Eighth Edition

# ANIMAL NUTRITION

P. McDonald, R.A. Edwards,
J.F.D. Greenhalgh, C.A. Morgan,
L.A. Sinclair, R.G. Wilkinson

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EIGHTH EDITION


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KAO Park
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Web: www.pearson.com/uk

First published by Oliver & Boyd 1966 (print)
Second edition published 1973 (print)
Third edition published 1981 (print)
Fourth edition published 1988 (print)
Fifth edition published 1995 (print)
Sixth edition published 2002 (print)
Seventh edition published 2011 (print)
**Eighth edition published** 2022 (print and electronic)



ISBN: 978-1-292-25166-0 (print)
978-1-292-25168-4 (PDF)
978-1-292-25167-7 (ePub)

**British Library Cataloguing-in-Publication Data**
A catalogue record for the print edition is available from the British Library

**Library of Congress Cataloging-in-Publication Data**
Names: McDonald, Peter, 1926-2018, author. | Edwards, R. A., author. | Greenhalgh, J. F. D., author. | Morgan, C. A. (Animal nutritionist), author. | Sinclair, L. A. (Liam A.), author. | Wilkinson, R. G. (Robert G.), author.
Title: Animal nutrition / P. McDonald, Formerly Reader in Agricultural Biochemistry, University of Edinburgh, and Head of the Department of Agricultural Biochemistry, Edinburgh School of Agriculture, R.A. Edwards, Formerly Head of the Department of Animal Nutrition, Edinburgh School of Agriculture, J.F.D. Greenhalgh, Emeritus Professor of Animal Production and Health, University of Aberdeen, C.A. Morgan, Scottish Agricultural College, L.A. Sinclair, Harper Adams University College, R.G. Wilkinson, Harper Adams University College.
Description: Eighth edition. | Harlow, England ; New York : Pearson, 2022. | Includes bibliographical references and index.
Identifiers: LCCN 2021044367 (print) | LCCN 2021044368 (ebook) | ISBN 9781292251660 (paperback) | ISBN 9781292251684 (ebook) | ISBN 9781292251677 (epub)
Subjects: LCSH: Animal nutrition. | Feeds.
Classification: LCC SF95 .M38 2022 (print) | LCC SF95 (ebook) | DDC 636.08/52--dc23
LC record available at https://lccn.loc.gov/2021044367
LC ebook record available at https://lccn.loc.gov/2021044368

10 9 8 7 6 5 4 3 2 1
26 25 24 23 22

Front cover image: Photos by R A Kearton/Moment/Getty Images
Cover design by Kelly Miller
Print edition typeset in 9.5/12pt Rotation LT Std by Straive
Printed by Ashford Colour Press Ltd, Gosport

NOTE THAT ANY PAGE CROSS REFERENCES REFER TO THE PRINT EDITION

# Contents

# Preface to the eighth edition

Recent research in the field of animal science has focused on advances in molecular biology, particularly in the study of gene expression, epigenetics and gene editing, and exciting advances have been made. However, knowledge of animal biochemistry and nutrition is still essential if we are to understand the significance and efficient application of these new findings to further improve animal production, health and welfare. The application of research and advice in animal nutrition continues to be at the centre of efficient animal production. Research in dog and cat nutrition has also progressed since the last edition and information in this area has been expanded in this new edition.

We have retained the early chapters on basic food chemistry and animal biochemistry to provide a quick reference to questions pertaining to the discipline of nutrition chemistry in later parts of the book. We have also taken the opportunity to introduce nutritional topics related to molecular biology and the environment. Each chapter now has a set of questions to assist with revision of the chapter topic and the Appendix tables have been revised where new data are available.

Three significant events have occurred since the last edition. In 2016, the British Society of Animal Science recognised the 50th anniversary of the publication of the first edition of *Animal Nutrition* by awarding framed certificates of congratulation to the original three authors, Peter McDonald, James Greenhalgh and Alun Edwards. Then, in 2018, came the sad news that Peter McDonald had died and in 2021 that Alun Edwards, too, had died. Although Peter and Alun had not been actively involved in the production of recent editions of the book, they had always shown great interest in its progress. Fittingly, Peter's funeral service was conducted by another eminent animal nutritionist, Rev. Dr Neville Suttle.

The production of this edition was assisted by comments and suggestions received from reviewers and we welcome comments from readers. As with previous editions, we are grateful to colleagues for their helpful discussions.

C A Morgan, L A Sinclair, R G Wilkinson and J F D Greenhalgh
2022

# PART 1

## The components of foods

This part describes the chemistry of foods and the components that supply nutrients to the animal.

Chapter 1 is concerned with the analysis of foods, from the early chemical analysis developed in the 1800s to categorise chemical and nutrient groups, through to the sophisticated physical and chemical methods used today to identify individual molecular components.

Chapters 2, 3 and 4 describe the major components of foods that supply energy and amino acids, i.e. the carbohydrates and lipids, and the proteins.

Chapters 5 and 6 give details of the nutrients required in smaller amounts, the vitamins and minerals which, nevertheless, are essential for the normal functions of the body and efficient animal production.

# 1 The animal and its food

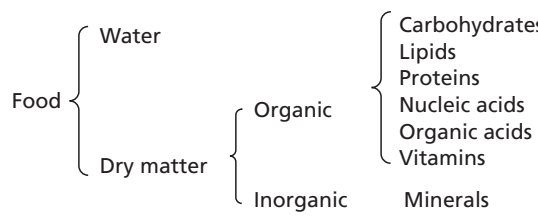


Food is material that, after ingestion by animals, is capable of being digested, absorbed and utilised. In a more general sense, we use the term 'food' to describe edible material. Grass and hay, for example, are described as foods, but not all their components are digestible. Where the term 'food' is used in the general sense, as in this text, those components capable of being utilised by animals are described as *nutrients*.

The animals associated with human beings cover the spectrum from herbivores – the plant eaters (ruminants, horses and small animals such as rabbits and guinea pigs); through omnivores, which eat all types of food (pigs and poultry); to carnivores, which eat chiefly meat (cats). Under the control of human beings, these major classes of animal still pertain, but the range of foods that animals are now offered is far greater than they might normally consume in the wild (for example, ruminants are given plant by-products of various human food industries and some dog foods contain appreciable amounts of cereals). Nevertheless, plants and plant products form the major source of nutrients in animal nutrition.

The diet of farm animals in particular consists of plants and plant products, although some foods of animal origin, such as fishmeal and milk, are used in limited amounts. Animals depend upon plants for their existence and consequently a study of animal nutrition must necessarily begin with the plant itself.

Plants are able to synthesise complex materials from simple substances, such as carbon dioxide from the air, and water and inorganic elements from the soil. By means of photosynthesis, energy from sunlight is trapped and used in these synthetic processes. The greater part of the energy, however, is stored as chemical energy within the plant itself and it is this energy that is used by the animal for the maintenance of life and synthesis of its own body tissues. Plants and animals contain similar types of chemical substances, and we can group these into classes according to constitution, properties and function. The main components of foods, plants and animals are:



## 1.1 WATER

The water content of the animal body varies with age. The newborn animal contains 750–800 g/kg water, but this falls to about 500 g/kg in the mature fat animal. It is vital to the life of the organism that the water content of the body be maintained: an animal will die more rapidly if deprived of water than if deprived of food. Water functions in the body as a solvent in which nutrients are transported about the body and in which waste products are excreted. Many of the chemical reactions brought about by enzymes take place in solution and involve hydrolysis. Because of the high specific heat of water, large changes in heat production can take place within the animal with very little alteration in body temperature. Water also has a high latent heat of evaporation, and its evaporation from the lungs and skin gives it a further role in the regulation of body temperature.

The animal obtains its water from three sources: drinking water, water present in its food and metabolic water, this last being formed during metabolism by the oxidation of hydrogen-containing organic nutrients. The water content of foods is variable and can range from as little as 60 g/kg in concentrates to over 900 g/kg in some root crops. Because of this great variation in water content, the composition of foods is often expressed on a dry matter basis, which allows a more valid comparison of nutrient content. This is illustrated in Table 1.1, which lists a few examples of plant and animal products.

The water content of growing plants is related to the stage of growth, being greater in younger plants than in older plants. In temperate climates, the acquisition of drinking water is not usually a problem and animals are provided with a continuous supply. There is no evidence that under normal conditions an excess of drinking water is harmful, and animals normally drink what they require.

**Table 1.1** Composition of some plant and animal products expressed on a fresh basis and a dry matter basis

| | Water | Carbohydrate | Lipid | Protein | Ash |
|---|---|---|---|---|---|
| **Fresh basis (g/kg)** | | | | | |
| Turnips | 910 | 70 | 2 | 11 | 7 |
| Grass (young) | 800 | 137 | 8 | 35 | 20 |
| Barley grain | 140 | 730 | 15 | 93 | 22 |
| Groundnuts | 60 | 201 | 449 | 268 | 22 |
| Dairy cow | 570 | 2 | 206 | 172 | 50 |
| Milk | 876 | 47 | 36 | 33 | 8 |
| Muscle | 720 | 6 | 44 | 215 | 15 |
| Egg | 667 | 8 | 100 | 118 | 107 |
| **Dry matter basis (g/kg)** | | | | | |
| Turnips | 0 | 778 | 22 | 122 | 78 |
| Grass (young) | 0 | 685 | 40 | 175 | 100 |
| Barley grain | 0 | 849 | 17 | 108 | 26 |
| Groundnuts | 0 | 214 | 478 | 285 | 23 |
| Dairy cow | 0 | 5 | 479 | 400 | 116 |
| Milk | 0 | 379 | 290 | 266 | 65 |
| Muscle | 0 | 21 | 157 | 768 | 54 |
| Egg | 0 | 24 | 300 | 355 | 321 |

## 1.2 DRY MATTER AND ITS COMPONENTS

The dry matter (DM) of foods is conveniently divided into organic and inorganic material, although in living organisms there is no such sharp distinction. Many organic compounds contain mineral elements as structural components. Proteins, for example, contain sulphur, and many lipids and carbohydrates contain phosphorus.

It can be seen from Table 1.1 that the main component of the DM of pasture grass is carbohydrate, and this is true of all plants and many seeds. The oilseeds, such as groundnuts, are exceptional in containing large amounts of protein and lipid material. In contrast, the carbohydrate content of the animal body is very low. One of the main reasons for the difference between plants and animals is that, whereas the cell walls of plants consist of carbohydrate material, mainly cellulose, the walls of animal cells are composed almost entirely of lipid and protein. Furthermore, plants store energy largely in the form of carbohydrates such as starch and fructans, whereas an animal's main energy store is in the form of lipid.

The lipid content of the animal body is variable and is related to age, the older animal containing a much greater proportion than the young animal. The lipid content of living plants is relatively low – that of pasture grass, for example, being 40–50 g/kg DM.

In both plants and animals, proteins are the major nitrogen-containing compounds. In plants, in which most of the protein is present as enzymes, the concentration is high in the young growing plant and falls as the plant matures. In animals, the muscle, skin, hair, feathers, wool and nails consist mainly of protein.

Like proteins, nucleic acids are also nitrogen-containing compounds and they play a basic role in the synthesis of proteins in all living organisms. They also carry the genetic information of the living cell.

The organic acids that occur in plants and animals include citric, malic, fumaric, succinic and pyruvic acids. Although these are normally present in small quantities, they nevertheless play an important role as intermediates in the general metabolism of the cell. Other organic acids occur as fermentation products in the rumen, or in silage, and these include acetic, propionic, butyric and lactic acids.

Vitamins are present in plants and animals in minute amounts, and many of them are important as components of enzyme systems. An important difference between plants and animals is that, whereas the former can synthesise all the vitamins they require for metabolism, animals cannot, or have very limited powers of synthesis, and are dependent upon an external supply.

The inorganic matter contains all those elements present in plants and animals other than carbon, hydrogen, oxygen and nitrogen. Calcium and phosphorus are the major inorganic components of animals, whereas potassium and silicon are the main inorganic elements in plants.

## 1.3 ANALYSIS AND COMPOSITION OF FOODS

Originally, the most extensive information about the composition of foods was based on a system of analysis described as the *proximate analysis of foods*, which was devised in the 19th century by two German scientists, Henneberg and Stohmann. More recently, new analytical techniques have been introduced, and the information about food composition is rapidly expanding (see the 'Modern analytical methods'

section). However, the system of proximate analysis still forms the basis for the statutory declaration of the composition of foods in Europe.

## Proximate analysis of foods

This system of analysis divides the food into six fractions: moisture, ash, crude protein, ether extract, crude fibre and nitrogen-free extractives.

The moisture content is determined as the loss in weight that results from drying a known weight of food to constant weight at 100 °C. This method is satisfactory for most foods, but with a few (such as silage), significant losses of volatile material (short-chain fatty acids and alcohols) may take place. Therefore, for silages, the moisture content can be determined directly by distilling the water from the sample under toluene. The distillate is measured and corrected for the presence of fermentation acids and alcohols. An estimate of the 'corrected' dry matter (g/kg) can be calculated from the oven dry matter (ODM g/kg) using a relationship such as that published by the Agriculture and Food Research Council:

$$\text{Corrected DM} = 0.99\ \text{ODM} + 18.9$$

The ash content is determined by ignition of a known weight of the food at 550 °C until all carbon has been removed. The residue is the ash and is taken to represent the inorganic constituents of the food. The major component of ash is silica but ash may, however, contain material of organic origin such as sulphur and phosphorus from proteins, and some loss of volatile material in the form of sodium, chloride, potassium, phosphorus and sulphur will take place during ignition. The ash content is thus not truly representative of the inorganic material in the food, either qualitatively or quantitatively. Animals do not have a requirement for ash *per se* but require the individual mineral elements that it contains and are determined by methods such as atomic absorption spectrometry or inductively coupled plasma spectroscopy (see p. 11).

The crude protein (CP) content is calculated from the nitrogen content of the food, determined by a modification of a technique originally devised by Kjeldahl over 100 years ago. In this method, the food is digested with sulphuric acid, which converts all nitrogen present, except that in the form of nitrate and nitrite, to ammonium sulphate. The ammonia is liberated by adding sodium hydroxide to the digest, distilled off and collected in standard acid, and the quantity collected is determined by titration or by an automated colorimetric method. It is assumed that the nitrogen is derived from protein containing 16 per cent nitrogen, and by multiplying the nitrogen figure by 6.25 (i.e. 100/16) an approximate protein value is obtained. This is not 'true protein', since the method determines nitrogen from sources other than protein, such as free amino acids, amines and nucleic acids, and the fraction is therefore designated 'crude protein'. Also, the nitrogen content of the protein varies according to its amino acid composition and 16 per cent is an average value.

The ether extract (EE) fraction is determined by subjecting the food to a continuous extraction with petroleum ether for a defined period. The residue, after evaporation of the solvent, is the ether extract. As well as lipids it contains organic acids, alcohol and pigments. This procedure is referred to as method A. In the current official method, the extraction with ether is preceded by hydrolysis of the sample with hydrochloric acid and the resultant residue is the acid ether extract (method B).

The carbohydrate of the food is contained in two fractions, the crude fibre (CF) and the nitrogen-free extractives (NFE). The former is determined by subjecting the residual food from ether extraction to successive treatments with boiling acid and alkali of defined concentration; the organic residue is the crude fibre.

When the sum of the amounts of moisture, ash, crude protein, ether extract and crude fibre (expressed in g/kg) is subtracted from 1,000, the difference is designated the nitrogen-free extractives. The nitrogen-free extractives fraction is a heterogeneous mixture of all those components not determined in the other fractions. The crude fibre fraction contains cellulose, lignin (an indigestible component of plant fibre) and hemicelluloses, but not necessarily the whole amounts of these that are present in the food: a variable proportion of the cell wall material, depending upon the species and stage of growth of the plant material, is dissolved during the crude fibre extraction and thus is contained in the nitrogen-free extractives. This leads to an underestimation of the fibre and an overestimation of the starch and sugars. Thus, the nitrogen-free extractive fraction includes starch, sugars, fructans, pectins, organic acids and pigments, in addition to those components mentioned above.

## Modern analytical methods

In recent years the proximate analysis procedure has been severely criticised by many nutritionists as being archaic and imprecise, and in the majority of laboratories it has been partially replaced by other analytical procedures. Most criticism has been focused on the crude fibre, ash and nitrogen-free extractives fractions for the reasons described above. The newer methods have been developed to characterise foods in terms of the methods used to express nutrient requirements. In this way, an attempt is made to use the analytical techniques to quantify the potential supply of nutrients from the food. For example, for ruminants, analytical methods are being developed that describe the supply of nutrients for the rumen microbes and the host digestive enzyme system (Fig. 1.1).

### *Starch and sugars*

Inadequacies in the nitrogen-free extractives fraction have been addressed by the development of methods to quantify the non-structural carbohydrates, which are mainly starches and sugars. Sugars can be determined colorimetrically after combination with a reagent such as anthrone. The official EC method involves extraction of sugars with dilute ethanol; the solution is then clarified and the ethanol removed. The sugars are then quantified before and after inversion (giving reducing and total sugars, respectively) by the Luff-Schoorl method. Starch is determined by dilute acid hydrolysis of the sample followed by polarimetric determination of the released sugars. This gives a figure for total sugars (i.e. those originating from the hydrolysed starch plus the simple sugars in the food). Sugars *per se* are determined by extracting the sample with ethanol, acidifying the filtrate and taking a second polarimeter reading. The starch content is calculated from the difference between the two readings multiplied by a known factor for the starch source. Starch can also be determined enzymically. For example, in cereals starch is converted to glucose using $\alpha$-amylase followed by amyloglucosidase and then the glucose is measured using the glucose oxidase-peroxidase reagent.

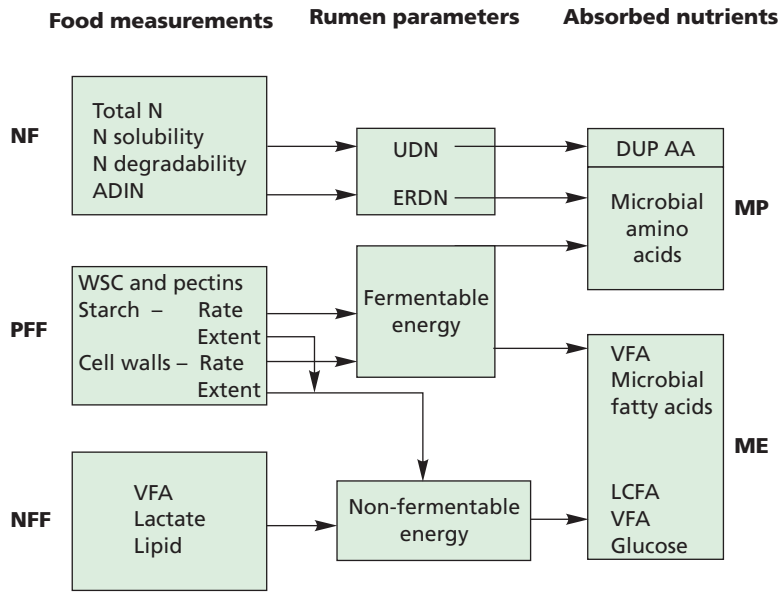



**Fig. 1.1** Proposed model for characterisation of foods for ruminants.
AA = amino acids, ADIN = acid detergent insoluble nitrogen, DUP = digestible undegradable protein, ERDN = effective rumen degradable nitrogen, LCFA = long-chain fatty acids, ME = metabolisable energy, MP = metabolisable protein, N = nitrogen, NF = nitrogen fraction, NFF = non-fermentable fraction, PFF = potentially fermentable fraction, UDN = undegradable nitrogen, VFA = volatile fatty acids, WSC = water-soluble carbohydrates.

From Agricultural and Food Research Council 1998 Technical Committee on Responses to Nutrients, report no. 11, Wallingford, CABI.

### *Fibre*

Alternative procedures for fibre have been developed by Van Soest (Table 1.2). The *neutral-detergent fibre* (NDF), which is the residue after extraction with boiling neutral solutions of sodium lauryl sulphate and ethylenediamine tetraacetic acid (EDTA), consists mainly of lignin, cellulose and hemicellulose and can be regarded as a measure of the plant cell wall material. The analytical method for determining NDF was originally devised for forages, but it can also be used for starch-containing foods provided that an amylase treatment is included in the procedure. By analogy with the nitrogen-free extractives fraction discussed above, the term *non-structural carbohydrate* (NSC) is sometimes used for the fraction obtained by subtracting the sum of the amounts (g/kg) of CP, EE, ash and NDF from 1,000.

The *acid-detergent fibre* (ADF) is the residue after refluxing with 0.5 M sulphuric acid and cetyltrimethyl-ammonium bromide, and represents the crude lignin and cellulose fractions of plant material but also includes silica.

The determination of ADF is particularly useful for forages as there is a good statistical correlation between it and the extent to which the food is digested (digestibility). In the UK, the ADF method has been modified slightly by altering the duration of boiling and acid strength. The term *modified acid-detergent fibre* (MADF) is used to describe this determination.

**Table 1.2** Classification of forage fractions using the detergent methods of Van Soest

| Fraction | Components |
|---|---|
| Cell contents (soluble in neutral detergent) | Lipids |
| | Sugars, organic acids and water-soluble matter |
| | Pectin, starch |
| | Non-protein nitrogen |
| | Soluble protein |
| Cell wall constituents (fibre insoluble in neutral detergent) | |
| Soluble in acid detergent | Hemicelluloses |
| | Fibre-bound protein |
| Acid-detergent fibre | Cellulose |
| | Lignin |
| | Lignified nitrogen |
| | Silica |

After Van Soest P J 1967 *Journal of Animal Science* **26**: 119.

The *acid-detergent lignin* determination involves the preparation of acid-detergent fibre as the preparatory step. The ADF is treated with 72 per cent sulphuric acid, which dissolves cellulose. Ashing the residue determines crude lignin, including cutin.

The Van Soest methods of fibre analysis and other standard techniques are used for fractionation of food carbohydrates for ruminants in the Cornell net carbohydrate and protein system (CNCPS) to derive the following fractions:

1. Total carbohydrate = 100 − (crude protein + fat + ash)
2. Non-structural carbohydrate (NSC) = 100 − (crude protein + fat + (NDF − NDF protein) + ash)
3. Sugar as a proportion of NSC
4. Starch, pectins, β-glucans, volatile fatty acids = NSC − sugar
5. Lignin

The carbohydrates are then classified according to their degradation rate by rumen microbes: fraction A – fast (comprising the sugars); fraction B1 – intermediate (starch, pectins, β-glucans); fraction B2 – slow (available cell wall material represented by lignin-free NDF); and fraction C – indigestible (unavailable cell wall in the form of lignin).

In monogastric, and particularly human, nutrition the term *dietary fibre* is often used and attention has been focused on its importance in relation to health. Dietary fibre (DF) was defined as lignin plus those polysaccharides that cannot be digested by monogastric endogenous enzymes. Initially, epidemiological studies linked a lack of DF to constipation, gut and bowel disorders, cardiovascular disease and type 2 diabetes; however, the causes of such diseases are multifactorial and in some cases

it is not just DF *per se* that has the beneficial effects but other aspects of the diet also (e.g. antioxidants). Nevertheless, DF is a major component related to health in human beings and it has equally important effects in animals.

The definition of DF has proved difficult, with definitions ranging through physiological/botanical (derived from cell walls of plants, which are poorly digested), chemical/botanical (non-starch polysaccharides (NSP) of plant cell walls), chemical (NSP and lignin) and nutritional/physiological (NSP not digested in the small intestine). The common features of DF definitions are carbohydrates (polysaccharides, oligosaccharides and lignin) resistant to digestion in the small intestine but that may be fermented in the large intestine and promote beneficial physiological effects. By virtue of its definition, DF is difficult to determine in the laboratory. The NSP in most foods, along with lignin, are considered to represent the major components of cell walls. Methods for measurement of NSP fall into two categories (with slight variations in the second category, depending on the research laboratory):

- Enzymic–gravimetric methods, which measure a variety of components and give no details of polysaccharide type. In the method of the Association of Official Analytical Chemists for total dietary fibre, samples are gelatinised by heating and treated with enzymes to remove starch and proteins. The total dietary fibre is precipitated with ethanol and the residue is dried and weighed.
- Enzymic–chromatographic methods, which identify the individual carbohydrates in the dietary NSP. The Englyst method can be used to determine total, soluble and insoluble dietary fibre. Measurement of NSP by this method involves removal of starch with the enzymes pullulanase and α-amylase. After precipitation with ethanol, the NSP residue is then hydrolysed with 12 M sulphuric acid. The individual monomeric neutral sugar constituents are determined by gas–liquid chromatography (see later) with separate determination of uronic acids. Alternatively, the total sugars are determined colorimetrically after reaction with dinitrosalicylate solution. Total NSP and insoluble NSP are determined directly by analysis of separate subsamples and the soluble NSP are calculated by difference. The major constituents of NSP are rhamnose, arabinose, xylose, glucose, galactose, mannose and glucuronic and galacturonic acids. Cellulose is the major source of glucose, and hemicellulose provides xylose, mannans and galactose. The degradation of pectins releases arabinose, galactose and uronic acids. Following the adoption of methods to determine NSP, it became apparent that non-digestible oligosaccharides and resistant starch also contributed to DF based on their physiological behaviour. In recognition of this, enzymic procedures have been developed to determine these components.

In recent years, attention has focused on the importance of both the soluble and insoluble forms of fibrous material in the human diet. Water-soluble NSP is known to lower serum cholesterol, and insoluble NSP increases faecal bulk and speeds up the rate of colonic transit. This last effect is thought to be beneficial in preventing a number of diseases, including cancer of the bowel.

The NSP of foods may be degraded in the gut of pigs by microbial fermentation, yielding volatile fatty acids, which are absorbed and contribute to the energy supply. A further benefit relates to the volatile fatty acid butyric acid, which is reported to be an important source of energy for the growth of cells in the epithelium of the colon; thus, the presence of this acid will promote development of the cells and enhance absorption. The extent of degradation depends on the conformation of the polymers

and their structural association with non-carbohydrate components, such as lignin. In addition, the physical properties of the NSP, such as water-holding capacity and ion exchange properties, can influence the extent of fermentation. The gel-forming NSPs, such as β-glucans. influence the viscosity of digesta and thereby reduce the absorption of other nutrients from the small intestine and depress digestibility and adversely affect faecal consistency in pigs and poultry (see the section on enzymes in Chapter 24). On a positive note, the water-holding properties lead to beneficial effects on the behaviour of pregnant sows by increasing time spent eating and resting, owing to increased gut fill, and by reducing inappropriate behaviour, such as bar chewing.

### *Minerals*

A simple ash determination provides very little information about the exact mineral make-up of the food and, when this is required, analytical techniques involving spectroscopy are generally used. In *atomic absorption spectroscopy*, an acid solution of the sample is heated in a flame and the vaporised atoms absorb energy, which brings about transitions from the ground state to higher energy levels. The source of energy for this transition is a cathode lamp, containing the element to be determined, which emits radiation at a characteristic wavelength. The radiation absorbed by the atoms in the flame is proportional to the concentration of the element in the food sample.

*Flame emission spectroscopy* measures the radiation from solutions of the sample heated in air/acetylene or oxygen/acetylene flames. Each element emits radiation at specific wavelengths and there are published tables of flame emission spectra. Atomic absorption and flame emission spectrometry are being replaced by *inductively coupled plasma emission spectroscopy*, as this has a greater sensitivity for the relatively inert elements and can be used to determine several elements simultaneously or sequentially. Energy from the inductively coupled plasma source is absorbed by argon ions and elements to form a conducting gaseous mixture at temperatures up to 10,000 °C. The electromagnetic radiation emitted from atoms and ions within the plasma is then measured. Alternatively, the ions can be separated and detected using a mass spectrometer.

Just as with other nutrients, a measure of the concentration of the element alone is not sufficient to describe its usefulness to the animal. Attempts have been made to assess the availability of minerals using chemical methods, such as solubility in water or dilute acids, but these have had little success. At present, animal experiments are the only reliable way to measure mineral availability (see Chapter 10).

### *Amino acids, fatty acids and sugars*

As an alternative to the standard Kjeldahl method for the determination of nitrogen (crude protein) described above, the Dumas method is also now used. In this method the sample is combusted in pure oxygen; the products are carbon dioxide, water, oxides of nitrogen and nitrogen. The carbon dioxide and water are absorbed on columns and the oxides of nitrogen are converted to nitrogen with a column packed with copper; the resulting total nitrogen is determined in a thermal conductivity detector. This method, although expensive in equipment, is rapid and does not rely on hazardous chemicals.

Knowledge of the crude protein content of a food is not a sufficient measure of its usefulness for non-ruminants. The amino acid composition of the protein is required

in order to assess how a food can meet the essential amino acid requirements (see Chapter 4). Similarly, the total ether extract content does not give sufficient information on this fraction since it is important to know its fatty acid composition. In non-ruminants, this has large effects on the composition of body fat and, if soft fat is to be avoided, the level of unsaturated fatty acids in the diet must be controlled. In ruminants, a high proportion of unsaturates will depress fibre digestion in the rumen. When detailed information on the amino acid composition of protein, the fatty acid composition of fat or the individual sugars in NSP is required, then techniques involving chromatographic separation can be used. In *gas–liquid chromatography*, the stationary phase is a liquid held in a porous solid, usually a resin, and the mobile phase is a gas. Volatile substances partition between the liquid and the vapour and can be effectively isolated. This form of chromatography is, however, usually a slow process; in order to speed up the separation procedure, *high-performance liquid chromatography* has been developed. In this technique, pressure is used to force a solution, containing the compounds to be separated, rapidly through the resin held in a strong metal column. In addition to speeding up the process, high resolution is also obtained. Gas–liquid chromatography and high-performance liquid chromatography can also be used for the determination of certain vitamins (e.g. A, E, $B_6$, K), but the measurement of available vitamins requires biological methods.

An example of the application of high-performance liquid chromatography is seen with food proteins, which are hydrolysed with acid and the released amino acids are then determined using one of the following methods:

- Ion-exchange chromatography – by which the amino acids are separated on the column, and then mixed with a derivatisation agent, which reacts to give a complex that is detected by a spectrophotometer or fluorimeter.
- Reverse-phase chromatography – in which the amino acids react with the reagent to form fluorescent or ultraviolet-absorbing derivatives, which are then separated using a more polar mobile phase (e.g. acetate buffer with a gradient of acetonitrile) and a less polar stationary phase (e.g. octadecyl-bonded silica). The availability of amino acids to the animal can be estimated by chemical methods. For example, for lysine there are colorimetric methods that depend on the formation of compounds between lysine and dyes (see Chapter 13).

### *Measurement of protein in foods for ruminants*

The new methods of expressing the protein requirements of ruminants (see Chapter 13) require more information than just the crude protein (nitrogen) content of the food. The unavailable nitrogen is measured as acid detergent insoluble nitrogen. Information on the rate of degradation in the rumen of the available nitrogen is also required and this can be estimated by biological methods. In the Cornell net carbohydrate and protein system, the neutral and acid detergent extractions of Van Soest, described previously, are used in combination with extraction with a borate–phosphate buffer and trichloracetic acid solution to derive several protein fractions. These fractions describe the components that are degraded in the rumen or digested in the small intestine (see Chapter 13).

### *Spectroscopy*

It is now common for laboratories to use *near-infrared reflectance spectroscopy* (NIRS) to estimate the composition of foods. The basis of this methodology lies

in the absorption of energy by hydrogen-containing functional groups in organic compounds present in the food (C–H, O–H, N–H and S–H). The reflected energy from the sample provides information on its composition but, unlike normal spectroscopy, is not related directly to concentration since the sample is heterogenous. Therefore, empirical relationships are derived by calibrating the reflected spectrum with samples of known composition, as determined by standard methods. In practice, energy in the wavelength range 1,100–2,500 nm is directed on to a cell containing the dried milled sample, and the diffuse reflected energy is measured across the spectrum. The spectral data are then related to the known chemical composition of the standard samples by multiple linear regression. The relationships are then validated with a second set of samples of known composition. Once satisfactory relationships have been derived, they can be applied to the spectra of samples of unknown composition. The technique has been extended to the analysis of fresh silage samples, eliminating the need to dry and mill the sample. NIRS has the advantages that it is rapid with minimal sample preparation, it gives instantaneous results and is non-destructive of the sample, it allows simultaneous measurement of several parameters with high precision, and it allows a high throughput of samples at low cost per sample. Nevertheless, it should be recognised that, as the technique is dependent on chemical or physical calibrations, it cannot be more accurate than the chemically determined values used to derive the calibration. NIRS is particularly useful in the context of compound food manufacture, where rapid analysis of raw materials and finished product is required for efficient mixing and quality control standards. With forages, particularly grass and cereal silages, NIRS is now routinely used to determine not only chemical composition but also a range of food characteristics, including those that are the resultant of a number of nutrient concentrations such as digestibility, metabolisable energy and nitrogen degradability in the rumen and potential silage intake (see Chapters 12, 13 and 17). Hand-held NIRS devices are now available for on-farm analysis of forages. Mineral elements do not have absorptions in the NIR spectral region and cannot be determined directly by NIRS. However, minerals can be measured when they are combined in organic complexes or chelates, or indirectly by their effects on hydrogen bonds. Accuracy of estimation depends on there being a close degree of correlation between the mineral and the organic compounds.

*Nuclear magnetic resonance spectroscopy* is a complex technique that is used to determine the constituents of foods. This method makes use of the fact that some compounds contain certain atomic nuclei that can be identified from a nuclear magnetic resonance spectrum, which measures variations in frequency of electromagnetic radiation absorbed. It provides more specific and detailed information of the conformational structure of compounds than, for example, NIRS but is more costly and requires more time and skill on the part of the operator. For these reasons, it is more suited to research work and for cases in which the results from simpler spectroscopy techniques require further investigation. Nuclear magnetic resonance spectroscopy has been useful in the investigation of the soluble and structural components of forages.

## SUMMARY

1. Water is an important component of animal foods. It contributes to the water requirements of animals and dilutes the nutrient content of foods. Water content varies widely between foods.
2. The constituents of dry matter comprise carbohydrates (sugars, starches, fibres), nitrogen-containing compounds (proteins, amino acids, non-protein nitrogen compounds), lipids (fatty acids, glycerides), minerals and vitamins.
3. Analytical techniques have been developed from simple chemical/gravimetric determinations.
4. Modern analytical techniques attempt to measure nutrients in foods in terms of the nutrient requirements of the animal.
5. Starch is determined by polarimetry.
6. Fibrous constituents can be determined by application of detergent solutions and weighing the residue, or by the use of enzymes followed by weighing or gas–liquid chromatography.
7. Individual mineral elements are measured by atomic absorption spectroscopy, flame photometry or inductively coupled plasma emission spectroscopy.
8. Gas–liquid chromatography is used to determine individual amino acids, fatty acids and certain vitamins.
9. Near-infrared reflectance spectroscopy is used routinely to determine food characteristics and to predict nutritive value. Nuclear magnetic resonance spectroscopy is a research technique for determining the chemical structure of food components.

## QUESTIONS

1.1 What are the major components of food dry matter? (p. 3)
1.2 What are the three major components of the organic matter of foods? (p. 5)
1.3 Grass has a dry matter content of 200 g/kg and 137 g carbohydrate/kg. What is the concentration of carbohydrate in the dry matter?
1.4 Barley grain has a dry matter content of 860 g/kg and 108 g protein/kg DM. What is the concentration of protein in the fresh matter?
1.5 What are the six fractions quantified by the proximate system of analysis of foods? (p. 6)
1.6 What is the usually assumed nitrogen content of crude protein?
1.7 What do the following abbreviations mean: NDF, ADF, WSC, NSP, NSC? (p. 8)
1.8 What is near infrared reflectance spectroscopy and how is it used to determine the composition of a food? (p. 12)

## FURTHER READING

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# 2 Carbohydrates



In general, carbohydrates are neutral chemical compounds containing the elements carbon, hydrogen and oxygen and have the empirical formula $(CH_2O)_n$, where $n$ is 3 or more. However, some compounds with general properties of the carbohydrates also contain phosphorus, nitrogen or sulphur; and others, e.g. deoxyribose ($C_5H_{10}O_4$), do not have hydrogen and oxygen in the same ratio as that found in water. The carbohydrate group contains polyhydroxy aldehydes, ketones, alcohols and acids, their simple derivatives, and any compound that may be hydrolysed to these.

## 2.1 CLASSIFICATION OF CARBOHYDRATES

The carbohydrates may be classified as shown in Fig. 2.1. The simplest sugars are the monosaccharides, which are divided into subgroups – trioses ($C_3H_6O_3$), tetroses ($C_4H_8O_4$), pentoses ($C_5H_{10}O_5$), hexoses ($C_6H_{12}O_6$) and heptoses ($C_7H_{14}O_7$) – depending on the number of carbon atoms present in the molecule. The trioses and tetroses occur as intermediates in the metabolism of other carbohydrates and their importance will be considered in Chapter 9. Monosaccharides may be linked together, with the elimination of one molecule of water at each linkage, to produce di-, tri-, tetra- or polysaccharides, containing, respectively, two, three, four or larger numbers of monosaccharide units.

The term *sugar* is generally restricted to those carbohydrates containing fewer than ten monosaccharide residues, while the name *oligosaccharides* (from the Greek *oligos*, a few) is frequently used to include all sugars other than the monosaccharides.

Polysaccharides, also called glycans, are polymers of monosaccharide units. They are classified into two groups, the homoglycans, which contain only a single type of monosaccharide unit, and the heteroglycans, which on hydrolysis yield mixtures

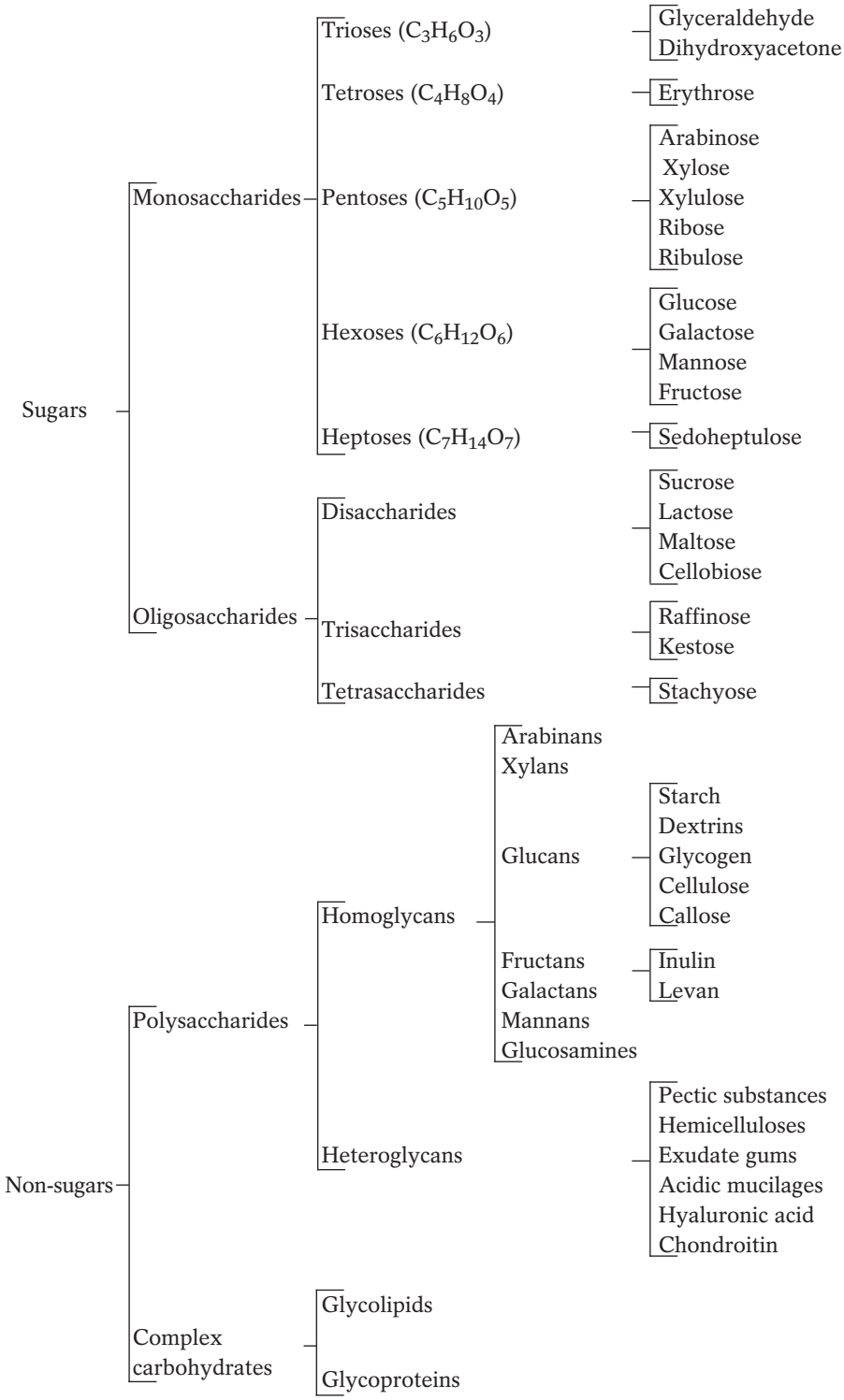



**Fig. 2.1** Classification of carbohydrates.

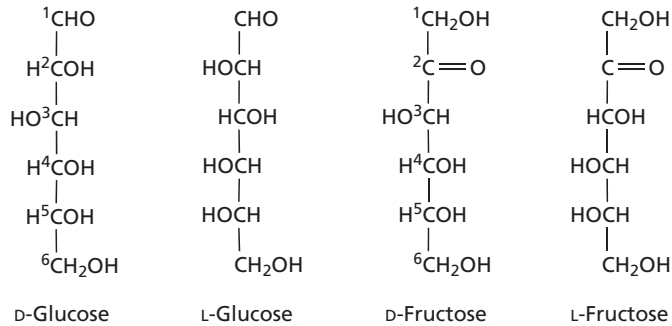
of monosaccharides and derived products. The molecular weight of polysaccharides varies from as little as about 8,000 daltons (Da) in some plant fructans to as high as 100 million in the amylopectin component of starch. Hydrolysis of these polymers to their constituent sugars can be affected by the action of either specific enzymes or acids.

The complex carbohydrates are an ill-defined group of compounds that contain carbohydrates in combination with non-carbohydrate molecules. They include the glycolipids and glycoproteins. The structure and biological importance of these two groups of compounds are discussed in Chapters 3 and 4, respectively.

## 2.2 MONOSACCHARIDES

### Structure

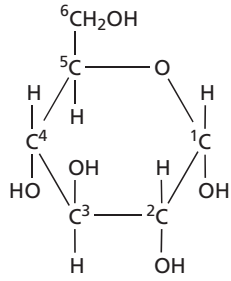
The monosaccharide sugars occur in a number of isomeric forms. Thus, glucose and fructose (both hexoses) are structural isomers, glucose having an aldehyde group and fructose having a ketone group. Both of these sugars occur in two mirror-image, stereoisomeric forms, dextro and laevo (D- and L-), according to the orientation of the OH group at carbon atom 5. Biologically, the D-forms are the more important.

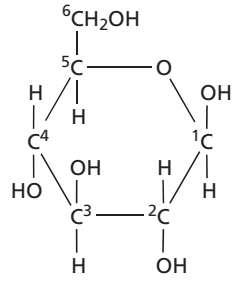


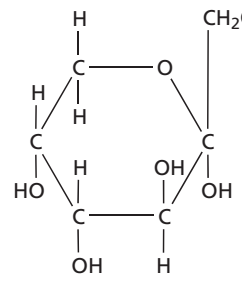

Under physiological conditions, sugars exist mainly in another isomeric form, as ring or cyclic structures rather than straight chains. For example, in solution, glucose and fructose exist mainly as a pyranose ring. However, in the biologically important molecules of which it is a part, fructose most commonly forms a furanose ring, as seen with the pentoses (e.g. ribose). Each ring structure can occur in two isomeric forms, designated α and β. Starch and glycogen are polymers of the α-form, while cellulose is a polymer of the β-form.

### Properties of the monosaccharides

Because of the presence of an active aldehyde (CHO) or ketone (C=O) grouping, the monosaccharides act as reducing substances. The reducing properties of these sugars are usually demonstrated by their ability to reduce certain metal ions, notably copper or silver, in alkaline solution. The aldehyde and ketone groups may also be reduced chemically, or enzymatically, to yield the corresponding sugar alcohols. Examples of oxidation and reduction products are given in the section dealing with monosaccharide derivatives (see the later section).

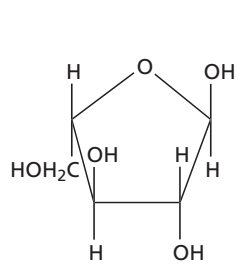

α-D-Glucose

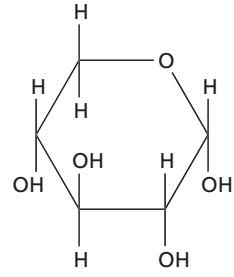

β-D-Glucose

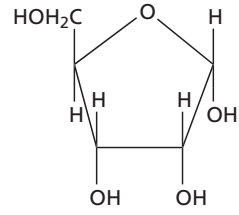

α-D-Fructose

## Pentoses

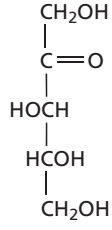
The most important members of this group of simple sugars are the aldoses L-arabinose, D-xylose and D-ribose, and the ketoses D-xylulose and D-ribulose.

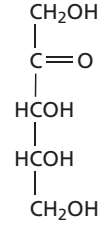

α-L-Arabinose


α-D-Xylose


α-D-Ribose

*L-Arabinose* occurs as pentosans in arabinans. It is a component of hemicelluloses and it is found in silage as a result of their hydrolysis. It is also a component of gum arabic and other gums. *D-Xylose* also occurs as pentosans in xylans. These compounds form the main chain in grass hemicelluloses. Xylose, along with arabinose, is produced in considerable quantities when herbage is hydrolysed with normal sulphuric acid. *D-Ribose* is present in all living cells as a constituent of ribonucleic acid (RNA), and it is also a component of several vitamins and coenzymes.


D-Xylulose


D-Ribulose

The phosphate derivatives of D-xylulose and D-ribulose occur as intermediates in the pentose phosphate metabolic pathway (see p. 211).

### Hexoses

Glucose and fructose are the most important naturally occurring hexose sugars, while mannose and galactose occur in plants in a polymerised form as mannans and galactans.

D-*Glucose* (grape sugar or dextrose) exists in the free state as well as in combined form. The sugar occurs free in plants, fruits, honey, blood, lymph and cerebrospinal fluid, and it is the sole or major component of many oligosaccharides, polysaccharides and glucosides. In the pure state, glucose is a white crystalline solid and, like all sugars, is soluble in water.

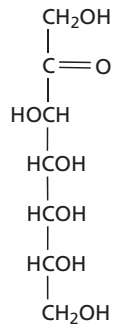
D-*Fructose* (fruit sugar or laevulose) occurs free in green leaves, fruits and honey. It also occurs in the disaccharide sucrose and in fructans. Green leafy crops usually contain appreciable amounts of this sugar, both free and in polymerised form. The free sugar is a white crystalline solid and has a sweeter taste than sucrose. The exceptionally sweet taste of honey is due to this sugar.

D-*Mannose* does not occur free in nature but exists in polymerised form as mannan and also as a component of glycoproteins. Mannans are found widely distributed in yeasts, moulds and bacteria.

D-*Galactose* does not occur free in nature except as a breakdown product during fermentation. It is present as a constituent of the disaccharide lactose, which occurs in milk. Galactose also occurs as a component of the anthocyanin pigments, galactolipids, gums and mucilages.

### Heptoses

D-*Sedoheptulose* is an important example of a monosaccharide containing seven carbon atoms and occurs, as the phosphate, as an intermediate in the pentose phosphate metabolic pathway (see p. 211).

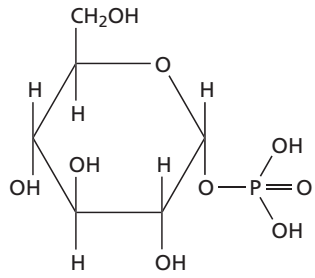



D-Sedoheptulose

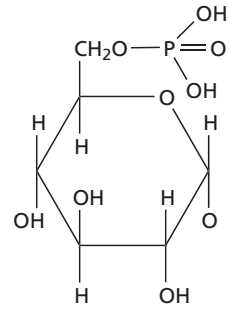
## 2.3 MONOSACCHARIDE DERIVATIVES

### Phosphoric acid esters

The phosphoric acid esters of sugars play an important role in a wide variety of metabolic reactions in living organisms (see Chapter 9). The most commonly occurring derivatives are those formed from glucose, the esterification occurring at either carbon atoms 1 or 6, or both.



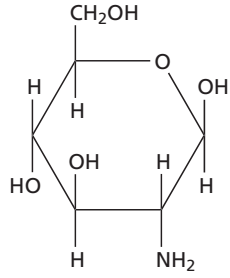

α-D-Glucose 1-phosphate



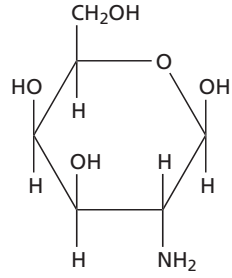

α-D-Glucose 6-phosphate

## Amino sugars

If the hydroxyl group on carbon atom 2 of an aldohexose is replaced by an amino group ($-NH_2$), the resulting compound is an amino sugar. Two such naturally occurring important compounds are D-glucosamine, a major component of chitin (see p. 28), and D-galactosamine, a component of the polysaccharide of cartilage.



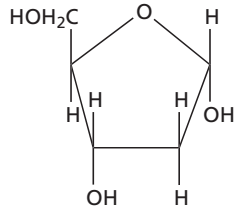

β-D-Glucosamine



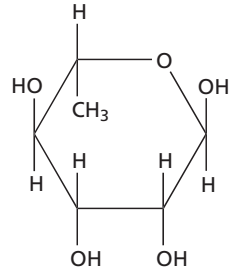

β-D-Galactosamine

## Deoxy sugars

Replacement of a hydroxyl group by hydrogen yields a deoxy sugar. The derivative of ribose, deoxyribose, is a component of deoxyribonucleic acid (DNA). Similarly, deoxy derivatives of the two hexoses, galactose and mannose, occur as fucose and rhamnose respectively, these being components of certain heteropolysaccharides.



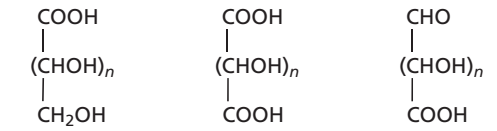

α-D-Deoxyribose




α-L-Rhamnose

## Sugar acids

The aldoses can be oxidised to produce a number of acids, of which the most important are illustrated here.

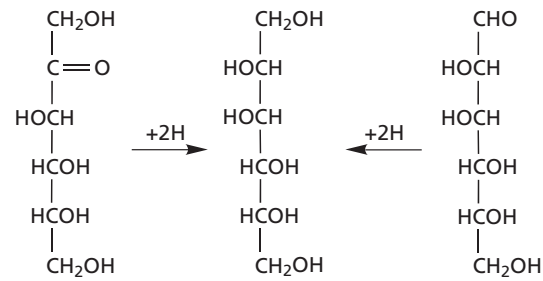



Aldonic acids Aldaric acids Uronic acids

In the case of glucose, the derivatives corresponding to these formulae are gluconic, glucaric and glucuronic acids respectively. Of these compounds, the uronic acids, particularly those derived from glucose and galactose, are important components of a number of heteropolysaccharides.

## Sugar alcohols

Simple sugars can be reduced to polyhydric alcohols; for example, glucose yields sorbitol, galactose yields dulcitol, and both mannose and fructose yield mannitol. Mannitol occurs in grass silage and is formed by the action of certain anaerobic bacteria on the fructose present in the grass.




D-Fructose D-Mannitol D-Mannose

## Glycosides

If the hydrogen of the hydroxyl group attached to the carbon 1 atom of glucose is replaced by esterification, or by condensation, with an alcohol (including a sugar molecule) or a phenol, the derivative so produced is termed a glucoside. Similarly, galactose forms galactosides and fructose forms fructosides. The general term glycoside is used collectively to describe these derivatives and the linkage is described as a 'glycosidic bond'.

Oligosaccharides and polysaccharides are classed as glycosides, and these compounds yield sugars or sugar derivatives on hydrolysis. Certain naturally occurring glycosides contain non-sugar residues. For example, the nucleosides contain a sugar combined with a heterocyclic nitrogenous base (see Chapter 4).

**Table 2.1** Some important naturally occurring cyanogenetic glycosides

| Name | Source | Hydrolytic products in addition to glucose and hydrogen cyanide |
|---|---|---|
| Linamarin (phaseolunatin) | Linseed (*Linum usitatissimum*), Java beans (*Phaseolus lunatus*), Cassava (*Manihot esculenta*) | Acetone |
| Vicianin | Seeds of wild vetch (*Vicia angustifolia*) | Arabinose, benzaldehyde |
| Amygdalin | Bitter almonds, kernels of peach, cherries, plums, apples and fruits of Rosaceae | Benzaldehyde |
| Dhurrin | Leaves of the great millet (*Sorghum vulgare*) | p-Hydroxy-benzaldehyde |
| Lotaustralin | Trefoil (*Lotus australis*), White clover (*Trifolium repens*) | Methylethyl ketone |

The cyanogenetic glycosides liberate hydrogen cyanide (HCN) on hydrolysis; because of the toxic nature of this compound, plants containing this type of glycoside are potentially dangerous to animals. The glycoside itself is not toxic and must be hydrolysed before poisoning occurs. However, the glycoside is easily broken down to its components by means of an enzyme that is usually present in the plant. An example of a cyanogenetic glycoside is linamarin (also called phaseolunatin), which occurs in linseed, Java beans and cassava. If wet mashes or gruels containing these foods are given to animals, it is advisable to boil them when mixing in order to inactivate any enzyme present. On hydrolysis, linamarin yields glucose, acetone and hydrogen cyanide. Examples of other cyanogenetic glycosides and their sources are shown in Table 2.1.

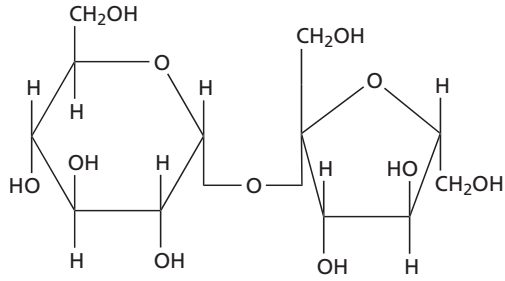
## 2.4 OLIGOSACCHARIDES

### Disaccharides

A large number of disaccharide compounds are theoretically possible, depending upon the monosaccharides present and the manner in which they are linked. The most nutritionally important disaccharides are sucrose, maltose, lactose and cellobiose, which on hydrolysis yield two molecules of hexoses:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$$

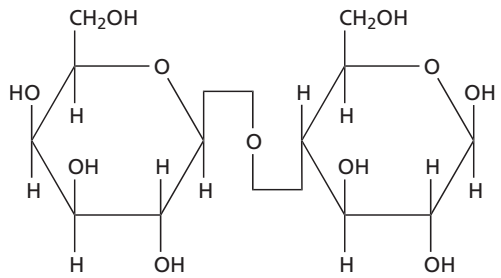
*Sucrose* is formed from one molecule of α-D-glucose and one molecule of β-D-fructose joined together through an oxygen bridge between their respective carbon atoms 1 and 2. As a consequence, sucrose has no active reducing group.




Sucrose

Sucrose is the most ubiquitous and abundantly occurring disaccharide in plants, where it is the main transport form of carbon. This disaccharide is found in high concentration in sugar cane (200 g/kg) and in sugar beet (150–200 g/kg); it is also present in other roots such as mangels and carrots, and it occurs in many fruits. Sucrose is easily hydrolysed by the enzyme sucrase or by dilute acids. When heated to a temperature of 160 °C it forms barley sugar and at a temperature of 200 °C it forms caramel.

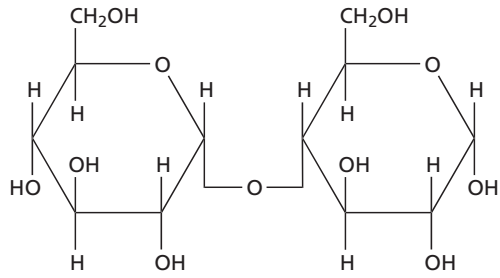
*Lactose*, or milk sugar, is a product of the mammary gland. Cow's milk contains 43–48 g/kg lactose. It is not as soluble as sucrose and is less sweet, imparting only a faint sweet taste to milk. Lactose is formed from one molecule of β-D-glucose joined to one of β-D-galactose in a β-(1:4)-linkage and has one active reducing group.




Lactose

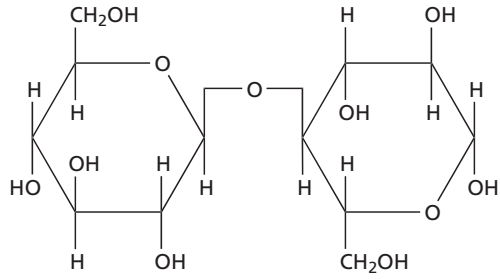
Lactose readily undergoes fermentation by a number of organisms, including *Streptococcus lactis*. This organism is responsible for souring milk by converting the lactose into lactic acid ($CH_3$.CHOH.COOH). If lactose is heated to 150 °C it turns yellow; at a temperature of 175 °C the sugar is changed into a brown compound, lactocaramel. On hydrolysis, lactose produces one molecule of glucose and one molecule of galactose.

*Maltose*, or malt sugar, is produced during the hydrolysis of starch and glycogen by dilute acids or enzymes. It is produced from starch during the germination of barley by the action of the enzyme amylase. The barley, after controlled germination and drying, is known as malt and is used in the manufacture of beer and Scotch malt whisky. Maltose is water-soluble, but it is not as sweet as sucrose. Structurally it consists of two α-D-glucose residues linked in the α-1,4 positions; it has one active reducing group.




Maltose

*Cellobiose* does not exist naturally as a free sugar, but it is the basic repeating unit of cellulose. It is composed of two β-D-glucose residues linked through a β-(1:4)-bond. This linkage cannot be split by mammalian digestive enzymes. It can, however, be split by microbial enzymes (see Chapter 8). Like maltose, cellobiose has one active reducing group.




Cellobiose

## Trisaccharides

*Raffinose* and *kestose* are two important naturally occurring trisaccharides. They are both non-reducing and on hydrolysis produce three molecules of hexose sugars:

$$C_{18}H_{32}O_{16} + 2H_2O \rightarrow 3C_6H_{12}O_6$$

Raffinose is the most common member of the group, occurring almost as widely as sucrose in plants. It exists in small amounts in sugar beet and accumulates in molasses during the commercial preparation of sucrose. Cotton seed contains about 80 g/kg of raffinose. On hydrolysis, this sugar produces glucose, fructose and galactose.

Kestose and its isomer isokestose occur in the vegetative parts and seeds of grasses. These two trisaccharides consist of a fructose residue attached to a sucrose molecule.

## Tetrasaccharides

Tetrasaccharides are made up of four monosaccharide residues. *Stachyose*, a member of this group, is almost as ubiquitous as raffinose in higher plants and has been isolated from about 165 species. It is a non-reducing sugar and on hydrolysis produces two molecules of galactose, one molecule of glucose and one of fructose:

$$C_{24}H_{42}O_{21} + 3H_2O \rightarrow 4C_6H_{12}O_6$$