### 10 Food Biochemistry

chromatography, protein mixtures are passed through an inert stationary phase containing beads having pores of known size. Thus, larger molecules will take a more direct (and hence faster) path relative to smaller molecules, which can fit into the beads, resulting in a more 'meandering', longer path, and therefore, elute slower (in comparison to larger molecules). Perhaps the most basic biochemical analysis is the determination of molecular mass. Polyacrylamide gel electrophoresis (PAGE) in the presence of a detergent and a reducing agent is typically used for this purpose (mass spectrometry is used for precise measurement where possible).

*Food allergenicity:* The primary role of the immune system is to distinguish between self and foreign biomolecules in order to defend the host against invading organisms. Antibody proteins that are produced in response to the foreign compounds are specifically referred to as immunoglobulins (Igs).

Allergic diseases, particularly in industrialised countries, have significantly increased in the last two decades. The most common causes of food allergic reactions for the young are cow's milk and egg, whereas adults are more likely to develop sensitivity to shellfish. Such reactions are thought to result from an abnormal response of the mucosal immune system towards normally harmless dietary proteins. Allergic reactions are distinct from food intolerances that do not involve the immune system.

One of the biggest problems with food allergy management is that avoidance of antigen is the primary means of preventing allergic reactions, however, minute amounts of so-called 'hidden allergens' in the form of nut, milk and egg contaminants occur in many processed foods. To avoid allergens entirely may pose the risk of avoiding nutritionally important foods, resulting in malnutrition, especially in the young, a problem highlighting the need for control of allergens in foods including the making of hypoallergenic food products. As a means of reducing the allergenicity of egg proteins, enzymatic treatments have been studied. The major limitations or potential hurdles to such an approach are the need for the allergen epitope(s) to be directly impacted, i.e. cleavage upon enzyme treatment, and the retaining of the unique functional properties of egg proteins in foods, e.g. foaming and gelling.

### FOOD LIPID BIOCHEMISTRY

The characterisation of the lipid fraction present in fats and oils became very important in the food industry. The composition of the lipid fraction has not only an influence on the nutritional value of the food but is also important for health related issues (digestion, prevention of heart diseases, etc.).

**Phospholipid:** Phospholipids are a class of lipids that are a major component of all cell membranes as they can form lipid bilayers. Most phospholipids contain a diglyceride, a phosphate group, and a simple organic molecule such as choline, one exception to this rule is sphingomyelin, which is derived from sphingosine instead of glycerol. The structure of the phospholipid molecule generally consists of hydrophobic tails and a hydrophilic head. Biological membranes in eukaryotes also contain another class of lipid, sterol, interspersed among the phospholipids and together they provide membrane fluidity and mechanical strength. Purified phospholipids are produced commercially and have found applications in nanotechnology and materials science.

Phospholipids have been widely used to prepare liposomal, ethosomal and other nanoformulations of topical, oral and parenteral drugs for differing reasons like improved bio-availability, reduced toxicity and increased penetration. Ethosomal formulation of ketoconazole using phospholipids showed good entrapment efficiency, stability profile and is a promising option for transdermal delivery with potential for topical application in fungal infections. Liposomes are often composed of phosphatidyl-choline-enriched phospholipids and may also contain mixed phospholipid chains with surfactant properties.

# Chapter 2

# Water Chemistry and Biochemistry

#### INTRODUCTION

Water is an essential part of all living organisms, making up 70% or more of the weight of most organisms. It consists of an oxygen atom connected to two hydrogen atoms by polar covalent bonds. It is considered to be the universal solvent for many reasons including its structural, chemical and physical properties. Water is capable of dissolving a variety of different substances, which is why it is such a good solvent. In fact, water is called the 'universal solvent' because it dissolves more substances than any other liquid. This is important to every living thing on Earth. It means that wherever water goes, either through the air, the ground, or through our bodies, it takes along valuable chemicals, minerals, and nutrients.

Water is the only substance that exists naturally on Earth in all three physical states of matter—gas, liquid, and solid—and it is always on the move among them. The Earth has oceans of liquid water and polar regions covered by solid water. Energy from the Sun is absorbed by liquid water in oceans, lakes, and rivers and gains enough energy for some of it to evaporate and enter the atmosphere as an invisible gas, water vapour. As the water vapour rises in the atmosphere it cools and condenses into tiny liquid droplets that scatter light and become visible as clouds. Under the proper conditions, these droplets further combine and become heavy enough to precipitate (fall out) as drops of liquid or if the air is cold enough, flakes of solid, thus returning to the surface of the Earth to continue this cycle of water between its condensed and vapour phases.

Water in all three states makes a large contribution to the planet's climate. Water vapour is a greenhouse gas that traps energy radiated from the surface of the planet and helps to keep the planet warm enough to sustain the complex life that has evolved in this environment. Water vapour is responsible for more than half the Earth greenhouse gas warming. On the other hand, clouds and ice fields on the surface reflect a good deal of the radiation from the Sun, so this radiation does not reach the surface and warm it. The reflectivity of clouds and ice has a cooling effect on the planet. However, where the Earth surface has been heated by solar radiation, clouds help trap energy radiated from the heated surface and thus have a warming effect as well. Variations in the amount and form of water in the atmosphere have a complex relationship to our climate that is difficult to model and predict. Pure water is colourless, odourless, and tasteless and so common that we probably never think about how unique it is and how

### 20 Food Biochemistry

But why does a simple molecule like water,  $H_2O$ , play so many important roles in food? It all has to do with something called 'hydrogen bonds' that occur between water molecules, and between water and other molecules that contain oxygen and nitrogen atoms, such as proteins, and carbohydrates. A molecule of water has a unique structure with two hydrogen atoms bonded to a single oxygen atom. Rather than being a linear molecule (H-O-H), the two oxygen-hydrogen bonds are separated by an angle of 104.5° due to the repulsion of other electrons (called non-bonded electrons) in the oxygen atom. But more importantly, oxygen is a very 'electronegative' atom. It has a strong affinity for electrons, while hydrogen does not. So the two electrons in each oxygen-hydrogen bond of a water molecule spend more time around the oxygen atom, giving the oxygen atom a partially negative electrical charge, while each hydrogen atom carries a partially positive electrical charge. This creates a strong 'electrostatic' attraction between the hydrogen atom of one water molecule with the oxygen atom of another water molecule, creating what is called a hydrogen bond between two molecules of water. Since there are two hydrogen atoms in each water molecule, then one water molecule can actually hydrogen bond with two other water molecules, and so on, thus creating an infinite network of hydrogen bonds between all the water molecules in a container of water.

Hydrogen bonds between water molecules are considered to be relatively weak bonds, being only about 5% as strong as the chemical bond formed between the oxygen and hydrogen atom in a molecule of water. Yet when all the hydrogen bonds are considered within a 'sea' of water molecules it becomes clear why it takes so much energy to separate molecules of water from each other so they can begin to move more rapidly as heat is applied. It takes twice as much energy to raise the temperature of water, by say 20°C, as it takes to raise olive oil by the same number of degrees. It also explains why it takes five times more energy to physically separate water molecules to the point that they can escape from each other as steam. When water molecules escape as steam they take all this extra energy with them, which results in the boiling point of water never rising above 100°C (212°F) at one atmosphere of pressure. If more heat is added to boiling water it just boils faster rather than increasing in temperature. But when water freezes there is no where for the molecules to escape, so ice can be cooled to virtually any temperature below 0°C (32°F). Ice cubes in a freezer will be the same temperature as the freezer.

Let's finish our discussion of the roles of water in food by examining how water influences the properties of proteins and polysaccharides. As mentioned earlier, foods with low moisture content (perhaps 10–20% or less) will be rigid and hard, while moist foods (perhaps 35% or more) will be flexible and soft. Proteins contain nitrogen atoms and polysaccharides contain oxygen atoms. Both of these atoms are electronegative, and therefore form hydrogen bonds with the hydrogen atoms in water molecules, resulting in water molecules being adsorbed to the surface of proteins and polysaccharides.

The adsorbed molecules of water lower the glass transition temperature of the proteins and polysaccharides making them more flexible at room temperature and above. In dry wheat flour the key proteins that form gluten, called gliadin and glutenin, are rigid and inflexible at room temperature. When water is added these two proteins become flexible at room temperature and are able to unfold and move about and bond with each other to form gluten. Kneading the dough helps to move the proteins around even more insuring enough bonding (called cross-linking) to form a strong gluten network. The same is true of the starch polysaccharide molecules, amylose and amylopectin. When water is added to starch the water begins to hydrogen bond with the starch molecules. In this case (usually at 35% or more moisture) the glass transition temperature of the starch molecules is above room temperature, so it takes heat to reach a temperature at which the granules of starch absorb water and swell. This is known as the gelatinisation temperature of starch. When dry pasta is cooked the gluten is hydrated,

When the biochemical reaction is over, the product of the reaction leaves the enzyme. The enzyme is then ready to effect the same reaction on another molecule again and again. Given the right conditions, the enzyme can go on and on for as long as needed. In some production processes, this lowers costs.

*Enzymes are biodegradable and environmentally friendly:* Most manufacturing produces industrial waste that can present a threat to nature if it includes chemicals. Enzymes can usually do the same job cheaper and do not threaten the environment. Enzymes are fully biodegradable. When industrial enzymes leave a production plant with the wastewater, the retired enzymes do not last long in the surrounding environment. Nature has many micro-organisms that easily break down enzymes into single amino acids, which are used to build up life around us.

*Enzymes consist of long strings of amino acids:* Like all other proteins, enzymes are made of amino acids. Each enzyme is made of between a hundred and up to a million amino acids placed like pearls on a string. Each amino acid is bonded to the next by chemical bonds. Each enzyme has its own unique sequence of amino acids, which is determined by the genes in the cells.

The vast majority of enzymes are made of only 20 different kinds of amino acid. The structure and function of the enzyme is determined by the order of the amino acids.

**Enzymes have a three-dimensional structure:** Enzymes consist of millions of amino acids placed one after the other like pearls on a long string, but most enzymes do not look like a long string. In most enzymes, the string is coiled and folded thousands of times to form a highly complex three-dimensional structure, which is unique to each enzyme. It is the chemical interactions between the amino acids that force the enzymes into their three-dimensional structure, which is held together by the many different links between the different amino acids.

Arrangement of amino acids determines the enzymes function: It is the unique three-dimensional structure of each enzyme that determines the function of the enzyme. Even slight changes in the sequence of the amino acids on the string have a huge impact on the structure and function of the protein. With just one or perhaps a few amino acids replaced or switched, an enzyme may not only look different, but also act differently and convert to working on other biological molecules or treating them differently.

*Enzymes have active sites that make them highly specific:* Enzymes are large molecules with hundreds of amino acids, but only a small part of the enzyme participates in the catalysis of biochemical reactions. This is called the active site. The three-dimensional structure of the enzyme determines the appearance of the active site. The active site precisely accommodates the shape of the biological substrate. The enzyme and substrate fit together like a key in a lock and only substrates with the right shape will be transformed by the enzyme. This is what makes enzymes specific in their action.

## **BIOLOGICAL CATALYSTS**

This section discusses shared properties with chemical catalysts and differences between enzymes and chemical catalysts.

- 1. Shared properties with chemical catalysts:
  - (a) Enzymes are neither consumed nor produced during the course of a reaction.
  - (b) Enzymes do not cause reactions to take place, but they greatly enhance the rate of reactions that would proceed much slower in their absence. They alter the rate but not the equilibrium constants of reactions that they catalyse.
- 2. Differences between enzymes and chemical catalysts:
  - (a) Enzymes are proteins.