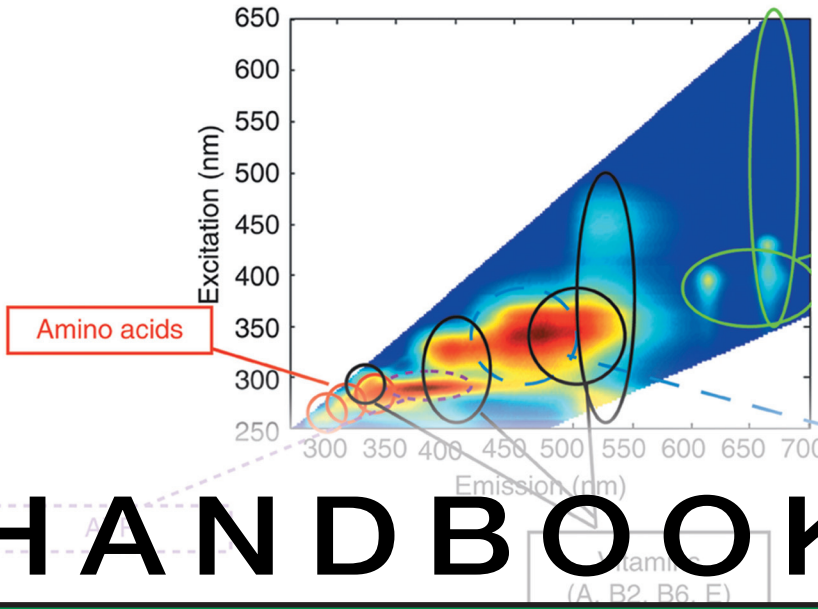

HANDBOOK OF

Analysis of Edible Animal By-Products

Edited by
LEO M.L. NOLLET
FIDEL TOLDRÁ

CRC Press
Taylor & Francis Group

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Contents

Preface

Offal or edible and inedible animal by-products comprise a wide variety of products like the skin, blood, bones, meat trimmings and mechanically separated meat, fatty tissues, horns, hoofs, feet, skull, and entrails and internal organs of a butchered animal. Depending on cultures and countries, edible by-products may be considered as waste material being thrown away or as delicacies commanding high prices. Offal not used directly for human or pet food is often processed as material that is used for animal feed, fertilizer, or fuel.

This book contains 23 chapters classified into 4 parts: Part I (Chemistry and Biochemistry— Chapters 1 through 5), Part II (Technological Quality— Chapter 6), Part III: (Nutritional Quality— Chapters 7 through 11), and Part IV (Safety— Chapters 12 through 23).

Chapter 1 introduces readers to the topic of the book. Chapters 2 through 5 focus on the analysis of chemical and biochemical compounds of animal by-products. The usage and detection of food-grade proteins and analysis of rendered fats and cholesterol are detailed. One chapter discusses oxidation in edible animal by-products. Chapter 6 describes the measurement methods of color in these types of products.

Chapters 7 through 11 deal with the analysis of composition and nutrients in animal by-products, such as essential amino acids, fatty acids, vitamins, minerals, and trace elements.

Chapters 12 through 23 deal with safety parameters, especially analytical tools for the detection of pathogens, toxins, and chemical toxic compounds usually found in muscle foods. Some chapters discuss tissues typically found in animal by-products, such as neuronal tissues, non-muscle tissues, and bone fragments.

This unique handbook is intended to provide readers with a full overview of the analytical tools available for the analysis of animal by-products and the role of these techniques and methodologies for the analysis of technological, nutritional, and sensory quality, as well as for safety aspects. In short, this book deals with the main types of analytical techniques and methodologies available worldwide for the analysis of animal by-products.

It was not an easy task to find authors for such chapters. We would like to thank all the contributing authors for their excellent efforts and hard work.

> It is better to keep your mouth closed and let people think you are a fool than to open it and remove all doubt.
>
> **Mark Twain**

Leo M.L. Nollet
Fidel Toldrá

Editors

Dr. Leo M.L. Nollet has been the editor and associate editor of numerous books. He edited for Marcel Dekker, New York (now CRC Press of the Taylor & Francis Group), the first and second editions of *Food Analysis by HPLC* (2000) and the *Handbook of Food Analysis* (2004). The last edition is a three-volume book. He also edited the *Handbook of Water Analysis, Chromatographic Analysis of the Environment*, third edition (CRC Press, 2005), and the second edition of the *Handbook of Water Analysis* (CRC Press, 2007).

With F. Toldra, he has coedited two books: *Advanced Technologies for Meat Processing* (CRC Press, 2006) and *Advances in Food Diagnostics* (Blackwell, 2007).

With M. Pöschl, he coedited *Radionuclide Concentrations in Foods and the Environment* (CRC Press, 2006).

He has coedited several books with Y. H. Hui and other colleagues: the *Handbook of Food Product Manufacturing* (Wiley, 2007); the *Handbook of Food Science, Technology, and Engineering* (CRC Press, 2005); *Food Biochemistry and Food Processing* (Blackwell, 2005); and the *Handbook of Flavors from Fruits and Vegetables* (Wiley, 2010).

Finally, he edited the *Handbook of Meat, Poultry and Seafood Quality* (Blackwell, 2007) and *Analysis of Endocrine Compounds in Foods* (Blackwell-Wiley, 2010).

With F. Toldra, he prepared or is preparing six books on meat analysis methodologies:

- *Handbook of Muscle Foods Analysis*
- *Handbook of Processed Meats and Poultry Analysis*
- *Handbook of Seafood and Seafood Products Analysis*
- *Handbook of Dairy Foods Analysis*
- *Handbook of Analysis of Edible Animal By-Products*
- *Handbook of Analysis of Active Compounds in Functional Foods*

With H. Rathore, he worked or is working on two books related to pesticide analysis: the *Handbook of Pesticides: Methods of Pesticides Residues Analysis* (CRC Press, 2009) and *Pesticides: Evaluation of Environmental Pollution*.

Dr. Nollet received his MS (1973) and PhD (1978) degrees in biology from the Katholieke Universiteit Leuven, Belgium. He is a professor at University College Ghent (Hogeschool Gent), a member of Ghent University Association, Faculty of Applied Engineering Sciences, Ghent, Belgium.

Dr. Fidel Toldrá, PhD, is a research professor at the Department of Food Science, Instituto de Agroquímica y Tecnología de Alimentos (CSIC), and serves as European editor of *Trends in Food Science & Technology*, editor in chief of *Current Nutrition & Food Science*, and member of CEF Panel at the European Food Safety Authority. He is a member of the editorial board of eight journals, including *Food Chemistry*, *Meat Science,* and *Food Analytical Methods*. He has acted as editor or associate editor of several books in recent years. He was the editor of *Research Advances in the Quality of Meat and Meat Products* (Research Signpost, 2002) and associate editor of the *Handbook of Food and Beverage Fermentation Technology* and the *Handbook of Food Science, Technology, and Engineering* published in 2004 and 2006, respectively, by CRC Press. In collaboration with L. Nollet, he coedited two books published in 2006: *Advanced Technologies for Meat Processing* (CRC Press) and *Advances in Food Diagnostics* (Blackwell Publishing). Both were also associate editors of the *Handbook of Food Product Manufacturing* published by John Wiley & Sons in 2007. Professor Toldrá edited the books *Meat Biotechnology* (2008, Springer) and *Safety of*

Meat and Processed Meat (2009, Springer) and also authored the book *Dry-Cured Meat Products* published by Food & Nutrition Press (now Blackwell) in 2002.

With F. Toldra, he prepared or is preparing five books on meat analysis methodologies:

- *Handbook of Muscle Foods Analysis*
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- *Handbook of Analysis of Edible Animal By-Products*

Both are also preparing the *Handbook of Analysis of Active Compounds in Functional Foods*, also for CRC Press.

Dr. Toldrá was awarded the 2002 International Prize for meat science and technology by the International Meat Secretariat and the Distinguished Research Award in 2010 by the American Meat Science Association. He was elected in 2008 as Fellow of the International Academy of Food Science & Technology (IAFOST) and in 2009 as Fellow of the Institute of Food Technologists.

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Part I

Chemistry and Biochemistry

1 Introduction—Offal Meat: Definitions, Regions, Cultures, and Generalities

Leo M.L. Nollet and Fidel Toldrá

CONTENTS

1.1 INTRODUCTION

Offal or edible and inedible animal by-products comprise a wide variety of products like the skin, blood, bones, meat trimmings and mechanically separated meat, fatty tissues, horns, hoofs, feet and skull, and entrails and internal organs of a butchered animal [1,2]. Depending on cultures and countries, edible by-products may be considered as waste material being thrown away, or as delicacies commanding high prices. Offal not used directly for human or pet food is often processed as material that is used for animal feed, fertilizer, or fuel [3]. The yield of edible by-products depends on species, sex, age, live weight, and others. This yield varies from 10% to 30% for beef, pork and lamb and from 5% to 6% for chicken. Yields of different by-products for different species are shown in Table 1.1.

Consumption of meat has increased in recent years; however, the use of its by-products for human consumption has decreased. Most consumed animal by-products are liver, heart, kidney, tongue, thymus or sweetbreads, brain, and tripe.

In some parts of Europe, scrotum, brain, chitterlings (the large intestine of a pig), trotters (feet), heart, head (of pigs, calves, sheep and lamb), kidney, liver, lights (lung), sweetbreads (thymus), fries (testicles), tongue, snout (nose), and tripe (stomach) from various mammals are common menu items.

TABLE 1.1
Weight of By-Products

	By-Product: Percentage of Live Weight			
	Beef	**Hog or Pig**	**Lamb**	**Chicken**
Blood	2.4–6	2–6	4–9	1.4–2.3 kg
Brain	0.08–0.12	0.08–0.1	0.26	0.2–0.3
Chitterlings	0.06			
Ears	0.02			
Feet	1.9–2.1	1.5–2.2	2.0	
Gizzard				1.9–2.3
Gullet	0.03	0.1		
Head		5.2	6.7	
Heart	0.3–0.5	0.15–0.35	0.3–1.1	0.3–0.8
Intestines		1.8	3.3	
Kidney	0.07–0.24	0.2–0.4	0.3–0.6	
Liver	1.0–4.5	1.1–2.4	0.9–2.2	1.6–2.3
Lungs	0.4–0.8	0.4–0.85	0.7–2.2	0.7
Pancreas	0.06	0.1	0.2	
Penis	0.18			
Spleen	0.1–0.27	0.1–0.16	0.1–0.4	0.15
Tail	0.1–0.25	0.1		
Tongue	0.25–0.5	0.3–0.4		
Tripe	0.75	0.6–0.7	2.9–4.6	

Source: Ockerman, H.W. and Basu, L., By-products/edible, for human consumption, in: Devine, C., Dikeman, M., and Jensen, W.K. (Eds.), *Encyclopedia of Meat Sciences*, Academic Press, New York, 2004, pp. 104–112. With permission.

Mammalian offal is slightly more popular in the southern parts of the United States, where some recipes include chitterlings, chicken gizzards and livers, and pig stomach (hog maw). Scrapple, sometimes made from pork offal, is more common in the northeast United States. Traditional recipes for turkey gravy include giblets of the bird.

In Australia, offal is most commonly consumed in meat pies, or in ethnic dishes. Addition of offal to food and labeling is regulated.

In China, different organs and other parts of animals are used for food or traditional Chinese medicine. Pork is the most consumed meat in China. Popular pork offal dishes include stir-fried cleaned pork kidneys or a spicy stew with pork intestine slices and pork blood cubes.

1.2 NUTRIENT CONTENT

Edible animal by-products are significant sources of nutrients. Examples of typical protein, fat, mineral, and vitamin contents of different organs of beef and pork are shown in Tables 1.2 and 1.3. In general, they have a good nutritional value due to the high protein and low fat levels as well as good content in vitamins and minerals. Liver contains the largest amounts of nutrients, especially B group vitamins, copper, and manganese. In the case of cholesterol, the brain is the organ with substantial larger amounts, while the contents in liver and kidneys are also relatively high [4].

TABLE 1.2
Components per 100 g of Beef Offal

	Protein (g)	Fat (g)	Ca (mg)	P (mg)	Fe (mg)	Na (mg)	K (mg)	Mg (mg)	Zn (mg)	Cu (mg)	Mn (mg)
Brain	10.4–11.5	8.6	10	312	2.1–2.4	125	219	13	1.22	0.20	0.04
Heart	14.9–28.5	3.6–20.0	5	195–230	4.0–4.9	86–95	193–320	23	2.38	0.36	0.04
Kidney	15.3–24.7	2.6–6.7	10–11	219–230	5.7–7.4	176–180	225–230	17	1.85	0.47	0.10
Liver	19.0–22.9	3.8–7.8	6–8	352–360	6.5–7.0	81–136	281–320	19	3.92	2.76	0.26
Pancreas	17.6–27.1	7.3	8	216–330	2.8–8.4	67	276	18	2.58	0.06	0.15
Tongue	15.3–22.2	10.4–14.6	6–8	170–182	2.1–2.9	73	197–250	16	2.47	0.17	0.03

	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenate (mg)	Biotin (µg)	Folacin (µg)	Vitamin B12 (µg)	Vitamin A (IU)	Ascorbic Acid (mg)	Cholesterol (mg)
Brain	0.07–0.23	0.22–0.26	3.0–4.7	0.10–0.26	2.5	2.0–6.1	4–12	7–4.7–10.9	Nihil	16.6–23.0	—
Heart	0.19–0.68	0.23–0.43	6.3–9.5	0.23–0.43	1.2–2.3	2.0–7.3	2–110	8.0–13.7	Traces-3.0	2.0–7.6	140
Kidney	0.28–0.38	0.32–0.44	5.4–7.9	0.32–0.44	3.4	24.0–92.0	41–77	8.5–31.0	264–880	8.9–15.0	285
Liver	0.23–0.28	0.74–0.94	12.8–21.0	0.74–0.94	5.5–8.3	33.0–100.0	81–330	65.0–110.0	12709–105032	2.6–31.0	354
Pancreas	0.14	0.20	3.1–5.8	0.20	3.8	14.0	—	4.8–5.0	Nihil	13.7–14.0	—
Tongue	0.12–0.17	0.13–0.31	3.9–4.9	0.13–0.31	2.0	1.0–3.3	4–7	3.8–7.0	Nihil	31–7.0	87

Sources: Ockerman, H.W. and Basu, L., By-products/edible, for human consumption, in: Devine, C., Dikeman, M., and Jensen, W.K. (Eds.), *Encyclopedia of Meat Sciences*, Academic Press, New York, 2004, pp. 104–112; Anderson, B.A., Composition and nutritional value of edible meat by-products, in: Pearson, A.M. and Dutson, T.R. (Eds.), *Edible Meat By-Products. Advances in Meat Research*, Elsevier Applied Science, London, U.K., 1988, vol. 5, pp. 15–45.

TABLE 1.3
Components per 100 g of Pork Offal

	Protein (g)	Fat (g)	Ca (mg)	P (mg)	Fe (mg)	Na (mg)	K (mg)	Mg (mg)	Zn (mg)	Cu (mg)	Mn (mg)
Brain	10.3–122	8.6–9.2	10	312	1.6–2.4	125	219	14	1.27	0.24	0.09
Heart	16.8–23.5	2.7–4.4	3–6	131–220	3.3–4.8	54–80	106–300	19	2.80	0.41	0.06
Kidney	15.4–25.4	2.7–3.6	8–11	218–270	5.0–6.7	115–190	178–290	17	2.75	0.62	0.12
Liver	18.9–21.6	2.4–6.8	6–10	356–370	19.2–21.0	73–87	271–320	18	5.76	0.68	0.34
Pancreas	28.5	4.0–15.0	—	—	18.9	—	—	17	2.62	0.09	0.16

	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenate (mg)	Biotin (µg)	Folacin (mg)	Vitamin B12 (µg)	Vitamin A (IU)	Ascorbic Acid (mg)	Cholesterol (mg)
Brain	0.16–0.23	0.26–0.28	4.3–4.4	0.19	2.8	—	6.0	2.2–2.8	Nihil	13.5–18.0	2195
Heart	0.13–0.16	0.81–1.24	6.6–9.6	0.29–0.39	2.5	4.0–18.0	2–4	2.4–8.0	Traces-106	3.0–5.3	131
Kidney	0.26–0.58	1.70–1.90	7.5–9.8	0.55	3.1	32.0–130	42	6.6–14.0	130–230	14.0–14.2	319
Liver	0.28–0.31	3.00	14.8–16.4	0.68–0.69	0.9	27.0	110–212	25.0–26.0	Nihil–10900	13.0–25.3	301
Pancreas	0.11	0.46	3.5	—	4.6	—	—	6.5–7.0	Nihil	15.0–15.3	—

Sources: Ockerman, H.W. and Basu, L., By-products/edible, for human consumption, in: Devine, C., Dikeman, M., and Jensen, W.K. (Eds.), *Encyclopedia of Meat Sciences*, Academic Press, New York, 2004, pp. 104–112; Anderson, B.A., Composition and nutritional value of edible meat by-products, in: Pearson, A.M. and Dutson, T.R. (Eds.), *Edible Meat By-Products. Advances in Meat Research*, Elsevier Applied Science, London, U.K., 1988, vol. 5, pp. 15–45.

1.3 MAIN EDIBLE ANIMAL BY-PRODUCTS AND ITS CONSUMPTION

1.3.1 Liver

The liver of beef, veal, lamb, and pork weights in average 5, 1.5, 1.4, and 1.4 kg, respectively. Liver is mostly thinly sliced and cooked. Further on it may be minced and incorporated in many preparations, e.g., braunschweiger, liver paste, and liverwurst.

Liver is one of the most nutritious parts of by-products and constitutes a rich source of vitamins B_{12} and A.

In the United Kingdom, Midlands faggots are made from ground or minced pig offal (mainly liver and cheek), bread, herbs, and onion wrapped in pig's caul. A similar dish, almôndega or meatball, is traditional in Portugal.

Ground chicken livers, mixed with chicken fat and onions, called chopped liver, is a popular Jewish dish.

1.3.2 Heart

The heart of beef, veal, pork, and lamb averages 1.4 kg, 227 g, 227 g, and 113 g, respectively. Hearts must be cooked for longer periods. So, they are diced and added to stews or other meat to add protein and color. In Perú, cow heart is used for anticuchos, a sort of brochettes. Anticuchos can be made of any type of meat, the most popular are made of beef heart (anticuchos de corazón). In Brazil, churrasco often includes chicken hearts, roasted on a big skewer.

1.3.3 Tongue

The tongue of beef, veal, pork, and lamb weighs ±2, 0.7, 0.3, and 0.2 kg, respectively. After blanching, the outer membrane is removed.

In some countries of South America, the tongue is usually boiled, sliced, and marinated with a mixture of oil, vinegar, salt, chopped peppers, and garlic.

1.3.4 Kidney

The pair of beef and veal kidneys are lobed and weigh ±0.5 kg each for beef or 340 g for veal. Sheep and pork kidneys have one lobe and weigh ±57 and 110 g, respectively. Kidneys may be added to meat casseroles, stews, and pies. Steak and kidney pie, typically with veal or beef kidneys, is widely known and enjoyed in the United Kingdom.

1.3.5 Sweetbreads

Sweetbreads are gathered from calves or lambs. Thymus or neck sweetbread (throat sweetbread) and heart sweetbread, degenerates in adult animals. Pancreas is called gut bread or stomach sweetbread. Thymus sweetbread is very likely to spoil.

1.3.6 Tripe

Beef tripe consists of the first and second stomachs of cattle. Stomachs of sheep and pork are also used for tripe. Beef first and second stomachs weigh ±3.9 kg; sheep stomach ±1 kg and pork stomach ±1.2 kg.

The traditional Scottish haggis consists of sheep stomach stuffed with a boiled mix of liver, heart, lungs, rolled oats, and other ingredients. Most modern commercial haggis is prepared in a casing rather than an actual stomach. Sometimes haggis is sold in tins, which can simply be microwaved or ovenbaked. Some supermarket haggis is largely made from pig, rather than sheep, offal.

Drob is in Romania a dish similar to haggis. It is served on Easter.

In Bulgaria, Republic of Macedonia and Turkey, Shkembe chorba is a widespread soup variety made from tripe.

Tripes are extensively used in Spain as casings for stuffing in the manufacture of traditional semidry and dry-fermented sausages. In some Latin American countries, tripe is used to make menudo and mondongo. The soup menudo is a traditional Mexican dish: a spicy soup made with tripe. Sopa de mondongo is a hearty traditional soup of Latin America and the Caribbean. It is made from slow-cooked diced tripe.

In the Chinese mainland, beef tripe is used as a cold appetizer.

Cooked buffalo tripe rolls were prepared from a combination of buffalo tripe (75%) and buffalo meat (25%) by using mincing and blade tenderization and their quality was then evaluated [5]. They were stored at 4°C ± 1°C and studied for various physicochemical, sensory and microbial qualities.

1.3.7 Chitterlings

Chitterlings are the intestines and rectum of a pig that have been prepared as food. As pigs are a common source of meat throughout the world, the dish known as chitterlings can be found in most pork-eating cultures. Chitterlings are popular in most parts of Europe, where pig intestines are also used as casing for sausages. In England, chitterlings remain especially popular in Yorkshire.

They are eaten as a dish in East Asian cuisines. In America, chitterlings are an African-American culinary tradition and a Southern culinary tradition sometimes called soul food cooking. In America, chitterlings are sometimes battered and fried after the stewing process.

Chitterlings are carefully cleaned and rinsed several times before they are boiled or stewed for several hours. Pajata is a traditional dish from Rome, Italy. It refers to the intestines of an unweaned calf, i.e., only fed on its mother's milk. The calf is killed soon after nursing. The intestines are cleaned, but the milk is left inside. When cooked, the combination of heat and the enzyme rennet in the intestines coagulates the milk and creates a sort of thick, creamy, cheese-like sauce.

In France, chitterlings sausage, a delicacy, is called andouillette.

Care must be taken when preparing chitterlings, due to the possibility of diseases. These diseases are caused by bacteria including *E. coli* and *Yersinia enterocolitica*, or *Salmonella*.

1.3.8 Brains

Beef brains weigh ±450 g; pork brains ±120 g and lamb brains ±130 g. Cattle brains belong to risk material and is thus forbidden because of its relation to bovine spongiform encephalopathy (BSE) or "mad cow" disease. Brains of other animals are edible but very perishable.

Fried-brain sandwiches are a specialty in the Ohio River Valley. Cow's brains, sesos, are used to make ravioli stuffing in some countries of South America. Sesos also constitute a typical and popular dish in Spain.

1.3.9 Testicles

Testicles of a bull or a ram weight ±0.25 kg; the testicles of a boar weigh ±130 g. Rocky Mountain oysters are a delicacy eaten in some cattle-raising parts of the western United States and Canada. Rocky Mountain oysters or prairie oysters is a North American culinary name for edible offal, specifically buffalo, boar, or bull testicles.

1.3.10 Blood

Beef contains 10–12 L of blood and a sheep ±1.5 L. Pork blood is used in Spain as an ingredient in a kind of typical cooked sausages called "morcilla." It is used in other cooked meat products due to

its good binding of fat and water. Plasma is the most interesting part of blood due to its color and functional properties. Albumin is the main component of the plasma fraction of blood and is the main responsible for plasma gel firmness upon heating [6]. Blood proteins also have an excellent foaming capacity.

1.3.11 Lard and Tallow

Lard is considered as the fat rendered from edible pork tissues while tallow is defined as the hard fat rendered from fatty tissues in cattle [7]. The fatty acid composition of these fats, especially lard, depends on the feed given to the animal before slaughter. Tallow may also contain some trans-fatty acids, including the conjugated linoleic acid (CLA), due to the action of rumen. There are many traditional uses for lard and tallow like deep-fat frying, use in margarine and shortenings, cover of dry-cured hams, etc.

1.3.12 Other Edible By-Products

Other parts suitable for human consumption are spleen, oxtail, bones for stock, trimmings such as beef diaphragm muscle, gullet or beef cheek papillae, pork jowl, pig tail, pigs' feet (trotters) and ears and poultry giblets.

In the United States, the giblets of chickens, turkeys and ducks are commonly consumed.

Brawn is a British English term for head cheese or the collection of meat and tissue found on an animal's skull (typically a pig) that is chilled and set in gelatin.

Iceland has its own version of both haggis and brawn. The Icelandic haggis, slátur, is made in two versions. Blóðmör or bloodlard is a stomach of a sheep stuffed with a mixture of sheep's blood, rolled oats and slices of sheep's fat, and lifrarpylsa or liver sausage consisting of sheep stomach stuffed with a mixture of ground lamb's liver, rolled oats, and cut up bits of sheep. The Icelandic brawn, svið, is made from singed sheep heads.

Romanian peasants make a kind of traditional sausages from pork offal, called caltabos. In Greece and in Turkey, splinantero consists of liver, spleen, and small intestine, roasted over an open fire. A festive variety is kokoretsi, made of pieces of lamb offal (liver, heart, lungs, spleen, kidney, and fat). These pieces are pierced on a spit and covered by washed small intestine wound around in a tube-like fashion. It is a traditional dish for Easter. Another traditional Easter food is mageiritsa: a soup made with lamb offal and lettuce in a white sauce.

An Armenian traditional dish is khash. The main ingredient in khash is pig's or cow's feet, although other animal parts, such as the ears and tripe may also be used. It is rich in cartilage and other connective tissues.

In Italy, consumption of entrails and internal organs is quite widespread, among the most popular preparations are fried or stewed brain, boiled intestines (trippa), lampredotto (the fourth stomach of the cow), liver, kidneys, heart and coronaries (coratella or animelle), head, eyes, testicles of pig; several preparations are based on chicken entrails.

In Sicily, many enjoy a type of sandwich pani ca meusa, or bread with spleen and caciocavallo cheese. In New York, it is named vastedda.

In Spain, the organs of the entrails are used in many traditional dishes: callos or cow tripe in Madrid and Asturias, liver, kidneys, criadillas or bull's testicles, cow's tongue, pork's head and feet in Catalonia and pork's ears in Galicia.

In the French city of Marseille lamb's trotters and a package of lamb tripe are a traditional food, pieds et paquets.

Feijoada is a stew of beans with beef and pork meats, ears, feet and tail, which is a typical Portuguese dish, also typical in Brazil, Angola and other former Portuguese colonies.

Lungen stew is a traditional dish among American Jews. In Argentina, Chile and Uruguay, the traditional asado is often made along with several offal types, achuras, like chinchulines and tripa gorda (chitterlings), mollejas (sweetbread) and riñón (kidney of a cow).

Pork tongue slices with salt and sesame oil is a common dish, especially in Sichuan province of China. Braised pork ear strips are available as street merchant food or in some supermarkets. Cleaned pork stomach roasted primarily in sugar and soy sauce then sliced is a popular food in Hong Kong. Pork liver slices served stir fried with onions or in soups is another hawker food. Pork blood soup is at least 1000 years old.

The offal of cattle, duck, and chicken is also used in traditional Chinese cooking. The Cantonese dish lou mei is made by simmering the organs and off-cuts of these animals in a soy-based sauce.

In Korea, offal usage is very similar to mainland China but less frequent. In Singapore, soup with organs of pigs is common for hawkers.

In Indonesia, goat's organs are very popular for soups and almost all of the parts of the animal are eaten. Babat or the stomach of cows and iso (intestines of cows) are popular in Javanese cuisine.

In Japan, chicken offal is often skewered and grilled over charcoal as yakitori. Offal originating from cattle is also an ingredient in certain dishes. However, Japanese culture mostly declines the use of offal from large animals due to the traditional Japanese preference for cleanliness, derived from Shinto purity beliefs.

In the Philippines, people eat practically every part of the pig, including snout, intestines, ears, and innards. Dinuguan is a particular type of blood-stew using pig intestines, pork meat and sometimes ears and cheeks. Bopis is a spicy dish with pork lungs and heart. Isaw is another course in the Philippines. It is a kebab made with pieces of the large intestine of a pork barbecued.

In India, Pakistan, Nepal, and Bangladesh, different parts of the goat, brain, feet, head, stomach, tongue, liver, kidney, udder, and testicles are eaten. The heart and liver of chickens are also enjoyed. One popular dish, Kata-Kat, is a combination of spices, brains, liver, kidneys and other organs.

Rakhti is a combination of heavily spiced porcine offal and cartilaginous tissue, consumed by the local Christian community in southern India.

In Lebanon, lamb brain is used in *nikhaat* dishes and sometimes as a sandwich filling. Another popular dish is korouch, rice-stuffed sheep intestine.

In Iran, sheep liver, heart, and kidneys are used as certain types of kebab and are frequently eaten, as well as sheep intestines and stomach. Sheep brains and tongue are eaten with traditional bread.

1.4 FOOD SAFETY ISSUES

Offal of certain animals may be unsafe to consume. Some animal intestines are very high in coliform bacteria and need to be washed and cooked thoroughly to be safe for eating. Wong et al. [8] discussed the presence of the pathogens *Salmonella* and *Escherichia coli* O157:H7, and *E. coli* biotype 1 on 100 New Zealand-produced pig carcasses and 110 imported pig meat samples.

Bhandare et al. [9] investigated the microbial contamination (*Staphylococcus—Bacillus—Enterococcus—Clostridium—Enterobacteriaceae—Pseudomonaceae—Faecal Coliforms—Salmonella*) on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops.

Nervous system tissue can be contaminated with transmissible spongiform encephalopathies (TSE) prions, which cause BSE or mad cow disease. Some tissues are classified as specified risk materials and are subject to special regulations. So, certain tissues like tonsils, intestine, brain, and spinal cord from cattle, sheep, and goats were controlled since 1989 in the United Kingdom and prevented to enter the human food chain and in 2000 were harmonized through the EU [10]. The production of mechanically recovered meat from all ruminant bones was also prohibited in the EU [10]. Adequate detection methodologies for the detection of BSE in meat and meat products, based on the detection of markers from the lipids, proteins, or nucleic acids fractions were developed [11]. Offal may contain high quantities of purines able to provoke an acute attack of gout.

Heterocyclic amines (HAs) are potent mutagens formed during intense heat-processing of proteinaceous food. PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) is the most ubiquitous and abundant mutagenic HA. In the study of Khan et al. [12], several offal products (beef liver, lamb

kidney and beef tongue) were thermally processed and analyzed for HAs. Norharman and harman were the amines most abundant, found at concentrations below 2 ng g^{-1}.

The practice of feeding raw offal to dogs on farms and ranches can spread echinococcosis, a potentially fatal parasitic disease of many animals, including wildlife, commercial livestock, and humans. The disease results from infection by tapeworm larvae of the genus *Echinococcus* (*E. granulosus*, *E. multilocularis*, *E. vogeli*, and *E. oligarthrus*).

Offal of bovine, ovine, and porcine may accumulate potentially toxic heavy metals, such as Cd and Pb, posing a risk for human health and making necessary the development of new methodologies like SF-ICP-MS for its quantification [13].

In summary, there is a wide variety of animal edible by-products with traditional consumption, sometimes high, in many countries worldwide. This book provides a full overview of the analytical tools available for the analysis of animal by-products and the role of these methodologies in the analysis of technological, nutritional, and sensory quality, as well as for safety aspects.

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2 Food-Grade Proteins from Animal By-Products: Their Usage and Detection Methods

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CONTENTS

2.1 INTRODUCTION

The parts of an animal that are not intended for direct use as human food are referred to as animal by-products. In the United States, the meat industry considers everything produced by or from an animal, with the exception of dressed meat, to be a by-product. These by-products include bones, skin, trimmed fat and connective tissues, feet, abdominal and intestinal contents, and blood. Biologically, however, most of the non-carcass material obtained after slaughtering the animal is edible once it has been properly cleansed, handled, and processed. Globally, the use of these non-carcass materials in the human food chain varies from region to region depending on such factors as religion and custom, with organs such as the liver, heart, kidney, and tongue being the most commonly used. Other edible parts include poultry feet, pig's feet, the brain, blood, and so on [1].

Animal by-products fall into two categories, namely edible and inedible, although the distinction is not always clear cut and may vary from situation to situation. For example, bovine liver is a beneficial by-product when passed as suitable for human consumption, but if it is infected with fascioliasis it is inedible [2]. Other animal by-products that are initially considered to be unfit for

human consumption when produced at the slaughter house may, after further processing, become fit for human consumption. For example, bones, connective tissues, hides, and skins can be processed to make gelatin and collagen, while sheep intestines can be turned into casings for use in sausage type products. Such products are referred to as edible co-products and must not be confused with edible by-products, which include parts of animals that are perfectly fit for human consumption but are not meant for such [3]. Animal by-products that are absolutely unfit for human consumption are used in animal feedstuffs such as meat-and-bone-meal, pet food, and in other technical products, such as glue, leathers, soaps, and fertilizers. The alternative is their destruction, often by incineration. This classification may change over time; whey, the watery part of milk that separates from the curd when milk is curdled in the production of cheese, used to be considered a by-product of the cheese industry and was disposed of as such, but is now widely used in the food industry. Whey is thus an animal by-product that for the purposes of this chapter will be included as a food grade protein source.

The combined livestock and poultry industry represents the largest agricultural businesses in the United States. According to the United States Department of Agriculture (USDA), it was forecast that 57,038 metric tons of beef and veal (carcass weight equivalent), 100,171 metric tons of pork (carcass weight equivalent), and 76,227 metric tons of broiler and turkey (ready to cook equivalent) meat would be consumed in 2009 [4]. As reported by the Food and Fertilizer Technology Center for the Asian and Pacific Region publication, by-products constitute 52%, 66%, and 68% of the live weight of pigs, cattle, and sheep, respectively; this implies that billions of kilograms of animal by-products are produced annually and a valuable source of revenue will be lost if these products are not well utilized. Also, disposing of unwanted by-products is expensive and continues to rise [1]; even worthless by-products must be disposed of in an environmentally responsible manner, which adds to the cost of meat production. As the selling price of the carcass (dressed meat) alone cannot compensate for the high cost of rearing the live animal, a great deal of effort has been devoted to finding ways to fully utilize these animal by-products, not only to increase profits and hence the viability of the meat industry, but also to address issues of environmental pollution associated with their inefficient disposal. However, despite the strenuous efforts that have already been made to fully utilize animal by-products, more than 2% of the weight of the carcass is still lost to effluent and there is therefore more room for improvement in this area [5].

This chapter will examine four food grade protein materials derived from major animal by-products, namely, blood, collagen, gelatin, and whey. Their use as ingredients in food production will be discussed, along with possible concerns associated with their use in food product formulation and the analytical methods for their detection that have been developed to address these concerns.

2.2 BLOOD

For many years, blood, which is the first by-product obtained after slaughter of an animal, used to be discarded as an unwanted by-product by slaughter houses in the United States. Blood is made up of two fractions, namely the cellular fraction which comprises the red blood cells, white blood cells and platelets, and the plasma fraction, with the former suspended in the latter. Plasma accounts for 65%–70% of the total volume of blood, with the cellular fraction accounting for the remainder [6]. Animal blood contains about 18% of protein, with hemoglobin, which is present in the red blood cells, accounting for more than half of the proteins present [7]. The typical nutrient composition of bovine blood consists of 80.9% water, 17.3% protein, 0.23% lipid, 0.07% carbohydrate, and 0.62% minerals. [8]. Plasma contains about 7.9% protein, consisting principally of immunoglobulins (4.2%), albumins (3.3%), and fibrinogen (0.4%) [9]. More than one hundred proteins have been well characterized from plasma. Selected specific proteins are listed in Table 2.1.

Of all the unwanted animal by-products, blood causes the most problems as a result of the huge volume produced and its high pollutant load. It is estimated that the annual blood waste in the United States is around 1.6 million tons. Given the large quantities of blood produced and its high solids

TABLE 2.1
Selected Well Characterized Plasma Proteins

Protein	Molecular Weight (kDa)	Amount in Serum (mg%)
Albumin	66	3500–5500
Antithrombin III	65	29
α_1-Acid glycoprotein	44.1	90
α_1-Antitripsin	54	290
α_1-Antichymotrypsin	68	45
α_1B-Glycoprotein	50	22
α_2-HS-Glycoprotein	49	60
α_2-Macroglobulin	820	240–290
β_2-Glycoprotein I	40	20
β_2-Glycoprotein III	35	10
C1 inactivator	104	24
C2	117	3
C3	185	110
C3 activator (β2II)	60	18
Ceruloplasmin	132	35
Cold insoluble globulin	350	33
Fibrinogen	341	300
Gc globulin	50.8	40
Hemopexin	57	80
Haptoglobulin 1-1	100	170–235
Histidine-rich 3.8 S α2-glycoprotein	58.5	9
Immunoglobulin A	158–162	90–450
Immunoglobulin M	800–950	60–250
Immunoglobulin D	175–180	<15
Immunoglobulin E	185–190	<0.06
Inter-α-trypsin inhibitor	160	45
Lipoproteins		
LDL	2500	350
HDL_2		40–90
HDL_3		225
Plasminogen	81	12
Prealbumin (thysoxine-binding)	54.98	25
Pregnancy-specific β1-glycoprotein	90	5–20
Prothrombin	72	6
Retinol-binding protein	21	4.5
Steroid-binding β-globulin	65	0.4–0.8
Thyrpxon-binding prealbumin	54.98	25
Thyroxine-binding globulin	60.7	1.5
Transcortin	55.7	4
Transferrin	80	295
Vitamin D-binding protein	52.8	0.5
Zn-α2-glycoprotein	41	5

Source: Selected from Heide, K. et al., Plasma protein fractionation, in: Putman, F.W. (Ed.), *The Plasma Proteins: Structure, Function, and Genetic Control*, 2nd edn., vol. III, Academic Press, Inc., New York, pp. 558–561, Table V. With permission.

content (18%), and chemical oxygen demand (COD) (500,000 mg O_2/L), the environmental problems caused by its disposal are enormous [10]. Hence efforts to fully utilize blood through the recovery of its proteins are both necessary and justified. The food industry currently uses about 30% of the blood products produced [11], mainly in meat products as a gelling agent and natural colorant. Other applications for blood products are the pet food industry (to increase the palatability of pet food), animal farming (as a feed ingredient), agriculture (as fertilizer), pharmaceutical diagnostic agents (especially bovine serum albumin [BSA] and immunoglobulins), and the paper industry (as glue). This practice of utilizing blood for a wide range of other applications avoids the cost of the environmental compatible disposal of these animal by-products, hence increasing the value of the animal to the farmer [12]. Here, we will focus on the use of blood as an ingredient in the food industry.

2.2.1 Use of Blood and Blood Products as Food Ingredients

Animal blood has long been used in Europe to make blood sausages, biscuits, bread, and blood pudding. It is also used widely in Asia in food products such as blood curd, blood cake, and blood pudding. Although the above-mentioned blood products are not common in the United States, blood finds its way into the human food chain in various forms. The U.S. Meat Inspection Act approves the use of blood in food provided that it is obtained by bleeding an animal that has been inspected and passed for use as meat (9CFR 310.20). Its uses include in sausage products to enhance the color and as an extender in meat products, with the primary purpose of lowering costs [13]. Blood from bovine and porcine origin is most commonly used in the formulation of meat products. The amount of whole blood that is used in meat products is very low however, as increasing the proportion has a detrimental effect on sensory qualities, particularly color and flavor [14]. In sausage products, the use of blood is restricted to 0.5%–2% of the sausage content, as levels above this have a negative impact on the sensory attributes of the final product. Restricting the addition of blood to within this range improves the overall perception of color and meat taste compared to reference samples with no blood added [15]. The adverse effect on sensory qualities associated with increased amounts of blood in meat products and food products in general is attributed to the presence of hemoglobin, which has an objectionable color and odor as a result of the heme component of the protein [7]. Plasma, which has a neutral taste and is devoid of the dark color normally associated with blood, is therefore preferred over whole blood and is more widely used in meat products [16]. Plasma proteins are good emulsifiers and are thus used in emulsified meat systems, where they serve as a source of large quantities of nutritionally beneficial protein [17]. They are also used as protein supplements and fat replacers in meat products such as sausages. Major muscle proteins such as myosin have the ability to cross-link with plasma proteins, enhancing resistance to endogenous protease degradation. Thus, dried plasma is used as an inhibitor for endogenous proteases in surimi-type products made from certain species of fish in order to inhibit degradation by endogenous proteases [18,19]. The predominant use of plasma in the meat industry, however, is as a binder because of its ability to form gels upon heating.

Plasma is produced by removing the blood cells from blood, but unfortunately this also removes the majority of the blood proteins present. This has led to a great deal of research into better ways of incorporating blood cells into food products to take advantage of the high protein content, while at the same time avoiding the undesirable sensory effects of hemoglobin. One approach has been to recover the proteins in the blood cells through decolorizing blood by removing the heme group from hemoglobin to produce what is known commercially as globin or decolorized blood, a more useful product in food formulations [8,20]. In addition to whole blood, plasma and decolorized blood, blood serum concentrates, and other proteins isolated from blood are now used as ingredients in food products. Examples of commercial food grade proteins produced from whole blood and blood fractions for use by the food industry are shown in Table 2.2.

Blood and blood products are used widely in the meat industry, but potential applications in other areas of the food industry have also been suggested by a number of studies for specific situations. For example, spray-dried plasma can be used as an egg substitute in bakery products because

TABLE 2.2
Examples of Food Grade Proteins Produced from Blood and Blood Fractions

Product	Company	Description	Usage
ImmunoLin	Proliant Inc., USA	Serum concentrate	Dietary supplement to be added to bars and drinks to boost immune system
NutraGammax	Proliant Inc., USA	Serum protein isolate	Dietary supplement target the sports nutrition world
Fibrimex	Harimex B.V., the Netherlands	Isolate of thrombin and fibrinogen precipitated from plasma	Natural binder for whole muscle processing
Harimix P	Sonac B.V., the Netherlands	Hemoglobin	Natural meat colorant
Plasma powder FG	Sonac B.V., the Netherlands	Plasma	A binder in meat products
Prietin	Lican Functional Protein Source, Chile	Whole blood	For making Morcilla (blood sausage)
Vepro globin	VEOS Group, Belgium	Globin	An emulsifier in meat products

of the foaming and leavening properties of blood plasma proteins [14,21]. Substituting spray-dried plasma for eggs, however, only produces cakes with desirable qualities, if the substitution is partial. Because egg products are among the more costly ingredients used in the bakery industry, the use of blood plasma would provide a low-cost substitute for some of the eggs in bakery products [21].

Food-based strategies remain the most sustainable means of combating iron deficiency anemia (IDA), which is the most widespread micro-nutrient deficiency disease globally, particularly in developing countries. Bovine blood, which has a high concentration of heme iron, has been found to be a suitable way to fortify commonly consumed food products in order to combat IDA. The heme iron content of bovine blood powder is estimated to be as high as 195.46 mg/100 g of bovine blood powder—more than 10 times that of bovine liver (17 mg/100 g of bovine liver) which is usually considered an iron rich food [22]. Studies by Walter and coworkers [23], where children were fed bovine hemoglobin fortified cookies through a nationwide school lunch program in Chile, revealed significant differences in hemoglobin concentrations and serum ferritin levels between the fortified and non-fortified groups. Thus, fortification of commonly consumed food items with blood products is a feasible and effective way to improve iron levels in iron-deficient populations. Other studies have shown that globin (hemoglobin without the heme group), which is deficient in isoleucine, serves as an excellent complement to widely consumed plant products such as corn and wheat that are low in lysine but rich in isoleucine, providing a nutritionally beneficial end product [24].

2.2.2 Need for Methods to Detect Blood and Blood Products in Food

Despite the nutritional, environmental, and economic benefits that can be derived from the use of animal blood, there is a concern about the widespread use of these products as a result of the advent of bovine spongiform encephalopathy (BSE), known colloquially as mad cow disease. There is strong evidence to suggest that blood from ruminant animals carries some level of infectivity for transmissible spongiform encephalopathies (TSEs) [25–29], a group of related fatal, progressive degenerative diseases, including BSE, which affect the central nervous system (CNS) in both humans and animals. In addition, certain individuals, for example Jews, Muslims, and vegetarians must avoid blood-tainted food products as a result of dietary restrictions imposed by their religion [30], or simply as a matter of preference [31]. Others restrict blood from their diets because of an allergy to blood

proteins such as serum albumin [32,33]. Yet other concerns regarding the use of animal blood relate to the labeling issue. Federal regulation requires that the percentage of meat or poultry in products identified as containing meat be declared on the label. Undeclared blood proteins in meat products would increase the nitrogen content of the product and hence, falsify the actual meat content, as meat content is usually estimated based on the nitrogen content of the product. Partial replacement of lean meat with blood plasma content thus offers great economic advantage to the manufacturer, as the addition of 2% of blood plasma to a meat product can boost yield by 4%–5% and substitute for up to 10% lean meat content [34]. Manufacturers may therefore be tempted to add more plasma to meat than permitted by law in order to boost profits. For all these reasons, it is necessary that effective methods be developed to detect blood in both raw and processed food as a product quality control measure to enforce labeling regulations and address consumer safety concerns. Accordingly, a great deal of effort has been devoted to developing effective ways to detect and quantify the amount of animal blood in food formulations. Some of the methods that have been developed for the purpose will be reviewed and their limitations discussed in the following section.

2.2.3 Detection Methods

Several methods, including spectrophotometric methods, ultra-thin layer isoelectric focusing, Kjeldahl, and immunological methods, have been used for detecting animal blood that is added to meat products. A spectrophotometric method developed by Maxstadt and Pollman [35] was designed to estimate the added blood in raw ground beef. This method is based on the detection of hemoglobin as a measure of the amount of added blood and involves the extraction of hemoglobin with water. Myoglobin is extracted alongside the hemoglobin from the meat sample, and these two heme pigments must then be separated by treating the extract with 85% $(NH_4)_2SO_4$ to precipitate the hemoglobin, leaving the myoglobin in solution. The hemoglobin is then converted to cyanomethemoglobin and quantified by its absorbance at 422 nm using a standard curve prepared using cyanomethemoglobin standards. The method has the advantage of being easy and fast to perform, with good repeatability. However, it is not species specific, and hence does not discriminate between blood of bovine origin and blood from other species, leading to BSE concerns, and the amount of hemoglobin extracted is the sum of that from both the added blood and the residual blood in the meat sample. Hence it is necessary to subtract a correction factor of 171 mg hemoglobin/100 g meat, which represents the normal residual hemoglobin content of ground beef, from the amount of hemoglobin measured to estimate the amount of hemoglobin (and hence blood) added. Since many factors may affect the quantity of residual blood in meat, this method cannot accurately determine the amount of added blood at low levels to enable effective regulatory decisions to be made.

The Kjeldahl method has been adopted as an alternative way to estimate the amount of hemoglobin in meat products [36]. This method uses hazardous chemicals to digest samples and measures the total nitrogen content to provide an estimate of the crude protein content. The crude protein content is determined by multiplying the nitrogen content by an appropriate conversion factor. Normally 6.25 is used as the average conversion factor, but the factors 5.94, 4.94, and 5.65 have been calculated based on biochemical data to estimate the amount of muscle protein, collagen, and hemoglobin, respectively, in the sample. This method is time consuming and at best gives only an approximation of the amount of blood present in the sample. Although it is the most commonly used method for crude protein determination in a wide range of samples, it does not distinguish between added blood and residual blood and is by no means either species- or tissue-specific.

Bauer and Stachelberger [37] employed ultra-thin layer isoelectric focusing to detect added blood in heat-treated meat, reporting that this method is not affected by heat-treatment, and has a detection limit of 0.2% of dry blood plasma in sausage. However, it is not able to discriminate between different species and requires a laborious gel chromatographic step to desalt the samples and then to concentrate the desalted samples by adsorption of the protein on hydroxyl apatite, followed by subsequent elution.

A liquid chromatography tandem mass spectrometric (LC-MS-MS) method has also been developed to detect the presence of a commercial bovine [38] and porcine [39] blood-based food binding agent in food products. The blood-based binding agent is sold under the brand name Fibrimex® and consists primarily of thrombin and fibrinogen. The method is based on the specific detection of fibrinopeptide A and B, which are cleaved by the blood protease thrombin from the N-terminus of the alpha and beta chains of fibrinogen, respectively. This method, however, suffers from matrix interference, as different matrices (fish, chicken, and meat) spiked with 5% (v/w) of the binding agent produced marked differences in the amount (peak size) of fibrinopeptides A and B detected. Although fibrinopeptides A and B could be detected when meat samples (beef, pork, and lamb) were spiked with these target peptides, neither was detected in 5% spiked cod fish samples. Detection in cod was only possible when spiking of the binding agent was above 10% level. Similarly the signal due to fibrinopeptide A in chicken samples spiked at 5% was considerably lower than that for comparable levels in meat samples. Although the authors asserted that this method was not hampered by the heat-treatment (80°C for 15 min) given to samples, it is questionable if the method will still be effective with samples that have undergone more severe or prolonged heat treatment. In addition, the fibrinopeptides could also be detected faintly in non-spiked control samples, indicating that the method may not adequately distinguish between added blood and residual blood.

Immunological methods have begun to emerge as important and effective methods for qualitative and quantitative analyses of numerous food ingredients due to the simplicity, specificity, and speed of the assays produced. These methods are all based on the specific binding between the antigen (the analyte) and its corresponding antibody and are available in many different formats, including immunodiffusion, immunoglutination, enzyme-linked immunosorbent assay (ELISA), immunoblot, and immunohistochromatography (lateral flow assay). The effectiveness of the assay mainly depends on the quality of the antibody used, which is either a polyclonal antibody (PAb) or a monoclonal antibody (MAb). Otto and Sinell [40] produced two antisera against extracts of dried bovine blood plasma and dried porcine plasma, for the detection of added dried bovine and porcine plasma in heat-treated meat mixtures, respectively. Plasma proteins were extracted with 7 M urea and tested against the antisera using either gel-diffusion or electro immune assays. Both methods proved useful for the detection of dried blood plasma, provided the tested samples had been properly diluted. The assay signal, however, was hampered by the heat treatment of the cooked samples. Thus, the concentration of plasma in a given sample can only be determined if the time–temperature regimen of the process experienced by the sample is known, and appropriate model samples tested for comparison. This method is therefore inadequate for most real life situations, where the details of the processing the sample has undergone are not available.

Blood and blood products used as the ingredients in food and feedstuffs could be produced from whole blood, plasma, serum, red blood cells, hemoglobin, and/or globin. Because of the easy availability of this wide range of blood products, the above-mentioned methods developed for detecting blood in food products that depend on detecting specific analytes may be useful for detecting only particular blood products. For example, the spectrophotometric method proposed by Maxstadt and Pollman [35], which is based on the specific detection of hemoglobin as a measure of the amount of blood that has been added to meat products, would not be useful for detecting the presence of non-hemoglobin-containing blood products such as plasma or serum in meat products. It is therefore necessary that the scope of these methods be better defined to streamline the application of these methods.

A competitive indirect enzyme-linked immunosorbent assay (ELISA) was recently developed for the detection of bovine blood in heat-processed meat [41]. This assay, which is based on a MAb, Bb1H9, that recognizes a 12 kDa thermal-stable antigenic protein in ruminant (bovine and ovine) red blood cells, is bovine and ovine blood specific and works effectively with severely heat-processed samples. It is also possible for the assay to distinguish between added blood and residual blood, with a detection limit of 0.5% bovine blood in beef. As mentioned earlier, decolorized blood, a useful blood product in food formulation, is produced through the removal of the heme group, which is responsible

for the negative sensory qualities of hemoglobin containing blood products. The 12 kDa antigenic protein recognized by the MAb Bb1H9 seems to be a monomer of the tetrameric hemoglobin molecule (Ofori and Hsieh, unpublished data). Consequently, this competitive ELISA can be used to detect any blood products containing hemoglobin or globin in a food sample, because MAb Bb1H9 has the ability to recognize hemoglobin both with and without the heme group. To the best of our knowledge, however, there is currently no single method available for the detection of globin in food products.

Ofori and Hsieh [42] have developed a sandwich ELISA for the detection of bovine blood in animal feed. This method is based on two MAbs, Bb6G12 and Bb3D6, that recognize an approximately 60 kDa thermal-stable antigenic protein in bovine blood. This assay also has the advantage of being bovine blood specific, tissue specific, and not hampered by any heat treatment the samples may have been subjected to. Further studies confirmed that the 60 kDa antigenic protein is present in the plasma fraction (Ofori and Hsieh, unpublished data) and that the assay has a detection limit of 0.3% bovine blood in autoclaved (121°C at 1.2 bar for 15 min) beef. These two methods [41,42] therefore complement each other and can be used together to detect the whole range of blood and blood products that may be used as ingredients both in food and feed. In addition, these two assays offer a specific and sensitive determination of added bovine blood in heat-processed products.

2.3 COLLAGEN

Collagen is an insoluble fibrous protein that constitutes the major component of connective tissues, skin, and bones. It is the raw material for the production of gelatin and is also used in the food industry as is, without processing into gelatin. It is the most abundant protein in vertebrates, accounting for about one-fourth of total proteins [43]. Unlike other mammalian proteins, collagen has high concentrations of 4-hydroxyproline, which is formed post-translationally from proline side chains [44]. Collagen has a triple helix structure composed of three almost identical polypeptide chains with a repeating triplet sequence (Gly-X-Y)*n*, where X and Y are usually proline or 4-hydroxyproline. The triple helical structure is stabilized by the presence of 4-hydroxyproline through the formation of hydrogen-bonded water bridges [45]. Over 90% of the different collagen types fall into three categories, namely Type I, Type II, and Type III, with Type I being the most abundant form, and the most widely distributed within the body. Each year, over 200,000 metric tons of collagen and gelatin are produced for use by the food, pharmaceutical, and cosmetic industry [46]. Most collagen is produced from bovine and porcine skin. A patented procedure of a collagen manufacturing process from calcified tissues such as animal skin, without a prior decalcification step is described in Figure 2.1.

2.3.1 Use of Collagen as a Food Ingredient

Collagen performs a wide range of functions in different food products. For example, collagen preparations are utilized in processed meat products to enhance the tenderness, texture, and yield of the final product [47]. Isinglass, a collagen fining agent obtained from the swim bladders of fish, has for years been used as a clarifying agent in the manufacture of both beer and wine. However, as a result of BSE-related fears and for religious reasons, raw materials from fish sources are now the consumer-preferred alternative for the production of collagen [48]. Collagen in the form of collagen hydrolyzate and collagen fiber preparation can also serve as a carrier of antioxidants when added to meat products in order to prevent meat lipid oxidation, which is responsible for the production of several chemical compounds that affect the nutritional and other quality attributes of meat products [49]. However, collagen is currently underutilized in the food industry because it suffers from low solubility, weak water-retention and emulsion-forming properties, and a poor amino acid profile, lacking the essential amino acid, tryptophan. In addition, collagen is poorly digested due to the low levels of collagenase in the human gastrointestinal tract [50]. Interestingly, individual fractions of collagen, which can be produced by various techniques, such as water-salt extraction or enzyme

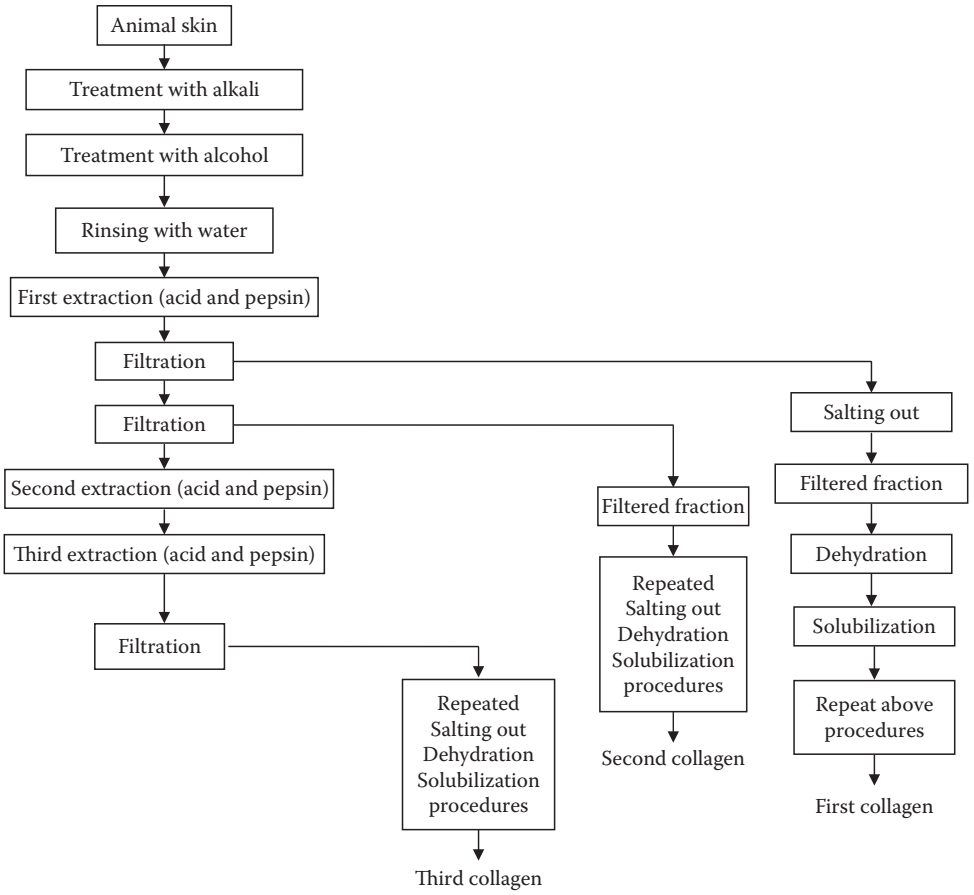

FIGURE 2.1 Manufacturing process of collagen from animal skin. (Adapted from Losso, J.N. et al., Extraction of collagen from calcified tissues, U.S. Patent 7109300, 2006. http://www.freepatentsonline.com/7109300.html. Accessed on November 28, 2010.)

preparation, exhibit a pronounced capacity for water retention and emulsion formation. It has therefore been suggested that these fractions be used as a replacement for gelatin, as research findings indicate that these collagen fractions have higher moisture-absorbing and gelling properties, and are also cheaper to produce than gelatin [51].

2.3.2 Need for Methods to Detect Collagen in Food

Methods have been developed to detect the presence of collagen in food products for a variety of reasons. For example, collagen serves as a measure of the quality of meat and meat products; poor quality meat-based products tend to be rich in connective tissues, which are high in collagen [52,53]. Accordingly, the amount of collagen gives an indication of the economic value of the raw material (meat) used in the manufacture of the meat-based product, as well as the nutritional value of the product [52,53]. The Food Safety and Inspection Service (FSIS) of the USDA thus considers estimation of the collagen content to be an easy and practical method for ascertaining the protein quality of meat products [54]. Many countries specify the maximum allowable level of collagen in comminuted meat products in order to prevent adulteration and partial substitution of economically high value meat portions with less expensive alternatives. Compared with other mammalian proteins, collagen has a high concentration of the imino acid 4-hydroxyproline; hence, estimating the content of 4-hydroxyproline provides an indirect measure of the collagen content.

2.3.3 Detection Methods

The most commonly used method for 4-hydroxyproline content determination as a measure of collagenous material in meat and meat products is the AOAC official method 990.26 [55]. The colorimetric method involves a time consuming (16 h) acid hydrolysis step. Hydroxyproline in the filtered and diluted hydrolysate is then oxidized to pyrrole by reacting with chloramines-T, followed by adding 4-dimethylaminobenzaldehyde to develop a red-purple color. The absorbance of the solution is measured spectrophotometrically and the amount of 4-hydroxyproline present in samples is calculated from a calibration curve obtained from absorbance readings of standard solutions of hydroxyproline. The amount of collagenous connective tissue present in the sample is computed as the hydroxyproline content multiplying a factor of 8, as the collagenous connective tissue contains 12.5% 4-hydroxyproline, if nitrogen to protein factor is 6.25. This calculation is different in some countries based on different nitrogen and hydroxhproline factors, and this method is not applicable to analysis of freeze-dried material. Recently, microwave hydrolysis of proteins combined with high performance anion exchange chromatography, and pulsed amperometric detection (HPAEC-PAD) analysis of 4-hydroxyproline, was reported as a new way to rapidly determine the collagen content in meat-based foods [52]. Briefly, an aliquot of sample corresponding to 25 mg of protein was subjected to hydrolysis in acid using a microwave digestion system. Filtered hydrolyzed samples evaporated to dryness and dissolved in 0.1 N HCl were then diluted with ultra-pure water, filtered and injected into the chromatographic system. Hydroxyproline quantification was then carried out alongside other amino acids using amino acid standards. The collagen content was also calculated by multiplying the 4-hydroxyproline content (g/100 g of sample) by 8. The use of microwave hydrolysis allowed the hydrolysis time to be reduced from 16 to 24 h typical of traditional hydrolysis to 20 min. The ratio of collagen to protein gives an indication of the quality of meat used, with higher ratios indicating the use of low quality meat and lower ratios indicating the use of high quality meat. However, there is an issue with the interpretation of the results for the above two methods. For example, a low collagen to protein ratio may not necessarily indicate the use of high quality meat but could be as a result of the use of non-meat protein in the preparation of the product.

Several instrumental methods have also been developed for the same purposes. The use of near infrared reflectance (NIR) spectroscopy has been reported for rapid estimation of components (fat, protein, collagen-free protein [CFP], moisture, and starch) in meat patties [56]. The instrument was first calibrated using results obtained from the analysis of each component from 50 meat patties using standard chemical reference methods. After calibration, the protein, water, fat, CFP, and starch contents of the meat patties were determined with the near infrared spectroscope and the results compared with the chemical data. The standard error between the two sets of data served as an estimate of the difference between the NIR and reference methods. After calibration, 43 additional meat patties were analyzed using both NIR and reference methods. The correlation coefficient (r), standard error of prediction (SEP), and coefficient of variation (CV) were then calculated to assess the accuracy of the NIR method. Correlation coefficients of 0.943 and 0.983 were recorded for CFP and crude protein, respectively. The collagen content of the sample was calculated as the difference between crude protein and CFP (crude protein minus CFP). The drawback of this method is that it suffers from a high prediction error due to the necessity to calculate SEP, and may thus be inaccurate. The researchers therefore recommended that samples be defatted and dehydrated to make the determination of CFP more accurate.

Autofluorescence spectroscopy is another instrumental method that has been proposed as a rapid way to quantify the hydroxyproline content of ground beef [53]. This method is based on the fact that connective tissue (such as collagen) is autofluorescent, giving off a bluish fluorescence [57]. Five excitation wavelengths, namely 300, 332, 365, 380, and 400 nm, were investigated (based on previous data) and autofluorescence spectra obtained using an optical system. The measurements were performed using an optical bench system and light from a 300 W Xenon light source. The light was focused onto samples (contained in specially designed cuvettes) at an angle of 45°.

Detection was via a detector coupled to the optical device. The emission intensities at 300, 365, and 380 nm increased as the concentration of collagen increased, while at 332 nm the samples exhibited emission spectra that provided information about both their fat and collagen contents. The emission spectra at 400 nm gave the least reliable results, as other chromophores influenced the spectra. This method was performed under ideal conditions and may therefore not be suitable under field conditions, given that autofluorescence is a very sensitive technique that may be influenced by several factors in the environment, including pH and color variations in the sample (i.e., dark or white meat) [53].

In addition to the above methods for quantifying the endogenous collagen in meat products as a measure of meat quality, methods are also developed to detect collagen that has been added to food products for the concern of the potential allergenicity of collagen proteins. For example, a semi-quantitative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)/densitometer has been used to detect trace amounts of native collagen in beer [45]. As the method does not detect denatured collagen, beer samples were first heat treated to denature any collagen that might be present. Some samples were then spiked with known amounts of acid soluble collagen in amounts ranging from 0.03 to 0.05 ppm to determine the detection limit, while other samples were tested without spiking. Supernatants obtained from samples that had undergone settling, centrifugation, treatment with acetic acid, and rotary evaporation were subjected to SDS-PAGE on pre-cast 7.5% or 4%–15% gels, and the resolved protein bands stained with Coomassie Stain for visualization. Gels were then scanned on an imaging densitometer. This method was based on the extraction of acid soluble collagen as fibers under elevated ionic strength brought about by the addition of salt. SDS-PAGE revealed three bands: α_1I and α_3I, co-eluted, while α_2I remained separate. The first band, α_1I/α_3I was easily visualized and also better resolved from contaminating proteins, so this was used for relative quantitative comparisons using the densitometer. The detection limit of the assay was reported to be 0.03 ppm for some beers and 0.05 ppm for others. The researchers noted that the recoveries of this method at different spiking levels were not always proportional to the amount of collagen added. Another disadvantage of this method is that background in the gel has a tendency to mask increases in the loading of samples, so it is important to establish correct loadings in order to produce a linear response to quantity on the gel.

In summary, all the above-mentioned methods developed for the detection of both endogenous and added collagen in food products suffer from shortcomings that limit their application. These problems include prediction errors, interference from environmental factors, errors in interpretation of results, low recoveries, and/or interference from background. In addition to their individual shortcomings, none of these methods is species-specific. Detection of native collagen is therefore still an analytical challenge.

2.4 GELATIN

Gelatin is a mixture of peptides commonly obtained through the heat dissolution at acidic or alkaline pH of collagen from animal skins, tendons, and bones [58]. As discussed in the previous section, collagen is the principal protein constituent of animal skins, bones, tendons, and loose connective tissues. The amount of collagen present, as well as its nature, varies considerably from tissue to tissue and also from one species to another, so differences in the gelatin manufacturing technology are dictated by the raw material (collagen), although the principle of gelatin production remains the same. The raw material is pre-treated, either with an acid or an alkaline solution, then subjected to increasing temperatures to extract the gelatin. The final product is obtained through centrifugation, filtration, or settling. The quality in terms of clarity, transparency, and purity of the gelatin produced depends on the type of extraction method (acid or alkaline), source, and history of the raw material, the thermal conditions used, and the subsequent processing [59]. Five types of gelatin are produced industrially for various applications, namely bovine gelatin produced by alkali

(BA gelatin) or acid (hydrochloric acid) treatment (BHA), porcine gelatin produced by alkali (PA) or acid (PHA) treatment, and fish gelatin (F gelatin) [60].

In Europe, about 80% of edible gelatin is derived from pig skin. Of the remaining 20%, 15% is obtained from bovine hide splits and 5% is extracted from the bones of bovine, porcine, poultry, or fish species [61]. In North America, pig skin is currently the major raw material source for the production of edible gelatin, while cattle hide is the least used raw material. Pig skins are obtained from slaughterhouses and meat processing plants already trimmed of fat, flesh, and hair, and supplied as either fresh or frozen [58]. However, the preferred source of collagen for producing high-quality gelatin is cow bone. Gelatin extracted from fish by-products has poor functional properties and therefore requires chemical or enzymatic modifications to improve its functionality [62]. Gelatins are sometimes referred to as edible, photographic, technical, or pharmaceutical grade, but these terms refer to their uses rather than the method of manufacture [58]. In the United States, gelatin manufactured for food, drug, and cosmetic uses must be produced from the skin and bones of healthy animals that have been slaughtered in plants inspected by the USDA. Such materials are also sourced to a small extent from other countries with comparable veterinary health services inspection systems. In Canada, gelatin manufactured for food, drug, and cosmetic usage is made solely from the skin of healthy pigs slaughtered in plants inspected by the Canadian Food Inspection Agency (CFIA) or in plants inspected by the USDA. Accordingly, in the United States, gelatin produced locally or imported from companies in countries that are affiliated to the Gelatin Manufacturers Institute of America (GMIA) should comply fully with the current recommendations of the U.S. Food and Drug Administration (FDA) Guidance [63].

2.4.1 Use of Gelatin in the Food Industry

Gelatin has traditionally been used in the food industry as a stabilizer, clarifier, or protective coating material, and is commonly found as an ingredient in food products such as candies, baked products, desserts, meat products, ice-cream, and dairy products. The major use of gelatin in the food industry is as a clarifying agent and as a stabilizer, for example, in drinks and beverages that contain tannins, where the gelatin reacts with the tannins to form a gelatin-tannin complex that precipitates out as sediment. In the United States, about 50% of edible gelatin produced is used for such purposes. The foaming capacity of gelatin is utilized in the manufacture of marshmallow, a common confectionery in the American diet, to produce a stable foam system that imparts an airy and light texture to the product. In the bakery industry, gelatin is widely used as a setting agent, a stabilizer, and as a foaming agent in products such as cakes, breads, and pies, and also as a stabilizer in different kinds of icing and dairy products. The meat industry makes use of a considerable amount of gelatin in products such as meat loaves, sausages, and meat jellies, where the principal function of added gelatin is to absorb the juices that separate out during cooking, or as a coating material [59]. Gelatin has also been suggested as a coating on meat products to extend the shelf life because the gelatin matrix is believed to act as a barrier to water and oxygen, which would slow down the oxidation of myoglobin and lipids and decrease water loss [64]. In developed countries, about one tenth of the edible gelatin produced goes into the pharmaceutical industry, primarily for use as capsules and emulsions. Gelatin has been subjected to several chemical modifications with the aim of producing food-grade gelatin with improved functional properties [59].

2.4.2 Need for Methods to Detect Gelatin and Sources of Gelatin in Food

It has become necessary for methods to be developed to detect the presence of gelatin in food products and also to differentiate between the species of origin of gelatin products. In some individuals, foods containing gelatin may trigger allergic reactions [65,66]. Most of these gelatin-sensitive patients develop allergic reactions to porcine and bovine gelatin but do not react to fish gelatin [67,68], so some countries require that the source of gelatin used in food products be appropriately

labeled. For example, the Ministry of Ordinance in Japan has recommended bovine and porcine gelatin to be clearly labeled. Labeling precautions notwithstanding, there is still a high possibility of gelatin (from bovine or porcine sources) inadvertently becoming present in processed foods as undisclosed allergens as a result of cross-contamination during processing, particularly in establishments that produce different products with various ingredients on the same production line. This is a serious issue, as even small amounts of bovine or porcine gelatin have the potential to cause severe reactions [60]. Thus, detection methods for bovine and porcine gelatin contaminants in food products are highly necessary. They are also important to address consumer concerns as certain individuals, for example Muslims, Jews, and Hindus, do not accept gelatin produced from porcine and/or bovine sources for religious reasons [69,70]. It has also been suggested that foods contaminated with the BSE prion may be responsible for variant Creutzfeldt-Jakob disease (vCJD), raising concerns about the use of any bovine-derived food ingredient. According to both the FDA and the European Food Safety Authority (EFSA), the manufacturing guidelines established for edible gelatin production that gelatin manufacturers are required to adhere to sufficiently reduce the likelihood of BSE infectivity to protect human health. Edible gelatins produced according to these guidelines can thus be assumed to be safe. This notwithstanding, there is still the need for methods to differentiate bovine gelatin from porcine or fish gelatin for labeling enforcement purposes, for the benefit of those individuals who may be concerned about the safety of bovine gelatin as a result of the BSE scare. Accordingly, immunological and DNA-based methods, as well as methods based on principal component analysis, have been designed for the speciation of gelatin, as outlined below.

2.4.3 Detection Methods

A sandwich ELISA has been developed for the detection of bovine and porcine gelatin in processed foods using different pairs of polyclonal antibodies [60]. Three polyclonal antibodies, PAb1, PAb2, and PAb3 were used for this assay. The antibodies PAb1 and PAb2 were obtained by immunizing rabbits with bovine gelatin, and PAb3 was obtained by immunizing goats with bovine gelatin. Several antibody pairs were evaluated and two antibody-pair systems, PAb2–PAb1 (PAb2 as first antibody and PAb1 as second antibody) and PAb3–PAb3 (with PAb3 as both first and second antibody) were selected for the ELISA. This ELISA, however, cross-reacted with heat-treated meat species and also with some sea foods tested, either raw or cooked, and also with gelatin from non-bovine species, although the nature of this cross-reaction varied, depending on the pair of PAbs used. The ELISA system made up of the antibody pair PAb2–PAb1 cross-reacted strongly with porcine gelatin and boiled squid, and slightly with fish gelatin, while the ELISA system made up of the antibody pair PAb3–PAb3 reacted strongly with porcine gelatin. Both antibody pairs reacted with different kinds of heat-treated meat samples. The researchers therefore pointed out the need for further studies to develop methods that are better able to discriminate between gelatin used as a food ingredient and gelatinized heated meat.

Another competitive ELISA method based on PAbs obtained by immunizing rabbits with tyrosylated bovine and porcine gelatins was reported by Venien and Levieux [70] to differentiate between bovine and porcine gelatin. Gelatin was tyrosylated to increase the immunogenicity of gelatin, which is traditionally known to be a weak immunogen. However, the antibodies were not species specific, as antibodies raised against porcine gelatin reacted with some of the bovine gelatins tested and vice versa. To circumvent this cross-reactivity, the authors used peptides synthesized from species-specific sequences of the bovine alpha 1(I) collagen chain as the immunogen to produce bovine-gelatin specific antibodies, reporting that this process was effective in producing antibodies capable of distinguishing bovine from porcine gelatin. However, neither the cross-reactivity of the antibodies with other proteins commonly used as ingredients in food nor the ability of the assay to differentiate between added gelatin and gelatin resulting from heat-treated meat samples was examined or discussed in this study.

Conventional and real-time PCR-based methods have also been used for the molecular detection and quantification of bovine species material in edible gelatin [61]. The method developed by Tasara et al. [61] involved the isolation of DNA from gelatin of bovine, fish, and porcine origin, followed by confirmation that sufficient amount and PCR-detectable template had been isolated given that the gelatin manufacturing process has a tendency to severely degrade nucleic acids. Several published species-specific PCR systems designed for bovine, porcine, and fish species detection were evaluated as potential tools for determining the species origin of the raw material used in the gelatin manufacture. A PCR system specific for bovine material in gelatin was selected after this preliminary evaluation, as most of the PCR systems tested were either incapable of identifying species of origin of gelatin or cross-reacted with DNA of other species. This bovine species-specific PCR primer set, which targets the ATPase 8 subunit gene in bovine mitochondrial DNA, was then optimized using both conventional and real-time PCR methodology. The conventional PCR assay had a detection limit of 0.1% and 0.5% bovine gelatin in porcine gelatin and fish gelatin, respectively. The real-time system had a better detection limit of 0.001% bovine gelatin in both pork and fish gelatin. However, this method only reveals the presence of bovine species material in gelatin, which does not necessarily mean it is gelatin, as DNA-based methods are not tissue specific. In addition, this method offers only an approximate estimate of bovine DNA, as absolute quantification of DNA in severely processed products such as gelatin is not feasible.

Principal component analysis (PCA), a technique that reduces the dimensionality of a data set while retaining the most significant information, and widely used in many classification studies, has also been used to differentiate between bovine and porcine gelatins [69]. Gelatin samples were hydrolyzed using hydrochloric acid (12 M HCl) to release their amino acid residues, then separated and analyzed using reversed-phase high performance liquid chromatography (RP-HPLC). Twenty peaks were detected by the HPLC for both bovine (14 samples) and porcine (5 samples) gelatin samples of high purity. PCA was then employed with the MATLAB® program using peak height, area, area percentage, and width to differentiate between bovine and porcine gelatin. PCA processes peak parameters, and extracts the principal components or significant variables (in this case, peaks), which are then used as the basis for classifying bovine and porcine gelatin. Twelve samples comprising of 9 bovine and 3 porcine gelatins, were first processed by PCA and presented in a two-dimensional graph. The remaining 7 samples, consisting of 5 bovine and 2 porcine gelatins, were employed as the prediction set and added to the first 12 samples. All 19 samples were then analyzed by PCA by comparing the two dimensional presentation graphs. Bovine and porcine gelatin was distinguished by a line from the PCA plot of HPLC data or peak height or peak width for bovine and porcine gelatins. However, one limitation of PCA is that finding the principal component direction becomes a problem when large numbers of data points are involved. In addition, it is not clear how to properly handle incomplete data sets in which some of the points are missing.

2.5 WHEY

Whey is a cheap by-product obtained during the production of cheese or casein from milk. It is the watery part of milk that separates from the curd when milk is curdled in the production of cheese. For every kilogram of cheese manufactured, about 9 kg of whey is produced [71]. According to a 2004 report, cheese whey was manufactured by over 200 whey plants in the United States and their total output represented 25% (935,000 metric tons) of the global production of cheese whey [72]. The composition of whey varies depending on the type of cheese produced, with lactose being the main constituent at 70–80 g/100 g of dry matter [73]. Whey is broadly categorized as sweet whey or acid whey, depending on the raw material used for the coagulation of the milk, that is, whether rennet (enzymatic) coagulation or acid coagulation, respectively, is used. A typical composition of the two types of whey is shown in Table 2.3. The major proteins found in whey are β-lactoglobulin

TABLE 2.3
Composition and pH of Fresh Whey

Component	Sweet Whey	Acid Whey
Water	93%–94%	94%–95%
Dry matter	6%–6.5%	5%–6%
Lactose	4.5%–5%	3.8%–4.3%
Lactic acid	Traces	Up to 0.8%
Total protein	0.8%–1.0%	0.8%–1.0%
Whey protein	0.6%–0.65%	0.6%–0.65%
Citric acid	0.1%	0.1%
Minerals	0.5%–0.7%	0.5%–0.7%
pH	6.4–6.2	5.0–4.6

Source: http://www.dairyforall.com/whey.php. Accessed on November 28, 2010.

TABLE 2.4
Whey Protein Composition

Protein	Abundance
β-Lactoglobulin	50%–55%
α-Lactalbumin	20%–25%
Immunoglobulins	10%–15%
Bovine serum albumin	5%–10%
Lactoferrin	1%–2%
Lactoperoxidase	0.5%
Lysozyme	<0.1%
Glycomacropeptide	ND

Source: http://www.wheyoflife.org/facts.cfm#8. Accessed on November 28, 2010.
ND, not determined.

(BLG), α-lactalbumin (ALA), bovine serum albumin (BSA), and immunoglobulins (IgG), with BLG being the most abundant (~55%) of the whey proteins. The various proteins found in whey and their relative abundance are shown in Table 2.4.

2.5.1 Use of Whey and Whey Proteins as Food Ingredients

The use of whey proteins in formulated foods has increased in recent years, but in the past whey was considered merely as a waste product and dumped in water bodies, on agricultural lands, or in any convenient location. The trend toward processing whey into valuable end products instead of its disposal as waste is at least partly as a result of present day pollution standards designed to protect water bodies from run-offs, which makes casual disposal unattractive [74]. Currently, whey products such as whey powder, whey protein isolates, whey protein concentrates, hydrolyzed whey protein isolates and concentrates, deproteinized whey, and lactose are used as ingredients in a wide range of food and dietary supplements because of certain qualities they possess that are highlighted below. Whey proteins are nutritionally valuable because of their complete amino acid composition; they are not deficient in any amino acid and also have high contents of the essential amino acids

tryptophan and lysine. They are particularly rich in cysteine, which is considered conditionally essential for individuals who do not synthesize it in sufficient amounts [75]. In addition to their nutritional quality, whey proteins have excellent solubility, water-binding, gel formation, foaming, and emulsifying capacities. They can also protect against syneresis in a product such as yoghurt, which impairs the organoleptic quality of the product and often causes consumers to believe that the product has gone bad [76], and are used as fat replacers in low-fat products such as cheese, pasta, and yoghurt [77,78]. The unique functional and nutritional attributes of whey proteins make them desirable as a functional ingredient in processed foods such as beverages, sauces, meat products, and baked goods [79].

The widespread use of whey as an ingredient in food products due to its excellent functional and nutritional qualities, coupled with improvements in processing technologies such as ultra-filtration, osmosis, and ion-exchange, means that several different whey products are now available for use in food products to address particular needs. These mostly take the form of powder obtained from the drying of liquid whey. Examples of the wide range of whey products that are available commercially are shown in Table 2.5. However, despite the multiple benefits that are derived from processing whey into valuable end products, whey is seldom fully utilized because of the high cost inherent in its processing into value added products. Drying or concentrating liquid whey is very costly and this adds to production costs [80]. Only a fraction of the tons of the fluid whey generated annually is currently utilized in food and feed production [81]. A study of 11 dairies in Serbia indicated that 78.75% of whey was discharged into river systems, contributing significantly to the organic pollution of the environment [82]. Whey is usually dumped as a result of a lack of the technology needed to process it, mainly due to the inherent cost of concentrating or drying [80]. Accordingly, efforts are now being directed toward utilization of liquid whey, which is currently not used because of its high water content, to circumvent the high cost of processing and environmental concerns over the dumping of excess whey, and to facilitate its full utilization. For example, research by Yetim et al. [80] has indicated that fresh liquid whey can be added to frankfurter-type sausages without compromising product quality. Further research in this area is necessary and should be encouraged to ensure maximum utilization of this valuable product.

TABLE 2.5
Examples of Commercially Available Whey Products

Product	Description
Whey Protein Concentrate 80% (WPC 80%)	Product obtained by removing non-protein constituents from whey such that the final dry product contains not less than 80% protein
Whey Protein Isolate (WPI)	Product obtained by removing non-protein constituents from whey such that the final dry product contains not less than 90% protein
Whey permeate	Dairy solids obtained by the removal of protein, lactose and some minerals from whey
Reduced lactose whey or mineral concentrated whey	Product produced by the partial removal of lactose from whey
Demineralized whey	Product obtained by removing a portion of the minerals from whey
Hydrolyzed whey	Product obtained by hydrolyzing whey with specific enzymes
Sweet whey powder	Product obtained by drying fresh whey obtained as by-product in the production of cheeses such as Cheddar, Mozzarella, and Swiss
Acid whey powder	Product obtained by drying fresh whey obtained as by-product in the production of cheeses such as cream cheese, cottage cheese and ricotta

2.5.2 Need for Methods to Detect Whey Products in Food

Because of the multiple benefits of whey proteins, they are beginning to be used as ingredients in food products that hitherto would not have contained whey proteins. For example, whey proteins are now added to fruit juices for protein fortification. However, although whey ingredients are assuming an increasingly important role in the food industry, they are known to be potent allergens and as such, their widespread use by the food industry poses a serious health threat to consumers who are allergic to whey proteins. While the most abundant whey protein, β-lactoglobulin (BLG), represents the major allergen in whey, studies using large populations indicate that even those minor whey proteins present in trace amounts, such as bovine serum albumin (BSA), lactoferrin, and immunoglobulins (IgG), are potential allergens [83]. The Food Allergen Labeling and Consumer Protection Act of 2006 (FALCPA) currently requires packaged foods to clearly label ingredients derived from the eight major allergen foods, namely milk, eggs, soybean, wheat, peanuts, tree nuts, fish, and shellfish, all of which are considered Class I allergens. There is therefore an urgent need for methods to be developed to detect the presence of whey proteins in food products as a regulatory tool to protect consumers who are allergic to whey proteins. This is particularly important given that several fruit juices and soft drinks that may contain undeclared whey proteins have recently been recalled in Canada and the United States [84], especially since these products typically do not contain whey products. Analytical methods for whey detection are necessary not only to protect consumers who are allergic to whey, but also to deter unscrupulous manufacturers from adulterating expensive whole milk-based products with low-priced whey products. Because of the large quantity of inexpensive whey that is available, the use of whey to adulterate more costly dairy products such as liquid milk and milk powder is economically very attractive [85]. Not only does whey cost about four to five times less than milk, adding it to milk does not compromise the sensory qualities of the final product [86].

2.5.3 Detection Methods

Several methods have therefore been developed to detect whey proteins in food as part of the effort to enforce food allergen labeling laws as a consumer safety measure, as well as to protect the consumer against fraud. One approach that has been tried uses liquid chromatography with mass spectrometric detection to quantify whey allergen traces in mixed-fruit juices [84]. Here the whey proteins ALA, BLG, and alpha lactoglobulin (ALG) were simultaneously extracted using solid phase extraction (SPE), and injected into the LC-MS (liquid chromatographic system coupled to a quadrupole mass spectrometer) system. The LC–MS system allows the identification and quantification of whey proteins from the spectrum based on the retention time of individual protein peaks. This method has the capacity to unambiguously detect intact whey proteins at levels as low as 1 μg/mL in fruit juices. However, this approach is only suitable for intact proteins and may not be suitable in situations where processing has affected the structural integrity of these proteins.

For those individuals that are allergic to cow's milk, soybean dairy-like products are often used as an alternative. However, in some cases, whey proteins are added to the soybean-based products for enrichment. A perfusion reversed-phase high-performance liquid chromatographic (RP-HPLC) method has therefore been developed to simultaneously separate soybean and bovine whey proteins [87]. Extracts obtained from samples, together with a soybean protein isolate standard, were injected into the chromatographic system. When bovine proteins were present at very low levels, the sample extracts had to first undergo an acidic precipitation step to concentrate the proteins. A linear binary gradient water-acetonitrile-0.1% trifluoroacetic acid procedure is used to separate the soybean and whey proteins, which were then detected by UV absorption at 254 nm, a wavelength at which sensitivity for soybean proteins is higher than at the wavelengths commonly used to detect whey proteins. Whey proteins in the powdered soybean milk were then quantified from calibration curves of standard solutions containing known concentrations of ALA or BLG. This method allows

soybean proteins and whey proteins to be rapidly separated in about 5 min, although high errors have been reported in the estimation of the concentration of BLG, which the authors ascribed to the shape of the peaks and poor resolution between peaks in the chromatogram.

A competitive ELISA has been developed as an easy-to-use alternative screening method for the detection of bovine rennet whey powder in milk powder and buttermilk powder [88]. This method uses an anti-bovine-κ-casein MAb 4G10 that recognizes bovine caseinomacropeptide CMP, a compound specific to whey. Because MAb 4G10 also binds to the κ-casein ordinarily present in milk, samples had to be treated with optimal concentrations of trichloroacetic acid (TCA) to selectively precipitate interfering casein and whey proteins without compromising the CMP recovery. The assay has a detection limit of 0.1% (w/w) whey powder in skimmed milk powder. The main drawback of this method is that it requires this optimal TCA concentration to be determined based on several trials using spiked samples to ensure that interfering proteins such as κ-casein are absent in the samples, and also to maximize the recovery of CMP. Thus, in the case of unknown samples it would be difficult to determine the optimum TCA concentration to use in each case, which ultimately affects the results obtained.

Other instrumental methods including capillary electrophoresis [89,90] and HPLC [91,92] have been reported for the detection of whey proteins in food, based on the detection of caseinomacropeptide (CMP). CMP (residues 106–109) is the smaller of two peptides and remains in the whey when the milk protein κ-casein is hydrolyzed by the enzyme chymosin in the making of cheese. The larger peptide (residues 1–105), known as para-κ-casein, remains with the curd [93]. However, in samples such as ultra high temperature (UHT) milk, the presence of CMP may not necessarily be an indicator of adulteration with whey proteins. This is because it has been found that certain proteases from psychotropic bacteria have the capacity to split CMP from κ-casein during storage of long-life products such as UHT milk [89]. Thus, the presence of CMP in UHT milk is an exception to the rule that it can be used to indicate that a product has been adulterated with whey solids.

There are ELISA kits available commercially for the detection of milk or milk-derived products such as whey, in food products. One such product is RIDASCREEN®β-lactoglobulin, a competitive ELISA kit manufactured by R-Biopharm AG, Germany, for the quantitative analysis of native and processed BLG in such products as hydrolyzed milk products, or foods containing whey, milk, or milk powder (http://www.r-biopharm.com/product_site.php?language=english&product_id= 273). Although the manufacturers report that this kit is suitable for both raw and processed foods, studies supporting this claim have not been reported in the literature. This ELISA kit shows trace cross-reaction with α-, β-, and κ-casein and hence cannot be said to be whey specific. The assay also shows cross-reaction with ALA and BSA. However, since these two proteins are present in the whey fraction of milk, cross-reaction with these two proteins is an advantage as far as detection of whey in food is concerned. Other commercial ELISA kits based on the detection of BLG have been developed by companies such as ELISA Systems Australia (http://www.elisas.com.au/allergens/allergen_2/index.htm) and Tepnel, U.K. (BioKits BLG) (http://www.tepnel.com/elisa-blg-assay-kit.asp). Both the kits from ELISA Systems and Tepnel are claimed to work as well with raw samples as with processed samples. The Tepnel kit also shows trace cross-reactivity with caseins. The cross reaction with non-whey proteins of these methods, so far as detection of whey products are concerned, is understandable, as they were primarily developed for the detection of milk, not just whey protein in food products. The above-mentioned assays have as yet not been validated by any research reports and hence their effectiveness in whey protein detection in different food products is not confirmed.

There is also a need for further work on developing better hydrolyzed whey protein detection methods. Hydrolyzed whey proteins are one of the fastest growing commercial products in the food industry. These hydrolyzed whey formulas are referred to as hypoallergenic formulas (HFs) and are often used in infant formula and medicinal dietary supplements. Their allergenic potential has been lowered through enzymatic hydrolysis, and they are considered a suitable alternative for infants or individuals that suffer from an allergy to cow's milk. HFs are classified based on the degree of hydrolysis as extensive (EHWFs) or partial (PHWF) protein hydrolysates. However, it

is important to note that hypoallergenic formulas may still contain residual allergenicity due to inadequate hydrolysis or filtration, which results in peptides that are large enough to induce allergic reactions. Even after extensive hydrolysis, peptides that are large enough to be antigenic may remain intact [94]. In addition, smaller peptides resulting from hydrolysis may aggregate into larger peptides with allergenic potential, which may explain the presence of higher molecular weight particles in HFs [95]. Hence, despite the use of HFs in child nutrition, cases of allergic reactions have been reported for EHWFs and, more commonly, for PHWFs [96]. Moreover, it has been reported that PHWFs may induce allergic reactions even in infants not previously sensitized to cow's milk [97]. Unfortunately, there are as yet no methods available for the detection of hydrolyzed whey proteins, and current methods based on intact protein molecules cannot be appropriated for the detection of hydrolyzed whey proteins. It is therefore imperative that new methods be developed for the detection of the presence of these modified whey proteins in a variety of food products.

2.6 CONCLUSIONS

In conclusion, the utilization of animal by-products as described in this chapter offers great economic, nutritional, and environmental benefits. As such, there is the need for more effort to be directed toward full utilization of these valuable by-products to ensure that maximum benefits are derived. However, there is also a need for methods to be developed to detect these by-products in both feed and food materials, to enforce labeling laws. This is necessary to protect the health of individuals who may be allergic to some of these by-products, or who avoid these by-products for cultural, religious, or personal reasons, and also to protect consumers from being cheated by rogue manufacturers who may be tempted to adulterate high priced food commodities with these lower priced by-products. Analytical methods are not only needed to detect animal by-products that are deliberately added to the food items, but also those that are present naturally in the food as a measure of the quality or economic value of the food. Despite the extensive work that has been done in the area of method development, there is still room for improvement; most of the methods developed so far suffer from one or more limitations that affect their suitability for the intended application. In some cases, as with hydrolyzed whey proteins, where processing has significantly altered the protein structure, as yet no methods are available that are capable of reliably detecting altered proteins that still possess allergenic potential. The availability of methods that can easily and quickly detect the whole range of by-products would enable the nutritional, environmental, and economic benefits of these products to be fully realized, while at the same time protecting the consumer from fraud or allergic reactions that may result from inadvertent exposure to these by-products.

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