylases, so fungal enzymes are added; also, xylanases increase elasticity of gluten in the dough by breaking down xylan polymers in the flour.
- i. Pharmaceuticals. 6-amino penicillanic acid (6-APA), a semisynthetic penicillin precursor (side chain removed by a fungal acylase). Fungi are used to convert readily available plant steroids into therapeutically useful compounds, and are also the source of many widely-used drugs.



Do you like stone-washed denim? Stone washing used to mean washing with small stones to 'distress' the fabric: but no more. Now fungal enzyme treatment randomly breaks cotton fibres giving the fashionable bleached look.

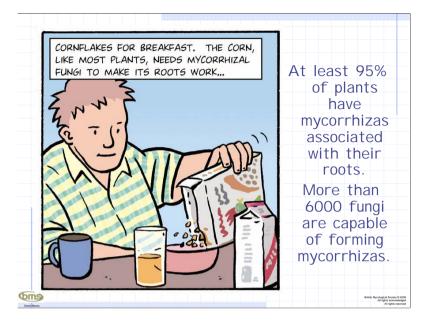
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"Stone washing" uses endo-cellulases: The effect used to be achieved by washing with small stones; now, fungal endocellulases randomly break cotton fibres giving bleached look because the broken fibres on the surface of the fabric scatter light and give the faded colour effect.

Restoration of fabrics using fabric conditioners containing exo-cellulases essentially does the reverse of this – wearing the cloth breaks cotton fibres and the broken ends reduce the vibrancy of the pigments. When the fabric is washed with a conditioner the conditioner trims away the broken ends that scatter the light, so the washed garment 'looks like new'.

Some other industrial uses for fungal enzymes include proteinases and lipases from *Aspergillus oryzae* used in detergents and proteinases used in hide processing in the tanning industry. The global value of enzymes from filamentous fermentations is in the region of US\$ one billion.

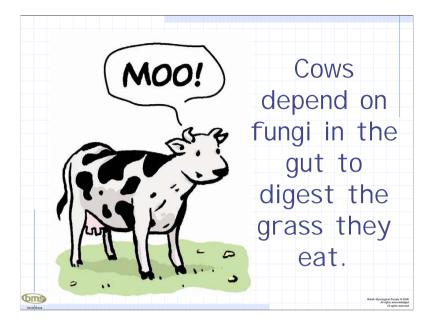
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Plants gain their nutrients by absorbing minerals and water from the soil using their roots. But they get quite a lot of help from certain species of fungi. The relationship appears to have started because the plant roots alone are not able to supply the plant with all the nutrients it needs. The fungi associated with plant roots are called mycorrhizas, which increase nutrient availability to the plant. The numerous hyphae of the fungi greatly increase the surface area available for absorbing minerals. The hyphae can also go looking for food; by growing into areas of fresh nutrients. The relationship between the plant and fungus is mutualistic; which means that both sides gain something from having the other present. The plant pays for the privilege of using this fungus to bring it nutrients by sharing up to 25% of the products of its own photosynthesis with the fungus. The fungus benefits by taking readily available sugars from the plant. Despite this 'tax' on its activities, the plant grows much better than it would without the mycorrhiza.

Some mycorrhizal fungi form a mat of fungal tissue around the root; the fungal cells grow between the cells of the plant root, but never actually cross the plant cell walls. These are called 'ectomycorrhizas'. In another mycorrhizal partnership (called endomycorrhizas) the fungal cells enter the plants cells. Inside the plant cells they make structures that exchange nutrients

with the plant cytoplasm. By greatly increasing the absorbing surface of a host plant's root system, mycorrhizas improve the plant's ability to tolerate drought and other extremes, like high and low temperatures and acidity. As many as 95% of all plants have mycorrhizal associations, showing just how important these types of fungi are for the growth of so many plants, including all the crop plants we need to feed the human population, and all the trees in all the forests.

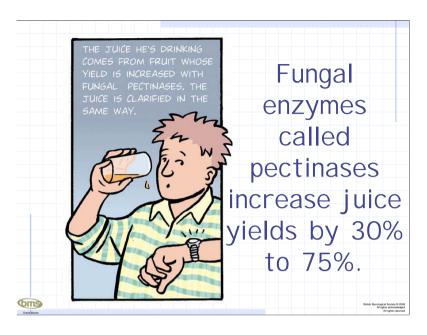


Many animals including cows, sheep, goats, deer, and even giraffes, are known as ruminants. This is because they have a specialised four-chambered stomach needed for the digestion of their exclusively vegetarian diet. The first chamber the food enters is called the rumen, hence the name ruminant. The ruminant we're most familiar with is the cow, and we all know that cows spend most of their time eating grass and hay.

Plant cell walls contain cellulose, which is an excellent source of fibre in the diet of most animals. However cows, like all animals, do not produce enzymes capable of digesting cellulose themselves; so without help they can't extract the nutrients the grass contains. The cow has special fungi in the rumen called chytrids; or more generally called rumen fungi. These fungi are anaerobic, meaning they are able to survive and digest plant cell walls without oxygen. The rumen acts like a large fermenter because the grass is stored there whilst the fungal enzymes from the chytrids break down the cellulose.

After the plant material is processed in the rumen, it is brought back up into the mouth of the cow. This material is now called 'cud' and the cow chews it again to grind it down further. When it is swallowed for the second time it passes through the next three chambers of the stomach.

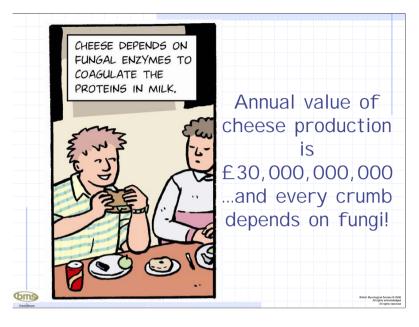
The relationship between chytrids and ruminants is said to be symbiotic. This means that both the fungi and the cow benefit from having the other present. In this case the cow benefits because plant material the animal can't degrade is digested and turned into materials the cow can absorb. In return, the fungi live off some of the nutrients obtained from the cow's food, and live out their lives in the cow's rumen.



Use of pectinolytic enzymes increases juice yields from 30 to 75%. The enzymes are produced by fungi to help them penetrate plant tissues. The enzymes degrade pectins that surround and link plant cells together; this makes it easier to squeeze out the juice. Pectinases are also used to enzymatically 'peel' fruit and vegetables prior to canning.



Bread structure depends on the gooey-gluey properties of the wheat gluten protein. Mix wheat flour with water, yeast, salt, sugar and some fat and the dough will stick to anything – it's the gluten that does the sticking. Put the dough in a warm place for at least an hour, ideally two, and the yeast produces carbon dioxide and alcohol. The carbon dioxide gas is trapped into bubbles by the gluten and as more and more bubbles are formed in the dough they make it 'rise'. After this fermentation period the dough is cooked, the alcohol evaporates (shame!) and the bubbly structure of the dough is turned into the open structure of bread. Try this website for information about bread: http://www.fabflour.co.uk/



Cheese production relies on the action of enzymes which coagulate the proteins in milk, forming solid curds (from which the cheese is made) and liquid whey. The enzyme Rennet (chymosin), is a protease that removes surface glycopeptides from soluble casein in milk and the treated casein (paracasein) then forms particles with calcium and precipitates (forming the curds).

Traditional cheese-making uses animal enzymes, but now around 80% of cheese making uses non-animal coagulants, especially enzymes from moulds like *Aspergillus* species and *Mucor miehei*, so most industrial cheese production still depends on enzymes from filamentous fungi for the coagulation step.

Mould ripening is a traditional method of flavouring cheeses which has been in use for at least two thousand years. Blue cheeses, like Roquefort, Gorgonzola, Stilton, Danish Blue, Blue Cheshire, use *Penicillium roquefortii* which is inoculated into the cheese. During storage at controlled temperature and humidity the fungus grows throughout the cheese, producing the major flavour and odour compounds.

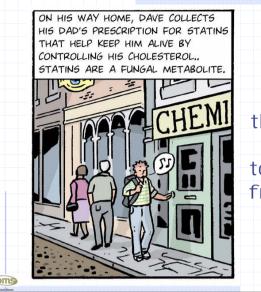
Camembert and Brie are ripened by *Penicillium camembertii*, which changes the texture of the cheese rather than its flavour. This fungus grows on the surface of the cheese producing enzymes which digest the cheese to a softer consistency from the outside towards the centre. http://www.cheese.com/



Apart from alcohol, the single most important chemical produced by fermentation is citric acid, more than 600,000 tonnes of which are produced (mostly by *Aspergillus niger* and *A. wentii*) each year to be used mainly in effervescent soft drinks. The standard production method is a fermentation of sugar beet or cane molasses or glucose syrup.

Citric acid is widely used as an acidity regulator and antioxidant in many foods and beverages and a stabiliser in medicines. It was originally isolated from lemons (expensive) but was found to be produced by *Aspergillus niger* in the 1920's and commercialised in early 1940's. It was, in fact the first biotechnology industry.

http://ejbiotechnology.ucv.cl/content/vol5/issue3/full/3/index.html



Some of the wonder drugs of today come from fungi.

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Most of us appreciate that if we have too much cholesterol the body is not able to use up the excess so it sticks to the inside walls of our blood vessels. This build up reduces the diameter of the vessels, and this restricts blood flow. If blood vessels that supply blood to the heart become clogged up like this it can cause a heart attack, because the heart muscle does not receive enough oxygen to function properly. To control heart disease it's important that humans regulate their cholesterol level.

The most effective cholesterol lowering-agents we have today are called Statins, and these are produced by fungi. The two fungi used to produce statins are called *Aspergillus terreus* and *Penicillium citrinum*. Statins inhibit enzymes needed to make cholesterol, so production of cholesterol is slowed down. With dietary control you can decrease the patient's cholesterol level.

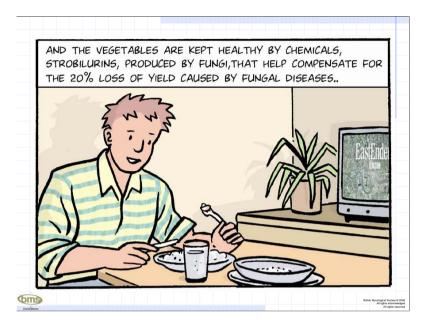
Today, many people rely on statins from fungi to help keep their cholesterol level normal; the drugs are credited with saving 7000 lives a year in the United Kingdom alone!

Cyclosporin is another crucial wonder drug of today. It makes successful long-term transplant of livers, kidneys, hearts and lungs possible. This compound is produced by the fungus *Tolypocladium inflatum*. The fungus was isolated from a soil sample and screened in a search for antibiotics. The compound cyclosporin was found to be a weak antibiotic, but to have strong activity at suppressing the immune system (= immunosuppressive). This is the crucial role of this drug now. Our bodies are programmed to eliminate foreign things, and the body will naturally reject a transplanted organ. Cyclosporin helps prevent rejection by reducing the production of lymphocytes. If lymphocytes are not able to increase in number there is a better chance that the transplant will continue to function normally. Cyclosporin is currently the most effective and widely used immunosuppressive drug.



Mushrooms are the most valuable horticultural crop sold in the UK; and, worldwide, mushroom cultivation is the next-biggest biotechnology industry after alcohol production. Mushrooms are available 365 days a year. The white *Agaricus bisporus* mushroom accounts for 95% of the total British market. The remaining 5% is shared between the cultivated brown/chestnut/portabello mushrooms (which are different varieties of *Agaricus bisporus*) and the so-called "exotics" like the cultivated oyster mushroom, shiitake (Chinese mushroom), blewit, enoki, horse mushroom, and hon-shimeji. Try the Mushroom Bureau website http://www.mushroom-uk.com

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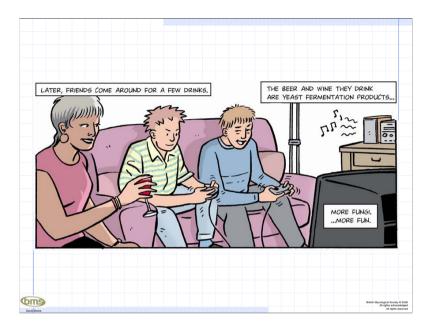


The raw material used in the manufacture of *Marmite* spread is brewer's yeast. For many years this by-product of the brewing process was seen as a nuisance rather than a potentially valuable food. Towards the end of the nineteenth century the German chemist, Liebig found that this yeast could be made into a concentrated food product which resembled meat extract in appearance, smell and colour, though it was vegetarian. In 1902, the Marmite Food Company Ltd, later changed to Marmite Limited was formed. This company perfected a form of yeast extract for popular consumption, originally in a rented disused malthouse in Burton-on-Trent. It's been spreading ever since! http://www.marmite.com/

Myco-protein is the name for a food product made from a filamentous fungus called *Fusarium venenatum*. The fungus is grown on food grade glucose as the carbon source in a 45 metre tall airlift fermenter. Medium is added continuously and mycelium + spent medium is harvested at a rate equal to the production of new fungus. The fungus is processed to reduce nucleic acid content to about 1%, then harvested to give a raw product containing about 30% solids. Myco-protein typically contains 44% protein, 18% dietary fibre and only 13% fat (the values for beef are 68%, 0% and 30% respectively). The sole use for myco-protein at the moment is as the primary ingredient of the Quorn range of 'meat-alternative' products. http://www.quorn.com/

In soy sauce production soybeans are soaked, cooked, mashed and fermented with two moulds called *Aspergillus oryzae* and *Aspergillus sojae*. When the soybean substrate has become overgrown with the fungus the material is mixed with salt and water and the fermentation is completed in the brine. The brine fermentation takes six to nine months to complete, after which the soy sauce is pressure-filtered, pasteurized and bottled.

http://www.japanweb.co.uk/listing/soy.htm



Beers and ales are also products of the fermentation of sugars from cereal grains by the yeast called *Saccharomyces cerevisiae*. All ales, beers and lagers are made from malted barley mashed into hot water and boiled with hops to add bitter flavours to the beer. The yeasts used for making ales tend to form froth and grow on the top of the mix. Lager yeasts do their fermenting at the bottom of the tank and they belong to the related species *Saccharomyces carlsbergensis*. http://www.brewingtechniques.com/

Wine yeasts are an elliptically-shaped variant of *Saccharomyces cerevisiae*. The classic wine grape has the scientific name *Vitis vinifera*. The important cultivars include Sauvignon (red Bordeaux); Pinot Noir (the main red Burgundy grape); Riesling and Silvaner (for German white wines); Barbera and Freisa (northern Italian wines); and Palomero, the main sherry grape. The whole of the black grape is crushed to make red wine; it's the grape skin pigment that makes it red. Black or white grapes can be used to make white wines but only the pressed juice is used and extraction of skin pigments is avoided. http://www.wines-411.com/; http://www.vinopolis.co.uk/; http://www.winepages.com/

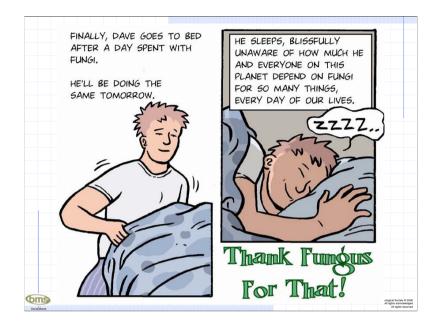
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Fermentations also play a role in the production of chocolate and coffee. In these cases the fermentation is responsible for the preparation of the bean, to separate it from the pod and mucilage in cocoa; or the cherry and mucilage in coffee. The fermentation of coffee is just to remove the mucilage so that coffee beans (two from each cherry) can be dried and further processed. In cocoa production the fermentation does breakdown the mucilage, but in addition the fermentation is absolutely essential for flavour development.

http://www.exploratorium.edu/chocolate/; http://www.realcoffee.co.uk/; http://www.nationalgeographic.com/coffee/

Interestingly, although tea leaves are also fermented, in this case the process involves only enzymes from the plant leaves themselves and neither fungi nor bacteria are involved.



You're never far from a fungus.

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Every hour of every day!