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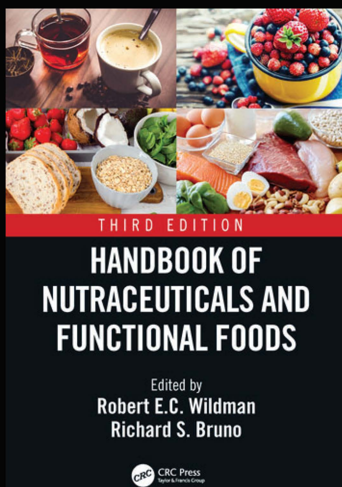

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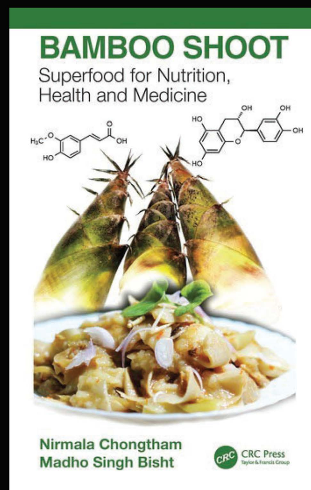

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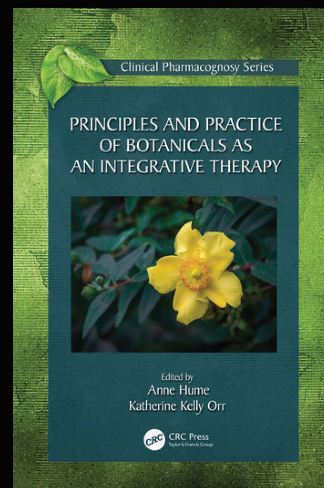

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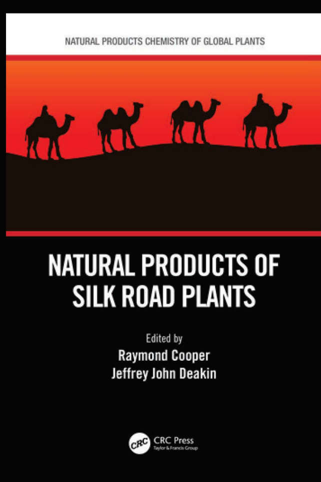

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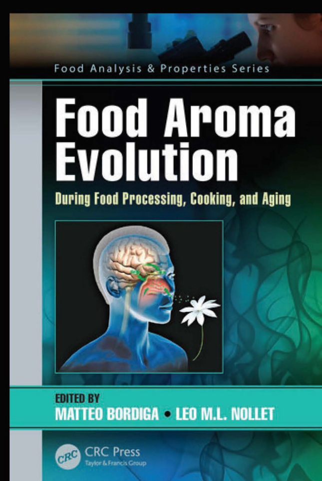

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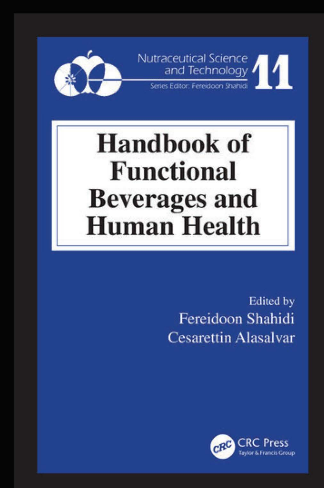

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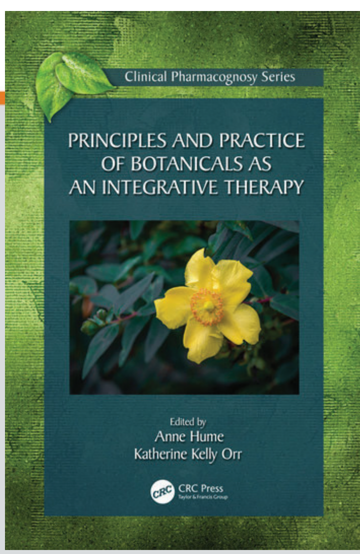
CHAPTER 1

BOTANICAL USE IN SPECIAL POPULATIONS



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Principles and Practice of Botanicals as an Integrative Therapy

by Anne Hume, Katherine Kelly Orr

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Botanical Use in Special Populations

Margaret M. Charpentier, Kelly L. Matson, Katherine Kelly Orr, and Anne Hume

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4.1 Introduction

The use of botanicals as an integrative therapy for health and wellness, as well as for the treatment of acute and chronic diseases is common worldwide. Unfortunately, healthcare providers continue to overlook or ignore the use of botanicals and other forms of integrative medicine by many individuals. This is especially important in populations such as older adults. Special populations may have unique considerations related to the use of botanicals. This chapter presents a brief overview of a few key issues related to the use of botanicals in selected special populations.

4.2 Pediatrics

4.2.1 Prevalence

An estimated 2% of children were reported using complementary and alternative medicine (CAM) in a 1996 survey (Kemper et al. 2008). Since that time, usage has increased substantially, as shown in a 2012 National Health interview survey of 10,000 children ages 4 to 17 years old, where 11.6% of children surveyed reported using a CAM practice in the past year (NCCIH 2012). The use of nonvitamin nonmineral dietary supplements were reported by about 5% and special diets by another 0.7% of respondents (NCCIH 2012). An estimated 9% of infants under one year of age have been given dietary supplements and teas by their mothers. Infant usage was more common among mothers who also used botanicals and among Hispanic individuals (Zhang et al. 2011). Overall, American families spent $1.9 billion on CAM for their children in 2012 (Nahin et al. 2016).

4.2.2 Clinical Considerations

4.2.2.1 PK/PD Issues

Multiple physiologic changes occur from birth through adolescence which result in pharmacokinetic (PK) and pharmacodynamic (PD) changes for many drugs. In pediatric populations, these changes are potentially most important in the neonate and infant. Some of the physiologic changes include reduced activity of intestinal cytpchrome 3A4 and drug transporters, as well as hepatic phase I enzyme systems (Lu and Rosenbaum 2014). In addition, the glomerular filtration rate, tubular reabsorption, and tubular secretion may also be reduced in the first year of life. Although many botanicals may have been used in neonates and infants, little information is available, especially among premature infants or those with comorbid onditions.

4.2.2.2 Chronic Diseases

Unlike the use of botanicals and other dietary supplements in adults for health maintenance, much of the use in pediatrics is for the treatment of chronic diseases. These conditions include pain, anxiety, attention-deficit hyperactive disorder (ADHD), autism, asthma, allergies, cystic fibrosis, irritable bowel disease, and insomnia (Kemper et al. 2008; NCCIH 2012; McClafferty et al. 2017). These conditions, especially autism and ADHD, can be difficult to treat pharmacologically. As a result, frustrated caregivers may want to explore all options, including botanicals and other supplements, as well as special diets such as the ketogenic diet for epilepsy. The supplements frequently used in children include fish oil/omega-3 fatty acids, melatonin, and pre- and probiotics (McClafferty et al. 2017). Among botanicals specifically, echinacea and flaxseed continue to be commonly reported.

Although data is available for fish oil/omega-3 fatty acids, melatonin, and pre- and probiotics in chronic conditions in children, alternative medicine practices such as the use of secretin or N, N-dimethylglycine for autism spectrum disorder are not supported by published evidence. Overall, autism and other challenging pediatric diseases are potential targets for dangerous interventions and fraud.

4.2.2.3 Safety Issues

Safety is a priority in considering the use of botanicals in children. One study evaluating the use of echinacea in 407 children for upper respiratory tract infections reported the development of rash in 7.1% in the echinacea group compared to 2.7% in the placebo group (Taylor et al. 2003). Whether this simply reflects the higher prevalence of atopic diseases in children is unclear, but this is of potential concern with plant-based therapies. In addition, since much of the use of botanicals in children is for the treatment of chronic disease, the potential for clinically relevant drug interactions is significant especially when the botanical is St. John's wort.

Adverse reactions from CAM products resulted in an estimated 4,965 pediatric emergency departments visits annually (Geller et al. 2015). Child resistant packaging is not required for supplements and the accidental ingestion by unsupervised young children is a unique concern in this population. Among children ages 5 to 18, weight loss and energy supplements were responsible for over 50% of the adverse events, with cardiac symptoms attributed to these products in adolescents (Geller et al. 2015). A report from U.S. Poison Control Centers on dietary supplement exposures between 2000 and 2012 similarly reported acute accidental ingestion of energy, yohimbine, and ma huang supplements in children under 6 years of age (Rao et al. 2017).

Selected botanicals used in children and adolescents are discussed in the Chapters 8 Women's Health, 11 Psychiatric Disorders, and 13 Dermatological Conditions.

4.3 Pregnancy and Lactation

The goal of every pregnancy is a healthy full term infant and a positive maternal experience. In addition, following the infant's birth, an increasing number of women are choosing to breastfeed with the World Health Organization (WHO) now recommending the practice exclusively for at least the first 6 months of life (WHO 2014).

4.3.1 Prevalence

An estimated 13%–78% of pregnant women use some form of CAM or integrative medicine, with this broad range likely due to varied study designs and definitions. For studies assessing botanical use, between 9% and 16% of pregnant women reported use and the actual usage might be significantly higher. Characteristics which predict the use of botanicals include a higher level of education and a history of use prior to becoming pregnant. Motivations vary, although many pregnant women use botanicals and integrative medicine as an adjunct to conventional care. Of note, many pregnant women do not discuss their botanical use with their healthcare providers due to fears of negative feedback or the provider not specifically asking about use (Hall et al. 2011).

4.3.2 Clinical Considerations

4.3.2.1 PK/PD Issues

Pregnancy and the postpartum period are known to have significant effects on the pharmacokinetic parameters of many drugs; these parameters include the volume of distribution, protein binding, and renal clearance (Feghali et al. 2015; Tasnif et al. 2016). Little evidence exists on the physiologic effects of pregnancy on botanical pharmacokinetics.

4.3.2.2 Safety Issues

Special considerations regarding the use of botanicals in pregnancy focuses on ensuring the product is safe without teratogenic or other adverse effects on the developing fetus. In addition, the risk of miscarriage or premature birth should be minimal with the supplement. Since most of the use of botanicals in pregnancy is based on historical use or cultural traditions, the available safety data is often limited, with the exception of ginger (Holst et al. 2011). As with most studies of botanicals in special populations, the literature is difficult to evaluate due to small populations, patient perception of risk and benefits of botanicals, influence and use due to one's cultural background, patterns of use over time during a pregnancy, and the role of the healthcare provider in guiding patient decisions (Adams et al. 2009).

Healthcare providers differ in their perspectives on the use of botanicals in pregnancy. Although some recommend avoidance of botanicals due to insufficient data and the potential for adulteration, other providers with experience and comfort in advising women on botanicals not only evaluate limited safety trials, but also include animal data, pharmacology, and historical use (Marcus and Snodgrass 2005; Low Dog 2009).

4.3.2.3 Lactation

Among breastfeeding women, the production of an adequate milk supply is common concern, with 78% reporting this in an online survey of 188 women. (Bazzano et al. 2017) Galactagogues such as fenugreek are recommended and used to address this concern. Many women have learned about these types of botanicals primarily through friends and lactation consultations, as well as the internet (Bazzano et al. 2017).

Use of botanicals during lactation also provides challenges in evaluating their ability to cross into breast milk and whether or not the infant will have a significant exposure. A systematic review of botanicals and breastfeeding found only 32 studies from the previous 40 years, types varying from safety, efficacy, and survey studies. Quality of safety and efficacy data was overall low, further demonstrating the lack of data (Budzynska et al. 2012).

Botanicals commonly used in pregnancy and lactation are discussed in Chapter 8 Women's Health.

4.4 Geriatrics

Older adults are a heterogeneous group. They range from healthy, independently living individuals even into their late 90s to much younger, but frail patients with multiple comorbid conditions and polypharmacy.

4.4.1 Prevalence

In 2015, 8.5% of the global population, or 617.1 million, were estimated to be over the age 65 years. By 2030, the number will likely increase to 12% of the world's population or about 1 billion people (He et al. 2015).

The reported use of dietary supplements has continued to increase among American adults age 65 and older based on longitudinal data from the 2012 National Health and Nutrition Examination Survey (NHANES), unlike younger age groups (Kantor et al. 2016). Data from the Ginkgo Evaluation of Memory (GEM) study involving over 3,000 independently living adults 75 years and older identified that 27.4% of the cohort were taking a nonvitamin, nonmineral dietary supplement at baseline (Nahin et al. 2009). Among the botanicals commonly reported were ginkgo, garlic, Echinacea, and saw palmetto in this study; use of botanical supplements was associated with having problems with muscle strength and with reading senior health magazines (Nahin et al. 2009). More recent population-based data on the use of specific botanical supplements in older adults is not available, but *Bacopa monnieri* and vinpocetine, for example, are present in many memory products promoted for use by older adults.

4.4.2 Clinical Considerations

4.4.2.1 PK/PD Issues

Two broad issues should be considered with the use of botanicals in older adults. The first is related to the potential PK and PD changes that have been associated with aging in many older adults. These changes may affect both the safety and potential efficacy of a given botanical. For example, renal function declines in manyolder adults especially if the individual has had poorly controlled hypertension or diabetes for a prolonged time. This might result in the botanical and its constituents potentially having reduced clearance resulting in higher concentrations. When this is combined with PD changes such as increased central nervous system sensitivity, more pronounced adverse effects may occur and the safety profile may be reduced.

The PK/PD changes might also increase the potential risk for clinically relevant botanical-drug interactions. As many older adults may have a significant burden of chronic diseases treated with multiple drugs, a potential for interactions may be possible. St. John's wort is well known to induce the metabolism of many drugs such as warfarin, generally resulting in less efficacy of the prescription drug. In terms of PD interactions, concern about an increased risk of bleeding is frequently raised about garlic and ginkgo if the older adult is also taking aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and other agents with antithrombotic properties. Of note, in the GEM study, an increased risk of bleeding was not identified with ginkgo compared with placebo (DeKosky et al. 2008).

4.4.2.2 Botanicals and Cognition

An additional concern with botanicals in older adults is that given their burden of chronic disease, their use may be promoted when limited, if any, clinical trial data exist. The best example is in the treatment of Alzheimer's disease and related disorders of cognition. Although huperzine and ginkgo have mixed efficacy data from clinical trials, other botanicals have much more limited data. These botanicals, however, are commonly included in proprietary blends for cognition.

4.5 Liver Disease

Liver disease encompasses a diverse group of conditions including, cirrhosis, alcohol-related hepatitis, nonalcoholic steatohepatitis, viral hepatitis, hepatocellular carcinoma, cholestatic, and autoimmune hepatitis, as well as drug-induced liver disease. In addition, metabolic liver diseases such as Wilson's disease exist among many others.

4.5.1 Prevalence

Precise statistics on the prevalence of cirrhosis, as one example, have been estimated to be between 4.5% and 9.5% of the global population (Sarin and Maiwall 2017). Unfortunately, many liver diseases are unreported and the available estimates may be low. In a population-based surveillance study of 1,040 individuals with newly diagnosed chronic liver disease, 16.8% reported the use of botanicals (Ferrucci et al. 2010). Predictors of botanical use in this study were the presence of alcohol-related liver disease and/or hepatitis C, as well as higher education and family income (Ferrucci et al. 2010). In a substudy of the population, milk thistle, St. John's wort, Echinacea and valerian were most commonly used by 43.8%, 10.7%, 8.9% and 4.5%, respectively (Ferrucci et al. 2010).

4.5.2 Clinical Considerations

Until recently, few safe and effective antiviral treatments were available for hepatitis C. The management of alcohol-related liver disease most importantly stressed alcohol abstinence, avoidance of hepatotoxins, and prevention of complications. Botanicals such as milk thistle and *Phyllanthus amarus* have been used by individuals with diverse liver diseases including specifically for the treatment of their liver disease. Constituents such as silymarin, catechins, glycyrrhizin, and phytosterols have been used worldwide. *Refer to Chapter 7 on Gastrointestinal Disease for further discussion.*

4.5.2.1 PK/PD Changes

Two broad issues should be considered with the use of botanicals in individuals with chronic liver diseases. The first is related to the PK changes in terms of reduced hepatic metabolism of many drugs and botanicals in end stage liver disease. Relatedly, botanicals that induce or inhibit hepatic enzyme systems may be expected to have fewer clinically significant effects on concomitant drugs and botanicals. The precise outcomes are difficult to predict given contributing factors such as polymorphisms in hepatic enzymes, physiologic, and environmental factors, as well as the severity of the liver disease (Palatini and DeMartin 2016). In addition, estimating how well the liver is functioning is challenging and is dependent on using multiple measures such as with the Child-Pugh classification.

4.5.2.2 Botanical-Induced Hepatotoxicity

Another consideration is the potential for botanical-induced hepatotoxicity. This is a complex topic for many reasons, as the evidence is usually from case reports such as with kava, black cohosh, and green tea. These are frequently missing important information such as presence or absence of underlying liver disease or other common causes of liver injury.

Data from the United States Drug Induced Liver Injury Network (DILIN) have suggested 15.5% of reported cases are due to botanicals and other dietary supplements (Navarro et al. 2014). The reporting of cases due to botanicals and other supplements increased from 7% to 20% over the first 10 years of the DILIN (Navarro et al. 2014). In some instances, the wrong plant may have been included in the supplement, either accidentally or for economic adulteration, and now case reports suggest the botanical as hepatotoxic when it may actually be due to a different plant. In addition, supplements may be formulated as proprietary blends so that if liver injury is suspected, the actual causative component may be difficult to establish especially if a mechanism for the adverse reaction has not been previously identified.

Pyrrolizidine alkaloids (PA) may be the compounds in botanicals associated with liver injury and their presence an indicator of a poor quality product. PAs have demonstrated hepatoxicity by causing veno-occlusive disease and may also be carcinogenic. Butterbur products, for example, may contain unacceptable levels of PAs when raw material is not processed appropriately to remove them. Only products specifically certified ast *PA-free* should be administered (Sutherland and Sweet 2010).

One common botanical that has been associated with hepatotoxicity is green tea. While drinking green tea is safe, concerns have been raised about the use of concentrated green tea extracts. The reported pattern is typically hepatocellular and is apparent around 3 months (LiverTox). Green tea extracts contain widely variable concentrations of catechins such as epigallocatechin-3-gallate (EGCG) which have been implicated in causing mitochondrial injury in an animal model (LiverTox). Women may be at increased risk, although this may also reflect higher usage in this subgroup. In addition, fasting has been identified as a risk factor for the development of liver injury in a safety review by the U.S. Pharmacopeia (Sarma et al. 2008).

Kava was identified as a potential hepatoxin almost 20 years ago. Several countries have restricted the sale of products containing kava due to risks of severe liver injury and case reports of liver failure associated with use. Several factors are still in question as to the origin of liver injury including aqueous versus acetone or ethanol extracts, continuous high doses, inappropriate raw products, and potential contaminants (Teschke and Lebot 2011). Since the initial consumer advisory report warning in the United States in 2002, there have been no updates issued.

4.6 Kidney Disease

Similar to liver disease, kidney diseases encompass diverse conditions affecting glomeruli, tubules and renal interstitium, as well as the renal vasculature. Causes can include genetic, immunologic, metabolic, neoplastic, or pharmacologic mechanisms among others.

4.6.1 Prevalence

An estimated 11%–13% of the global population has chronic kidney disease (CKD), with stage 3 CKD being the most common (Hill et al. 2016). Published estimates of botanical use in CKD, hemodialysis, and in people who have received renal transplants have varied widely. A study of Swiss renal transplant recipients reported 11.8% used some form of CAM, particularly homeopathy and Chinese medicine (Hess et al. 2009). An Egyptian study reported CAM use by 52% of patients with end stage renal disease (ESRD) or a renal transplant, with 78% of this comprised of botanicals and other natural products (Osman et al. 2015).

4.6.2 Clinical Considerations

4.6.2.1 PK/PD Changes

Multiple issues should be considered with the use of botanicals in individuals with CKD, patients receiving hemodialysis, and renal transplant recipients. Pharmacokinetic changes resulting in reduced renal clearance of botanicals especially in CKD and potentially in hemodialysis may be possible. Patients with ESRD receiving hemodialysis are particularly sensitive to CNS effects of drugs and possibly by botanicals, although data is limited.

4.6.2.2 Electrolyte Abnormalities and Other Complications

Electrolyte and mineral disorders occur as CKD progresses and ESRD develops. Both hyperkalemia and hyperphosphatemia are common and require careful management in ESRD and while the patient is on hemodialysis. Some botanicals may contain potassium or phosphorus and should be avoided. Botanicals that contain potassium include dandelion root and turmeric rhizome among many others (NKF 2015). Phosphorus-containing botanicals include milk thistle, borage leaf, evening primrose, feverfew, flaxseed seed, and stinging nettle leaf among many others (NKF 2015). In addition, botanicals that possess antithrombotic or hypertensive effects might theoretically increase the risk of bleeding or cardiovascular events in patients with CKD.

4.6.2.3 Botanical-Induced Nephrotoxicity

Various renal syndromes have been reported after the use of medicinal plants including tubular necrosis, acute interstitial nephritis, Fanconi's syndrome, hyperkalemia/hypokalemia, hypertension, papillary necrosis, chronic interstitial nephritis, nephrolithiasis, and cancer of urinary tract.

The nephropathy associated with the use of aristolochic acid (AA) from plants in the Aristolochia genus is perhaps the best known example. The first reports of AA nephropathy consisted of previously healthy young women who had used a Chinese slimming formula for weight loss which resulted in the rapid development of ESRD. It was later determined that *Aristolochia fangchi* had been substituted for *Stephania tetranda* in the formulation. The mechanisms for developing AA nephropathy are unclear, but acute proximal tubular necrosis develops followed by interstitial and tubular fibrosis (Luciano and Perazella 2015). The presentation, diagnosis, prognosis, treatment and link to urinary tract cancers has been discussed in an excellent review (Luciano and Perazella 2015).

4.6.2.4 Botanical-Drug Interactions in Renal Transplants

An estimated 70,000 renal transplants were performed globally in 2008 (WHO). A major component of a successful transplant is the use of immunosuppressive therapy such as tacrolimus, cyclosporine, mycophenolate, and sirolimus, among other agents. Many of these drugs are metabolized through CYP 3A4 and pharmacogenetic differences are well established for this isoenzyme. Concomitant use of botanicals that are CYP 3 A4 inducers such as St. John's wort might result in rejection of the transplanted organ, while inhibitors might significantly increase the risk of toxicity (Moschella and Jaber 2001; Columbo et al. 2014).

4.7 Cancer

Cancer is a diverse collection of diseases in which genetic changes alter the normal functioning of cells. The changes may be due to many factors including environmental causes such as from cigarette smoking and radiation exposure. The treatment of diverse cancers has undergone fundamental changes in recent years to focus on the underlying genetic mutation instead of solely on the type or location of the cancer (NCI).

4.7.1 Prevalence

CAM is widely used by cancer patients both in the United States and globally. A 1998 survey revealed that greater than 95% of patients had heard of CAM, and over 2/3 were using some form of CAM to treat their cancer, or symptoms related to cancer. Almost 40% were using a botanical product (Dy et al. 2004). The rationale for using CAM includes seeking more control over their disease and relief of cancer symptoms (Richardson et al. 2000; Dy et al. 2004).

4.7.2 Clinical Considerations

4.7.2.1 PK/PD Issues

Three major areas should be considered with the use of botanicals in oncology. Similar to other sections in this chapter, PK-related drug interactions may be important since many of the new targeted therapies are metabolized through cytochrome P450 enzymes, most notably 3A4. A botanical that interferes with this may affect the drug efficacy and/or toxicity. The lack of information on drug interactions is concerning, especially since a higher toxicity is accepted with cancer therapy, and higher dosages experienced due to inhibition of metabolism, may increase toxicity which can be life-threatening or even potentially fatal.

4.7.2.2 Considerations as Adjunctive Therapy

One of the most common uses for botanicals and other natural products in oncology is as adjunctive therapy to decrease the frequency and severity of chemotherapy or radiation therapy induced adverse events. Of note, multiple studies on this role have been published over the years with mixed outcomes. The interpretation of these studies requires the consideration of several factors. Studies evaluating decreased adverse events with botanicals as an adjunct require fewer patients than trials determining if the botanical interferes with the efficacy of the chemotherapy. As an example, a drug may cause an adverse event in 30% of patients, yet the difference in cancer recurrence maybe only 5%–10% from the chemotherapy agent. To investigate this, assuming an absolute survival benefit of 5% from chemotherapy, and assuming that the botanical interferes in this drug completely, a total of 2000 patients would need to be enrolled to determine this (Lawenda et al. 2008). Further, the chemotherapy agent is administered for a few cycles over a few months, while cancer recurrence is measured in years, requiring a long duration of follow up to determine the outcome of the botanical agent on the efficacy of the chemotherapy regimen.

4.7.2.3 Considerations in Prevention and Treatment of Cancer

4.7.2.3.1 Therapy-Related Issues

Experience from the cancer prevention trials illustrates another concern with use of some botanicals. While consumption of dietary levels of a supplement may be beneficial in prevention of cancer, in clinical trials where larger quantities may be used, an increase in cancer risk has been identified in the worst case or a lack of any benefit in the best case (Bairati et al. 2005; Ferreira et al. 2004; Bairati et al. 2006).

Complex tumor physiology makes interpretation of the risks and benefits of botanicals and other dietary supplements frequently challenging. As an example, tumors express a higher level of reactive oxygen species (ROS), relying on higher ROS activity for survival. Decreasing ROS through the use of dietary supplement with antioxidant properties has been proposed to prevent and treat cancer. However, the data has demonstrated a complex interplay of the balance of ROS and cancer progression and survival (Kasiappan and Safe 2016). In addition, radiation therapy and some chemotherapy agents increase ROS activity, tipping this balance to increase apoptosis (Lawenda et al. 2008). In these cases, botanicals with antioxidant properties might potentially decrease the benefit of chemotherapy agents.

In addition to the potential interference of botanicals with established cancer treatments, clinical evidence indicating a benefit with botanicals is often based on comparators that are either no longer in use or do not include a comparator. The available cancer treatments have expanded exponentially during the past decade, with improvements in survival observed for many cancers. Older studies of botanicals demonstrating benefit may not be reflective of current practice.

4.7.2.3.2 Disease-Related Issues

Another consideration with botanicals is which cancers to use an agent. As mentioned, cancer is a general term that encompasses many different diseases. Cancer of the breast is a completely different disease than cancer of the lung. Within each specific tumor type, for example lung cancer, the aggressiveness of the cancer and the response to treatment will vary based on tumor pathophysiology and other markers for disease, such as tumor markers for Epidermal Growth Factor, or Anaplase Lymphoma Kinase.

An additional consideration is the stage of the disease. Early cancers such as stages I, II, and III are often treated differently than advanced, stage IV cancers. Therapies demonstrating efficacy in one stage may not confer a benefit in an earlier stage such as with the use of bevacizumab in colorectal cancer. Therefore, when a treatment is studied for cancer, clear definitions of cancer type, including identified tumor markers, staging, previous treatments, time since diagnosis, patient characteristics such as organ function, age and performance status, are required to evaluate the investigational regimen for efficacy and safety. Since many studies evaluating botanicals are small trials of short durations, information regarding these parameters is often not available, and even if described, the study lacks the power to determine significance. Finally, botanical studies may use outcomes that are of unclear clinical relevance and not in routine use.

Botanicals used in cancer therapy and supportive care are discussed in Chapters 16 Cancer Prevention and 17 Treatment and Supportive and Palliative Care for Cancer.

4.8 Summary

Botanicals and other dietary supplements are used by an increasingly diverse population including those with significant comorbid conditions. Their usage may be both for health maintenance as well as treatment of underlying conditions or mitigation of adverse effects from conventional therapies. An essential element in using botanicals in special populations is open communication between healthcare providers and patients.

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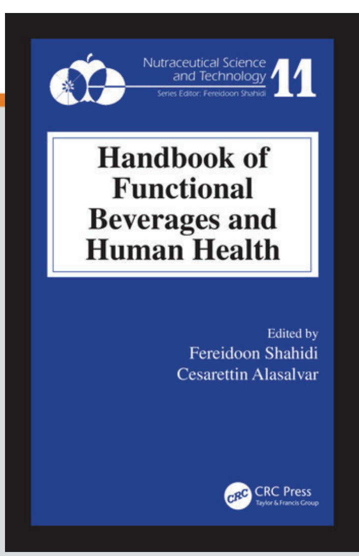
CHAPTER

2

HANDBOOK OF FUNCTIONAL BEVERAGES AND HUMAN HEALTH



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Handbook of Functional Beverages and Human Health

by Fereidoon Shahidi, Cesarettin Alasalvar

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1

Functional Beverages: Market Trends and Market-Oriented New Product Designs

Joe Bogue and Amy Jane Troy

CONTENTS

1.1 Introduction

There have been many changes and innovations in the beverage market over the past years as consumers seek new benefits from their beverages. One of the most important benefits sought by consumers is health and wellness. The market for new functional beverages with added ingredients and related health benefits has grown rapidly with positioning strategies linked to energy, digestion, aging, satiety, cognitive ability, hydration, weight management, and fatigue, among others. While the opportunity for developing functional beverages is high, manufacturers often struggle to achieve market success, and the challenges new functional beverage developers face include the technological challenge of developing and marketing new products with new ingredients; differentiating brands in ultracompetitive markets; identifying the most appropriate positioning platforms of convenience; and marketing science and technology to consumers, as well as health, natural, and legal obstacles. The high failure rates suggest an inability to understand consumer preferences and choice motives in relation to the purchase of functional beverages.

This chapter examines the main trends in the functional beverage market and identifies the key issues related to the consumer and the functional beverage market. Following this, a case study is introduced that focuses on developing market-oriented functional beverages based on consumer insights. It examines the importance of market-oriented approaches in developing new functional beverages and views how firms can use the *voice of the consumer* information to design products that more closely meet consumer needs. The case study examines utilizing qualitative research techniques to generate information at the early stages of the new product development (NPD) process. In particular, it looks at using semiotics to generate information on beverage packaging as it can greatly influence consumers' first purchase of a new product and repeat purchases.

1.2 Global Functional Beverage Market

By 2017, the global market for beverages will be worth approximately US$1347 billion. Market growth drivers include urbanization, expansion in the middle-class population, and an increase in double income families [1]. Moreover, there are also significant opportunities for functional food and beverages in emerging markets, such as in China, Brazil, Asia Pacific, and Latin America, where companies can successfully innovate by observing closely studied trends, understanding local consumers, and differentiating their products from their competitors [2]. Global trends that will impact the development of new functional food and beverages include age complexity, gender complexity, life stage complexity, income complexity, convenience, health, sensory, comfort, and individualism [3].

The global retail value of health and wellness beverages reached US$274 billion in 2011. This was approximately 44% of the retail value sales of nonalcoholic beverages [4]. The growth of health and wellness beverages was set to outperform the wider soft and hot beverage industry over the period 2012–2016 [5]. In addition, concerns continue to grow around the link between carbonated beverages and specific health issues such as obesity, diabetes, and coronary heart disease [6,7]. Global market trends indicate that demand for health beverages, specifically the low or no calories ones, has shown signs of decline, while there is an increased demand for beverages that help address health conditions by including *natural ingredients* and those focused on specific health benefits, such as digestive or heart health [4,8]. The inclusion of functional foods and beverages into consumers' diets can also be viewed as part of future public health prevention strategies that aim at reducing expenditure on health care [9].

Often, consumers associated low-calorie beverages with artificial ingredients, and this finding created the opportunity for functional beverages to be considered as naturally healthy [10]. In addition, 48% of consumers believed a natural product was one that was produced in adherence with strict regulations, while 46% of consumers aligned natural products closely with organic products [5]. Consumers believed ingredients were key to the concept of a natural beverage with beverages labeled as *100% juice*, *all natural*, and *containing no artificial colors*, perceived as healthier beverages.

Drivers of innovation in the beverage industry over the period 2012–2017 include new functional ingredients, advances in technology in relation to taste masking and encapsulation, reduction of sugar content, and wider channels of distribution [11]. Specifically, in relation to future functional beverage innovation, the focus will be on beverages targeted at specific parts of the body such as bones, joints, eyes, as well as sleep improvement, weight management, cholesterol management, maintenance of healthy teeth, energy, the elderly market segment, and beverages with omega-3 fatty acids [12]. This innovation focus will lead to market opportunities across demographic groups, as beverages are developed that are tailored more and more to the rapidly changing needs of individuals.

It has been suggested that beverage developers should follow a product development strategy that combines health, convenience, modern packaging, and affordable prices [5]. Firms can use different strategies, such as using natural ingredients, or novel ingredients, or adding natural and/or low-calorie sweeteners to beverages [13]. Alternatively, they can incorporate ingredients into beverages such as omega-3 oils, fiber, or probiotics. However, products should be built on what consumers believe is *inherent nutritional functionality*, and foods or beverages should be developed based on what is culturally relevant as a delivery medium [14]. In addition, consumers in developed regions made the switch to low-calorie soft beverages a number of years ago, and as these markets had entered into maturity, consumers were actively seeking healthier soft beverages [5]. This offers opportunities to develop added value through functionality, in the form of energy beverages and those with associated health claims. Increased use of vitamins, in the energy beverage sector, indicated the shift in consumer attitudes toward products that had more natural appeal [15].

Consumption of superfruit juice is also on the rise globally, and with increasing consumer awareness, there are opportunities to appeal to more mass-market consumers [5]. China leads in the consumption of superfruit juice at 1587 million liters per annum, followed by the United States and Japan. As superfruit juices move to a wider range of beverage categories, more novel flavors and ingredients are likely to be available to consumers. Blueberry, pomegranate, and aloe vera are consumer favorites worldwide, but more unusual varieties will be used in future beverage manufacture such as acai berry, baobab, mangosteen, goji berry, and sea buckthorn [5].

A key part of developing novel beverages with new ingredients will be consumer acceptance of new ingredients, their knowledge of the potential health benefits of such ingredients, and how the specific science and technology is marketed to consumers. In addition, consumers' interest in health is related to what health benefits are relevant to them and their lifestyles and, thus, are highly individualized [16]. This suggests that beverage developers need to generate deep insights into consumers' perceptions and how these beverages fit in with their lifestyles.

1.3 Consumer-Oriented New Product Development Case Study: Functional Beverages

The success of food and beverage firms is dependent on their ability to develop and market new products that provide consumers with superior value to that of their competitors. However, the driver of innovation within the functional food sector is often based on R&D within the food firm, rather than the consumer. This provides a *science push* rather than a *consumer pull* focus to the innovation and has often been attributed to failures within the functional beverage sector, where new products frequently do not meet consumer needs or expectations [17]. Reasons for functional food failures, which are relevant to functional beverages, include too many benefits from a single brand, benefits that are often not relevant to the consumer, relying on the selling power of the ingredient rather than the benefit, and using a nonrelevant carrier [16]. For example, many omega-3 fatty acid–fortified products have had little impact on the global market as they provide benefits that consumers cannot quickly see or feel [16].

There is a strong positive relationship between market orientation and the NPD activities of firms [18,19]. Market-oriented NPD entails generating information on consumers' needs and choice motives, integrating this information with the early stages of the NPD process, and developing an optimal product with attributes that maximize consumer acceptance. Information gathered for functional beverage development may include consumer perceptions of functional beverages; identifying consumer segments that are functionally driven, for example, consumers that will pay a premium for beverages with added functional benefits; understanding consumer knowledge of the health benefits associated with functional ingredients and health claims; identifying the key intrinsic (texture, mouth feel, and flavor) and extrinsic (packaging, brand, and health claim) product attributes that will influence consumer acceptance; and how consumers interpret the message (through images, colors, and icons) communicated by functional beverage packaging and the type of delivery method (shots, stick packs, and ready to drink).

Generation of consumer information is an important part of the NPD process that helps identify new ideas, defines the target market more explicitly, and then aids in the design of specific marketing strategies that position new products on markets [20]. Qualitative techniques are important for information generation at the early stages of the NPD process, and many studies acknowledge that both in-depth interviews and focus groups are particularly good at exploring concepts, generating ideas, and eliciting opinions on packaging [19,21–23]. They facilitate the integration of consumer insights at the earliest stages of the NPD process, and thus consumers act as codesigners in NPD.

1.4 Semiotic Approach to Market-Oriented Product Concept Optimization

Differentiation of a product or brand is seen as one of the key factors for developing successful functional foods. One of the ways of achieving this differentiation is through packaging design [16]. Semiotics can play an important role in the process of designing differentiated product packaging for functional beverages. Semiotics is an abductive method of analyzing meanings by examining signs that communicate information [24,25].

Research estimates that approximately 70% of purchase decisions are usually made at point of sale, and that for approximately 40 weeks of the year, packaging is the main attribute that leads to the sale of a product [26,27]. For low-involvement purchases, the package is the product, particularly because impressions formed during initial contact can have lasting effects. Beyond providing pure information, the emotional aspects of packaging graphics are more subliminal, which evolve from the styling of various graphical elements, including logo styling, symbols, icons, colors, textures, photography, and illustrations [28].

Semiotics can be used to understand the important role of packaging design in motivating consumer purchase of functional beverages. This entails an analysis of consumer perceptions of all icons, colors, and images on the package and how these combined might influence the purchase decision. Good packaging can support a brand in highlighting its difference in the marketplace [16].

In the following case study, some key activities at the early design stages of the NPD process are outlined in terms of the use of qualitative techniques to understand consumer requirements for functional beverages.

1.5 Consumer Insights on Functional Beverages

In this first step of developing new functional beverage concepts, a total of 12 in-depth interviews and 3 focus groups were conducted to generate consumer information on functional beverages. A convenient sampling technique was used to select participants for the in-depth interviews and focus groups [29]. Majority of the samples were middle-aged, educated to third level, in full-time employment, and lived in Cork City, Ireland. Both interview and focus group guides consisted of semistructured questions, and participants were rewarded with €30 for their time and effort in line with best practice [30]. Results were tape-recorded and the data were analyzed using the QSR N6 software package [31].

Focus group participants felt that the concept of a functional beverage was relatively new to the market and were positive toward the product idea. Five functional beverage concepts were generated from the participants. Most participants had a positive attitude toward a symbiotic beverage that consisted of the combined benefit of fiber and a probiotic culture in a functional beverage. Focus Group 2, which consisted of younger females, was very interested in the health benefits of such beverages. These consumers were familiar with the fermentation process, which they felt was traditional and were positive toward the concept of fermented functional beverages. They were also familiar with many other fermented food and drink products that they consumed on a daily basis. However, there were mixed attitudes toward certain functional beverage concepts, where they were unsure of the health benefits of certain ingredients and also the high retail prices charged for such beverages. In addition, the ingredient carrier, whether juice or dairy, for example, was seen as having an important influence on the purchase of functional beverages.

Some interviewees felt that a functional beverage could be marketed as a health drink and positioned as such in the marketplace. These interviewees felt that since some functional beverages were lactose-free, they could also be marketed as a nondairy probiotic category and had the potential to rival soy and probiotic dairy beverages. Focus group participants also suggested that functional beverages could be targeted at the lactose-intolerant consumer segment. Participants' comments included the following:

> I think functional beverages are suitable for those who do not like dairy products or are allergic to lactose. (Focus Group 1, male, 18–24 years)

> I know that there are many probiotic beverages on the market and most of them are dairy-based. I think a non-dairy probiotic beverage may be of interest to consumers. (Interviewee 9, male, 35–44 years)

Some young participants, across both focus groups and interviews, suggested that functional beverages could be positioned as healthy meal replacements or, more particularly, breakfast meal replacements.

They felt that those with busy lifestyles could consume functional beverages at the breakfast meal occasion as they were convenient and could be consumed on the go based on the method of delivery. These products could contain the nutritional and vitamin profile of a healthy breakfast and be positioned as an on-the-go beverage. Moreover, they suggested that functional beverages could also be marketed to different demographic groups as healthy alternatives to carbonated sports or energy beverages with a natural positioning strategy. However, these consumers were also concerned about the high calorie content of functional beverages. They might not consume a functional beverage as a meal component due to its perceived high calorie content. Examples of participants' comments were as follows:

> A meal replacement at breakfast is an option and would be welcomed by people with busy lifestyles. (Focus Group 1, male, 18–24 years)

> I think it is a good idea that you can take one (functional beverage) in the morning instead of breakfast, but I would not have it with a meal because of its high calorie content. (Interviewee 11, female, 45–54 years)

The health benefits of functional beverages were considered valuable marketing cues by both focus group participants and interviewees. The most important health benefits that would encourage purchase of functional beverages were *enhancing the immune system*, *aiding the digestion*, *lowering cholesterol*, and having *high fiber* and *reduced sugar*. Older focus group participants were interested in disease-preventing benefits, such as cholesterol reduction or cancer prevention. Generally, males were more interested in the specific health claims of the product. However, female participants focused more on their knowledge of functional ingredients and their health benefits. From a marketing perspective, this illustrates the significance of correctly identifying a suitable target market and then positioning an optimal functional beverage toward the target market.

Many participants also indicated that product information had a strong influence on their willingness to purchase functional beverages. They suggested that product descriptions on functional beverage packaging should contain information on the functional ingredients, the health benefits, the suggested daily dosage, and the reference daily intake. The following was a typical comment:

> I want to know whether I have to be careful of the dosage I consume daily. (Focus Group 1, female, over 55 years)

These participants also mentioned that the brand name, logo, images, and color on the packaging would influence their motivation to purchase functional beverages. In addition, many of the respondents perceived that beverages that utilized a single-serve packaging format were healthier than other formats.

Fifteen product attributes were generated from consumer interviews that would strongly influence participants' purchase of functional beverages. It also revealed that consumers' purchase decision in relation to functional foods was complex and influenced by many intrinsic and extrinsic attributes such as taste, the added ingredients to the products, health benefits, price, product volume, packaging (color, images, typeface, and product shape), brand, and label information. Label information and packaging were seen by many participants as being central to the purchase decision particularly with respect to reduced risk in relation to new ingredients, new products, or new brands. Packaging has frequently been mentioned as a direct aid for consumers for evaluating product quality and, therefore, should be carefully designed to effectively convey product attributes to the consumer.

1.6 Semiotics and Functional Beverages

The next stage in the process was to conduct a semiotic analysis of functional beverages to see how the information can be used to inform decisions on packaging design. Semiotics, a market-oriented methodology, consisted of two separate stages and complemented the information generated from the focus groups and interviews in the first part of this case study.

1.6.1 Stage 1: The Semiotic Sort

Stage 1, the semiotic sort, was conducted and classified information attributes (brand, color, and logo) not only by things they had in common but also by any shared meanings [32]. An electronic sort consisted of an electronic collection of the most prominent functional beverages on the market. Any functional beverage that was positioned on the basis of health was included in the sort. A sort is a gathering of digital images and products, in this case, of functional beverages. Signs most prevalent on these packages were recorded such as color, brand, health/nutritional claims, iconic symbols, and images. From this, the main sign system for the semiotic analysis of functional beverages was developed and can be seen in Table 1.1.

The images gathered in the sort were utilized in a primary semiotic analysis of functional beverage packaging. The primary semiotic analysis required the researcher to interpret signs and encoded meanings from all functional beverage packages collected during the sort. All signs and encoded meanings were decoded based on the existing literature. Existing literature included manufacturers' design specifications, manufacturers' intended meanings, and interpretation of color and images. This information was readily available through semiotic journals, corporate reports, and corporate websites. Literature focused on color interpretation, shape and typography, and consumer perceptions of various package designs and types. This first stage identified suitable visual stimuli that would be presented to consumers in the second stage of the study in conjunction with an in-depth interview guide.

1.6.2 Stage 2: Interpretation of Package Signs by Respondents

To ensure the methodology remained market oriented, Stage 2 focused on the interpretation of package signs by respondents. Twelve respondents, five males and seven females, were recruited through the use of convenience sampling [33]. Information was generated that focused on consumers' attitudes toward functional beverages, the purchase experience when buying functional beverages, and functional beverage packaging. A semistructured interview was utilized to gather the information [34].

Thirty-six visual stimuli were presented to respondents through a PowerPoint presentation at the interview. The stimuli chosen were informed by the findings of the focus group and interviews conducted in the earlier part of the case study, in addition to the results of the semiotic sort. This ensured that the research followed a market-oriented methodology. The 36 visual stimuli consisted of 12 full packaging concepts with all external stimuli removed, 12 brands and logos separate from the packaging concepts, 6 images found on packaging concepts, and 6 full packaging concepts complete with all external stimuli. The PowerPoint presentation ensured that all the images were displayed on a neutral background and were presented to consumers in the same sequence. This process illustrated how the signified concept of an individual sign (a brand logo) might change due to its paradigmatic relationship with other signs (how the brand logo might interact with other colors and icons on the packaging). Studies have shown

TABLE 1.1
Main Sign System for Semiotic Analysis of Functional Beverages

Main Sign System Headings	Examples
Brand	Innocent, Actimel, and Red Bull.
Signifier	Any material thing used to signify something: use of a wheat kernel and heart symbol to signify health.
Signified	The concept that the signifier refers to: healthy ingredients, energy, and naturalness.
Code	A set of conventions understood in a given society: modern lifestyles, healthiness, and wellness.
Metaphor	Expressing the unfamiliar in terms of the familiar: the use of phrases or images to imply that something is healthy and natural such as the use of a *halo* to imply pure.
Connotation	The cultural meaning of signs: the use of the color *green* to mean organic or natural.
Imagery	The use of graphics to convey a meaning: a picture of an active person to convey the meaning that the product is for active people.

that graphic elicitation, such as PowerPoint presentations, encouraged contributions from consumers that were difficult to obtain by other means and this facilitated a more market-oriented methodology [35–37].

The semiotic interviews were transcribed from the audiotapes and analyzed using the software package QSR N6 [31]. Various codes were allocated and assigned to key segments of information within the data [30]. A detailed analysis of the codes identified signs that when incorporated onto a product package could motivate the purchase of new or existing functional beverages.

1.7 Semiotic Results

1.7.1 Stage 1: Results

The codes present from the interaction of multiple signs allowed for an initial semiotic analysis to be performed for six selected functional beverages. The main sign system for each of these functional beverages is outlined in Tables 1.2 through 1.7.

The individual signs prevalent on functional beverages were then analyzed. The semiotic sort revealed that the colors *white* and *green* were synonymous with certain functional beverages. The use of the color *white* was used primarily on dairy-based functional beverages such as *Actimel* and *Yakult*. The color *green* was primarily used by juice-based functional beverages such as *Naked* and *Tropicana*. It was found that the colors *blue* and *yellow* were most utilized for products that were trying to convey the benefit of energy. Transparent packaging was common for a large number of beverages, such as *Innocent*, *Naked*, *Ribena*, and *Yakult*. The transparent packaging allowed for the beverage color to be seen by the purchaser and so generally denoted the flavor of the beverage. Iconic imagery was frequently used to convey the ingredients used within the beverage. These images were often used in conjunction with images associated with nature such as *sun*, *grass*, *leaves*, or *growing crops*.

TABLE 1.2

Semiotic Analysis of Functional Beverage 1

Beverage Brand	***Innocent* Pure Fruit Smoothie**
Signifier	Brand and image of a head with a halo.
Signified	Healthy ingredients, no artificial ingredients.
Code	Wellness, dietary behavior, convenience, and modern lifestyles.
Metaphor	The signs transfer the qualities of the signified for another, thus creating a metaphorical sign that offers the meaning that the beverage can easily be incorporated into the everyday diet to enhance its healthiness.
Connotation	The use of the white label, the brand name, and the image with a halo suggests that the beverage is healthy to the consumer and does not contain negative ingredients.
Imagery	Face with a halo to portray the perception of healthiness.

TABLE 1.3

Semiotic Analysis of Functional Beverage 2

Beverage Brand	***Naked* Juice Smoothie**
Signifier	Brand and logo, dominant use of the ingredient color (green).
Signified	Naturally healthy juice, energy giving.
Code	Natural, organic, and healthy.
Metaphor	The signs transfer the qualities of the signified for another, thus creating a metaphorical sign that offers the meaning that the beverage is a natural product with natural health-enhancing benefits.
Connotation	The dominant use of the color green offers a connotative relationship between the green ingredients and the green color utilized in the packaging.
Imagery	Fruit and vegetables to represent the ingredients used within the product and the use of leaves to convey the perception of naturalness.

TABLE 1.4

Semiotic Analysis of Functional Beverage 3

Beverage Brand	***Actimel Original***
Signifier	Brand and image of rising sun.
Signified	Fun, energy giving, and natural.
Code	Wellness, naturalness, health, and convenience.
Metaphor	The signs transfer the qualities of the signified for another, thus creating a metaphorical sign that offers the meaning that consumption of this beverage would improve the healthiness of the diet.
Connotation	The dominant use of the color *white* offers a connotative relationship between the dairy carrier used and the perception of natural ingredients.
Imagery	Rising sun over a green pasture offers the perception of energy, freshness, and naturalness.

TABLE 1.5

Semiotic Analysis of Functional Beverage 4

Beverage Brand	***Ribena Plus Summer Fruits***
Signifier	Brand and descriptor: plus.
Signified	Rich in fruits, high in vitamins and antioxidants.
Code	Healthy and thirst quenching.
Metaphor	The signs transfer the qualities of the signified for another, thus creating a metaphorical sign that offers the meaning that the juice is high in fruits and therefore high in vitamins.
Connotation	The use of primary color in conjunction with the blue sky and clouds offers the perception of a refreshing drink.
Imagery	Medallion illustrating the calcium content and fruits reflecting the ingredients of the product.

TABLE 1.6

Semiotic Analysis of Functional Beverage 5

Beverage Brand	***Yakult***
Signifier	Brand: dominant use of transparent packaging with red typeface for the brand.
Signified	Small and powerful.
Code	Convenience, innovation, wellness, simple, and effective.
Metaphor	The signs transfer the qualities of the signified for another, thus creating a metaphorical sign that offers the meaning that the beverage is novel, healthy, and powerful.
Connotation	The use of the red typeface for the brand on the transparent packaging offers the idea that the beverage is simple and effective.
Imagery	Not applicable.

TABLE 1.7

Semiotic Analysis of Functional Beverage 6

Beverage Brand	***Tropicana Essentials* Orange Juice and Omega 3**
Signifier	Brand and descriptor (essentials) in green typeface.
Signified	Necessary for healthy living and natural.
Code	Convenience, health, and wellness.
Metaphor	The signs transfer the qualities of the signified for another, thus creating a metaphorical sign that offers the meaning that the beverage is essential to everyday health.
Connotation	The use of the green typeface for the brand on the white packaging gives the perception of a natural beverage with no artificial ingredients.
Imagery	Faded image of male and female on the center of the package illustrating the product is essential for every person. Iconic images of oranges reaffirming the flavor and content of the beverage.

1.7.2 Stage 2: Results

Respondents were first asked a variety of questions related to their functional beverage purchase habits and preferences. They then viewed 36 images and were asked questions on each image to ascertain what messages they decoded from the information given to them.

1.8 Preferences and Purchase Habits toward Functional Beverages

The interviews revealed that the packaging color played a significant part in consumer acceptance of functional beverages. For dairy beverages, packaging that was primarily *white* and contained iconic images of the ingredients were perceived as most healthy by respondents. For juices, packaging that was transparent was most preferred by respondents. Most interviewees admitted they made presumptions about the flavor of a beverage based on the color of the base ingredient. These presumptions varied based on whether the beverage was a dairy-based product or a juice-based product. Respondents felt that the carrier had a very important influence on product purchase, whether the carrier was juice, water, cereal, or dairy based. In addition, noncarbonated beverages with more natural ingredients were perceived as healthier by respondents.

For dairy-based products, *light pink* was associated with strawberry, *light yellow* was associated with banana, *purple* was associated with berry, and *white* was mostly associated with a vanilla-type flavor. For juice-based beverages, *pink* was associated with grapefruit, and *yellow and orange* colors were used interchangeably to describe the flavor for orange juice, although tropical juice was also mentioned. *Purple* was used to denote black currant and *green* was mostly associated with apple flavors. Most females indicated they preferred strawberry or vanilla flavors, while males did not have any particular preferences. Some females, who regularly purchased functional juice beverages for health benefits, mentioned the sugar content and indicated they paid particular attention to the label to ensure they chose the healthiest product available. In addition, respondents believed that both juice-based and dairy-based products, which contained images of fruits, were healthiest.

The packaging on functional beverages in the retail environment was also mentioned by respondents as an important influence on purchase. In addition, the location where the beverage was merchandised in the retail outlet also impacted on the purchase decision. If a beverage was available from the refrigerated section of the retail outlet, respondents were more likely to purchase the product. They also mentioned the need for healthy on-the-go beverages, explained by some as "those beverages that keep you going." These beverages were described as those that had extra caffeine, or other ingredients, such as ginseng or guarana, that provided a *boost*. These products were associated with strong and bright colors, in particular orange and red. There was a strong interest in noncarbonated energy beverages with more natural ingredients.

Dairy-based functional beverages, such as *Danone Actimel* and *Danone Activia*, were the most frequently purchased brands mentioned by interviewees. However, it was evident that a large proportion of respondents tried to minimize the quantity of dairy consumed in their diets, due to either health or weight concerns. These respondents frequently mentioned fruit juices, such as *Innocent*, as healthy beverages. Juice-based carriers gave the perception of a healthy beverage. A small number of respondents purchased own-brand functional beverages, such as *Tesco* yogurt or *Tesco* juice beverages, with a wide variety of added vitamins.

The consumption occasion also had a significant influence on the beverage brand purchased. A number of respondents were willing to purchase own-brand juice beverages for breakfast consumption. However, when beverages were consumed outside the home, a brand name was nearly always preferred.

> I just get the Tesco orange juice with added calcium but I always buy Actimel to take to work.
> (R2, male, 41–50 years)

A majority of interviewees gave significant consideration to the preferences of the overall family unit in the purchase of healthy beverages. Interestingly, a large number of female consumers indicated they would purchase additional single-serve beverages to meet their own specific health and wellness needs. These beverages were nearly always a popular branded product that reflected a premium price.

> I will buy a multi-vitamin juice for everyone so they are getting all the vitamins they need but I will have my own shot every morning. My kids would not drink those. (R10, female, 31–40 years)

The packaging type was also of particular importance to consumers. The majority of respondents indicated that a resealable package that was easy to open and close was essential, in addition to being lightweight and rectangular in shape, so that it could easily fit on the refrigerator shelf. For products that were positioned on the basis of single-serve portions, a number of consumers mentioned the need for compact packaging so that a number of portions could fit in the fridge. A small number of female consumers also mentioned how these products should also have durable packaging for on-the-go consumption. The importance of on-the-go consumption to the purchase decision was evident across many of the demographic groups.

The font size of nutritional and labeling information on packaging was an important issue for respondents. They noted that food firms used smaller font sizes on the back of pack labeling, and, in some cases, the additional information provided to support a health claim or logo. Importantly, these respondents were reluctant to try any new products that displayed difficult to read text.

> I find that the writing explaining ingredients has become very small and I hate having to look for my glasses. So I will just pick up another one [beverage] instead. (R11, male, 61+ years)

Most respondents believed that juice and dairy beverages they regularly purchased were healthy and positively contributed to the overall wellness of their diet. In particular, dairy was perceived to be a naturally healthy product category, and older consumers mentioned a number of health benefits including *calcium*, *phosphorus*, and *protein*. However, younger respondents were more positive toward water and juice-based beverages particularly with energy, hydration, and beauty benefits.

Respondents were unsure how *cholesterol reduction* could be effectively conveyed through an image. The majority of respondents revealed that a *heart* image would contribute to the perception of reduced cholesterol levels, while images that conveyed the idea of natural ingredients and active individuals were also deemed appropriate. In addition, a smaller number of respondents emphasized the need for key words to accompany the image in order to avoid confusion at the retail point of purchase for the consumer. Similar responses were found for the health benefits of omega-3 fatty acids. Respondents identified the image of a *heart* as appropriate to describe the benefits of this ingredient, and they stressed the need for text that clearly outlined omega-3 benefits. Typical comments included the following:

> I would like to see an active person and the healthy heart. (R2, male, 41–50 years)
>
> Natural ingredients and a heart like before, but I would have to see the words Omega-3 to know for sure. (R3, female, 51–60 years)

The image of a *wheat shaft* or *grain kernel* was suggested as a symbol for fiber in functional beverages. Respondents also associated these images with whole grain. The most common health benefit for this type of product was gut health and digestion. The use of images of *leaves* and *grass* on a beverage were associated with fresh organic products. Although not a specific health benefit, respondents indicated that these images were successful in attracting their attention to products in the retail environment.

1.9 Semiotics and New Product Development

The semiotics revealed that consumers preferred partially transparent packaging for beverages they were unfamiliar with or for beverages that claimed a certain health and wellness benefit. This allowed consumers to make assumptions about the ingredients and flavors of the product they were unfamiliar with.

Moreover, opaque packaging was associated with very familiar beverages that made no specific health and wellness claims. Transparent packaging has been associated with reduction of the uncertainty or purchase risk associated with novel products [38].

Typographical elements are often used to intentionally signify specific things, namely, the subcultural context, the strategy of the food manufacturer, and the target group of consumers [39]. This was evident for a number of beverage packages that were examined in the semiotic analysis. It was clear the respondents held a preference for typeface that was simple and clearly legible for beverages that claimed to have a functional benefit or positioned as a healthier product. Overemphasis on the depth and curvature of the *type* gave the perception of a cheaper quality product for the vast majority of respondents. For this reason, functional beverage packaging needs to incorporate plain, possibly Antigua-style text, as this text is mostly associated with healthier products [26].

The consumer interviews also provided an insight into how the presence of a claim could interact with other aspects of packaging to influence consumers' attitudes and purchase intentions. The strategic use of color is regarded as a fundamental tool in corporate marketing strategies and provides a means of product and brand differentiation [40,41]. Importantly, this case study revealed that color was the primary sign that attracted consumers to a brand on the market that they were unfamiliar with. In addition, consumers made assumptions about the taste of the product based on the primary color used in the packaging.

It was also evident that the use of certain colors in smaller amounts on product packaging, that is, other than the main package color, encouraged the consumer to interpret metonymic relationships. The most common of which was the use of the colors *green*, *white*, and *yellow*: *green* to portray the meanings of natural, healthy, organic, and fresh; *white* to portray the meanings of natural, fresh, and free-from; and *yellow* to portray the meanings of sunlight, morning, energy, and fresh. In addition, the use of pictures and images also attracted consumer attention to brands they were unfamiliar with on the market [37].

1.10 Lessons from the Case Study

The aim of the case study in this chapter is to explore market-oriented design issues of new functional beverages. Information was generated about new functional beverages from the consumers' perspective. In addition, a semiotic analysis was conducted to inform the design of product packaging that would encourage first purchase, and repeat purchase, by consumers of functional beverages. A key role of information is to reduce market uncertainties and then to create, build, and maintain competitive advantage, through an in-depth understanding of consumers' needs during the NPD process.

The case study illustrated the important role that consumers can play in the design and marketing of functional beverages in terms of the development of new product concepts and associated new product packaging and also the identification of suitable target markets. The case study showed the importance of generating information on functional beverages in relation to how consumers perceive them and how different attributes, such as the carrier, taste, packaging, and price, may influence purchase. It illustrated the importance of consumer knowledge of functional beverages and how these products fit in with consumers' healthy lifestyles.

The importance of packaging to the purchase decision was clear from this case study in terms of attracting consumer attention to functional beverages. Involving consumers at the early stages of the NPD process to assist in codesigning product packaging can ensure that benefits of the product are communicated effectively through various signs, symbols, and colors. This may result in increased acceptance of new functional beverages and repeat purchase of existing functional beverages. Consumers' initial interaction with novel products is through the medium of packaging. Therefore, consideration should be given to the development of product packages that successfully communicate intrinsic attributes of the product through the use of appropriate signs and codes. This case study found that colors such as *white*, *green*, and *yellow/orange* were most synonymous with healthy beverages. In addition, the inclusion of images such as *wheat shafts*, *healthy hearts*, and *leaves* further added to the overall perception of increased health and wellness.

The information generated in this case study can then be used with quantitative techniques, such as conjoint analysis, to identify optimal product attributes. Conjoint analysis is a multivariate concept optimization research technique that is used to measure consumer preferences, through utility trade-offs, for product concepts to understand preferences for products [42]. It is premised on the idea that consumers evaluate the value of an object by combining the separate amounts of value provided by each attribute. This enables the development of an optimal functional beverage that creates the most value for consumers.

1.11 Conclusion

The market for functional beverages continues to grow as consumer demand for traditional carbonated beverages falls, in line with changing consumer health and wellness lifestyles. This market offers huge opportunities for firms that develop market-oriented beverages, where the intrinsic and extrinsic attributes are designed to closely meet consumer expectations, offering benefits as part of a healthy lifestyle. A market-oriented approach to the development of new functional beverages incorporates the *voice of the consumer* information at the early stages of the NPD process in order to increase the likelihood of consumer acceptance of such beverages. This is particularly important in the very competitive functional beverage sector where consumers are faced with new choices, innovations, and brands on a very regular basis.

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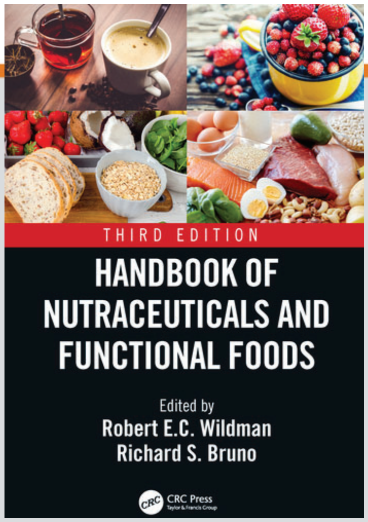
CHAPTER

3

PROTEIN AS A FUNCTIONAL FOOD INGREDIENT FOR OPTIMIZING WEIGHT LOSS AND BODY COMPOSITION



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Learn more

14 Protein as a Functional Food Ingredient for Optimizing Weight Loss and Body Composition

Paul J. Arciero, Michael J. Ormsbee, Robert E.C. Wildman, and Donald K. Layman

CONTENTS

14.1 INTRODUCTION

Obesity continues to plague our society as a major public health issue. The latest obesity prevalence statistics show over 70% of U.S. adults are overweight and 40% of U.S. adults and 20% of youth are obese. Unfortunately, the prevalence of obesity among non-Hispanic blacks and Hispanic adults is even higher at 47%.[1] Healthcare costs associated with obesity-related disease are in excess of $190 billion per year.[2,3] In addition, the economic impact expands past healthcare costs and includes indirect costs associated with wages lost due to employee absenteeism, decreased work productivity, poor academic performance, impaired cognition, and low morale both in the present and future.

These staggering numbers clearly highlight the necessity for immediate intervention among all groups and ages of our population. While progress has occurred in illuminating certain pathways controlling energy homeostasis,[4,5] scientific advances have thus far failed to prevent the worldwide spread of obesity. The current definition of overweight for an adult is a BMI between 25–29.9 kg/m^2 and for obese $\geq$30 kg/m^2.

To date, lifestyle intervention programs including healthy nutritional intake and fitness training remain the foundation of obesity treatment and prevention, as well as for attaining optimal body composition levels. Despite the abundance of both research-based and trending popular dietary and fitness interventions available, controversy remains regarding the most effective nutrition and fitness lifestyle strategies to combat obesity and its related complications, as well as to optimize body composition. This chapter will focus on the plethora of new research supporting the role of increased dietary protein intake as a first line of defense for both obesity prevention and treatment (weight loss) as well as optimizing body composition. In addition, combining the benefits of increased protein intake with an appropriate and effective exercise program further improves health (and performance) outcomes.

There are a myriad of causes of overweight and obesity, including a genetic link. However, the epidemic increase in overweight and obesity during the past 40 years is primarily the result of lack of proper quality, quantity, and timing of nutrients and physical activity.[6,7] The purpose of this chapter is to provide a basic overview of protein, followed by current research findings pertaining to the impact of protein quality, quantity, and timing on optimizing body weight control (weight loss) and composition, as well as physical performance outcomes.

14.2 OBESITY, MACRONUTRIENTS, AND WEIGHT LOSS

Given the current epidemic of overweight and obesity among all age groups, 20% in children and >40% in adults, and the overwhelming societal and media focus on obesity, it's no surprise that most people worldwide admit to trying to lose weight or being on a diet at any given time.[8–10] Thus, the question is: What is the best type of meal plan to optimize weight loss and body composition? Most experts agree that, in general, for weight loss to be successful, energy (calorie) intake needs to be below energy (calorie) expenditure. However, to what extent does the deficit need to occur and for how long? Perhaps more importantly, what role does variation in macronutrient intake play on the degree of weight loss and weight loss maintenance, as well as optimal body composition? Finally, what impact does the interplay of nutrition and exercise have on weight loss and body composition?

There are three primary "macronutrients" required to nourish the body: Protein, carbohydrates, and fats. To date, a growing body of scientific evidence is showing that macronutrient distribution combined with a negative energy balance (caloric deficit) plays a vital role in both the prevention and treatment of overweight/obesity.[11–13] Dating back to the 1970s, dietary fat came under heightened scrutiny for its purported association with obesity and even heart disease.[14,15] As such, government agencies, certain health organizations, and the food industry began ostracizing dietary fat, and hence the low-/no-fat craze began.[16] In turn, weight loss recommendations included reducing dietary fat intake to less than 60 g per day. The fallout from this "fat-phobia" reduction of dietary fat intake, by default, left Americans with a disproportionate excess of carbohydrate intake, including too many simple sugars, as well as a surplus of an additional 250 calories per day increase that still exists today, nearly five decades later![6] Another interesting "side effect" from this low-/no-fat diet craze, along with the massive increase in carbohydrate and total energy (calorie) intake, was a significant reduction in dietary protein intake that has persisted since the 1970s.[6,7] It's interesting to make note of the alarming rise in the overweight and obesity epidemic during this exact same time period.[17,18] The metabolic consequences of this major shift in dietary intake of reducing fat and protein intake and increasing carbohydrate and total calorie intake included a reduction in body fat oxidation,[19] an increase in blood triglycerides,[20] and a reduction in satiety,[21] which continues to have most people either completely unaware or utterly confused as to where to find the most accurate information regarding the best food choices to prevent or treat obesity and optimize body composition.

The above trend has coincided with the increasing prevalence of obesity and its association with excess calories in a high-carbohydrate, particularly high–simple sugar and high–glycemic index, meal plan.[22] It's well known that a high carbohydrate intake (simple sugars, glycemic index) increases blood glucose levels and subsequent elevated secretion of insulin into the blood to increase tissue uptake of glucose and/or decrease the amount of glucose in the blood (circulation). Interestingly, the increased insulin output and potential postprandial hypoglycemic response may be contributing factors to excess energy consumption and positive energy balance because of the high simple sugar intake.

A growing body of scientific research suggests that a higher intake of dietary protein may be an effective strategy to promote weight loss and improve body composition by increasing postprandial thermogenesis,[11] increasing satiety,[23] and enhancing protein synthesis.[24] Dietary protein has relatively no effect on blood glucose levels and minimal effect on insulin response. Recent evidence demonstrates that a higher protein intake evenly distributed throughout the day, termed "protein pacing®," favorably alters blood glucose, insulin and energy homeostasis hormones, body weight, and composition.[11] Furthermore, combining protein pacing with a comprehensive exercise training program extends these benefits by enhancing physical performance outcomes as well.[25–28] Collectively, this new research has led to rethinking dietary recommendations for weight loss and optimization of body composition.

14.3 PROTEIN OVERVIEW

The term "protein" originates from the Greek word *proteios* meaning "primary or first," and it is vital to human life. The three major macronutrients are carbohydrates, proteins, and lipids, all of which contain carbon, oxygen, and hydrogen. However, protein is unique because it also contains nitrogen and sulfur. The energy content from protein is approximately 4 kilocalories per gram, like carbohydrate, and less than half that of fat (9 kilocalories per gram). Unlike carbohydrates and fats, protein is not regarded as a readily available fuel source or energy-contributing macronutrient because it's not readily stored as a source of energy inside the body, although it can be made available as a limited source of energy during periods of energy deficit, stress, trauma, disease, and so on (Figure 14.1).

Increasingly, protein is regarded and even marketed as the premier macronutrient as a functional food ingredient in the areas of weight loss and diabetes, and for good reason. The weight loss market annual revenue is nearly $70 billion and will likely continue to increase in concert with the growing number of people who are overweight and obese.[29,30] Despite an abundance of misinformation, fad diets, and marketing hyperbole, higher-protein diets have clearly emerged as a science-based efficacious lifestyle strategy to both prevent and combat overweight and obesity and improve body composition. This makes protein an ideal macronutrient to incorporate as a foundational ingredient for weight loss products.

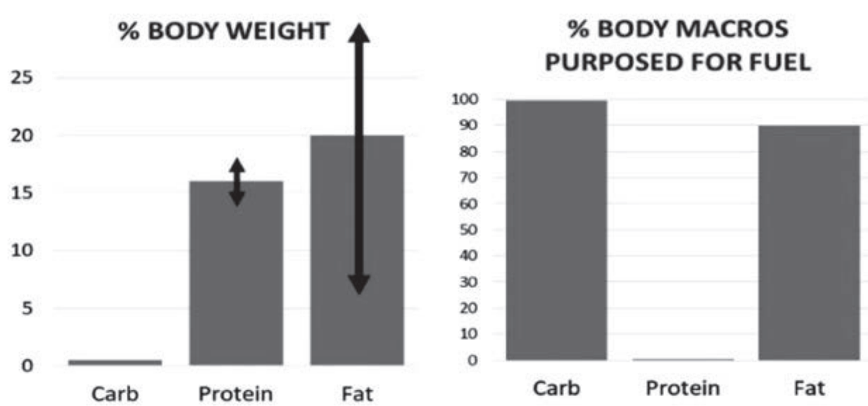

FIGURE 14.1 Bars on left represent percentage of total body weight contributed by each nutrient. Bars on right represent percentage of available fuel contributed by each macro-nutrient. Bars based on typical healthy, lean adult. Directional arrows on left indicate variability. (Adapted and used with permission from www.internationalproteinboard.com.)

14.4 AMINO ACIDS AND PROTEINS

Within the body, a protein molecule contains varying amounts of amino acids, ranging from 51 amino acids for the hormone insulin to as many as 6100 amino acids for the structural protein myosin found in a single skeletal muscle fiber. Amino acids are referred to as the "building blocks" of proteins. Although the human body contains a wide variety of proteins as structures, hormones, enzymes, cells, and so on, each protein is built from just 20 individual amino acids assembled with different amounts and sequences. This allows each individual protein to have its own unique size, shape, and function inside the body.

Amino acids are categorized in two groups, as shown in Table 14.1. There are nine *essential* amino acids, as they must be consumed (eaten) on a daily basis, and 11 *nonessential* amino acids, because the body is capable of making them in sufficient amounts and therefore they are not required to be consumed on a daily basis. Although the majority of amino acids inside the human body are part of protein or used to synthesize protein, a few amino acids, such as citrulline, ornithine, homocysteine, and beta-alanine, play other critical roles.

Amino acids and the resulting proteins have multiple bodily functions, including serving as:

- Structures in cell membranes, muscles, and bones
- Enzymes to help regulate chemical reactions
- Antibodies for the immune system
- Hormones as regulators of metabolic processes
- Clotting factors in the blood
- Blood proteins for transporting nutrients and oxygen
- Receptors on cells
- Enzymes for digestion and absorption of food
- Unique metabolic regulators (such as leucine) in protein synthesis and arginine in nitrous oxide
- An important energy source for muscle, liver, and the intestine

Perhaps the most significant amino acids for optimizing weight loss and body composition are the branched chain amino acids (BCAAs) of leucine, isoleucine, and valine. Of these, leucine plays the vital role. Obtained through the diet with subsequent uptake into skeletal muscle, leucine performs the highly regarded roles of chief regulator of initiating translation of protein synthesis, modulator of the insulin-PI3 signal cascade, and nitrogen donor for muscle production of alanine and glutamine. The quality and quantity of protein differ among food sources due to the amino acid amounts and

TABLE 14.1
Essential and Nonessential Amino Acids

Essential Amino Acids[9]	Nonessential Amino Acids[11]
Leucine	Alanine
Isoleucine	Glutamic acid
Valine	Asparagine
Tryptophan	Aspartic acid
Threonine	Arginine
Lysine	Serine
Phenylalanine	Glutamine
Methionine	Proline
Histidine	Glycine
	Tyrosine
	Cysteine

TABLE 14.2
Sources of Protein and Leucine by Weight

Food Item (1 oz)	Protein (g)	Leucine (g)	% Leucine	% BCAA
Whey, powder[a]	24.00	2.53	10.54	26.38
Pork, lean	8.83	0.71	8.04	21.63
Chicken, breast	8.79	0.66	7.51	21.27
Beef, lean	8.66	0.69	7.97	20.90
Tuna	8.50	0.69	8.12	21.88
Halibut	7.57	0.61	8.06	21.80
Peanut butter	7.11	0.46	6.47	13.50
Cheese, low-fat[b]	6.75	0.61	9.04	22.81
Nuts, peanut	6.71	0.43	6.41	13.41
Turkey, breast[b]	5.00	0.40	8.00	22.40
Soybean, cooked[b]	3.78	0.38	10.10	24.34
Egg	3.57	0.3	8.40	20.73
Cottage cheese, 1%	3.51	0.32	9.12	21.65
Egg whites	3.09	0.27	8.74	21.04
Hummus[b]	2.24	0.14	6.25	15.18
Bread, white	2.17	0.15	6.91	13.36
Tofu, firm	1.98	0.15	7.58	18.18
Yogurt, low-fat[b]	1.75	0.15	8.57	20.57
Black beans[b]	1.73	0.14	8.09	19.65
Milk, skim	0.96	0.09	9.38	20.83
Rice, white	0.76	0.06	7.89	15.79
Potato, baked	0.71	0.04	5.63	15.49

Source: ESHA Research, Professional Nutrition Analysis Software and Databases v. 9.6.1 2002–2003 ESHA Research. Adapted from Layman.

[a] Commercially available whey protein powders.

[b] USDA National Nutrient Database for Standard Reference. Release 18 (2005).

types present in each protein. In general, foods from animal sources contain more protein and leucine and thus provide a more complete amino acid mixture than foods from plant sources (Table 14.2). A *complete protein* such as whey protein contains adequate amounts of each of the essential amino acids in proper ratios, whereas an *incomplete protein* such as a rice does not have all the essential amino acids in adequate amounts or correct proportions.

14.5 PROTEIN REQUIREMENTS

Initially introduced in 1943, the recommended daily allowances have been used as the standard nutrition guidelines. The RDAs were set as minimal levels of intake to prevent deficiency in otherwise healthy people. The reality is, the RDA for protein (0.8 g/kg) is likely too low for most people. The International Protein Board (www.internationalproteinboard.com), composed of the leading protein scientists in the world, is dedicated to providing key insight, research, and opinions on all matters related to dietary protein, health, and performance. They've concluded that "a re-evaluation of the current RDA for protein requirements is necessary to identify areas of improvement, application and globalization and be based on the most current information and research available." The RDA for protein serves as a minimum requirement for certain age groups, and during pregnancy and lactation. However, evidence suggests that this may be too low for older people and vegetarians, as well as that required for weight loss, exercise/sport, sickness, and disease, which collectively make up a significant portion of the adult population. In response to these limitations of meeting the "requirement" to prevent deficiency versus the optimal "recommendation" for protein, the Food and Nutrition Board (FNB) of the National Academy of Science developed the Dietary Reference Intakes for macronutrients. In the case of protein, it ranges from 10%–35% of energy intake or roughly 0.8 to 2.0 g/kg. The challenge, of course, is understanding how to appropriately apply this range of protein in terms of quality, quantity, and timing/frequency to produce the greatest health benefit in various settings. One example would be if 10% of energy for protein was targeted for diet planning for weight loss or athletic performance, this would mean 90% of the remaining energy would need to be split between carbohydrate and fat, which may not be ideal from an optimal health standpoint. More specifically, in a society struggling with an expanding obesity (and cardiometabolic disease) epidemic mostly driven by a high (refined) carbohydrate intake, menu/meal planning providing the minimum amount of protein to prevent deficiency (RDA and/or lower limit of DRI at 10% energy intake) is not consistent with current scientific research or optimal health across the lifespan.

14.6 DIETARY PROTEIN SOURCES

Dietary protein sources vary within different foods, such as gluten in wheat, albumin in eggs, and casein and whey in milk. Specifically, these proteins are made up of a group of proteins or chemically associated protein molecules. The protein in egg albumin includes ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme. In the case of milk whey protein, it includes β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, and lactoperoxidase, as well as glycomacropeptide (GMP), a casein-derived protein in cheese whey, whereas the principal milk casein fractions are α(s1) and α(s2)-caseins, β-casein, and kappa-casein.

Of all the animal proteins, milk has evolved as the most popular form of protein powder due to its protein composition. Casein, the main protein in milk, makes up roughly 80% of total milk protein and is deemed a slow-acting protein. Because of the complex chemical nature of casein protein, digestion and absorption of its amino acids can take up to a few hours, depending on the amount consumed.[31] Therefore, casein would provide a slow, steady rise in blood levels and uptake of amino acids into circulation. Whey, on the other hand, is more readily digested, allowing for a quick increase in blood amino acid levels and an increase in protein synthesis.[32] Therefore, the combination of whey and casein protein (fast- and slow-acting proteins) has been shown to be beneficial during muscle recovery, especially the time that immediately follows a vigorous exercise training session.

Whey protein has proven very effective and versatile. For example, it is ideal for fitness enthusiasts to help with lean muscle mass support, yet it is also ideal to support weight loss by increasing postprandial thermogenesis, satiety, and protein synthesis. Interestingly, whey is also a major part of infant formulas due to its high nutrient value, by providing calcium, phosphorus, lactose, water, magnesium, fat, and, of course, protein.[33] One advantage of whey versus casein is the greater satiety due to higher levels of circulating amino acids after a meal is consumed.[34] Whey is rich in the essential amino acids, particularly the branched-chained amino acids, leucine, isoleucine, and valine. These amino acids are major contributors to skeletal muscle replenishment after exercise[35] or short-term periods of food restriction, such as overnight fasting. If the meal plan is adequate in leucine, then the muscles can build or maintain muscle protein. However, recent evidence has focused on the use of casein protein consumed pre-sleep in an effort to have a slow and steady release of amino acids into the blood stream while sleeping to aid in overnight muscle protein synthesis,[36] metabolism,[37] and general health.[37]

14.7 PROTEIN DIGESTION AND ABSORPTION

The process of converting dietary protein into amino acids for use in the body is a complex process involving the stomach, small intestine, and liver. Although the process is complex, it is highly efficient, with nearly 100% of dietary protein digested and absorbed into the intestinal cells, known as *enterocytes*. Protein digestion begins in the stomach as gastric acids denature complex protein structures, and pepsin begins to cleave protein chains. The resulting polypeptides are released into the small intestine, where proteases derived from the pancreas and enterocytes continue protein digestion. Ultimately, protein digestion produces a mixture of free amino acids and di- and tri-peptides in ratios of approximately 1:1:1. These amino acid mixtures and small peptides are absorbed into the enterocytes by amino acid and peptide transporters. Once inside enterocytes, the remaining peptides are hydrolyzed to amino acids before being released into portal circulation and delivered to the liver (Figure 14.2).

Amino acids within the enterocyte can be used for intestinal enzyme synthesis, for example, proteases, used for energy, or transported to portal blood for use by the rest of the body. Use of amino acids by the intestine varies greatly among amino acids. Dietary glutamine and glutamate are completely removed by the enterocyte to provide nourishment and optimal function; neither one of these amino acids, from a meal (or supplements), reaches the blood. In total, the enterocytes remove approximately 25% of dietary amino acids before they reach the blood and become available to other tissues. This is a major reason glutamine, and protein in general, are critically important for the overall health and function of the intestinal tract and immune system.

Amino acids leave the intestine via the portal blood to the liver. The liver is the most active amino acid metabolism tissue in the body. Amino acids that reach the liver can be used for protein synthesis, as an energy source, or released to the blood to serve the needs of other tissues, including the brain, muscles, and vital organs. Similar to the small intestine, the liver removes a significant portion of ingested amino acids and releases approximately 30% of the total amino acids ingested. Interestingly, the primary energy source for both the intestine and the liver is amino acids. As a result, only about a third of dietary amino acids reach the blood for delivery to other tissues.

Although the liver and intestines use amino acids for energy, amino acids are not removed uniformly. The enterocyte is active in removing glutamine, glutamate, asparagine, and aspartate, whereas the liver is capable of metabolizing most of the remaining amino acids, with the exception of the BCAAs. The liver lacks the necessary enzymes to metabolize BCAAs, so the net result is that BCAAs appear in the blood in nearly the exact amounts present in a meal. This serves the body very

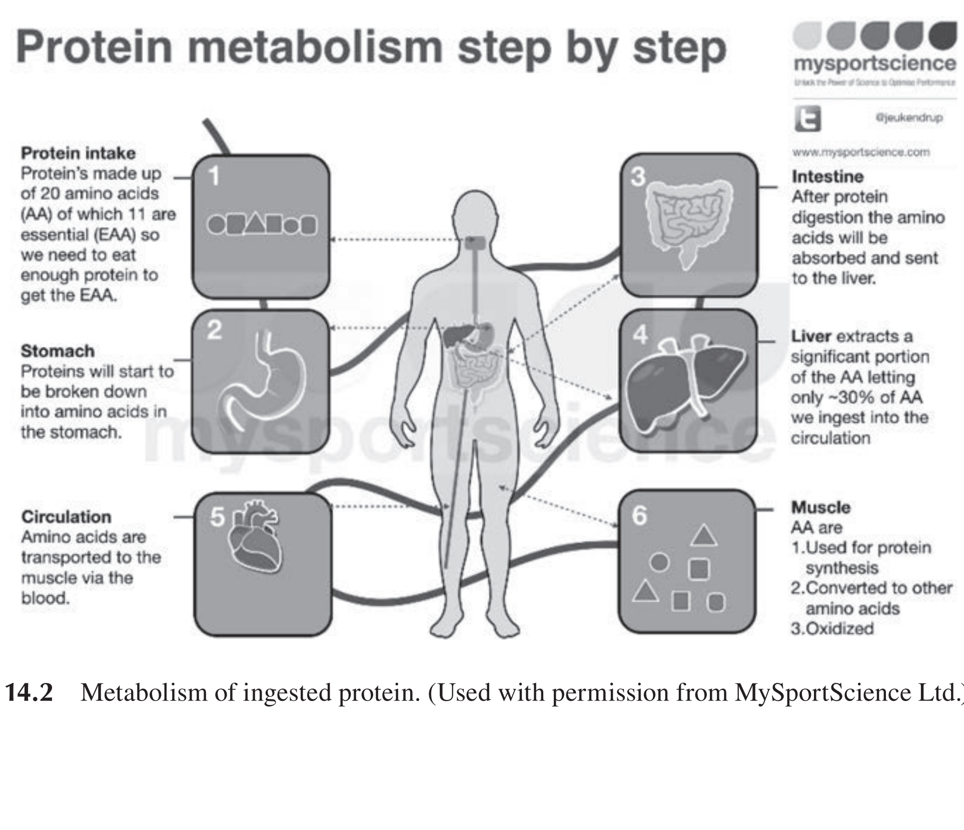

FIGURE 14.2 Metabolism of ingested protein. (Used with permission from MySportScience Ltd.)

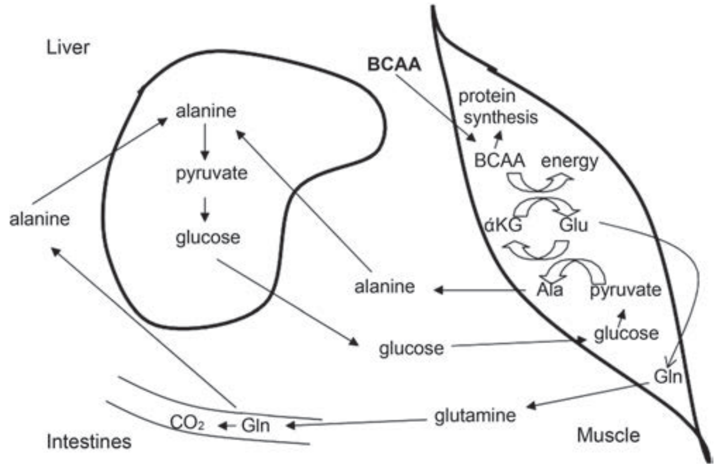

FIGURE 14.3 Metabolism of branched-chain amino acids. (Used with permission from Layman et al.)

well because BCAAs are a valuable energy source for the muscles during times of stress, prolonged vigorous exercise, low energy intake, and recovery from exercise to aid in repair and rebuilding of muscle protein synthesis (Figure 14.3).

14.8 PROTEIN TURNOVER

Approximately 16% and 24% of a lean adult woman and man's body mass, respectively, is composed of protein. Of this amount of body protein, the vast majority (~99%) makes up body structures such as lean muscle mass, enzymes, hormones, bones, and so on, and the remaining 1% is circulating in the blood and within cells as free amino acids. Amino acids that enter the blood move throughout the body, referred to as the amino acid pool, are transported into cells, and become available for synthesis of new proteins (see Figure 14.4). This is important because proteins are continually being broken down (degraded) and built (synthesized). Some proteins such as enzymes have a lifespan of only a few hours, whereas other structural proteins such as connective tissues are retained for as long as 6 months. The process of synthesis and degradation of proteins is called *protein turnover*. Each day, the body makes and degrades over 250 g of protein. The magnitude of this turnover is surprising, as few people consume more than 100 g of protein per day. The lack of direct relationship between the amount of dietary protein and the level of daily protein turnover emphasizes the difficulty in defining protein requirements. Body protein quantity is largely determined by the balance of protein synthesis and degradation. Although the daily turnover is greater than 250 g/d, the actual potential to accumulate new proteins is very limited. During maximum growth, protein turnover is positive; that is, synthesis is greater than degradation, but the net balance is less than 10 g/d. Protein turnover balance appears to be largely regulated by protein synthesis change.

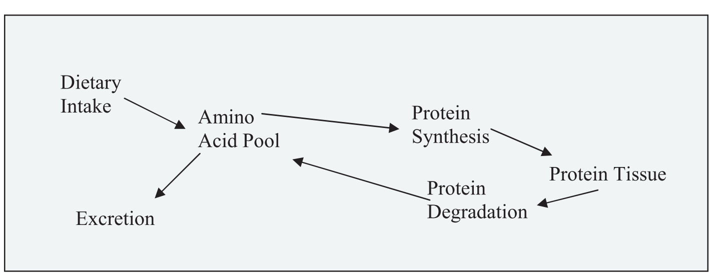

FIGURE 14.4 Protein turnover.

14.9 PROTEIN INTAKE, WEIGHT LOSS, AND BODY COMPOSITION

Protein is arguably the most crucial nutrient for weight loss and body composition because of its role in protein synthesis, energy metabolism, immune support, and satiation. There is little doubt there are more "diet" types available today than any other point in time. Further confusing this diet landscape is the ongoing media propaganda of fad diets, resulting in a quagmire of conflicting misinformation disseminated to the general public. Thus, it is paramount to closely and systematically scrutinize the peer-reviewed scientific literature to generate objective and credible nutrition recommendations to help coaches, trainers, dietitians, nutritionists, athletes, healthcare practitioners, and the general public. Dietary guidelines have consistently recommended a higher carbohydrate (CHO) intake (up to 65% of total kcals), moderate fat (20–35% of total kcals), and 10%–35% of intake as protein (PRO) for proper weight control.[38] Applying this approach to a calorie-restricted diet (e.g., 1200 calories) using the commonly prescribed protein percentage of total calories of 15%, the daily protein level would be 45 grams, or approximately the RDA. Clearly, this would not be an ideal protein intake to support healthy weight loss for overweight and obese conditions. Distributing this amount of protein over three to five meals would result in a significantly inadequate amount of protein on a per-meal basis (9–15 grams/meal). Given the valuable contribution protein plays in satiety, thermogenesis, and body protein, this would be counterproductive to an effective weight loss program. In light of this, recent data suggests that protein consumption at the upper range of acceptable intake (~25%–35% of total kcals or 1.6 vs. 0.8 grams per kilogram of body weight) enhances weight loss, energy expenditure,[39–42] and body composition to a greater degree than the lower RDA,[11,25,43–45] and may enhance metabolism and body composition independent of inducing weight loss.[46] This is great news for those interested in improving health outcomes without wanting to undergo caloric restriction and weight reduction. Moreover, recent evidence demonstrates the combined effects of increased protein intake, including protein timing/frequency, with low-glycemic index diets to improve weight loss maintenance[12,47] and body composition.[12,48,49] Of all the diet types currently available, including very-low and low-energy diet (VLED, LED), low-fat diet (LFD), low-carbohydrate diet (LCD), ketogenic diet (KD), higher-protein diet (HPD), and intermittent-fasting (IF), the HPD appears most effective.[50] The prevailing thought on weight loss is the "energy in/energy out" (EIEO), also known as "calories in/calories out" (CICO) concept based on the laws of thermodynamics. Unfortunately, the public health message has been an oversimplification of the CICO/EIEO concept via encouraging an "eat less, move more" approach to the obesity pandemic. This posits that weight loss or gain is dictated solely by either a caloric (energy) deficit or surplus. In a closed circuit environment, these laws hold true. However, in free-living humans, these laws break down due to the dynamic influence of changes in body composition, neuro-pyscho-hormonal-biological factors that drive eating and physical activity behaviors, and the significant influence of varying postprandial thermogenic cost of macronutrients. A recent meta-analysis supports the benefit of higher protein intakes for reducing body weight, fat mass, triglycerides, and waist circumference, and preserving fat-free mass and resting energy expenditure.[41] Moreover, a recent comprehensive position stand concluded that increasing dietary protein above the recommended dietary allowance may improve body composition.[50] Taken together, the scientific evidence supports higher protein intake as an effective lifestyle strategy to enhance weight loss and body composition.

14.10 PROTEIN PACING, ENERGY METABOLISM, AND BODY COMPOSITION

Following consumption of a meal, a series of metabolic, physiologic, and digestive processes occur that transition the body from a catabolic to an anabolic state, including an increase in protein synthesis and decrease of protein breakdown. It's well established that energy expenditure and metabolism differ greatly in response to macronutrient intake of isoenergetic meals. Specifically, protein has the highest metabolic cost (thermic response, expressed as a percentage of energy content of the food) of all macronutrients at 25%–30%, carbohydrate at 6%–8%, and fat at 2%–3%.[51] In support of this, both higher protein intakes as well as timed-daily protein feedings throughout the day have been shown to enhance energy metabolism (postprandial thermogenesis) and maximize protein synthesis and thus lean muscle mass accretion compared to other dietary approaches.[11,52–54] In addition, meals containing whey protein have a higher thermic effect than casein, which has a higher thermic response than soy protein.[55] In addition, compelling evidence favors dietary proteins containing a full complement of essential amino acids, including a high level of the branched-chain amino acid leucine, to maximally stimulate muscle protein synthesis.[56–58] In this case, whey protein is considered the ideal protein source because it has a high content of leucine per serving to maximize protein synthesis and serve as a fuel source for skeletal muscle and as a nitrogen donor for production of alanine and glutamine in skeletal muscles. Leucine's ability to fulfill each of these important roles is dependent upon the amount consumed in the diet. This is especially relevant during energy restriction. Intakes of 10 grams of leucine per day, equivalent to approximately 125 grams of protein intake per day, have been shown to stimulate protein synthesis during energy restriction to a greater degree than commonly recommended protein intakes.[59] (See Table 14.2 for the breakdown of protein and leucine content in various food sources.) Thus, the precise mechanism responsible for enhanced energy expenditure following macronutrient intake is partly due to an increase in muscle protein synthesis (MPS) that is triggered by protein (especially leucine) ingestion. In addition, there is evidence that a frequent macronutrient intake of protein-containing meals favors an anabolic state resulting in an increase in protein synthesis and lean body mass accretion.[52,60] Specifically, distributing protein feedings in 20 grams of whey protein every 3 hours following resistance exercise maximizes MPS, including increased signaling proteins and transcriptional activity of muscle cells compared to either smaller (10 grams every 1.5 hours) or larger (40 grams every 6 hours) feeding.[52] Indeed, not only does this have beneficial implications for enhanced functional capacity of muscles and an increase in lean body mass, but also for increased energy expenditure, all of which lead to improved body weight control and optimal body composition.

The frequency and timing of meals eaten is another important factor for optimization of energy metabolism and body composition. Several studies have suggested meal frequency is inversely related to body weight.[61,62] Expanding on the body of literature of these major diet types is work by Arciero et al. on Protein pacing. Protein pacing involves the consumption of 4–6 high-quality protein-based mini-meals/day, evenly spaced approximately every 3–4 hours, containing 20–40 grams (or 0.25–0.4 grams of protein/kg BW) at each meal. The protein pacing meal plan has consistently shown superiority over conventional, low-fat, LED, and lower-frequency meal patterns (three meals/day) for enhancing energy metabolism and body composition under both eu-caloric and hypocaloric conditions.[11,12,25,28,63]

Arciero et al. recently compared a protein pacing (~35% of total kcals as protein) meal pattern (~50% from whey protein and 50% from both plant and animal sources), moderate in CHO (~40% of total kcals), consumed as either three (HP3) or six (HP6) meals/day versus a traditional diet (~15% of total kcals as protein; TD3), higher in CHO (~60% of kcals), consumed at three meals/day, consumed throughout 28 days of energy balance (weight maintenance) and deficit (75% of energy requirements resulting in a negative energy balance of 25%), respectively (56 days total).[11] The results demonstrated that postprandial thermogenesis during both weight maintenance and weight loss was significantly elevated by 67%–128% in HP6 compared to both HP3 and TD3 groups (Figure 14.5). The increased thermic response in HP6 may partly explain the significant total and abdominal fat loss and increased lean body mass that also occurred in the HP6 group. Layman et al.[64] proposed that enhanced quality and quantity of protein during weight loss increases plasma leucine, which in turn stimulates muscle PRO synthesis and may increase fat oxidation, both of which support the findings of increased lean muscle mass in HP6 versus TD3. However, the study design does not rule out the possibility of eating frequency or an interaction of the two as playing a major role in mediating these responses. In a follow-up study, Arciero and colleagues demonstrated that overweight/obese men and women respond equally to a hypocaloric (1500 kcals

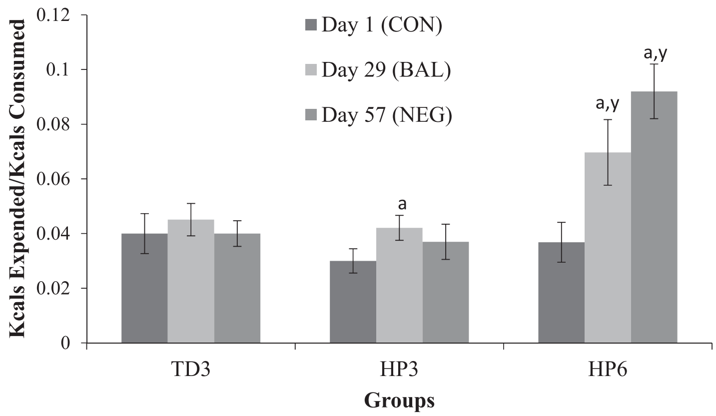

FIGURE 14.5 Change in postprandial thermogenesis following meal consumption. Letter a denotes significantly different from CON ($P < 0.05$); letter y denotes significantly different from TD3 and PP3 ($P < 0.05$). TD3, traditional diet, three meals/day; HP3, protein pacing, three meals/day; HP6, protein pacing, six meals/day. CON, control phase of standardized meal pattern; BAL, energy balance; NEG, negative energy balance of 75% of calculated energy needs.

men; 1200 kcals women) protein pacing meal pattern (four to six meals/day) combined with a 1 day/week intermittent fast (450 kcals men; 350 kcals women) over a 12-week weight loss phase. As a group, they lost 11 kg (24 lbs), of which 9.5 kg (21 lbs.) was fat mass, and 0.8 kg (1.8 lbs.) of visceral fat and the proportion of lean body mass (LBM/BW) increased by 9%.[12] In this study, following the initial 12-week weight loss phase, subjects were then divided into two groups, a protein pacing and heart-healthy group, and followed an additional 52 weeks. The heart-healthy group observed the dietary guidelines that are in compliance with the National Cholesterol Education Program Therapeutic Lifestyle Changes (TLC) diet (i.e., <35% of kcal as fat, 50–60% of kcal as carbohydrates, <200 mg/dL of dietary cholesterol, and 20–30 g/day of fiber). At the end of the 52-week weight maintenance phase, the protein pacing group maintained body weight and composition, whereas the heart healthy group regained body weight (12.1 lbs./5.5 kg) and fat mass (3.9 lbs./1.8 kg) and lost a greater proportion of lean body mass (2%). Collectively, these findings indicate that macronutrient composition (increased dietary protein), nutrient quality (high quality whey protein, low glycemic index carbohydrates), and frequency of eating (6× per day) using the protein pacing meal pattern are more impactful than total energy intake to enhance energy metabolism (post prandial thermogenesis) and body composition (reduced total and abdominal obesity and increased lean body mass) during both weight loss and weight loss maintenance. In support of these intervention studies, a recent position stand on nutrient timing summarizes consuming a 20–40 g protein dose (0.25–0.40 g/kg body mass/dose) of a high-quality source every 3 to 4 hours favorably increases MPS rates when compared to other dietary patterns and is associated with improved body composition (and performance) outcomes.[65]

From a practical application standpoint, protein pacing from both animal (mostly whey protein sources) and plant (mostly from legumes) food sources, more often (four to six meals/day) throughout the day (every 3–4 hours) increases postprandial thermogenesis and lean body mass and reduces total and abdominal fat mass compared to traditional protein (15% of total kcals as protein) and meal frequency (three meals/day) intakes. These favorable metabolic and body composition changes may directly lead to enhanced health improvement. Importantly, these beneficial improvements were achieved even though total kcals consumed were identical among all groups and in the absence of any physical activity/exercise training. These data indicate that macronutrient distribution (protein pacing), nutrient quality (high-quality protein, low GI, essential fats) may be more important than total energy intake to improve energy metabolism (postprandial thermogenesis) and body composition, and thus overall health.

14.11 PROTEIN INTAKE, EXERCISE, AND GLYCEMIC CONTROL

The ability to control blood glucose and insulin in both the fasted and fed state is crucial for losing and maintaining weight. This is especially important for individuals suffering from overweight/obesity with insulin resistance or type 2 diabetes mellitus (DM), with impaired glucose tolerance and disposal pathways. Following a mixed meal containing carbohydrate, fat, and protein, blood glucose increases, causing an increase in insulin production and secretion from the pancreas. Elevated insulin levels in the blood activate cell membrane receptors, of which muscle cells are a primary target, and cause GLUT 4 receptors to translocate from the cytoplasm to the surface of the plasma membrane for glucose uptake into the muscle cell. This, in turn, suppresses hepatic glucose production in the liver and increases glycogen synthesis and storage in both muscle and liver. Interestingly, an increase in dietary protein intake concomitant with a reduction in carbohydrate, fat, or total calorie intake has been shown to favorably influence glucose and insulin levels by significantly improving insulin sensitivity.[12,44,66,67] Arciero and colleagues have demonstrated both short-term hypocaloric (10 days) and longer-term ad libitum (3 months) interventions of higher dietary intake of protein (25%–40% of total kcals) results in a significant improvement of glucose disposal and insulin sensitivity, in some cases similar to that obtained with moderate-higher protein intake combined with exercise training[44,68] (Figure 14.6). Although the precise mechanism(s) is unclear, the reversal of insulin resistance in response to dietary manipulation appears to be induced by a combination of reduced calories and/or dietary carbohydrate[69] and increased protein intake leading to reduced blood glucose, a negative fat balance, and/or an increased supply of gluconeogenic substrates for the liver. Increased dietary protein intake provides valuable gluconeogenic substrates, in the form of amino acids, to the liver during calorie restriction.[70] The amino acids are produced by protein breakdown in the muscle. Therefore, dietary intake of protein is vital to provide a continual supply of these amino acids. Alanine and glutamine, derived from BCAA breakdown, are the most common gluconeogenic substrates. Once the muscle releases these amino acids, they travel to the liver, undergo deamination, and provide carbon skeletons for gluconeogenesis, producing pyruvate and glutamate. The fasted state is accompanied with a decrease in insulin and an increase in glucagon, which causes an increase in hepatic glucose production (including gluconeogenesis) and degradation of glycogen, via a series of dephosphorylations, to produce fuel for the body. The increased glucose production by the liver serves the primary role of maintenance of blood glucose during caloric restriction.[71] An increased dietary protein intake, particularly during caloric restriction, is an effective and proven strategy to manage glucose/insulin levels for the overweight, obese, and those with type 2 diabetes by providing

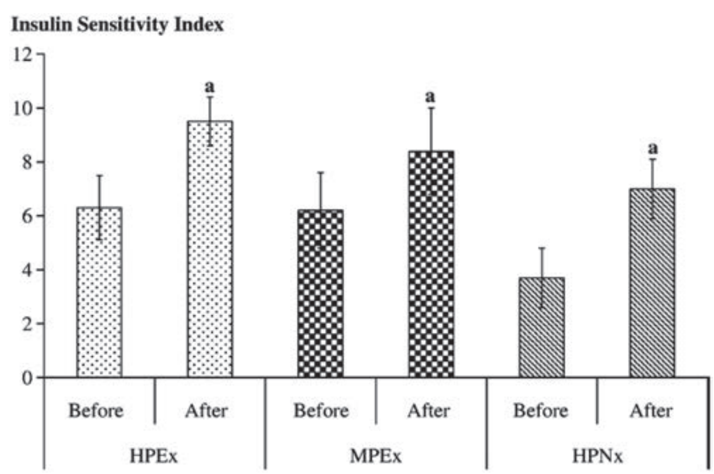

FIGURE 14.6 The insulin sensitivity index in high protein and exercise (HPEx), moderate protein and exercise (MPEx), and high protein and no-exercise (HPNx) before and after 3-month interventions. Each value is mean ± SE. $^aP \leq 0.05$.

greater substrate availability for gluconeogenesis and subsequent hepatic glucose production.[72] With regard to the addition of exercise training to increased dietary protein, it's now well established that the increase in skeletal muscle insulin sensitivity and glucose uptake after a bout of exercise is due to both the acute insulin-independent stimulation of glucose transport and an exercise-induced increase in GLUT-4 glucose transporters. An increase in muscle GLUT-4 content is associated with increases in insulin-stimulated glucose transport[73] and physical inactivity with an abrupt reduction in GLUT-4 activity.[74] Thus, the combined effects of higher protein intakes (25%–40% of total kcals) with exercise training may very well be supra-additive to glycemic control.

14.12 NIGHTTIME PRE-SLEEP PROTEIN FEEDING, METABOLISM, AND BODY COMPOSITION

Nighttime pre-sleep eating is historically considered controversial in terms of its influence on metabolism and body composition.[75–77] Much of the original data from investigation of this topic used and contributed to the controversy for this window of feeding used high calorie mixed-macronutrient meals and populations such as night-shift workers or individuals with night eating syndrome.[37] It is also documented that the thermic response to identical large mixed meals is lower in the evening compared to the morning.[78] However, recent evidence has identified small (∼150 kcals) pre-sleep protein (30–40 g) consumption to be supportive of overnight muscle protein synthesis[79,80] and advantageous to limiting morning hunger, improving satiety, and improving resting metabolic rate.[81–83] Interestingly, when 30 g of whey protein, 30 g of casein protein, and 33 g of carbohydrate were provided 30 minutes pre-sleep, resting metabolic rate was elevated compared to a non-caloric placebo.[81] Similarly, although not statistically significant, morning increases in resting metabolic rate were reported in young overweight and/or obese women.[82] It is important to note that most of the research to date with regard to pre-sleep feeding has used protein shakes as opposed to whole-food options. Recently, Lay et al.[84] examined the influence of a bedtime milk snack on next-morning resting metabolic rate, substrate utilization, and appetite in overweight men, and Leyh et al.[85] examined the influence of cottage cheese compared to an energy- and macronutrient-matched powdered supplement in young healthy women. In both instances, there were no significant changes to any outcome measures, which further supports that pre-sleep protein-centric meals (supplement or whole foods) do not interfere with metabolism or change the ability to use fat as a fuel while sleeping or the next morning.

Despite the data, some concern for fat gain as a result of increased calorie intake and/or decreased lipolysis as a result of pre-sleep protein intake remains. In the previous work, it is important to note that there were no differences in respiratory quotient the morning after pre-sleep feeding of either placebo or casein, while both carbohydrate and whey protein were increased compared to placebo.[81,82,86] This suggests that casein protein consumed pre-sleep maintains overnight lipolysis and fat oxidation. In a follow-up study, overnight microdialysis was used to measure interstitial glycerol concentrations as an indicator of lipolysis after pre-sleep feeding of 30 g of casein or a flavor- and sensory-matched noncaloric placebo in obese men.[87] It was reported that no differences in overnight or next-morning lipolysis or fat oxidation existed despite the consumption of calories. In addition, data supports that exercise performed in the evening augments the overnight muscle protein synthesis response in both younger and older men.[36,88,89] In active individuals and athletes, pre-sleep feeding may be even more important as a mechanism to ensure optimal calories and protein for recovery and training. The influence of pre-sleep protein on next-day performance is a new area of investigation with only one study to date reporting no change in running performance following pre-sleep milk consumption.[77]

Relatively little data exist for chronic nighttime pre-sleep protein consumption. The first study to investigate this randomly assigned young men (22 ± 1 years) to consume a drink containing 27.5 g of casein protein, 15 g of carbohydrate, and 0.1 g of fat or a noncaloric placebo every night before sleep for 12 weeks, while also completing a progressive resistance exercise training program (three times per week). Pre-sleep protein resulted in greater improvements in muscle mass and strength at the end of the study. Of note, this study was not nitrogen balanced and the protein group received approximately 1.9 g/kg/day of protein compared to 1.3 g/kg/day in the placebo group. On the other hand, these robust responses to protein-centric beverages ingested both post-exercise and pre-sleep are blunted in older (70 ± 1 years) men.[90]

More recently, nitrogen-balanced study designs have been implemented to see if the pre-sleep feeding was critical or just consuming more daily protein was the key. Antonio et al. (2017) gave casein supplementation (54 g) for 8 weeks in the morning (any time before 12 pm) compared to evening supplementation (90 minutes or less prior to sleep) to investigate the influence on body composition and strength performance outcomes in free-living men and women.[91] The authors reported no differences in body composition or performance between the morning and evening casein supplementation groups. In addition, Joy et al.,[92] designed a similar study and reported that after 12 weeks of pre-sleep vs. daytime casein, there were no differences in outcome measures between groups. In contrast, data from Burk et al.[93] indicate that casein-based protein consumed in the morning (10 am) and evening (10:30 pm) was more beneficial for fat-free mass than consuming morning (10 am) and afternoon (~3:50 pm) protein when young, high school–aged boys were resistance training. Thus, it appears that daily protein needs are most critical; however, protein consumption in the evening before sleep is also an underutilized time to take advantage of a protein feeding opportunity that (1) will not blunt lipolysis or fat oxidation and (2) may improve muscle protein synthesis and metabolism. Indeed, taking advantage of the pre-sleep window to consuming protein is a nutrition approach that may improve overall protein intake and meet recent recommendations for protein consumption[94] and nutrient timing.[65]

14.13 PROTEIN AND SATIETY

Increased dietary protein intakes are associated with increased satiety, particularly during negative energy balance of weight loss and long-term weight maintenance and therefore serve as an effective dietary strategy.[45,95] Protein is the most satiating macronutrient, followed by carbohydrate, then fat.[96] The difference between appetite and hunger is appetite is associated with our desire to consume food and is often influenced by emotional, behavioral, environmental, and certain biological cues. Hunger is our physiological need to eat, and this drives food consumption. Foods that inhibit further consumption produce satiety and a delay in the onset of the next meal. A food that is considered to have a high level of satiety is one that produces a long period of time between feelings of hunger. Diets containing increased protein are associated with reducing appetite and thus reduced caloric consumption. Two studies conducted in a respiration chamber by the same investigators directly assessed increased protein intake and satiety. The first study showed that consuming a higher protein and carbohydrate and lower fat (29%, 61%, and 10% of energy intake, respectively) compared to a lower protein and carbohydrate and higher fat (9%, 30%, and 61% of energy, respectively) diet, both equal in total kcals, reported higher levels of satiety.[97] In the second study, healthy women consuming higher protein (30% of total intake) versus lower protein (10% of total intake) resulted in significant increases in metabolic rate, postprandial thermogenesis, and satiety, and less food consumption over a 24-hour period.[98] Taken together, increased dietary protein intakes, and not the so-called "low-carb diet," are responsible for the enhanced satiety and energy metabolism in energy deficit and energy balance leading to greater weight loss and maintenance.[45]

14.14 PROTEIN, EXERCISE, WEIGHT LOSS, AND BODY COMPOSITION

A growing body of scientific evidence suggests that RDA for protein may not be ideal for specific populations and conditions, including older people, vegetarians, weight loss, exercise/fitness and sport performance, sickness and disease. The International Protein Board addresses several key points of debate related to current protein requirements. First is the reliance on data from nitrogen balance assessments, the original methodology used to determine protein requirements over a century ago, which may not be the most accurate and comprehensive method.[99–101] Indeed, more current and sophisticated methodologies, such as indispensable amino acid oxidation (IAAO), suggest base protein requirements may be higher, especially for older people.[102] Second is whether current protein requirement standards were even designed for dietary planning. In the case of RDAs, they were established to provide a standard for minimum levels of dietary protein intake without susceptibility to nutritional inadequacy and the development of signs of protein deficiency, and not necessarily dietary planning. Minimum protein requirements are typically only meant to cover age groups as well as pregnancy and lactation. But, it is now clear that current protein requirements are likely too low for certain conditions such as older people and those deriving dietary protein from plant and other non-animal sources, as well as those with sickness and disease or involved in weight loss, exercise, and sport performance (https://www.internationalproteinboard.org/protein-matters/protein-requirements.htm). Diets deficient in dietary protein, especially leucine, often lead to a decrease in muscle mass and physical performance.[103] During and after exercise, leucine is a favored amino acid oxidized and used to promote protein synthesis by the muscles, respectively.[104,105] A compilation of recent data strongly supports the benefit of higher protein intakes during resistance exercise training to improve body composition[106–109] and does so with no harmful effects.[110,111]

An exciting area of new research has examined the combined effects of higher protein intakes, using protein pacing, with various exercise training interventions on body weight control and composition. In two separate 12-week intervention studies,[43,44] investigators systematically examined the effects of protein pacing (27%–40% of total kcals as protein) compared to normal protein intake (19% of total kcals) with or without exercise training in 87 overweight/obese men and women on body weight and composition. Meal plans consisted of protein pacing (four to six meals/day of 27%–40% of total kcals; 1.3–2.2 g of protein per kilogram body weight per day) or normal (19% of total kcals; 1.1 g of protein per kg BW/day) protein. The exercise training interventions consisted of either combined resistance (4 days/week) and interval (2 days/week) exercise training with protein pacing or cardiovascular/aerobic exercise (6 days/week) training in accordance with the American Heart Association exercise guidelines with normal protein intake. In both studies, the exercise treatment groups all lost weight and body fat, but the protein pacing groups with and without resistance/interval exercise training resulted in significantly greater weight loss and improved body composition compared to the normal protein intake with cardiovascular exercise training. In fact, the protein pacing and exercise groups lost twice as much weight and total and abdominal body fat compared to the normal protein and exercise group, which corroborates previous research.[112] As an extension to these experiments, the same authors conducted a series of studies incorporating protein pacing with a multi-component fitness program of resistance, high-intensity intervals, stretching, and endurance exercise, termed PRISE® compared to other commonly recommended exercise programs. In the first,[25] overweight/obese men and women were divided into three groups: (a) PRISE, (b) protein pacing and resistance (P-RT), and (c) protein pacing only (P), and followed for 16 weeks. The protein intake for all three groups was approximately 1.6 g/kg BW per day. At the end of the 16-week trial, all groups lost significant weight and total and abdominal body fat, with PRISE losing the most. In addition, PRISE and P-RT groups had significant increases in the proportion of lean body mass, with the PRISE group gaining significantly more (Figure 14.7).

It is important to highlight that protein pacing as part of the PRISE protocol is equally effective when consumed as either whole food sources or supplemented with whey protein in terms of weight loss and improved body composition and physical performance outcomes in both men and women.[28] The final two studies involved highly trained men and women utilizing the PRISE protocol with (PRISE) and without protein pacing (RISE) over a 12-week trial.[26,27] The PRISE group's protein intake was 2.1 g of protein per kg/BW per day for both men and women, whereas the RISE group's protein intake was 1.1 g of protein per kg/BW per day for both men and women. At the end of the intervention, all groups had significant improvements in all body composition measures (loss of

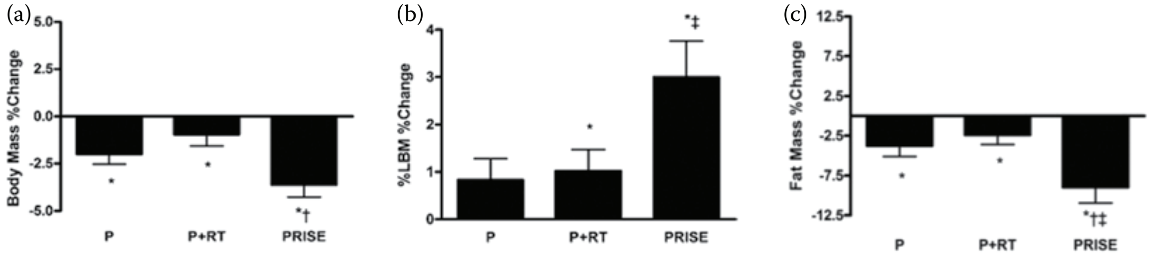

FIGURE 14.7 Changes in body mass, lean body mass, and fat mass following 16 weeks of protein pacing and exercise training. (a) Body mass percent change, (b) percent lean body mass (LBM) change, and (c) fat mass percent change.

body fat both men and women, increased lean body mass in women). But the greatest effect was observed in physical performance outcomes. The PRISE groups (men and women) significantly outperformed the RISE only group on nearly every performance test of muscular strength (men, upper body), power (women, upper body; men, lower body), and endurance (women), core strength (women), as well as aerobic power (men). Taken together, these findings have valuable implications for enhanced body composition as well as general fitness and sport/athletic performance, suggesting that combined protein pacing (~1.6–2.1 g of protein per kg/BW per day) and multi-component fitness training (PRISE) provides superior body composition and physical performance in overweight/obese and normal-weight trained men and women. These data corroborate recent work by Ormsbee et al. (2018),[113] who had young sedentary men and women complete 5 days/week of concurrent training (strength and endurance) while consuming either 84 g/d of protein or 84 g/d of a carbohydrate placebo (42 g twice per day). In support of these findings, Ormsbee et al.[113] had young sedentary men and women complete 5 days/week of concurrent training (strength and endurance) while consuming either 84 g/d of protein or 84 g/d of a carbohydrate placebo (42 g twice per day).

14.15 CONCLUSIONS

The current recommended dietary allowance for protein of 0.8 g/kg/d is too low for most individuals, and a target value for protein intake should begin around 1.2–1.6 g/kg/d based on the current evidence. These targeted protein intake values are the top priority for protein needs. However, once the daily protein needs are met, data support protein pacing to (1) practically encourage regular protein intake and (2) provide a stimulus for muscle protein synthesis, enhanced postprandial thermogenesis, and appetite suppression for individuals looking to optimize body composition and, potentially, enhanced physical performance (strength, power, endurance, etc.). In addition, if total daily protein intake is difficult to achieve, recent literature suggests that the addition of a pre-sleep protein-centric beverage or small meal will not only help to increase total daily protein consumption but may also improve overnight muscle protein synthesis and recovery as well as metabolism without causing fat gain. Lastly, combining protein pacing with a multi-component fitness training regimen such as RISE (PRISE) produces additional body composition, cardiometabolic, and physical performance benefit compared to standard protein intakes and traditional resistance and/or cardiovascular (aerobic) exercise training. In sum, the bulk of the existing evidence suggests that protein can and should be used as a functional food ingredient to optimizing weight loss, body composition, and physical performance.

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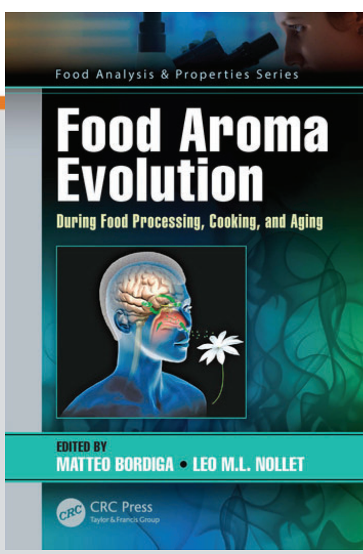
CHAPTER 4

EXTRACTION METHODS OF VOLATILE COMPOUNDS FROM FOOD MATRICES



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CHAPTER 6

Extraction Methods of Volatile Compounds from Food Matrices

Arthur Luiz Baião Dias, Francisco Manuel Barrales, and Philipe dos Santos

CONTENTS

6.1 INTRODUCTION

Volatile compound fractions in food matrices are associated with the aromas and flavors that are constructed during the preparation, cooking, and consumption of the food. A multifaceted series of factors can affect the perception, quantification, and analysis of the food's flavor. Generally, the analysis involves the identification and quantification of an analyte or a group of analytes. According to Sides, Robards, and Helliwell (2000), odor analysis involves the determination of a physiological concept via physicochemical measurements of a complex system aimed to the detection of a wide range of compounds and this introduces some unique analytical constraints. Generally, the food flavor is composed of volatile organic compounds (VOCs), as well as their derivatives, and other nonvolatile compounds. Several factors can complicate the analysis of the food's flavor. First, a recurrent problem is identifying the compounds contributing to the food aroma, which is generally represented by many classes of compounds and may be present in low or trace concentrations (Sides, Robards, and Helliwell, 2000). Another group of factors that can influence an analysis are the differences in the physical proprieties of those compounds, since each class of compound or each compound has specific thermodynamic proprieties and can interact with the matrix and solvents in many ways, which affects the extraction and separation. However, the use of an adequate technique can facilitate the separation of a specific compound or a group. Thus, the objective of this chapter is to provide an overview of the different methodologies to extract, isolate, and purify volatile compounds from food matrices. The methods are arranged according to the sample's physical state.

6.2 EXTRACTION METHODS FOR LIQUID SAMPLES

6.2.1 Liquid–Liquid Extraction

Liquid–liquid extraction (LLE) is based on the relative solubility of two different immiscible liquids, usually a polar and a nonpolar solvent; therefore, this technique has also been referred to as immiscible solvent extraction. Usually, one phase is aqueous, and the other is an organic solvent, where the analyte is transferred from one phase to the other liquid immiscible phase, due to the chemical potential of the solutes. The immiscibility of the solvents allows for an easy separation of the phases in a separatory funnel, where the lower layer can be collected through the bottom, and the upper layer can be removed through the top, since the position of each phase in the funnel depends on the densities of the liquids (Wells, 2003). The LLE method has disadvantages compared to other food flavor extraction methods, such as the exposure to large volumes of organic solvents and the formation of emulsions. Moreover, this method has a limitation when extracting and isolating flavor compounds from food matrices containing a significant content of lipids, because this method also extracts lipids. Therefore, in these cases, further purification steps are required for the separation and isolation of the flavor compounds. Despite the simplicity of the method, there is a tendency to replace LLE with other techniques, due to, on the one hand, trace analysis requiring expensive high-purity solvents, and on the other hand the elevated use of environmentally damaging and unhealthy solvents associated with this technique (Augusto, Lopes, and Zini, 2003; Wells, 2003). To overcome these drawbacks, other sample preparation techniques have been developed and implemented; for example, solid-phase extraction (SPE) and solid-phase microextraction (SPME). Meanwhile, there are several papers that have used this technique for food aroma analysis, such as for wine and grape varieties (López and Gómez, 2000; Rocha et al., 2000; Mestres, Busto, and Guasch, 2000), and for the isolation of volatile compounds in fruits and vegetables, for example cherry tomatoes (Selli et al., 2014), and oranges (Kelebek and Selli, 2011), and for the extraction of rose water (Canbay, 2017). The wide applications of this technique is evidence of its success for food aroma analyses; however, according to Varlet, Prost, and Serot (2007), LLE is not recommended for recovering the volatile aldehydes in smoked fish. Moreover, according to Marsili (2016), researchers identified 80 neutral volatiles in raw milk from different species using vacuum distillation and liquid–liquid extraction followed by high-resolution gas chromatography (HRGC). Also, despite the significant number of volatiles identified, the results of their work did not identify which compounds were the most important to good milk flavor. Therefore, this method has some restrictions and limitations. However, it is an excellent preliminary test for research of food flavoring compounds due to the simplicity and low cost.

6.2.2 Liquid–Solid Extraction

Liquid–solid extractions (LSEs) were used to concentrate semi-volatile compounds from liquids into a solid. In summary, the liquid sample is placed in contact with the bulk solid extracting phase, and after a determined period an equilibrium is established between the two phases. Next, the physical separation of the solid and liquid phases is performed, by decanting or filtering, and finally the extraction from the solid is conducted with a suitable solvent to isolate the volatile compounds. Different modifications to the LSE of volatile organic compounds have developed several extraction techniques; for example, solid-phase extraction, solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE) (Wells, 2003).

6.2.3 Solid-Phase Extraction

Solid-phase extraction was a technique developed in the mid-1970s and was introduced in the market in the 1980s (Panighel and Flamini, 2015). This methodology of sample preparation is based on the sorption of the analyte onto a cartridge and its recovery by elution using a suitable solvent, resulting in a concentration and purification/isolation of the target compound or a class of compounds. The capability of the cartridge to extract some target compound depends on the bed sorbent, sample volume loaded, and the characteristics and volume of the solvents and eluents used in the analysis. The performance of the method can also be affected by the breakthrough volume, which is defined as the maximum volume of sample that can be introduced into the sorbent (Panighel and Flamini, 2015; Wells, 2003). The SPE technique is composed of four stages: (i) column preparation; (ii) sample loading; (iii) column post-wash; and (iv) sample desorption, as shown in Figure 6.1. However, recent advances in sorbent technology removed the column preparation step. The objective of the pre-wash or column preparation and post-wash

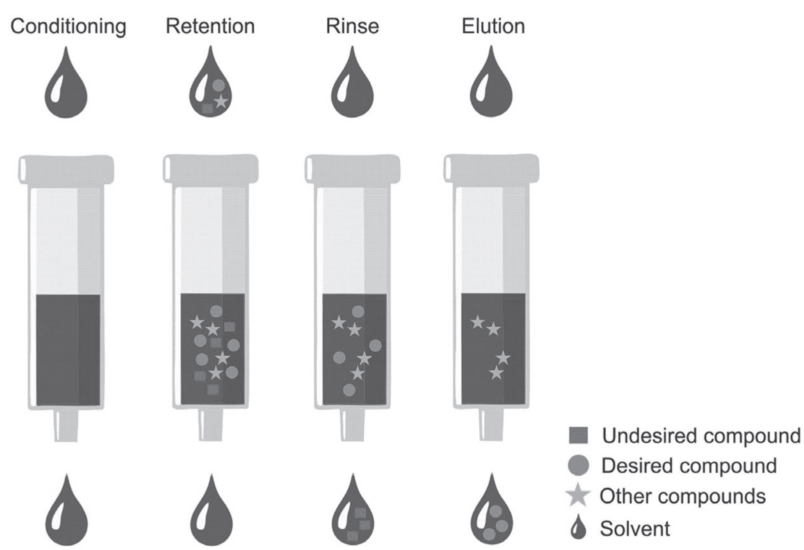

FIGURE 6.1 Four basic steps for solid-phase extraction: (1) Conditioning the sorbent prior to sample application ensures reproducible retention of the compound of interest; (2) Retention of the adsorbed isolate, undesired matrix, and other undesired matrix compounds; (3) Rinse the column(s) to remove undesired components; (4) Elution of desired components remain.

stage is a condition of the stationary phase and removal of undesirable contaminants, respectively. Generally, the target compounds are retained on the sorbent, the interferers go through the stationary phase, and an elution solvent with an appropriate solvent recovers the adsorbed analytes. Usually, in analytical procedures, SPE is carried out using small columns or cartridges containing the solid stationary phase (sorbent). In volatile compounds analysis, the most common sorbent used is octadecyl (C18); this material allows a reversed-phase extraction of mid-polar to nonpolar analytes (Wells, 2003). SPE has been widely used in grape and wine volatiles analysis (Campone et al., 2018; Picard et al., 2018; Weldegergis et al., 2011; Williams et al., 1982). SPE has also been applied to characterize butter flavor (Vreuls et al., 1999), to aromatic compounds from fruit pulps (Boulanger and Crouzet, 2000), to the analysis of flavor-related to alkylbenzenes in cigarette smoke (Stanfill and Ashley, 2000), and to other nonvolatile compounds present in food matrices. For more details and examples, the papers published by Andrade-Eiroa and collaborators (Andrade-Eiroa et al., 2016a,b) give an excellent review of SPEs.

6.3 EXTRACTION METHODS FOR LIQUID OR SOLID SAMPLES

6.3.1 Static Headspace Extraction

This technique is over 30 years old and has been applied to a variety of food matrices for the extraction of volatile organic compounds, such as fish products (Fukami et al., 2002; Girard and Nakai, 1991; Duflos et al., 2006; Li et al., 2013) and vegetables (Colina-Coca et al., 2013; Molina-Calle, Capote and de Castro, 2007), among others. The versatility of this method allows the sample to be solid or liquid. Meanwhile, its low sensitivity does not allow for the analysis of trace or high boiling point compounds (Pico et al., 2016). The most significant advantage of the static headspace extraction technique is the simplicity of the liquid samples preparation. The preparation involves only transferring the sample into the vial, typically of 10 to 20 mL, and sealing it immediately. Meanwhile, for solids, the sample must be ground to increase the superficial area available for the analyte volatilization to the headspace, and occasionally the solid sample might be dissolved or suspended in a liquid to attain equilibrium inside the vial faster. Once the sample is inside the vial and hermetically sealed, it is incubated at a controlled temperature. The volatile analytes diffuse to the headspace of the vial. When the equilibrium between the gas and liquid (or solid) phase is achieved, an aliquot of the headspace gas is injected into a gas chromatograph (GC) for analysis, as illustrated in Figure 6.2a. The last step may be manual or automatic (Slack, Snow, and Kou, 2003). The automatic system consists of three stages: (i) Equilibrium; (ii) Pressurization; and (iii) Sampling, as illustrated in Figure 6.2b.

(i) *Equilibrium*: This is the most critical stage. Special attention must be given to equilibrium temperature and time when developing a method. The equilibrium time is considered once the sample is inserted into the vial and sealed, until the insertion of the sample needle into the vial. Each compound migrates from the sample matrix to the gas phase at its own rate, according to the temperature. Therefore, the minimum equilibrium time depends on the slowest diffusion compound. High volatility compounds might start the migration to the gas phase during the vial preparation, occasioning the loss of some volatile compounds before they arrive in the vial. In these cases, it is recommended to keep the preparation of the samples at a low temperature. In addition, there are different migration

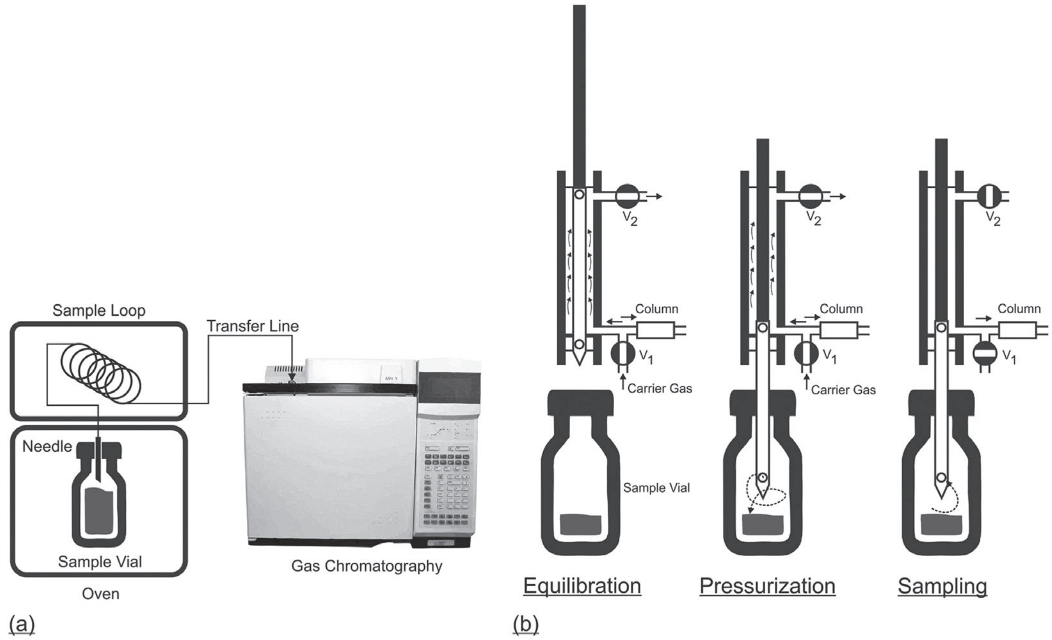

FIGURE 6.2 Schematic diagram of headspace extraction autosampler (a) and the steps for balanced pressure sampling in GC headspace analysis (b). (Adapted from Kolb 1999.)

rates from the sample matrix to the headspace, since each compound has its own solubility in the sample matrix, which also depends on the temperature. Usually, the rise of temperature diminishes the solubility of the VOCs, increasing the concentration of the analyte in the headspace at higher temperatures. It is recommended to use a temperature of at least 15°C over room temperature to ensure the correct temperature control. Special attention must be given to higher temperatures, which may cause degradation of thermolabile compounds.

(ii) *Pressurization*: Once equilibrium is attained, the headspace gas is ready to be transferred into a GC. The most common transference mechanism involves the pressurization of the vial headspace with an inert gas, using a heated hollow needle, followed by a pressure release into the pneumatic sampler, through the same needle. Another strategy is to use a system with a sampler loop. In this case, the pressure inside the vial will transfer the sample into an internal sampler loop, from where the sample accesses the GC inlet by actioning the sampler valve.

(iii) *Sample transference*: After pressurization, the gas sample into the vial flows to the pneumatic sampler through the needle, moved by the pressure gradient established during pressurization.

6.3.2 Dynamic Headspace Extraction (Purge and Trap)

Dynamic headspace extraction (DHE) may be used for liquid as well as for solid samples. It is mainly used when there is a small quantity of the analyte in the sample, at trace level, or when an exhaustive extraction is needed, and it has been applied for a variety of matrices, such as sponge cake (Pozo-Bayón et al., 2007), fruit (Mamede and Pastore, 2006), and meat (Madruga et al., 2009), among others. However, it must be considered that DHE only allows for measurement of the ratio of specific volatile compounds

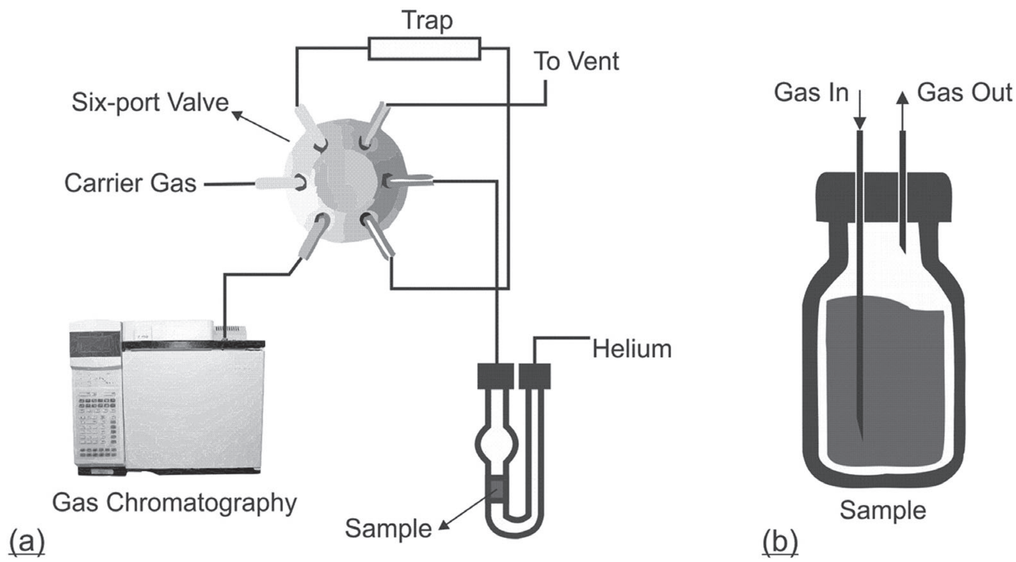

FIGURE 6.3 Schematic diagram of a typical purge and trap–GC system (a), and the needle sparger for purge and trap (b). (Adapted from Zang et al. 2017.)

concentrated over the sorbent surface. Meanwhile, static headspace extraction (SHS) allows the measurement of the ratio of all of the volatile compounds contained in the gaseous phase inside the vial (Pico et al., 2016). DHS is similar to SHS; however, the VOCs are removed continuously from the sample by a continuous inert gas flow. In this way, a concentration gradient that favors the process exists. The system is composed of a purge vessel, a sorbent trap (usually Tenax-TA®), a six-way valve, and transference lines, as illustrated in Figure 6.3a. The sample is inserted into the purge vessel, and a purge gas (usual helium) passes through the sample continuously carrying the VOCs into the trap, where the sorbents retain them. When the purge ends, the trap is heated to desorb the analytes into the GC (Soria, García-Sarrió, and Sanz, 2015). There are three kinds of purge vessels: (i) frit spargers; (ii) fritless spargers; and (iii) needle spargers. Frit spargers (Figure 6.3a) are used for liquids that are relatively clear, that are not prone to foam, and that have solid particles that may clog the system. Fritless spargers and needle spargers (Figure 6.3b) are recommended for particulate systems and liquids with proteins that are prone to foam. The trap is usually a stainless-steel tube of 3 mm (ID) and 25 mm long, packed with different layers of sorbents. The trap must retain the analyte and not introduce impurities. The system operation consists of a series of steps: (i) Purge, a period of 10 to 15 minutes, wherein the carrier gas extracts the VOCs and leads them to the trap, the samples might be heated by an electrical heater or by microwaves to accelerate the mass transference from the matrix to the gas phase; (ii) Dry purge, wherein the carrier gas only passes through the trap, and not through the sample in order to eliminate the water that might be carried out into the trap along with the analyte; (iii) Desorption preheating, wherein the carrier gas is turned off and the trap is heated to 5 to 10°C below the desorption temperature, to accelerate desorption; (iv) Desorption, wherein the trap is heated to 180 to 250°C and the GC gas carrier is turned on in a reversed flow, for about 1 to 4 min. The gas carrying the VOCs is conducted into a GC inlet; (v) Trap bake, the gas flow returns to the initial direction and the trap temperature rises to 15°C above the desorption temperature to eliminate possible contamination in the trap and gets the system ready for the next sample (Slack, Snow, and Kou, 2003).

6.3.3 Solid-Phase Microextraction

This technique was first developed by Belardi and Pawaliszyn (1989) while looking to reduce the costs and time employed to perform liquid–liquid extraction and solid-phase extraction. This method has been commercially available since 1993 and has been widely applied since then on several matrices, such as fish products (Jiménez-Martín et al., 2015), fruit (Chen et al., 2018; Sdiri et al., 2017), off-flavors (Marsili, 1999; Matsushita et al., 2017), and spices (Korkmaz, Hayaloglu, and Atasoy, 2017), among others. Solid-phase microextraction (SPME) is a microscale approach to solid-phase extraction for the extraction and preconcentration of analytes. The SPME methodology is based on the partitioning of analytes between a reusable coated fiber (a stationary phase) and a sample. In SPME, the analyte molecules must migrate from the sample and penetrate into the fiber coating, so the mass transfer resistance must be overcome to reach the equilibrium or adsorption equilibrium, and then the fiber is inserted into the GC inlet to desorb and analyze the analytes (Merkle, Kleeberg, and Fritsche, 2015). There are two possible approaches for the analyte adsorption process into the fiber, direct and headspace (HS-SPME). In direct adsorption, the fiber is immersed into the sample matrix or in a solution containing the sample. Thus, the fiber might be exposed to nonvolatile compounds that will contaminate the sample and may affect the chromatography and reduce the number of times that the fiber might be reused. On the other hand, in HS-SPME, the fiber is inserted into the vial's headspace, which contains the sample, the vial is heated, and the volatile compounds are transferred from the liquid phase to the gaseous phase, and then they are absorbed into the fiber. Therefore, only volatile compounds reach for the fiber, avoiding undesirable compounds (Slack, Snow, and Kou, 2003). There are several fiber coatings on the market, which may be arranged in three groups, polar, semi-polar, and nonpolar. To choose a fiber coating, one has to consider the nature of the analytes. A fiber coating with similar polarity to that of the analyte will favor its adsorption. Thus, the extraction will be selective, reducing the chance of extracting contaminant compounds (Valente and Augusto, 2000).

SPME exhibits several advantages over traditional extraction methods, such as to be a rapid, simple, sensitive, and solvent-free method, and have linear results for a wide range of concentrations and analytes (Nerín et al., 2009). However, the disadvantages of SPME are the limited number of commercially available stationary phases (fiber coatings), low recommended operating temperature (240–80°C), the instability and swelling in organic solvents, breakage of the fiber, stripping of coatings, bending of the needle, and the cost. Other disadvantages are the limited lifetime of the fiber and the low extraction efficiencies (Nerín et al., 2009; Merkle, Kleeberg, and Fritsche, 2015). SPME has been extensively applied to the sampling and analysis of aroma in several raw materials and food products. Optimization processes for this method involve the selection of the fiber coating, as well as the fiber diameter, and the extraction conditions, stirring, temperature, direct immersion or headspace, vial volume, sample volume, equilibrium time, fiber exposure time, and fiber preparation and conservation.

6.4 EXTRACTION METHODS FOR SOLID SAMPLES

In general, analysis of aromas or volatile compounds from plants or foods involves two steps: extraction and analytical methods. Extraction of analytes aims to separate the target compounds from the matrix and increase their concentration level. The sample preparation is an important step which determines the quality of the analysis, and it is also the primary source of systematic errors and the lack of precision of analytical methods (Armenta, Garrigues, and de la Guardia, 2015). A pre-treatment is required before extraction, aiming at the reduction of the particle size and an increase in the diffusion of analytes from the sample to the solvent. The choice of appropriate parameters (e.g., solvent, sample size, pressure, temperature, number of cycles, and extraction time) is necessary to optimize the process. Moreover, extraction efficiency is mainly influenced by three factors: the solubility of an analyte on a solvent, the mass transfer properties, and the matrix effects (Kou and Mitra, 2003).

6.4.1 Conventional Methods

Conventional extraction methods, such as hydro-distillation (HD), simultaneous distillation extraction (SDE), Soxhlet extraction, and ultrasound-assisted extraction (UAE) are operated under atmospheric pressure and under heating. These methods consume a large amount of solvent and may have a long extraction time. On the other hand, there are other "green" and "innovative" extraction methods, such as supercritical fluid extraction (SFE), pressurized fluid extraction (PLE), and microwave-assisted extraction (MAE) that are faster, demand less consumption of solvent, and require less energy to operate (Kou and Mitra, 2003). Soxhlet is a benchmark method used for the extraction of semi-volatile organics from solid samples. The main advantages are the independence of the matrix, the low cost of the equipment, and that further filtration is not required. However, the disadvantages are the long extraction time and the relatively large amount of solvent consumed. Study of this methodology to quantify aroma compounds of promising application in food industries has been conducted over the years. Recent works have identified volatile substances from wheat breadcrumb and gluten-free flours (Pico et al., 2016) and cornstarch (Pico et al., 2018). Soxhlet extraction was also used to identify volatile compounds from grape seed oils (Al Juhaimi and Özcan, 2018).

6.4.2 Ultrasound-Assisted Extraction

UAE can enhance the extraction yield since it increases the mass transfer between the solvent and plant matrix. The cavitation bubbles lead to a cell disruption near the solid surface, which improves the solvent penetration and can also break the cell walls. Among the advantages of UAE are less dependency of the solvent, better solvent penetration, extraction at lower temperatures, reduced extraction time, a higher yield of extracted compounds, and faster start-up. Disadvantages are the amount of solvent required and possible extraction evaporation. A study compared the extraction of volatile compounds from tea leaves using Soxhlet, ultrasound-assisted extraction, and simultaneous distillation extraction. It was observed that Soxhlet obtained the highest extraction yield (Gao et al., 2017). In another study, the quantification of volatile compounds from *Schinus terebinthifolius* Raddi fruits was performed by UAC. The authors noticed that high yields of the extracts might be due to the extraction of high-molecular weight compounds (e.g., triterpenes and carotenoids) (Silva et al., 2017). Ultrasonic waves are mechanical vibrations applied to solids, liquids, or gases with frequencies exceeding 20 kHz. Such waves are different from electromagnetic waves since they need matter to propagate. Their frequency and wavelength characterize them, and the mathematical product of these parameters results in the wave velocity through the medium (Wang and Weller, 2006).

Ultrasonic waves cause effects of expansion and compression on the matter. The expansion can create bubbles in a liquid and produce negative pressure, while the collapse of the formed bubbles can cause cavitation. The collapse of bubbles near the cellular walls produces cellular disruption, and as a result, there is better penetration of the solvent into the cells, and consequently an increase in the mass transfer (Esclapez et al., 2011). Figure 6.4 is a schematic representation of the effects of the ultrasound process on the interface of a plant matrix.

In Figure 6.4, the formation of bubbles can be seen in the first stage, (a). Such bubbles undergo expansion and compression (b), which will cause their collapse or implosion (c). Eventually, should this collapse occur near the array interface, it can generate shock waves that will disturb the wall of the matrix, consequently releasing the intraparticular material into the solvent (d) (Esclapez et al., 2011). An extremely important factor in ultrasonic-assisted extraction is the extractor configuration, which may have the transducer coupled to the extraction vessel or have an ultrasonic probe immersed in the solvent/matrix medium. Figure 6.5 shows some extractor geometries schematically with ultrasonic waves.

Currently, the most widely used laboratory scale extraction systems are ultrasonic baths or direct sonification. Ultrasonic baths consist of a transducer coupled to a tank containing a liquid responsible for transferring the waves to a container containing the extractive matrix, according to Figure 6.5a. The disadvantages of this geometry are the lack of uniformity of ultrasound wave distribution. Thus, direct sonification emerged as an alternative to improve the distribution of the ultrasonic waves through the extraction medium. This system consists of inserting an ultrasonic probe directly into the solvent/matrix mixture, as shown in Figure 6.5b and c (Esclapez et al., 2011). The use of ultrasound was conducted in the extraction of aroma compounds from aged brandies, tea, wine, and garlic, in the extraction of antioxidants from pomegranate peel, and carotenoids from tomato waste (Chemat et al., 2017). Moreover, some studies extracted lycopene from tomatoes,

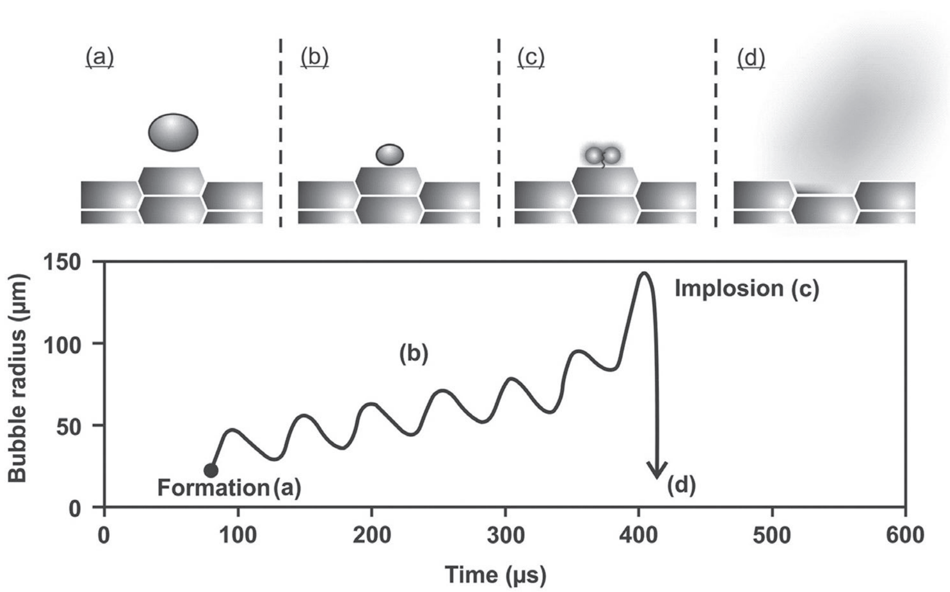

FIGURE 6.4 Schematic representation of the effects of the ultrasound process on the matrix vegetable. (Adapted from Esclapez et al., 2011; and Capote and de Castro, 2007.)

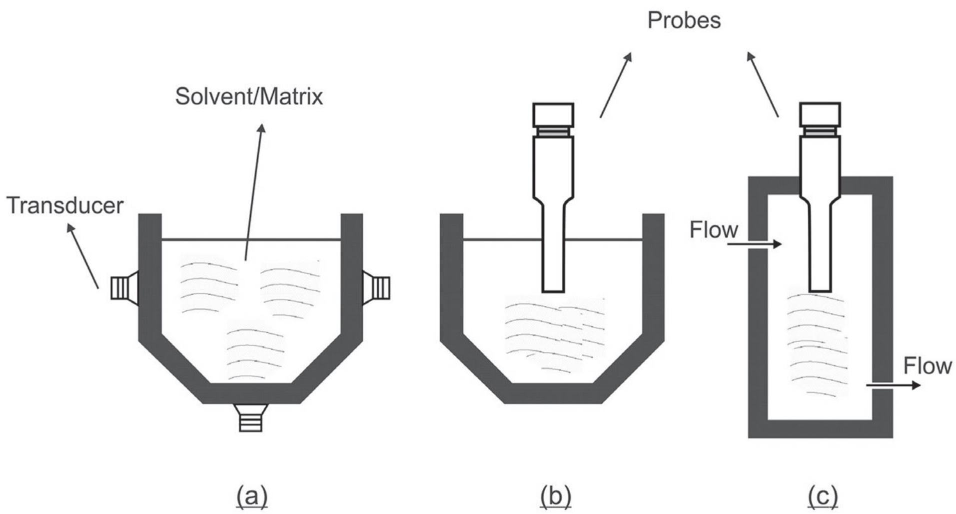

FIGURE 6.5 Typical extractor configurations: (a) transducer coupled to a vessel; transducer (probe) immersed in solvent/plant matrix medium, (b) in batch and in continuous (considering the fluid/solvent) (c). (Adapted from Esclapez et al., 2011.)

phenolics from strawberries, citrus peel, and coconut shell powder, anthocyanins from red raspberries, and capsaicinoids from peppers (Chemat and Khan, 2011).

6.4.3 Microwave-Assisted Extraction

Microwave-assisted extraction works with the dissipation of the electromagnetic waves in the irradiated medium through heat. Among the main advantages of this technique are the reduced costs, easy equipment manipulation, higher purity of the final products, reduced extraction time, and energy consumption. However, MAE requires a post-extraction step (e.g., cooling and filtration) which can extend the process and it is quite an exhaustive process including interfering species that need cleanup before the analysis. For food production, it is worth mentioning that this method allows the reduction of the equipment size, a faster response to heating processes, and increases of the production and elimination of post-treatment steps (Chemat et al., 2017). Solvent-free microwave extraction (SFME) is an adaption of MAE that uses a combination of microwave heating and dry distillation under atmospheric pressure without the use of solvent or water. This process has been used to extract aroma compounds from citrus fruit (e.g., orange [Ferhat et al., 2006] and lemon [Ferhat et al., 2007a]), aromatic herbs (e.g., basil, mint, and thyme) and spices (e.g., cumin and anise [Lucchesi, Chemat, and Smadja, 2004]). In a recent study comparing SFME with a conventional hydro-distillation extraction to obtain essential oil from *Origanum vulgare* L, it was observed that SFME was more efficient with a higher extraction yield, and in a shorter extraction time (Bayramoglu, Sahin, and Sumnu, 2008). Another study compared microwave-assisted simultaneous distillation-solvent extraction (MW-SDE) with conventional SDE in the extraction of volatile compounds from fresh aromatic herb (*Zygophyllum album* L.). It was noticed that MW-SDE was faster, required less solvent amounts and less energy in comparison to conventional SDE extraction (Ferhat et al., 2007b).

6.4.4 Supercritical and Pressurized Fluid Extraction

Among the innovative extraction processes, SFE appears as an alternative to the use of conventional methods. SFE is considered a green methodology that aims to obtain compounds with reduced energy requirements, shorter extraction times, and using solvents generally recognized as safe (GRAS). Supercritical fluids enhance transport properties, due to their high diffusivity and low viscosity, being able to readily diffuse through solid materials with faster extraction rates. Supercritical carbon dioxide (SC-CO_2) is the most common solvent used since it is a non-toxic, non-flammable, non-polluting, and low-cost solvent; it is relatively inert and can be recovered (Brunner, 2005). Moreover, it works at moderate critical conditions (Tc = 31.05°C and Pc = 7.38 MPa) that are crucial in the preservation of aroma compounds from extracts. However, the equipment costs can be a disadvantage of this technique when scale-up is aimed for. Another SC-CO_2 limitation is its low polarity making the process more appropriate for extracting nonpolar compounds.

The process of extraction with a supercritical fluid, specifically using CO_2, occurs in two stages: the extraction of the solutes and the separation of the solute from the solvent. The first step is to manipulate the carbon dioxide in the binomial pressure/temperature in order to obtain the highest solvation of the target solutes. The solvent flows into the extractor and through the entire plant matrix, solubilizing the solutes. The solvent/solute then mixture goes to the second step, in which the pressure is reduced below the critical point value. In this way, the solvent changes its state of supercritical aggregation to gas, reducing its power of solvation, and consequently precipitation of the solute occurs. Thus, the solute is recovered, and the gas is redirected into a recycle (Raventós, Duarte, and Alarcón, 2002; Martínez, 2007). Figure 6.6 shows the supercritical fluid extraction process. The recycle conducts the gaseous carbon dioxide into a condenser, where the gas

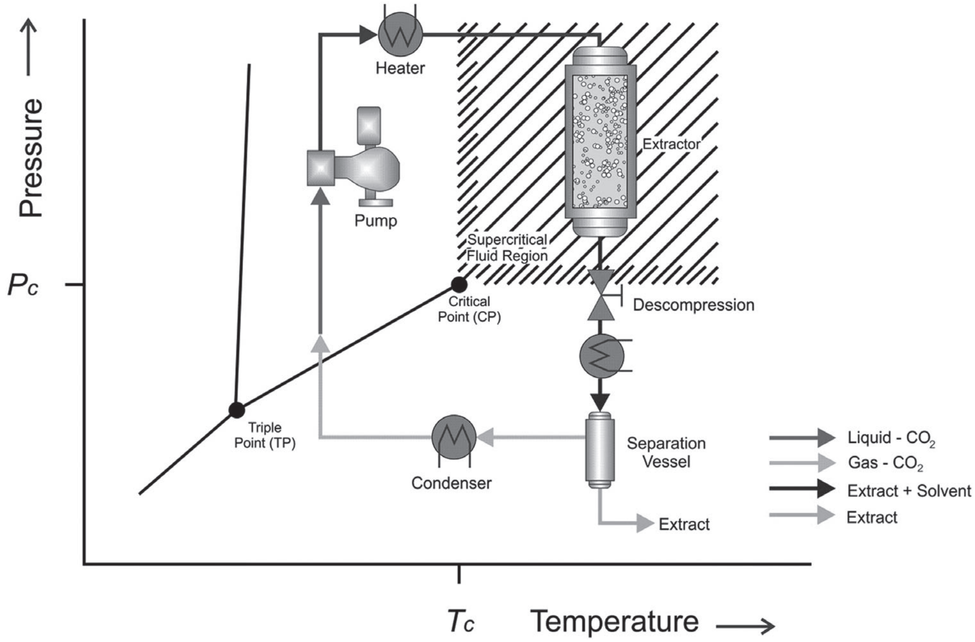

FIGURE 6.6 Flow diagram of a supercritical extraction process from solid matrices. (Adapted from Rosa et al., 2008.)

is liquefied. Thereafter, the CO_2 has its pressure increased above the pressure of the critical point, as a result of the work of a pump, and its elevated temperature up to the desired operating temperature by a heater (Rosa et al., 2008).

The use of SFE to obtain extracts enriched in a specific compound of interest for the food industries has been carried out throughout the years, such as the extraction of caffeine from green coffee beans, free fatty acids from rice bran oil, tocopherol from wheat germ oil, and beta-carotene from crude palm oil. SFE has been used in the fractionation of fatty acid ethyl esters from fish oil, extraction of essential oils from seeds of a pomegranate, walnut oil, carrot fruit, and carotenoids from pumpkins. The application of SFE was also studied before analysis of volatile compounds in beverages and sugar cane (Herrero et al., 2010). The use of SFE assisted by ultrasound (SFE-US) has emerged as an alternative for extracting and quantifying bioactive compounds from food samples, since the ultrasound waves can increase the rupture of the cell walls of the matrix enhancing solvent penetration and extraction yield. SFE-US was studied for the extraction of aroma compounds from ginger (Balachandran et al., 2006), essential oil from almond (Riera et al., 2004), antioxidants from blackberry (Pasquel Reátegui et al., 2014), tocopherol and tocotrienols from passion fruit (Barrales, Rezende, and Martínez, 2015), and capsaicinoids from peppers (Santos et al., 2015; Dias et al., 2016). Pressurized liquid extraction (PLE) is an alternative technique that uses liquid, like ethanol or/and water, as solvents at elevated pressure and temperature, enhancing the solubility and mass transfer properties of an analyte in a solvent. Moreover, the higher temperature diminishes the solvent's viscosity and surface tension, improving the solvent penetration in the matrix sample. PLE works with a reduced extraction time and has less solvent consumption, and it does not require a filtration step when compared to conventional processes, which is important taking into account the use of automated and online systems. Furthermore, the use of polar solvents, such as water and ethanol, covers the extraction of compounds of high and intermediate polarity. The main drawback of this technique is the cost of equipment. The use of PLE to obtain food aroma compounds was recently performed in the quantification of anthocyanins, phenolics, antioxidants, isoflavonoids, carotenoids, and capsaicinoids (Mustafa and Turner, 2011).

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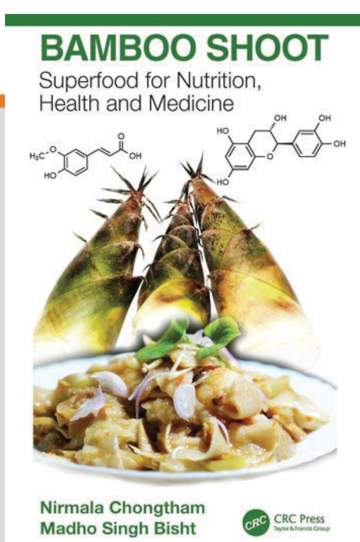
CHAPTER

5

BAMBOO SHOOTS AS FUNCTIONAL FOODS AND NUTRACEUTICALS



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8 Bamboo Shoots as Functional Foods and Nutraceuticals

Most foods are considered functional in terms of providing nutrients and/or energy to sustain basic life. However, in the last decade, consumer demands in the field of food have changed considerably. Rapid economic growth, urbanization and globalization are the main drivers for these changes. Different lifestyles and purchasing patterns have resulted in a change in diet which has modified production, distribution and marketing trends. With the economic development and the improvement of people's living standards, demand for natural foods, especially healthy and organic food, has greatly increased. At the same time, an increased number of working women caused a shift towards processed food, which requires less time for preparation. Moreover, increasing public awareness of the link between diet and health has boosted the consumption of these foods to unparalleled levels, particularly in countries where the population is aging and health care costs are rising. Scientific evidence and a growing awareness of the correlation between diet and health, coupled with sedentary lifestyles, an aging population, and ever-increasing health care costs have driven the interest in healthier food products (Malla et al. 2014). These products include functional or fortified foods and nutraceuticals that confer positive health benefits to consumers. Nutraceuticals and functional foods have not only captured the world food market but also the psyche of average consumers through the supply of rich nutrients to the body even by the simple popping of different supplement formats, for example, dietary supplements, capsules or pills. Both are intensively researched for their role in maintaining health and prevention of diseases. Functional foods are defined as products that resemble traditional foods but possess demonstrated physiological benefits. However, nutraceuticals are commodities derived from foods but are mostly used in the medicinal form of pills, capsules or liquids and again render demonstrated physiological benefits. In some countries, however, the term functional foods and nutraceuticals are used interchangeably. Regardless, the main focus of such products is to improve health and reduce disease risk through prevention. There is an increased interest both in functional foods and nutraceuticals, as they provide physiological and metabolic benefits by boosting the immune system and counteracting diseases and degenerative disorders. One of the most important trends in the food industry is the demand for all-natural food ingredients that are free of chemical additives and they are usually obtained from edible plants and bamboo is one such plant that can be utilized for this purpose.

8.1 FOOD FORTIFICATION AND FUNCTIONAL FOODS

Functional foods are defined as whole foods along with fortified, enriched, or enhanced foods that have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels (Crowe 2013). Consumers today demand foods that are sustainably produced and processed, deemed safe and have nutritional value and food producers have invested resources in the development of functional foods that may provide added benefits to consumer well-being (Granato et al 2020). Such types of food include foods supplemented with bioactive and mineral substances (e.g. probiotics, anti-oxidants, iodized salt) and derived food ingredients introduced to conventional foods (e.g. prebiotics). According to FAO report, it is estimated that 2 billion people worldwide suffer from micronutrient malnutrition and micronutrient deficiencies account for approximately 7.3% of global diseases. The most effective way of combating malnutrition is food fortification which is one of the many public health interventions towards mitigating micronutrient malnutrition as well as poor growth and development of children (Alina et al. 2019, Okeyo 2019). Fortification or enrichment refers to the addition of one or several essential nutritional elements to a food product towards the prevention or correction of proven deficiencies in one or more nutrients. In many cases, fortification targets restoring nutrients and/or enhancing nutrients lost during processing, enhancing nutrient levels of food vehicles that have limited content than what is required, and adding nutrients not usually present in food to some commonly consumed food vehicles for the purposes of boosting intake of that particular nutrient (Ottaway 2008). There is a large interest in fortifying food with vitamins, minerals, organic acids, dietary fibres, phenolic compounds, essential amino acids and anti-oxidants. Although a huge world population suffers from various nutritional deficiencies, people with low income are the most affected, particularly in developing countries. The most efficient and accessible way of providing nutrients is additional fortification using these substances in popular consumer food products especially flour and bakery products.

Food fortification and supplementation with nutrients are not new and have been carried out for centuries even before the scientific rationale became available. Nutrient supplementation of food was mentioned for the first time in the year 400 BCE by the Persian physician Melanpus who suggested adding iron filings to wine to increase soldier's potency (Meija 1994). However, it was between the first and second world wars (1924–1944) that supplementation was established as a measure to prevent nutritional deficiencies in populations or to restore nutrients lost during the processing of foods. Thus, during this period, the adding of iodine to salts, vitamins A and D to margarine, vitamin D to milk and vitamins B1, B2, niacin and iron to flours and bread was established (Murphy 1996). In Central Europe during the Middle Ages, mothers were known to push iron nails into apples, leave them for a while and then feed the apples to their ailing daughters. In 1824, the indigenous population of Columbia and South America treated goiter with a special type of salt which was later found to have a high content of iodine. In Mexico, while making traditional tortillas, the corn was first soaked in lime water and a pinch of ground limestone was added to the tortilla to enrich it with calcium. In 1831, French physician Bossingault urged adding iodine to salt to prevent goiter. The first official addition of iodide to domestic salt started in Switzerland in 1900 which later spread to other countries also. Currently, food fortification encompasses a broader concept and is done for several reasons: as a tool to correct or prevent widespread nutrients inadequacies and hence, correct associated micronutrient deficiencies to balance the total nutrient profile of diets, to restore nutrients lost during processing, to add nutrients that may not be naturally present in food, to make products more appealing to consumers and to provide certain technological functions in food processing such as adding a preservative or colouring agent to processed food. Food fortification is now one of the most effective methods of preventing nutritional deficiencies and has contributed to the virtual elimination of many diseases of goiter, rickets, beriberi and pellagra. The most commonly used fortified food products include ready-to-eat breakfast cereals, cereal bars, fat spreads, bread, milk and milk products and juices.

8.2 FORTIFYING FOOD PRODUCTS WITH BAMBOO SHOOTS

The quest for finding suitable natural ingredients to make popular healthy food items is gaining momentum. In recent years, attention is being paid to foods that have valuable amounts of minerals, vitamins, micronutrients and other bioactive compounds such as fibre and anti-oxidants. The most important elements used for food fortification are iron, iodine, vitamins, calcium, selenium, fibres, proteins and fatty acids (Alina et al. 2019). The concern for healthy diets and cost-effective health care among people has prompted the food industry to search for plants rich in nutrients and have nutraceutical properties and desirable functional characteristics. Bamboo shoots have been frequently assessed for various bioactivities due to their nutritional and therapeutic importance and overall application in the food industry. The juvenile bamboo shoots being rich in nutrients, health promoting bioactive compounds (phenols, phytosterols, dietary fibre), vitamins (vitamin A, vitamin B1, vitamin B3, vitamin B6, vitamin C, vitamin E), amino acids and minerals, hence, play a significant role in maintaining good health and is being projected as health food (Hiromichi 2007, Kumbhare and Bhargava 2007, Park and Jhon 2009, Chongtham et al. 2011). Nowadays, consumer's interest has inclined towards food products that can impart health benefits, increase longevity and reduce the risks of, or delay the onset of, diseases and disorders. Thus, formulation of novel food products by incorporating nutrients, bioactive compounds, dietary fibre and anti-oxidants from bamboo shoots, as ingredients for physiological benefits or disease prevention and control has drawn the attention of researchers, nutritionists, consumers and industrialists.

Bamboo shoot dietary fibre possesses a wide range of structural diversity and favorable functional properties that makes it an ideal ingredient for various functional and health foods and is gaining popularity in the food and nutraceutical industry. The utilization of bamboo fibre in fortifying bakery products, meat, sausage, beverages, spices, pasta and ketchup have been reported (Chongtham et al. 2011). Inclusion of bamboo dietary fibre in the diet has a beneficial effect on healthy digestion and lowering of lipid profile. Fortification of dietary fibre in food also lowers the fat content in deep-fried products, which will also solve the problems of obesity and various cardiovascular diseases due to the over-ingestion of high fat-containing food items. Ge et al. (2017) analyzed the effect of extrusion on various physiochemical and functional properties of bamboo shoot dietary fibre. It was observed that extrusion cooking enhanced the functional properties of bamboo shoot dietary fibre viz. swelling capacity, water holding capacity, cholesterol absorption capacity and fat absorption capacity. Several scientific studies have been conducted to analyze the effect of bamboo shoot dietary fibre on the physical and functional properties of food fortified with bamboo shoot dietary fibre. Felisberto et al. (2017a, 2017b) analyzed the nutritional and physiochemical properties of flour prepared from *Dendrocalamus asper* young culms and reported significantly higher quantities of fibre (67–79 g/100 g) and starch (6–16 g/100 g), thus highlighting the potential of young bamboo culm flour utilization in fibre enriched bakery and other food products. Zheng et al. (2017) analyzed the effect of the addition of bamboo shoot dietary fibre (BSDF) extracted from *Dendrocalamus latiflorus* on rheological behavior, microstructure and textural properties of milk pudding. They observed that the addition of 2 g BSDF was effective in improving all these features of milk pudding which is helpful in enhancing its food-industrial value. Zhang et al. (2017) investigated the effect of bamboo shoot dietary fibre (1.0%, 1.5% and 2%) on the mechanical properties, moisture distribution and microstructure of frozen dough. Results indicated that the addition of BSDF can improve the viscoelasticity, extensibility and plasticity of frozen dough and enhance its processing qualities thereby making it more suitable for the production of various processed food products such as bread and dumplings. Hemicellulose components of *Sasa senanensis*, a mixture of xylose and xylo-oligosaccharides (XOS) isolated by steaming and subsequent water extraction are reported to be a potential raw material in the development of functional food and pharmaceutical industries. Miura et al. (2013) reported the presence of xylitol in *Phyllostachys pubescens* which is converted from hemicelluloses through microbial activity. Since the compound has several health benefits such as anti-caries, anti-inflammatory and sweetening properties, it is of great interest in the food industries.

Interest in utilizing bamboo shoot which is rich in nutrients, bioactive compounds and minerals for the production of natural functional food is gaining popularity in the food industries (Chongtham et al. 2011, Santosh et al. 2019). Processing of shoots for long term preservation and removal of anti-nutrients for the production of value-added food products have been reported (Choudhury et al. 2012, Santosh et al. 2019). Bamboo shoots are now being used for food fortification to incorporate the nutrients and bioactive compounds to make (Table 8.1) several value-added products such as pickles, candies, nuggets, crackers, chutney, chips, cookies, chappatis and buns (Pandey et al. 2012, Bisht et al. 2012, Mustafa et al. 2016, Sood et al. 2013, Choudhary et al. 2012). Shoots can be used as fresh, dried or fermented for making various food items as described next.

8.2.1 Biscuits/Cookies

It is well established that malnutrition in early life can compromise the development potential of children and their later health and productivity as adults. To address the nutritional needs of young school going children and provide social protection to families, global efforts have largely focused on school feeding and school-based

TABLE 8.1
Contemporary Food Items Fortified with Bamboo Shoots

Fortified Product	Bamboo Species	Form Used	References
Amaretti cookies	Not mentioned	Bamboo fibre	Farris and Piergiovanni 2008
Crackers, Nugget, Pickle	*Bambusa bambos, B. tulda, Dendrocalamus asper, D. strictus*	Brine-treated boiled shoot	Pandey et al. 2012
Chicken nuggets	*B. auriculata*	Fermented for two months	Das et al. 2013
Candy, Chutney, Chukh, Cracker, Nugget	*D. hamiltonii*	Boiled shoot	Sood et al. 2013
Pork Nuggets	*B. polymorpha*	Brine-treated boiled and fermented for six months	Thomas et al. 2014
Biscuit	*B. balcooa*	Boiled, dried and powdered	Choudhury et al. 2015
Chips	*B. vulgaris*	Shoot boiled for two hours	Maroma 2015
Pork Pickles	Not mentioned	Minced shoot exposed to sun and fermented for 21 days, dried and powdered	Chavhan et al. 2015
Candies	Not mentioned	Boiled shoot	Nimisha et al. 2015
Cookies	Not mentioned	Boiled shoot, dried and powdered	Mustafa et al. 2016
Pork Nuggets	*B. polymorpha*	Brine-treated boiled shoot extract	Thomas et al. 2016
Battered and breaded fish balls	*Not mentioned*	Bamboo shoot fibre	Zeng et al. 2016
Fried potato chips	*Bambusa balcooa*	Bamboo shoot powder and bamboo shoot extract	Shanmgam et al. 2016
Frozen dough	Not mentioned	Bamboo shoot fibre	Zhang et al. 2017
Milk pudding	*D. latiflorus*	Shoot fibre extracted with cellulase and papain enzyme method	Zheng et al. 2017
Biscuit	*D. hamiltonii*	Freeze-dried powder of fresh, boiled and soaked shoots	Santosh et al. 2018
Biscuit	*D. hamiltonii*	Fresh, boiled and soaked shoot paste	Santosh et al. 2019
Cookies	*D. asper*	Bamboo culm treated with meta-bisulphite, dried and powdered	Felisberto et al. 2019

fortification approaches (Adams et al. 2017). Biscuits represent a good candidate for the addition of functional ingredients because they are popular, daily consumed bakery items and have long shelf-life. Many functional biscuits have been formulated with anti-oxidant and/or prebiotic properties (Pasqualone et al. 2015, Obafaye and Omoba 2018). Daily consumption of fortified biscuits by primary school children had a significant positive impact on mean levels of iron, folic acid, vitamin B12, retinol and vitamin D. Bamboo shoots with its high nutrient content and health promoting bioactive compounds have been used for fortifying biscuits (Bisht et al. 2015, Choudhury et al. 2015, Mustafa et al. 2016, Santosh et al. 2018, 2019). Influence of bamboo shoot powder fortification on physico-chemical, textural and organoleptic characteristics of biscuits have been studied (Choudhury et al. 2015). Shoots of *B. balcooa* were sliced, boiled, dried, powdered, analyzed for nutritional status and used for making biscuits. Different concentrations of bamboo shoot powder (BSP) along with other necessary ingredients were mixed to make biscuits followed by an analysis of protein, fibre, fat, ash, phenolics, anti-oxidant activity, hardness, colour and sensory acceptability. Biscuits enriched with BSP showed higher phenolics, fibres and protein contents than the control. It was concluded that up to 10% BSP could be incorporated in the formulation of biscuits without affecting the overall quality.

Effect of ingredients and process conditions on important characteristics of bamboo fibre fortified Amaretti cookies, a popular Italian food product was studied by Farris et al. (2008). Fructose, bamboo fibre and egg white were used as ingredients and their effects along with baking time and baking temperature on quality responses such as hardness, water activity, moisture content and colour of cookies were measured by using a fractional factorial design in a screening test. It was observed that the addition of bamboo fibre contributed to beneficial textural properties and improved mouthfeel. Choudhury et al. (2015) investigated the influence of fortifying biscuits with the shoots of *Bambusa balcooa* for physicochemical, texture and organoleptic characteristics. The shoot was processed by blanching 30 mins, which was then dried and the powder was used for the formulation of 5%, 10% and 15% shoot-fortified biscuit. The study observed an increased level of water absorption capacity whereas gluten content decreased with the increased fortification level in the dough sample. Fortified biscuits also showed an increase in moisture, fibre, protein, fat and ash content with an increase in fortification level. There was a decrease in the phenolic and anti-oxidant properties of bamboo shoot powder during processing, however, the increase was observed in the fortified products with increasing level of formulation. Sensory observation for appearance, colour, texture, flavor, mouthfeel and overall acceptability was also analyzed and a decrease in acceptability was found with increasing the percentage of fortification. Incorporation of 10% shoot powder was recommended due to overall consumer acceptability.

Mustafa et al. (2016) studied the effect of bamboo powder supplementation on the physicochemical and organoleptic characteristics of fortified cookies. They studied physical characteristics like diameter, thickness and spread factor and observed a significant reduction in the spread factor with an increase in bamboo shoot powder. Physical characteristics depicted exceptional dough making characteristics when mixed with wheat flour. Sensory evaluation revealed that the cookies prepared with 6% and lower concentration of bamboo shoot powder was acceptable.

Santosh et al. (2018, 2019) developed bamboo shoot–fortified functional biscuits by using freeze-dried bamboo shoot powder and paste from *Dendrocalamus hamiltonii* (Figure 8.1). Nutrients, bioactive compounds and minerals were increased in bamboo shoot–fortified biscuits as compared to control biscuits. Nutritional content was observed to be maximum in fresh freeze-dried fortified biscuits with 0.30 g/100 g amino acids, 1.27 g/100 g protein, 20.45 g/100 g carbohydrate, 0.22 g/100 g phenol, 0.18 g/100 g phytosterol, 62.44 g/100 g neutral detergent fibre (NDF) and 5.16 g/100 g acid detergent fibre (ADF). Anti-nutrient content in all the fortified biscuits was much below the permissible level. Both the bamboo shoot paste and freeze-dried powder fortified biscuits scored high on sensory analysis and overall acceptability indicating that bamboo shoot supplementation in biscuits is a convenient vehicle for imparting nutrients and health promoting bioactive compounds into people's diet.

8.2.2 Nuggets

Value-added edible products such as nuggets, pickle and papad have been made from fresh bamboo shoots of four species *Bambusa bambos*, *B. tulda*, *Dendrocalamus strictus* and *D. asper* (Pandey et al. 2012). The nuggets were made by mixing boiled shoots, soaked chickpea, green gram, soybean and black gram with spices and salt as per taste. The mixture was then ground to a coarse paste and small equal sized balls were made and dried in oven for three days at 45–50°C. Organoleptic, sensory and chemical evaluation revealed that the products were good in taste, texture and quality for six months from the date of processing at ambient conditions and polypropylene and glass containers. Sood et al. (2013) modified this method and made nuggets by mixing bamboo shoots with soaked green gram and black gram along with spices

FIGURE 8.1 Bamboo shoot–fortified biscuits developed in the laboratory of Department of Botany, Panjab University and Department of Environmental Studies, North-Eastern Hill University, India.

in a different proportion and ground it to make a coarse paste. This paste was made into small equal sized balls and transferred directly to preheated and greased oven trays and dried in an oven for 24 hrs at 45–50°C.

Das et al. (2013) prepared nuggets from the relatively tough and fibrous meat of desi "spent" hen using fermented bamboo shoot (FBS) as a phyto-preservative in order to enhance the physicochemical, microbiological and keeping quality of the nuggets. The meat was minced and blended along with other non-meat ingredients and 10% fermented bamboo shoot. The emulsion was filled in metallic moulds and steam cooked and cut into pieces. Ready-to-eat nuggets thus prepared were packed in sterilized LDPE zip bags and stored at 4 ± 1°C for up to 15 days for quality evaluation. Emulsion stability (%), cooking yield (%) and proximate composition were studied on the day of preparation, while estimation of pH, TBA values, microbial load and sensory evaluation were carried out at an interval of five days and up to 15 days of storage. The emulsion stability (%), cooking yield (%), moisture (%), crude protein (%) and total ash (%) of FBS treated nuggets differed significantly from the control products. It was concluded that FBS fortified nuggets were superior to the control product and addition of 10% FBS gave the most promising physicochemical, microbial and sensory qualities in the nuggets and could be suitably stored for 15 days under refrigeration.

Pork nuggets were made by incorporating fermented bamboo shoot mince (FBSM) and their physicochemical, microbiological and sensory characteristics evaluated during 35 days' storage at refrigeration temperature (Thomas et al. 2014, 2016). The addition of fermented bamboo shoot significantly affected the pH, moisture, protein, fat fibre and texture profiles of nuggets. Addition of FBSM significantly retarded quality deterioration during refrigeration temperature storage, especially lipid oxidation and microbiological characteristics. Also, incorporation of 8% FBSM increased the storage life of pork nuggets by two weeks without impairing different physicochemical and sensory attributes and improved their microbiological characteristics. Chavhan et al. (2015) used fermented bamboo shoot as a preservative for the preparation of pork pickles and analyzed the physicochemical, microbial and shelf-life qualities of the products. Bamboo shoot–fortified pork pickle was studied in different formulations using fermented bamboo shoot extract, fermented bamboo shoot paste, dried powder of fermented bamboo shoots at the level of 50 to 100% with or without vinegar. The shelf-life of all the products was recorded maximum up to 90 days without any physicochemical and microbial effects except for the formulation of pork pickle with dried fermented powder for 30 days.

8.2.3 Candies

Tender bamboo shoots were used for preparing candies by Sood et al. (2013) and Nimisha et al. (2015). Fresh bamboo shoots were washed with tap water and the outer sheath was removed using a sharp stainless-steel knife. The inner edible portion was cut into uniform size cubes (1.5 cm). The cut cubes were washed thoroughly with tap water and subjected to boiling in steam-jacketed kettles for 30 mins followed by changing the water two to three times to ensure complete removal of HCN. Cubes were dipped in sugar syrup of required solute (sugar) strength and citric acid.

Thereafter, cubes candy was prepared by gradually increasing the solute concentration in the aqueous medium by heating 40°brix to 70°brix with 0.25% citric acid till equilibrium persists. Candy cubes to hypertonic sugar syrup ratio 1:2 were considered best because it gave complete dispersion of tender bamboo shoot (TBS) cubes into the sugar syrup and was easy to stir. Some desired flavors were also added to the syrup after equilibrium. Thereafter, preservative (KMS, 40 ppm) was added to have better preserving effect for candies cubes for the prolonged storage period. The equilibrated candy was dried at 55°C until 24–26% moisture content was retained and then packed in a pre-sterilized glass bottle.

8.2.4 Chips

Young shoots of *Bambusa vulgaris* were used for making chips with the aim to make a healthy snack by Maroma (2015). Shoots were harvested, washed and then sanitized in chlorinated water for 1 hr. The bamboo shoots were cut into half, and each sheath separated from one another and washed thoroughly. Then it was boiled in two litres of water for 1 hr. After boiling, the shoots were cut into 50 mm × 250 mm strips, coated with cornstarch and deep fried in 150°C palm oil until golden brown. The product was subjected to microbiological analysis and sensory evaluation to determine the safety and acceptability of the product and was found to be safe and liked by consumers. Such chips can also be prepared using gram flour (Figure 8.2).

FIGURE 8.2 Bamboo shoot chips coated with gram flour.

8.2.5 Crackers

Papad is a thin, circular, crispy wafer-like a popular snack in India. It is generally roasted or fried and served with a traditional Indian meal. Bamboo papad was made mixing one part (200 g) of boiled shoots, one part (200 g) of boiled potatoes, ½ teaspoon red chili powder, ½ teaspoon black pepper powder, ½ teaspoon cumin seeds and salt to taste (Pandey et al. 2012). The mixture was then ground and the dough was prepared. Equal sized balls were made from dough and rolled on a rolling board with the help of rolling pins in circular movements to make round papads. Papads were then dried in an oven for 2 days at 45–50°C. Dried papads were then stored in an airtight container. A modified version was also made by Sood et al. (2013). The papads had higher nutritive value than the control.

8.2.6 Pickles

Pickling is one of the most popular preservation methods that not only extends a food's shelf-life but also enhances its taste and flavor. Foods that are pickled include meats, fruits, eggs and vegetables. Bamboo shoot pickles (Figure 8.3) are quite popular in some parts of India. Pandey et al. (2012) made pickles from tender shoots of *B. bambos*, *B. tulda*, *D. strictus* and *D. asper*. Shoots were cut into small pieces, boiled and dried in air for about 1 hr and mixed with appropriate spices. The shoots were then transferred to a sterile glass container and hot mustard oil was poured over it before putting on the lid and kept unopened for a week.

High perishability of meat and meat products is a serious problem in tropical countries due to the prevailing climatic conditions and pickling is a suitable method for preserving these products. Chavhan et al. (2015) studied the effect of incorporation of the fermented bamboo shoot on physiochemical and microbial quality of pork pickle. Fermented bamboo shoot (FBS) extracts, paste and powder were incorporated in the pork pickle at the level of 50% to 100% with or without vinegar and stored at room temperature for 90 days.

FIGURE 8.3 Bamboo shoot pickle prepared in North Eastern Hill University, Shillong, India.

No significant differences were observed with respect to proximate composition, that is percentage of moisture, protein, fat and ash contents among the products except with 100% FBS powder which had significantly lower moisture content. Except for the product with 100% FBS powder which could be stored for 30 days only, other products could be stored up to 90 days without and physiochemical and microbial problems thereby indicating that natural and organic FBS extract and paste can be used successfully replacing the conventional chemical preservative like vinegar for preparation of pork pickle and preserved for more than 90 days at room temperature.

8.3 NUTRACEUTICALS

Nutraceutical is a broad term that describes a substance extracted from food sources with additional health benefits along with basic nutritional value already present in them. The term nutraceutical was coined in 1989 by Stephen DeFelice, founder and chairman of Foundation for Innovation in Medicine, Cranford, New Jersey, by combining nutrition and pharmaceutical for any substance that is a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease. The health ministry of Canada later modified the meaning of nutraceutical as ‘a product isolated or purified from the food, generally sold in a medicinal form not associated with food and demonstrated to have a physiological benefit’. So, in essence, nutraceuticals differ from functional foods mainly in the manner of their consumption. Although both are consumed for their perceived health benefits courtesy to their bioactive compounds, nutraceuticals are utilized mainly in the form of pills, tablets, capsules, powders and tinctures, whereas the functional foods are always consumed as normal food formulations. Apart from capsule and pill forms, nutraceuticals are also marketed in the form of herbal and dietary supplements. With ever-increasing public health consciousness, demand for nutraceutical and related health products has grown tremendously over the past few decades. This rise in the popularity of nutraceuticals has been backed by the modern scientific research which has provided conclusive evidence toward the beneficial role of nutraceuticals against several chronic diseases such as cancer, heart diseases, hypertension, high cholesterol, osteoporosis, diabetes, arthritis, indigestion, macular degeneration, cataracts, treating headaches and migraines resulting from stress. The nutraceuticals hold the key for the food industries to create reliable health products in the future, providing a suitable and more natural alternative to consumers dissatisfied with drug cost and conventional health care systems for the prevention and treatment of chronic diseases.

The modern nutraceutical market began to develop in Japan during the 1980s. In contrast to the natural herbs and spices used as a folk medicine for centuries throughout Asia, the nutraceutical industry has grown alongside the expansion and exploration of modern technology. The global nutraceutical market is growing rapidly and is expected to grow from USD 198.1 billion in 2016 to USD 285.0 billion by 2022 as per recent reports. India has also started to strengthen its position in the functional food and nutraceutical sector, with its nutraceutical industry set to cross USD 6 billion from the current USD 2.8 billion by 2020 (ASSOCHAM, 2017).

This rise in the popularity of nutraceuticals has been backed by the modern scientific research which has provided conclusive evidence towards the beneficial role of nutraceuticals against several chronic diseases such as cancer, heart diseases, hypertension, high cholesterol, osteoporosis, diabetes, arthritis, indigestion, muscular degeneration, cataracts, headaches and migraines resulting from stress. Bioactive compounds comprise an excellent pool of molecules for the production of nutraceuticals, functional foods and food additives (Joana Gil-Chávez et al. 2013). Natural bioactive compounds are being searched for the treatment and prevention of human diseases because of their health benefits (Table 8.2). These compounds efficiently interact with proteins, DNA and other biological molecules to produce desired results, which can then be used for designing natural therapeutic agents. Hence, pharmaceutical and food domains have a common interest to obtain new natural bioactive components that can be used as drugs, functional food ingredients, or nutraceuticals (Joana Gil-Chávez et al. 2013).

8.4 BAMBOO SHOOTS AS NUTRACEUTICALS

Bamboo shoots, which make a delicious vegetable, apart from being highly nutritious are also a rich source of various bioactive compounds like phytosterols, dietary fibres and phenolic compounds as discussed in Chapter 4. The presence of these bioactive compounds makes bamboo shoots an excellent source for developing natural nutraceutical products. These bioactive compounds have been shown to be

TABLE 8.2
Bioactive Compounds Used in Nutraceuticals and Their Health Benefits

Bioactive Compounds	Health Benefits
Alkaloids (e.g. Quinine, Coumarin etc.)	Anti-malarial, hypoglycaemic, anti-depressant, heart tonic
Carotenoid terpenoids/ Isoprenoids (e.g. α-carotene, Lycopene, Lutein etc.)	Anti-oxidants, anti-carcinogenic, anti-cataract
Non-carotenoid terpenoids (e.g. Saponins, Terpenol etc.)	Anti-carcinogenic Reduces cholesterol levels in blood
Flavonoid polyphenolics (e.g. Anthocyanins, Catechins, Quercetin etc.)	Anti-oxidants, anti-sitaminic, reduces blood cholesterol
Phenolic acids (e.g. Ellagic acids)	Anti-oxidant, anti-cancer, anti-microbial, lower blood glucose
Phytosterols (β-sitosterol, stigmasterol, campestanol etc.)	Decrease LDL cholesterol level, anti-cancer activity, modulate the immune function and inflammation
Vitamins	Anti-oxidant, anti-tumor, hypocholesterolemic potential Prevention of cardiovascular disease and angiogenic disorders
Proteins and peptide	Anti-hypertensive, anti-microbial, anti-inflammatory and immune-stimulating activities, anti-oxidant, anti-cancerous properties
Dietary fibres	Prevention of cardiovascular diseases, hypertension, diabetes, obesity, cancer and gastrointestinal disorders and improvement of bowl movement

effective against several chronic disorders like cancer, diabetes, obesity and cataracts. Phytosterols from bamboo shoots have been found to be very effective in lowering the LDL and serum cholesterol levels. Bamboo shoot fibre has several health benefits and is useful in the management of hypertension and obesity and is associated with decreased low-density lipoprotein cholesterol, increased stool bulk, increased laxative properties and so on. Li et al. (2016) reported that bamboo shoot dietary fibre prevented obesity in mice by modulating the gut microbiota and also improved the host metabolism. Recent studies have indicated the nutraceutical potentials of bamboo (Table 8.3) and many nutraceutical products have been developed (Table 8.4).

Fresh bamboo shoots are also rich in potassium, which helps prevent cardiovascular diseases and blockage of blood vessels. A healthy diet including bamboo shoots is also key to blood sugar management and preventing or treating diabetes. We conducted studies on shoot extracts of the edible bamboo *Dendrocalamus*

TABLE 8.3

Nutraceutical Potential of Bamboo Proven by Scientific Experiments in Laboratories

Potential Benefit	Species	References
Anti-cancerous	*Sasa sinensis, Pseudosasa japonica, Caulis bambusae, S. quelpaertensis, Phyllostachys pubescens*	Tsunoda et al. 1998, Panee 2009, Seki et al. 2010, Hong et al. 2010, Kim et al. 2013, Hiromichi 2007
Anti-diabetic	*Phyllostachys pubescens, Sasa borealis, Pseudosasa japonica, Bambusa vulgaris*	Ding et al. 2008, Oh and Lim 2009, Senthilkumar et al. 2011, Koide et al. 2011, Nam et al. 2013
Anti-obesity	*Sasa borealis, S. quelpaertensis*	Yang et al. 2010, Li et al. 2016
Anti-inflammatory	*Bambusa arundinacea, S. quelpaertensis*	Hu et al. 2000, Lu et al. 2005, Muniappan and Sundararaj 2003, Hwang et al. 2007, Carey et al. 2009
Anti-fatigue	*Bambusa tuldoides, Phyllostachys nigra, Pseudosasa japonica,*	Zhang and Tang 1997, You et al. 2006, Zhang et al. 2006
Anti-hyperlipidemic	*Phyllostachys pubescens*	Ding et al. 2010
Anti-hypertensive	*Phyllostachys pubescens*	Liu et al. 2013
Anti-microbial	*Bambusa arundinacea, Phyllostachys pubescens, P. nigra, P. heterocycla*	Fujimura et al. 2005, Park and Jhon 2010, Tanaka et al. 2011, Singh et al. 2010, Zhanwang et al. 2005, Jin et al. 2011
Cardiovascular diseases	*Phyllostachys pubescens, P. nigra*	Fu et al. 2006, Liu et al. 2012, 2013
Cholesterol-lowering	*P. pubescens, P. nigra, P. edulis, B. oldhamii, D. latiflorus*	Park and Jhon 2009, Lachance and He 1998

TABLE 8.4
Nutraceutical Products from Bamboo

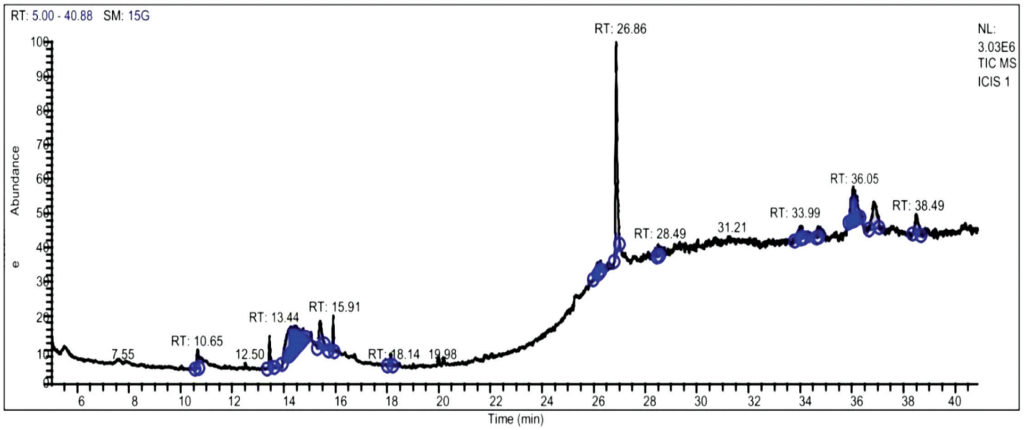
Product Name	Content	Health Benefits/Health Claims
Bamboo Silica/Silice de Bambou®	Bamboo Silica	Anti-aging, build healthy bones, nails, teeth and skin wrinkle free and beautiful
World Organic Bamboo Extract Capsule®	Bamboo Silica, Vegetable, Gelatin, Rice Flour, Magnesium Stearate	Prevent premature aging, preserves skin youthfulness, builds healthy bones and teeth and promotes growth of strong hair
Solaray Bamboo Extract®	Bamboo Silica, Cellulose and Magnesium Stearate	Dietary supplement, stimulates collagen synthesis in bone and connective tissue, remineralization effect
My Kind Organics® Organic Plant Collagen Builder	Bamboo Silica, Pomegranate, Green and Rooibos Tea, Citrus Bioflavonoids and Turmeric	Helps restore and maintain youthful levels of collagen, keratin and elastin, three proteins responsible for promoting body's healthy, natural glow
Congshengzhu Bamboo Juice®	Fresh Bamboo Shoot Juice (Zhuli), contains essential amino acids and flavonoids	Dietary supplement, nourishes blood and improves eyesight
Congshengzhu Green Bamboo Dietary FibrePowder®	Green Bamboo Dietary Fibre Powder	Dietary supplement, improve the digestive system
Lesen® Bamboo Shoot Extract Powder	Food Grade Bamboo Shoot Extract	Dietary supplement, improve digestive system
Nutra Green® Bamboo Shoot Extract Powder	Bamboo Shoot Dietary Fibre	Dietary supplement, treatment for cough and fever, dissipate phlegm
Just Fibre®	Vegetable Fibre Derived From Bamboo	Herbal tonic, reduces fat and calories
Sanacel bamboo®	Bamboo dietary fibre	Dietary supplement, improve digestive system
GuoZhen Bamboo Leaf Essence®	Bamboo Leaf Extract	Regulates blood fat, purifies blood, strengthen heart and protect brain
Fenioux Bambou Tabashir	Bamboo Silica	Maintains balance of connective tissue, strengthens bones and improves endurance, increases immunity and fights fatigue
Lamberts® Natural Source Silica	Bamboo Silica	Contributes to the structure and resilience of connective tissue, synthesis of bone collagen and cartilage, recommended for healthy skin
Hawlik® Cappilary active capsules	Bamboo Shoot Extract and Polypore Umbellate Fungal Extract	Improves hair health by restructuring hair surface layer

(*Continued*)

TABLE 8.4 (CONTINUED)
Nutraceutical Products from Bamboo

Product Name	Content	Health Benefits/Health Claims
Vigorous® Bamboo Shoot Extract	Bamboo Shoot Powder/ Fibre	Stimulate appetite, relieve constipation, improve immune system
Yancheng Goodex Bamboo shoot powder	Bamboo Shoot Powder/ Fibre	Improve skin health, protect the blood vessel of brain and heart, adjust blood lipid, low the blood viscidity, enhance immunity, anti-fatigue action
Aladdin® Bamboo shoot extract	Bamboo Shoot Extract	Promote intestinal peristalsis, help digestion, prevent constipation and has the effect of prevention of colorectal cancer, useful for weight loss
Herblink® Bamboo Shoot Extract Powder	Bamboo Shoot Powder	Reduce fever and dissipate phlegm, relieve pressure and prevent or arrest vomiting, improve blood cholesterol level, improve digestive health
Health Ingredients® Natural Silica	Bamboo Leaf, Shoot Extract	Organic silica plays an important role in keeping the skin and hair healthy
Organic Bamboo Tea®	Bamboo Leaves	Bamboo leaf tea is rich in silica, fibre and anti-oxidants, useful to improve bone health, strengthen hair and nails, improve dental health and make skin more elastic and healthier
Bonusan Forte®	Tabashir Exudates	Reduces fatigue, supports energy, metabolism, good for nervous system
Bamboolex	Bamboo Leaves	Anti-oxidant, anti-bacterial, anti-acrylamide

hamiltonii. When ethanol extract of shoots was analyzed using GC-MS spectroscopy (Figure 8.4), it showed the highest presence of fatty acid amide namely 13-Docosenamide (Z) (25.02%), which has anti-microbial activity. It also acts as an anti-static agent and is used in the manufacturing of food packaging material, personal daily care products like perfumes, deodorant, lotions, moisturizers, talcum powder, soaps, toothpaste, etc. Other main components were 4H-Cyclopropa [5',6'] benz [1',2':7,8] azuleno [5,6] oxiren-4-one,8,8a-bis(acetyloxy)-2a-[(acetyloxy) methyl]-1,1a,1b, 1c,2a,3,3a,6a, 6b,7,8,8a-dodecahydro-6b-0 hydroxy-3a- methoxy-1,1,5,7-tetramethyl- (9.18%), Propanoic acid, 3-chloro,4-formylphenyl ester (8.63%), ç-Sitosterol (4.43%) and 1-Monolinoleoylglycerol trimethylsilyl ether (2.43%). 1-Monolinoleoylglycerol trimethylsilyl ether has anti-microbial, anti-oxidant, anti-inflammatory, anti-arthritic and anti-asthmatic activity. The health beneficial properties of 9, 12, 15-Octadecatrienoic acid, 2, 3-bis [(trimethylsilyl)oxy] propyl ester, (Z,Z,Z)-(Linolenic acid ester) include anti-inflammatory, hypocholesterolemic, anti-arthritic, nematicide and hepatoprotective. Similarly, ç-Sitosterol, an important steroid found in shoots has cholesterol-lowering and anti-inflammatory activity (Table 8.5).



Qual Peak Table

RT	Peak Area	Area %	Peak Height
10.65	876095.92	2.52	172599.40
13.44	1258257.19	3.62	287805.18
14.19	2999709.93	8.63	250838.52
14.27	1011559.29	2.91	250452.30
14.33	971689.75	2.80	228351.38
14.41	986083.20	2.84	218053.61
14.48	816434.77	2.35	196753.69
14.57	736601.88	2.12	155332.15
14.66	482240.48	1.39	107799.21
14.74	415234.05	1.20	110657.15
15.41	1719015.49	4.95	234190.33
15.91	863565.41	2.49	322622.77
18.14	405946.14	1.17	115208.56
26.14	845785.03	2.43	118084.69
26.26	241192.24	0.69	103457.90
26.86	8691582.74	25.02	1901516.20
28.49	372798.27	1.07	110490.64
33.99	1019214.64	2.93	120396.67
34.07	368146.60	1.06	111999.62
34.68	303211.76	0.87	102784.10
35.97	832363.68	2.40	216876.22
36.05	1538626.49	4.43	304454.14
36.11	699944.95	2.01	262119.43
36.19	1369209.86	3.94	208936.19
36.84	3187703.59	9.18	241184.12
38.49	1726771.21	4.97	183825.58

FIGURE 8.4 GC-MS spectra of ethanol extract of juvenile shoots of *D. hamiltonii*.

8.4.1 Anti-Oxidant Activity of Bamboo

Phenolic compounds from bamboo shoots are known to possess potent anti-oxidant properties. Anti-oxidant rich foods have generated a lot of interest and attention as it plays an important role in disease prevention. Anti-oxidants are substances or compounds which inhibit the oxidation of other molecules in our body and prevent the formation of free radicals by scavenging them. Most of the health benefits of anti-oxidants arise from their anti-inflammatory properties within the body. The important role of anti-oxidants is to promote cardiovascular health, to inhibit the growth of cancerous tumors, to slow the aging process in the brain and nervous system and to lessen the risk and severity of neurodegenerative disease including Alzheimer

TABLE 8.5
Major Phytochemicals Identified in the Ethanolic Extract of Juvenile Shoots of *D. hamiltonii* by GC-MS

Retention Time	Peak Area (%)	Molecular Formula	Name of the Compound	Health Benefits
10.65	2.52	$C_{11}H_{24}O$	1-Undecanol	Anti-fungal
13.44	3.62	$C_{16}H_{32}O$	Hexadecen-1-ol, Trans-9-	Anti-inflammatory
14.74	1.20	$C_{14}H_{42}O_7Si_7$	Cycloheptasiloxane, tetradecamethyl-	Anti-inflammatory, anti-psychotics, anti-neoplastics
15.91	2.49	$C_{13}H_{26}$	Cyclotridecane	Anti-asthmatics
18.14	1.17	$C_{17}H_{36}O$	n-Heptadecanol-1	Anti-bacterial
26.14	2.43	$C_{27}H_{54}O_4Si_2$	1-Monolinoleoylglycerol trimethylsilyl ether	Anti-microbial, anti-oxidant, anti-inflammatory, anti-arthritic, anti-asthma, Diuretic
26.26	0.69	$C_{27}H_{52}O_4Si_2$	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester, (Z,Z,Z)-	Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Anti-histaminic, Anti-arthritic, Anti-coronary, Anti-eczemic, Anti-acne, 5-Alpha reductase inhibitor Anti-androgenic
26.86	25.02	$C_{22}H_{43}NO$	13-Docosenamide, (Z)-	Anti-microbial
33.99	2.93	$C_{30}H_{50}O_6$	Olean-12-ene-3,15,16,21,22,28-hexol	Anti-cancer, anti-inflammatory, anti-diabetogenic, anti-microbial
36.05	4.43	$C_{29}H_{50}O$	ç-Sitosterol	Anti-cancer, anti-inflammatory, anti-diabetogenic, anti-microbial
36.19	3.94	$C_{32}H_{50}O_6$	Dodecanoic acid, 1a,2,5,5a,6,9, 10,10a-octahydro-5,5a-di hydroxy-4-(h ydroxymethyl)-1,1,7,9-tetramet hyl-11-oxo-1H-2,8a-methanocyclopenta[a] cyclopropa[e]cyclodecen-6-yl ester	Anti-obesity, cholesterol-lowering

disease and Parkinson disease. Anti-oxidants are also of immense importance in industries dealing with petrochemicals, food, cosmetics and medicine where they are used for stabilization of polymeric products. In the food and pharmaceutical industries, anti-oxidants are used to prevent deterioration, rancidity and discolouration caused by oxidation during processing and storage. There are several known natural compounds with anti-oxidant properties that can be extracted from plants, which are mainly phenols, polyphenols, vitamin C, vitamin E, beta-carotene, flavonoids, amino acids and amines that are known to have the potential to reduce disease risk. However, due to the lack of natural anti-oxidants, nowadays most food and pharmaceutical products contain synthetic anti-oxidants that cause concerns about their adverse effect on health. Hence, more emphasis is given to the use of natural anti-oxidants (Schillaci et al. 2013, Chongtham et al. 2018).

Although bamboo is known for its therapeutic properties, it is rarely considered for its anti-oxidant properties. Studies have revealed that bamboo is a rich source of anti-oxidants and regular consumption of bamboo-based products may reduce the risk of age-related chronic diseases including cardiovascular diseases, Alzheimer disease, Parkinson disease, cancer and diabetes. The main anti-oxidants in bamboo leaves and shoots are phenols, flavonoids, vitamin C and E and mineral elements such as selenium, copper, zinc, iron and manganese (Chongtham et al. 2018). Several identified anti-oxidants derived from bamboo shoot and leaves, display certain biological roles, including anti-oxidative (Hu et al. 2000), anti-cancer (Shi and Yang 1992), anti-hypertensive (Akao et al. 2004) and anti-bacterial (Fujimura et al. 2005) functions. Research on bamboo shoot anti-oxidants began with a study by Ishii and Hiroi (1990) that identified a compound namely, diferuloyl arabinoxylan hexasaccharide containing 5-5-linked diferulic acid from bamboo shoots and reported that ferulic acid is a naturally occurring anti-oxidant present in the plant-based products. At present, natural anti-oxidants are in great demand as synthetic anti-oxidants being used in food and pharmaceuticals may be deleterious to health. Hence, bamboo a fast-growing plant with huge biomass can serve as an alternative for the production of natural anti-oxidants.

Trace elements in bamboo shoots associated with anti-oxidant defense systems are selenium, zinc, copper, iron and manganese. Selenium is an essential trace element and co-factor for an enzyme, glutathione peroxidase. Chinese scientists discovered a disease, namely ‘Keshen disease’, that occurs due to the severe deficiency of dietary selenium (Yang et al. 1983). Copper, zinc, iron and manganese are other indispensable metals, which are required for the activities of anti-oxidant enzymes such as superoxide dismutase (SOD). Iron is the most abundant trace element in the body and almost all iron occurs bound to proteins. Dietary deficiency of proteins promotes reactive oxygen species production, lipid peroxidation and oxidative stress (Dabbagh et al. 1994). There have been only a few studies that evaluated the content of selenium in bamboo shoots (see Chapter 3).

It is well known that phenolic compounds in the plants are very important anti-oxidants and bamboo shoots are one of the best sources of phenolic compounds in the plants. Several direct and indirect health benefits have been attributed to the presence of phenolic compounds in bamboo shoots. Their action on the regulation of apoptosis, neuroprotective activity and anti-oxidant properties has been studied in detail. Several bamboo species have also been analyzed for their total phenolic content and anti-oxidant activities such as *Dendrocalamus asper*, *D. hamiltonii*, *Bambusa tulda*, *B. vulgaris*, *B. balcooa*, *B. pallida* and *Phyllostachys* species, using different anti-oxidative assays to acquire comprehensive information about the anti-oxidant capacity of bamboo shoots (Satya et al. 2009, Park and Jhon 2010, Nemenyi et al. 2015). Soesanto (2016) analyzed the total flavonoids, total polyphenols, vitamin E content and anti-oxidant activity of shoots of two bamboo species viz. *Bambusa vulgaris* and *Gigantochloa apus* and found that one- to two-week-old freeze-dried shoots of *G. apus* had the highest concentration of total flavonoids, total polyphenols and vitamin E content. It was also observed that the anti-oxidant activity of one- to two-week-old freeze-dried shoots of *G. apus* is higher as compared to the shoots of *Bambusa vulgaris*. In one of the studies, it was found that bamboo shoots contributed 46% of the daily anti-oxidant activity intake among different vegetables consumed in China (Yang et al. 2005). Anti-oxidant activity is represented as IC_{50} of DPPH/ABTS (μg/ml). The value signifies the concentration of the test sample that can scavenge 50% reduction of the DPPH/ABTS free radicals. With the increased value of IC_{50}, the decrease in anti-oxidant activity was indicated, since it required a higher test sample to achieve a 50% reduction of the DPPH/ABTS. Thus, with the decease in IC_{50} values, it indicates an increased potential of anti-oxidant activity as lower concentration or amount of test sample is able to achieve a 50% reduction of DPPH/ABTS solution. Juvenile shoots of *Dendrocalamus hamiltonii* were analyzed for their total phenols, total flavonoids and in-vitro and in-vivo anti-oxidant activities (Bajwa et al. 2018). In-vitro anti-oxidant activity of the aqueous extract of young shoots (Figure 8.5) was measured by DPPH free radical and ABTS radical cation assay. The IC_{50} values were

FIGURE 8.5 Bamboo shoot extract preparation for determining anti-oxidant activity.

568.18 μg/ml and 66.48 μg/ml, respectively. Butylated 4-hydroxytoluene (BHT) was used as standard. Its IC_{50} value for DPPH free radical and ABTS radical cation was 277.28 μg/ml and 12.67 μg/ml, respectively (Table 8.6).

8.4.2 *In-vivo* Studies in Balb/c Mice

Several in-vivo studies have shown that regular consumption of bamboo shoots is an effective and safe way to meet all anti-oxidant requirements. Shoots reduce the risk of arteriosclerosis, cardiovascular problems and some forms of cancer. Our team conducted studies to evaluate the effect of bamboo shoot extract on body and organ weight, blood glucose level, lipid profile, hepatic function, kidney function and anti-oxidant defense system in Balb/c mice. Mice were randomly assigned into two groups (N = 6). Group-1 served as a control group and to another group, an aqueous extract of *D. hamiltonii* shoots (BSE) was administered at the concentration of 800 mg/kg body weight for six weeks (Figure 8.6). The control group was given tap water and feed *ad libitum*. The results obtained are discussed next.

8.4.3 Effect of Bamboo Shoots on Body and Organ Weight

Involuntary weight gain worsens all elements of the cardiovascular risk profile, including dyslipidemia, hypertension, insulin-resistant glucose intolerance, left-ventricular hypertrophy, hyperuricemia and elevated fibrinogen. The degree of overweight is related to the rate of development of the cardiovascular disease. Diet plays a very important role in weight management. In the present study, individual body weight was recorded once a week during the experimental period. Mean body weight gain was calculated for each group. The body weight of both the groups increased normally but the highest increase in the body weight during the experimental period was observed in the control group. However, no significant change was observed in the weight of the liver and kidney of the BSE-treated group when compared with the control group (Table 8.7).

TABLE 8.6
Phenols, Flavonoids and Anti-Oxidant Activity of Aqueous Extract of Shoots of *D. hamiltonii*

Parameter	Bamboo Shoot	BHT (Standard)
Total phenols (mg of GAE/g of extract)	67.50 ± 0.01	–
Total flavonoid (mg of QUE/g of extract)	7.92 ± 0.05	–
ABTS, IC_{50} (μg/ml)	66.48 ± 3.11	12.67 ± 0.60
DPPH, IC_{50} (μg/ml)	568.18 ± 0.02	277.28 ± 0.02

Values reported are measurement replication means ± standard deviation (n = 3 replicates). BHT-Butylated Hydroxytoluene, GAE- Galic Acid Equivalent, QUE- Quercetin, ABTS- 2,2 – zino- bis (3-ethylbenzothiazolin- 6- sulfonic acid), DPPH- 2,2- diphenyl- 1- picrylhydrazyl.

TABLE 8.7
Effect of Bamboo Shoot Extract on Body and Organ Weight of Balb/c Mice

Weight (g)	Control	BSE (800 mg/kg b.w.) Treated Group
Initial body weight	31 ± 0.58	29 ± 0.55
First week	34 ± 0.93	27 ± 0.33
Second week	35 ± 0.37	28 ± 0.98
Third week	34 ± 0.88	28 ± 1.00
Fourth week	36 ± 0.91	30 ± 1.31
Fifth week	36 ± 0.11	31 ± 0.76
Sixth week	37 ± 0.43	31 ± 1.27
Liver weight	1.31 ± 0.25	1.32 ± 0.31
Kidney weight	0.31 ± 0.09	0.27 ± 0.04

Values reported are measurement replication means ± standard deviation (n = 3 replicates).
BSE = Bamboo shoot extract, b.w.—body weight

8.4.4 Effect of Bamboo Shoots on the Anti-Oxidant Defense System

In the present study, the activity levels of GSH, SOD, CAT, GR and GPx were detected. In the normal control mice, the level of reduced glutathione (GSH) content in the serum was 0.897 ± 0.07 (nmol/mg proteins). But the level of GSH increased significantly after administering the animals with bamboo shoot extract. The activity of enzyme glutathione reductase (GR) and catalase (CAT) was decreased while; a significant increase was seen in the activity of superoxide dismutase (SOD) in the treatment group. In contrast, no significant change was seen in the activity of glutathione peroxidase (GPx) in the treatment group when compared with the control group (Table 8.8). Bamboo anti-oxidants may thus offer promising avenues to prevent and control oxidative-stress related chronic and degenerative diseases.

8.4.5 Effect of Bamboo Shoots on Glucose and Lipid Profile

Blood glucose level is the amount of sugar present in the blood of a human or animal. The digestive system breaks the food into glucose, which is a primary source of energy for the body. Glucose then travels in the bloodstream to cells throughout the body. This causes a rise in blood sugar levels. The pancreas releases insulin to help cells in absorbing glucose for energy. When the body is in a fasting state, it relies on stored energy. The body naturally regulates blood glucose levels as a part of metabolic homeostasis. Many factors affect blood sugar levels. A healthy diet is key to blood sugar management and preventing or treating diabetes. The results of the present study confirmed the hypoglycemic properties of aqueous extract of *D. hamiltonii* shoots. In the normal control mice, non-fasting serum glucose level was 97 ± 1.56 (mg/dl) while, fasting serum glucose level was 68 ± 2.14 (mg/dl). Treatment

TABLE 8.8
Effect of Aqueous Extract of Bamboo Shoots on Anti-Oxidant Defense System in Balb/c Mice

Parameter	Group I	Group II
GSH (nmol/mg protein)	0.897 ± 0.07	1.03 ± 0.06
Glutathione reductase (nmol of NADPH consumed/min/mg protein)	19.86 ± 2.31	15.76 ± 2.63
Glutathione peroxidase (nmol of NADPH oxidized/min/mg protein)	14.87± 2.00	14.89 ± 1.25
Catalase (μmol H_2O_2 reduced/min/mg proteins)	1.53 ± 0.23	0.569 ± 0.04
Superoxide dismutase (IU/mg protein)	2.73 ± 0.68	4.21 ± 0.82

Values reported are measurement replication means ± standard deviation (n = 3 replicates).
Group I: Control, Group II: Bamboo shoot treated, GSH—Glutathione reductase, H_2O_2—hydrogen peroxide, NADPH—Nicotinamide adenine dinucleotide phosphate

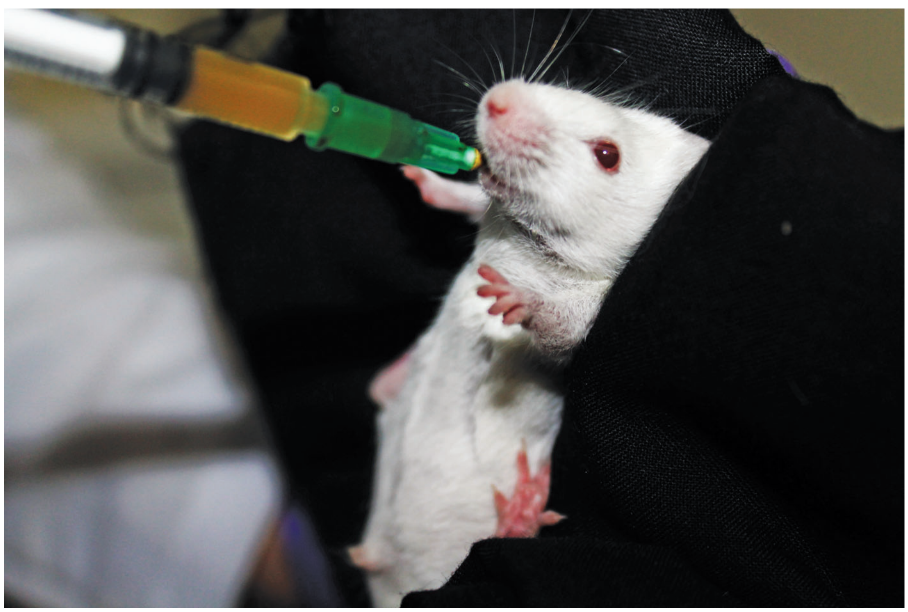


FIGURE 8.6 Administration of aqueous extract of bamboo shoot in Balb/c mice.

with aqueous extract of bamboo shoots at concentration 800 mg/kg, body weight for six consecutive weeks resulted in a slight increase in serum glucose level (72 ± 2.38 mg/dl) in fasting group while a significant decrease was observed in non-fasting animals (81 ± 1.77 mg/dl) when compared with the control group (Figure 8.6, Table 8.9). Less fall in blood sugar level in fasting mice after administration of BSE as compared to control mice which were receiving normal animal diet might be due to high fibre content present in bamboo shoots. According to Haber et al. (1977) the removal of fibre from food and also its physical disruption, can result in faster and easier ingestion, decreased satiety and disturbed glucose homeostasis which is

TABLE 8.9
Effect of Fresh Shoot Extract on Glucose Level and Lipid Profile

Parameter (mg/dl)	Control	BSE (800 mg/kg b.w.) Treated Group
Glucose	97 ± 1.56 (Non-fasting) 68 ± 2.14 (Fasting)	81 ± 1.77 (Non-fasting) 72 ± 2.38 (Fasting)
Total cholesterol	124 ± 2.71	98 ± 2.65
HDL	88 ± 0.74	94 ± 0.63
LDL	19 ± 2.24	13 ± 1.42
Triglycerides	221 ± 0.64	161 ± 1.15

Values reported are measurement replication means ± standard deviation (n = 3 replicates).
BSE = Bamboo shoot extract, HDL—High Density Lipoprotein, LDL—Low Density Lipoprotein

probably due to inappropriate insulin release. These effects favor overnutrition and if often repeated, might lead to diabetes mellitus. This is based on the findings when ten normal subjects were provided with three kinds of apple-based diet (fibre-free apple juice, apple puree and intact apples), plasma-glucose rose to similar levels. But there was a striking rebound fall in blood sugar level after apple juice and to a lesser extent after puree but was not seen after consuming intact apples because serum-insulin rose to higher levels after juice and puree than after apples. It has also been proved that juice could be consumed eleven times faster than intact apples and four times faster than fibre-disrupted puree. This indicates that the regular consumption of a high-fibre diet like bamboo shoots could help in maintaining the serum-insulin level and also the energy level of the body even in the fasting state.

The levels of total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) in the serum of animal groups were also tested to evaluate the anti-oxidant effect of bamboo shoots (Table 8.9). It was found that the treatment groups that received the aqueous extract of bamboo shoots had decreased levels of TC, TG and LDL as compared to the control group. The level of HDL in the treatment groups was slightly higher than that of the control group. The cholesterol-lowering effects of bamboo shoots have been attributed to inhibition of cholesterol absorption and increase of cholesterol excretion (Lu et al. 2010).

8.4.6 Effect of Bamboo Shoots on Liver Function

The effect of bamboo shoots was also studied on various parameters that determine the health of the liver. Liver functions were monitored by analyzing the levels of serum bilirubin, total proteins, albumin, globulin, alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH) (Table 8.10). Bilirubin is an endogenous

TABLE. 8.10

Effect of Fresh Shoot Extract on the Liver Function in Balb/c Mice

Parameter	Control	BSE (800 mg/kg b.w.) Treated Group
Billirubin (mg/dl)	0.235 ± 0.08	0.236 ± 0.05
Proteins (mg/dl)	81 ± 2.52	79 ± 1.72
Albumin (mg/dl)	19 ± 1.31	19 ± 1.08
Globulin (mg/dl)	63 ± 0.96	52 ± 1.12
Alkaline phosphatase (U/L)	59 ± 3.32	73 ± 4.52
SGOT (U/L)	108 ± 3.22	93 ± 3.40
SGPT (U/L)	53 ± 3.68	44 ± 2.90
LDH (U/L)	991 ± 21.5	984 ± 31.2

Values reported are measurement replication means ± standard deviation (n = 3 replicates).

anion derived from hemoglobin degradation from the RBC. When the liver function tests are abnormal and the serum bilirubin levels more than 17μmol/L, it suggests underlying liver disease (Friedman et al. 2003). The measurement of proteins is another useful indicator of hepatic functions because the liver is the major source of most of the serum proteins. A total serum protein test measures the total amount of protein, albumin and globulin in the blood. Albumin is quantitatively the most important protein in plasma synthesized only by the liver. Albumin synthesis is affected not only in liver disease but also by nutritional status, hormonal balance and osmotic pressure (Rosalki and Mcintyre 1999). Globulins are produced by the liver and the immune system. High serum globulin levels may be indicative of some liver problems. The serum glutamic pyruvate transaminase (SGPT) and serum glutamate oxaloacetic transaminase (SGOT) is the most widely used liver enzymes that are sensitive to abnormalities in the liver. These liver enzymes form a major constituent of the liver cells and are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes catalyze the transfer of the α-amino acids of aspartate and alanine, respectively, to the α-keto group of ketoglutaric acid. SGPT is primarily localized to the liver but the SGOT is present in a wide variety of tissue like the heart, skeletal muscle, kidney, brain and liver (Rosen and Keeffe 2000; Friedman et al. 2003). The SGPT and SGOT levels are increased to some extent in almost all liver diseases. Similarly, elevated levels of LDH, in the blood indicate acute or chronic cell damage. Results of the study revealed that bilirubin, protein and albumin content of the BSE-treated animals remained unchanged while, some alterations were seen in the level of globulin, alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and lactate dehydrogenase (LDH) as compared to the control group.

Similarly, phytomodulatory effects of fresh and processed shoots of *Dendrocalamus hamiltonii* on the anti-oxidant defense system in mouse liver were studied by Bajwa et al. (2019a). The study revealed a significant increase in glutathione content in the liver tissues of all the groups administered with extract of boiled and fermented bamboo shoots except the mice that received brine preserved shoot extract, where content decreased by 19%. Glutathione reductase (21%), glutathione peroxidase (20%) and catalase (69%) activity decreased significantly in the group administered with the extract of fresh shoots. The fermentation process was recognized as the most valuable process to improve the activity of hepatic superoxide dismutase (23%), glutathione peroxidase (27%) and catalase (29%). The results indicated that fresh, boiled and 5% brine preserved shoots significantly decreased the anti-oxidant status of the liver. Conversely, the activity of anti-oxidant enzymes increased remarkably after the administration of fermented shoot extract. It was thereby concluded that fermented shoots are best for improving the nutritional and pharmaceutical qualities and also effective utilization of bamboo shoots to their full potential as food and medicine.

8.4.7 Effect of Bamboo Shoots on Kidney Function

Kidney functions in all the experimental groups were monitored by analyzing the levels of serum creatinine, blood urea and blood urea nitrogen (BUN) which are commonly measured to determine kidney health. Creatinine is a breakdown product of creatine phosphate in muscles and is usually produced at a fairly constant rate by the body. It passes into the bloodstream and is usually passed out in the urine. Urea is also a waste product formed from the breakdown of proteins and passed out in the urine. BUN tests measure the amount of nitrogen in the blood. Urea nitrogen is a breakdown product of protein. Generally, a high blood level of creatinine, urea and BUN indicate that the kidneys may not be working properly. In a study carried out by Bajwa et al (2017), the nonsignificant increase was observed in the level of serum creatinine, blood urea and blood urea nitrogen as compared to the control group (Table 8.11). This might be due to the presence of high-protein content in

TABLE 8.11
Effect of Fresh Shoot Extract on the Kidney Function in Balb/c Mice

Parameter (mg/dl)	Control	FBSE (800 mg/kg b.w.) Treated Group
Creatinine	0.332 ± 0.02	0.348 ± 0.05
Blood urea	51± 0.34	55 ± 0.92
BUN	25 ± 0.09	27 ± 0.31

Values reported are measurement replication means ± standard deviation (n = 6 replicates).
BUN—Blood urea nitrogen

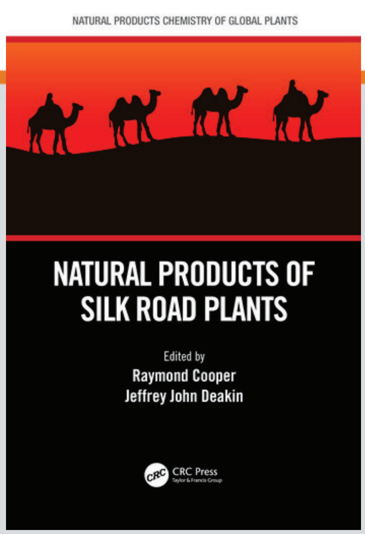
juvenile bamboo shoots. It has been reported that a high-protein diet is associated with increased glomerular filtration rate (GFR), serum creatinine, urea, urinary calcium excretion and serum concentrations of uric acid.

It is concluded that bamboo shoot being rich in nutrients, anti-oxidants and bioactive compounds has all the ideal characteristics for being used as a food additive and also exhibit potential as raw materials for the pharmaceutical, nutraceutical and food industries (Chongtham et al. 2018). In recent years, there has been considerable emphasis on the use of natural products as preservatives in foods, cosmetics and medical products as synthetic chemical compounds have caused health concerns to consumers. Bamboo is endowed with several health beneficial properties due to which its application in the pharmaceutical industry is gaining much importance. Anti-oxidants have a long history of use in nutrition, health and in the food industry (Chongtham et al. 2018). In the past, anti-oxidants were used to control oxidation and retard spoilage but today, many are used because of putative health benefits. The understanding is that anti-oxidants improve health by removing reactive species that may otherwise exert harmful metabolic effects. Many dietary compounds are capable of negating the danger of ROS—vitamin C, tocopherols, carotenoids, polyphenols, etc. It has been suggested that including these compounds in foods and medicine will enhance their capacities to support protection against ROS damage and reduce the risk of chronic diseases. The emergence of natural products with less harmful effects has become highly desirable. Natural anti-oxidants derived from plant products have been proposed as replacements for synthetic anti-oxidants to prevent spoilage. In addition, anti-oxidant activities of different agents have claimed to have potential health functions for reducing aging and possible prevention of cancer and heart diseases. Phenolic compounds are known for their anti-oxidant activity and bamboo is rich in these compounds.

CHAPTER

6

RESOURCES ALONG THE SILK ROAD IN CENTRAL ASIA: LAGOCHILUS INEBRIANS BUNGE (TURKENSTAN MINT) AND MEDICAGO SATIVA L.



This chapter is excerpted from
Natural Products of Silk Road Plants
by Raymond Cooper, Jeffrey John Deakin

Learn more

6 Resources along the Silk Road in Central Asia: *Lagochilus inebrians* Bunge (Turkestan Mint) and *Medicago sativa* L. (Alfalfa)

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CONTENTS

The Genus *Lagochilus* and *L. inebrians* (Turkestan Mint) in Central Asia

The Silk Road was a network of caravan routes connecting East Asia with the Mediterranean region in times of antiquity. One of the commodities moved along the road was silk exported from China, hence the name. As early as the 2nd century BCE, this route crossed Central Asia starting from the ancient city Xi'an (Shaanxi Province, central China) through Lanzhou to Dunhuang (Gansu Province, western China), where it bifurcated. The northern road passed through Turpan (Xinjiang Uygur Autonomous Region in western China), then crossed the Pamir and went to Ferghana and crossed the Kazakh steppes. The southern road passed the Lop Nor Lake, on the southern outskirts of the Taklamakan desert, through Yarkant and along the Pamir mountain ranges, eventually crossing the Central Asian region known as Bactria. From there, the road led to Parthia (part of modern Iran), the Middle East, and the Mediterranean Sea.

During the long journey across this vast region, travelers would inevitably experience a variety of injuries. Local people used infusions and decoctions of the plant species, *Lagochilus inebrians* Bunge (Lamiaceae), as a hemostatic remedy. This species is still used in this way today in Central Asia. Each year, large amounts of plant material are collected, especially in the Samarqand and Jizzax Provinces of Uzbekistan. The plant also has a long history of traditional use as an inebriant and a sedative by local tribesmen, hence its species epithet, *inebrians* and the colloquial names inebriating mint, intoxicating mint, or Turkestan mint. Species of the genus, *Lagochilus* Bunge ex Benth., are found primarily on dry slopes, in valleys and deserts from Iran through northern Pakistan, Central Asia, south central Russia to Mongolia, and northwest China. The number of species recorded in the genus varies due to differences among regional texts on flora and a lack of recent monographic revision. According to Jamzad (1988), the genus contains ~60 species worldwide, while Tsukervanik (1985) recognized 44 species, which he arranged in two sections and six subsections. It is agreed upon that the greatest species diversity occurs in Central Asia. A total of 34 species can be found in the region with around 20 being endemic (Ikramov, 1976).

The genus consists of shrubs and sub-shrubs, which have bilabiate flowers arranged in verticillasters. The most widely utilized species in the genus is *L. inebrians* (Figures 6.1 and 6.2). This species is 20–60(80) cm tall, with numerous simple or branched, erect stems that are woody at the base. The shrub is densely leafy and covered with long, one to three segmented horizontally spreading hairs interspersed with many glandular, capitate, sessile hairs. The leaves are broad-ovate, cuneate at the base, with three to five broad-ovate lobes, entire or dentate margins, and petioles on the lower leaves. The flowers, arranged in verticillasters, are sessile and clustered in groups of four to six in the axils of the upper leaves, forming spike-shaped inflorescences. The bracts are stiff, reclinate, triangular-awl-shaped, and covered with long, two to three segmented hairs and glandular sessile capitate hairs. The calyx is bell-shaped with a

FIGURE 6.1 *Lagochilus inebrians* Bunge growing wild in Uzbekistan. (Photograph by Buston Islamov.)

FIGURE 6.2 *Lagochilus inebrians* flowers. (Photograph by Buston Islamov.)

funnel-shaped neck, pubescent and has five recurved teeth that are 5–6 mm long and spine tipped. There are four stamens, and the corolla is bilabiate, white or pale pink, and 1–1.5 times the length of the calyx. The fruit consists of four, 4–5 mm long glabrous, brownish nuts. This species generally flowers in May through September and produces fruits in July through September.

The genus *Lagochilus* occupies a relatively wide ecological range, occurring from lowland plains to the upper zones of mountain ranges. The plants in Central Asia generally grow in the hot, dry foothills and the middle mountain zone. However, some species are also found at an altitude of 3,200 m above sea level. The range of *Lagochilus* extends through the Tien Shan and Pamir-Alai mountain systems where the plants grow on foothill plains and low foothills on clayey, rocky, or gravelly slopes, and along dried up waterways. *Lagochilus* species are quite drought tolerant and have a growing season from late April to November. The plants often maintain a green color through the intense summer season when surrounding vegetation has dried out and senesced. *Lagochilus* can also be found in the dry *Artemisia*-grassland ecosystems of foothill steppes in the Samarkand, Bukhoro, and Qashqadaryo Provinces of Uzbekistan, along dry channels in the Chardzhou region of Turkmenistan, as well as in Kazakhstan, Kyrgyzstan, Tajikistan, and adjacent areas (Khalmatov et al., 1984; Akopov, 1990). In Uzbekistan, the most common species is *L. inebrians*, which can be found in the Samarqand, Buxoro, Qashqadaryo, and Surxandaryo Provinces.

Use of *Lagochilus* Species in Folk Medicine

Plants in the genus *Lagochilus* have a long history of use as both a therapeutic and an intoxicant. In particular, the peoples of Central Asia have used *L. inebrians* as hemostatic to stop bleeding. A decoction or tincture made from a dry mixture of flowers and leaves is widely used as a hemostatic agent after childbirth as well to treat nose bleeds and hemorrhoidal bleeding (Khalmatov et al., 1984; Grinkevich, 1991). Traditionally, the above-ground plant material is harvested during the flowering period and then air-dried in the shade. It has also been used as a treatment for allergies and skin disorders, as well as a hypotensive, an antispasmodic, and a sedative (Schultes, 1970; Schultes and Hoffman, 1979). Similarly, *Lagochilus platyacanthus* Rupr. has been used as a styptic folk medicine to treat hemorrhage and coronary heart disease in the Xinjiang Uygur Autonomous Region of China (Zhang et al., 2015). Additionally, *L. inebrians* has a long history of use as a psychotropic, inducing a mild state of euphoria. The dried leaves, and sometimes the stems and flowering tops, are used to prepare tea, which is mixed with honey or sugar to mask the very bitter taste (Bunge, 1847; Ratsch, 2005).

Documented Biological Activity of *Lagochilus* Species

As early as 1955, the Ministry of Health in the USSR allowed the use of an infusion of the plant as a hemostatic agent and as a sedative. In clinical studies, preparations from the aerial parts of plants have been shown to have significant biological activity by increasing the blood coagulation process and reducing vascular permeability, as well as having a sedative effect and lowering blood pressure (Khalmatov et al., 1984; Akopov, 1990). Preparations of the plant have been used to treat various types of bleeding including traumatic, uterine, hemorrhoidal, pulmonary, nasal, etc. It has also been used in the treatment of hemophilia, immune thrombocytopenic purpura (formerly referred to as Werlhof's disease), Henoch-Schonlein purpura, in cases of functional nervous disorders, allergic skin reactions, various forms of dermatitis (eczema, hives, neurodermatitis, etc.), as well as for stage 1–2 hypertension, glaucoma, and in surgeries to prevent severe bleeding (Khalmatov et al., 1984). Part of the hemostatic effect of the preparations is due to the presence of vitamin K and tannins (Grinkevich, 1991). When an infusion of an extract of the plant containing the drug, lagochilin, is administered to animals, the fibrinolytic activity of blood is found to be inhibited by the activation of plasma inhibitors and suppression of fibrinolysis pro-activators. Aqueous extracts of the plant have sedative, hypotensive, and hyposensitizing activities, and stimulate the contractile ability of the smooth muscles of the uterus, and the contractile and motor functions of the stomach and intestines. In experiments with animals given a single sub-lethal treatment of X-ray irradiation, daily subcutaneous administration of the plant infusion contributed to the rapid restoration of vital activity. The preparations also had a sedative effect and enhanced inhibitory processes in the cerebral cortex and helped to eliminate experimental neurosis and seizures caused by treatment with strychnine or caffeine in animal models. A noticeable decrease in the arteriole and capillary wall permeability was observed in experiments where animals were treated with plant preparations (Sokolov and Zamotaev, 1989). The known active compounds from *L. inebrians* are a diterpenoid, tetrahydric alcohol called lagochilin (Figure 6.3) and its acetyl derivatives, which are poorly soluble in water. Based on these compounds, a number of hemostatic drugs have been created for both intravenous and oral uses. Other forms of application have been employed including a hemostatic gel, a bandage, and a collagen film. In experiments with animals, an intravenous injection of a 10% infusion of the plant extract of the closely related species *Lagochilus gypsaceus* Vved., accelerated coagulation of the blood by 30% in 30 minutes, decreased the time of recalcification by 38%, increased plasma tolerance to hepatitis by 35%, and decreased blood pressure by 7% (Zaurov et al., 2013). Treatment with an alcoholic tincture of the plant increased blood coagulation and induced a significant increase in antihemophilic globulin in children with hemophilia A. Clinical observations showed that hemophilia patients treated with *L. inebrians* preparations had improvement in their general condition, remission periods increased, bleeding time was shortened, the resorption time of hematomas and hemarthroses was shortened, and organ soreness

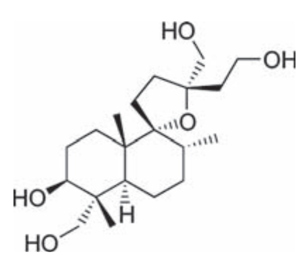

FIGURE 6.3 Molecular structure of lagochilin.

was reduced. When applied topically, the tincture also had a hemostatic effect. In instances where a side effect of an increased heart rate was observed, it was deemed necessary to reduce the dosage of the drug (Sokolov and Zamotaev, 1989).

According to I.E. Akopov (1981), more than ten species of plants of the genus *Lagochilus* have a stimulating effect on blood coagulation, while five species do not stimulate, but slow down the coagulation process. *Lagochilus* preparations increased the coagulation ability of blood by activating plasma and cellular coagulation factors, depressing the anticoagulant system, and reducing plasma fibrinolytic activity. Aimenova et al. (2016) investigated the effects of treatment with a *Lagochilus setulosus* Vved. extract (called "Setulin") on the process of blood hemostasis in rabbits with heparin-induced hypocoagulation. Oral introduction of the extract (50 mg/kg) increased the hemostatic effect associated with thromboplastin formation and transformation of prothrombin to thrombin. The extract completely negated the hypocoagulative effect of heparin in 60–90 minutes.

Phytochemistry of *Lagochilus* Species

The above-ground plant parts of *L. inebrians*, collected during flowering time in the vicinity of Samarkand (Uzbekistan), contained 0.60%–1.978% lagochilin, 0.20% stachydrine, 9.66%–12.42% resins, 0.068%–0.217% essential oil, 2.58%–2.78% tannins, 3.94%–6.41% sugar, 6.0%–7.025% total titrated organic acids, 0.67% flavonoid glycosides, 5.08%–8.04 mg% of carotene, 44%–77 mg% of vitamins C and K, calcium and iron salts, and 20 different microelements including cobalt, strontium, titanium, gold, arsenic, etc. (Khalmatov, 1964; Akopov, 1990). Flavonoids including quercimeritrin, rutin, quercetrin, and acacetin have been isolated from the plant among others, and the roots contain 2% tannins (Khalmatov et al., 1984; Chikov, 1989; Zhang et al., 2014). In a study of wild and cultivated *L. inebrians* plants, Zainutdinov et al. (2011) found that plants cultivated in foothill areas of the Navoi Region in Uzbekistan had 18%–20% more lagochilin than wild-growing plants.

A detailed, systematic phytochemical study of the genus *Lagochilus* was begun in 1971 by A.S. Sadykov (Uzbekistan Academy of Sciences). As a result of the study of about 10 *Lagochilus* species, more than 25 new diterpenoids of the labdane series (type) were isolated. On the basis of one of them, an effective hemostatic preparation, "Lagoden", was created for intravenous administration. A second drug, "Inebrin", was created on the basis of extractive substances of *L. inebrians* and was recommended in the form of tablets for the treatment of chronic uterine, nasal, gastrointestinal, and other bleeding conditions (Zainutdinov, 1993). Zainutdinov et al. (2002) studied 12 Central Asian *Lagochilus* species from which they isolated 25 diterpenoids with a 9,13-epoxylabdane skeleton, 20 of which were novel compounds. Previously identified hydrocarbons, and steroids were found, as well as flavonoids and iridoids, which were identified in nearly all the species investigated.

Akramov et al. (2019) analyzed the chemical composition of *L. gypsaceus*, *L. inebrians*, and *L. setulosus* essential oils from Uzbekistan. The essential oil of *L. gypsaceus* contained linalool, β-ionone, trans-chrysanthenyl acetate, and α-terpineol as the primary components; *L. inebrians* contained trans-chrysanthenyl acetate, eugenol, trans-verbenol, bicyclo[3.1.1]hept-3-en-2-one, and pinocarvone in the greatest abundance, and *L. setulosus* contained 2,4-bis(1,1-dimethylethyl)phenol, bicyclo[3.1.1]hept-2-en-4-ol, hexadecanoic acid, limonene, and 2-hexenal as dominant components. The essential oil of *L. inebrians* exhibited the best antioxidant and tyrosinase inhibitory activities, while *L. setulosus* essential oil exhibited the strongest inhibitory effect against amylase. The chemical constituents from the ethanolic extract of *L. platyacanthus* were analyzed, and 21 compounds (including 15 flavonoids, 3 lignans, 2 iridoids, and 1 phenylethanoid glycoside) were isolated from the plant for the first time with many being new to the genus, as well. In a study on the chemical constituents of *L. platyacanthus*, Zhang et al. (2015) identified five new diterpenoids (lagoditerpenes A–E) and ten known compounds. The known compounds were identified as diterpenoids (13E)-labd-13-ene-8α, 15-diol, leojaponins B, leoheteronin D, enantio-agathic acid, isocupressic acid, 7β, 13 S-dihydroxylabda-8(17), 14-dien-19-oic acid, 8α, 13(R), 14(S/R), 15-tetrahydroxylabdane, 15-nor-14-oxolabda-8(17), 12E-diene-18-oic acid, 12β, 19-dihydroxymanoyl oxide, and ent-12α, 19-dihydroxy-13-epi-manoyl oxide. Three of the compounds showed moderate hemostatic activity *in vitro*.

Summary

The unique medicinal properties of the *Lagochilus* species have been utilized over centuries in the folk medicine of Central Asia as a hemostatic, sedative, and recreational intoxicant. Significant pharmacological activities have been documented during the 20th and 21st centuries. The genus still requires detailed examination in order to improve definition of its taxonomy and delineation. New phytochemical discoveries are still being made – fully justifying further research on the biochemical and biological activities of the *Lagochilus* species.

Alfalfa (*Medicago sativa* L.) in Central Asia and beyond

During the journey from east to west, commodities passed through many hands. Routes crossed mountains, deserts, and other areas with limited vegetation. Donkeys, horses, and camels were the primary modes of transportation for both goods and people. Pack animals need an efficient and sufficient supply of fodder – one of the main sources being alfalfa (*Medicago sativa* L.), which grows wild in these regions. The plant also appeared to influence the condition and vigor of animals. Alfalfa is a plant of great value for ruminants as it is a significant source of high-protein roughage while being highly digestible (Morris et al., 1992). It has been called the "queen of forage crops" because of its remarkable ability to produce high yields of palatable, nutritious forage under a wide range of soil and climatic conditions across much of the world (Lubenets, 1936, Sinskaya, 1950; Barnes et al., 1988).

Alfalfa was one of the first domesticated fodder plants to be cultivated having been used for over three millennia. According to Vavilov (1951), *M. sativa* has a center of diversity in the Middle East (Asia Minor, Transcaucasia, Iran, and the Turkmen highlands). Some theorize that the domestication of alfalfa coincided with the domestication of the horse around 5000–6000 BC, although others have estimated that domestication occurred as early as 8000 BC (Small, 2011). The range of *M. sativa* has been expanded over the millennia. Historical records provide evidence for the distribution of alfalfa in Turkey and in Media (NW Iran) during the first millennium BC. The name "alfalfa" has been traced to an ancient Iranian word meaning "horse fodder". Alfalfa was known as the "median herb" by Romans which is celebrated in the scientific name of the genus *Medicago* (Sinskaya, 1950, 1969; Bolton et al., 1972). The oldest historical reference to the occurrence of alfalfa comes from stone tablets found in Turkey. Hittite (Anatolian) brick tablets (1400–1200 BC) discovered in Turkey indicate that animals were fed alfalfa all through the winter season since alfalfa was regarded as a highly nutritious animal feed (Lubenets, 1956).

It is widely accepted that travel by sea was well established in the eastern Mediterranean region as early as 4000 B.C. and significantly influenced the distribution of *M. sativa*. In addition, many regions (e.g., the Mesopotamian plain, Iraq) were a meeting place for many trade routes of the peoples of Asia, Africa, and Europe. Hence, the expansion of alfalfa's distribution occurred early and followed in the path of historic civilizations from east to west (Sinskaya, 1950; Bolton et al., 1972).

Alfalfa soon gained importance in Greek agriculture and was acquired by the Romans from Greek civilization in the 2nd century BCE. The practice of cultivating alfalfa migrated to what is now southern Spain in the 1st century. From Spain, it slowly spread to other regions such as modern France, Belgium, Holland, England, Germany, Austria, Sweden, and Russia during the 16th and 18th centuries. In the 18th century, the distribution of alfalfa was expanded across the world with the Spanish and Portuguese taking it from Europe to America and colonists introducing it to Australia and South Africa in the 19th century (Sinskaya, 1950; Bolton et al., 1972). It can currently be found growing wild, whether native or naturalized, from China to Spain and from Sweden to North Africa, and has been introduced in North America, Australia, and Africa.

Biogeography of Alfalfa

M. sativa L. is found growing in the wild in Asia and Europe, and is cultivated on six continents. The area of wild alfalfa extends from western Turkey to the Dzungarian Ala-Tau, Tibet, and Western India.

The occurrence of *M. sativa* to the west of this area (Germany, France, Italy, Spain, and Portugal) is not native most likely because it has escaped from cultivated areas. Populations in the Balkans region remain under some doubt. Various species of *Medicago* grow in the wild and are thought to be native in this area (Sinskaya, 1950; Zhukovsky, 1971; Bolton et al., 1972). Alfalfa is widely cultivated in temperate regions; the area under production gradually decreases toward the Arctic and the tropics. When grown in tropical climates, alfalfa becomes short-lived and usually thins out and dies after 1–2 years. The most ancient areas of alfalfa cultivation are concentrated in the Asia Minor, Central and Southwest Asia. From here, even in ancient times, alfalfa began to gradually spread to the west (Belov, 1931; Bolton et al., 1972). By at least the end of the 5th century BC, alfalfa was being grown in Greece, where it was brought from the original Asian-Iranian mountainous regions. The ancient East, in very distant times, had close ties with the ancient world of Eastern Europe (Gafurov, 1977, 1989). Aristophanes and Aristotle mentioned the cultivation of alfalfa in ancient Greece. From Greece, alfalfa passed to the Italian Peninsula around 200 BC and was introduced to the oases of North Africa as well as Spain (Zhukovsky, 1971).

At the beginning of the 16th century, alfalfa was brought to Mexico by the Spanish, and subsequently to Peru and Chile, and then Uruguay and Argentina. Thus, in the Western Hemisphere, alfalfa was first established in South and Central America. The practice of alfalfa cultivation came to North America in two ways: from South America and directly from Europe. In the mid-19th century, alfalfa, under the name "Chilean clover", was brought in to the country by gold miners traveling from Chile to California. It later spread to Colorado, possibly from Mexico (Bolton et al., 1972). Alfalfa came to India from Afghanistan, where it is generally confined to oases as in Arabia and North Africa. Alfalfa was imported into the European territory of the former USSR from Central Asia, mainly from the Khorezm (Khiva) Region (*Medicago asiatica* subsp. *khivinica* and its hybrid populations). Currently, alfalfa is widely produced in the United States, Canada, Argentina, Chile, Peru, southern France, northern Italy, Asia Minor, Central Asia, Iran, Australia, and New Zealand. Alfalfa cultivation spread to China from Central Asia, and the greatest area of production is concentrated in western Xinjiang Province.

Botanical Description

The genus *Medicago* L. contains ~87 annual and perennial species (Small, 2011). *M. sativa* is a difficult species to define as it has been complicated by polyploidy and the influences of hybridization and domestication. This has led to complex circumscriptions with *M. sativa* being split into numerous species with many infraspecific taxa (Sinskaya, 1935a,b, 1948, 1950, 1960; Maisuryan, 1970; Lubenets, 1972, among others). Some authors, such as A.I. Belov (1929), described ecological and geographical groups of *M. sativa*. More recently, recognition of a single broadly circumscribed species has been adopted in lieu of highly segregated treatments (Quiros and Bauchan, 1988). In a recent monograph, Small (2011) recognized the following infraspecific taxa: *Medicago sativa* subsp. *sativa*, *Medicago sativa* subsp. *caerulea* (Less. ex Ledeb.) Schmalh., *Medicago sativa* subsp. *falcata* (L.) Arcang. var. *falcata*, *Medicago sativa* subsp. *falcata* (L.) Arcang. var. *viscosa* (Rchb.) Posp., *Medicago sativa* subsp. ×*varia* (T. Martyn) Arcang., and *Medicago sativa* subsp. *glomerata* (Balbis) Rouy. In Central Asia, the two most common taxa are recognized as independent species *M. sativa* L. (Figure 6.4) and *Medicago falcata* L. (Figure 6.5). The morphological differences of these species are described in Table 6.1.

M. sativa is an herbaceous perennial plant with a taproot and strongly developed lateral roots. In the first year, the roots penetrate to a depth of 2–3 m, and in subsequent years can grow as deep as 10 m. The stem is herbaceous, up to 1 (1.5) m in height and strongly branched. A mature plant will branch from the base, sending up multiple stems that are ascending or erect and that occasionally branch. The stems are usually hairless, particularly as they become older. The alternate compound leaves are olive-green and trifoliate. Each leaflet is oblanceolate or obovate, wedge-shaped at the base, and nearly truncate at its outer edge. The margin is smooth, except for some dentate teeth toward the apex. A typical leaflet is about 2–2.5 cm long and 8 mm wide. At the base of each compound leaf are two small lanceolate stipules. Some stems have terminal inflorescences consisting of many-flowered racemes about 2–5 cm in length. Each flower is about up to 1 cm long, consisting of five petals that are lavender or purple, ten stamens, a single pistil, and a green calyx. The flowers have the typical papilionaceous flower structure,

FIGURE 6.4 *Medicago sativa* L. (Photograph by Oimahmad Rahmonov.)

FIGURE 6.5 *Medicago falcata* L. (Photograph by Vladimir Yanov.)

TABLE 6.1

Morphological Differences between *Medicago sativa* and *Medicago falcata*

Character	*Medicago sativa* L.	*Medicago falcata* L.
Flower color	Purple	Yellow
Fruit shape	Spiral (twisted 1–5 times)	Sickle-shaped or straight
Leaflet size	Medium and large	Small
Leaflet shape	Elongate elliptic or obovate, rarely narrow	Narrow, almost lanceolate
Leaflet pubescence	Weakly and moderately pubescent with short, rarely long hairs	Strongly pubescent with long hairs

with a large standard, a keel, and two small side petals. The standard and keel are somewhat spread apart, exposing the throat of the flower. The calyx has five long teeth, and it often has scattered white hairs. The blooming period usually occurs during the summer and lasts about 1–2 months. However, some plants may bloom during the late spring or early fall. The flowers are replaced by tightly coiled fruits (legumes) that are about 8–9 mm in length. They are flattened with a reticulate surface, sometimes with stiff hairs along the outer edges. Each fruit contains several yellowish-brown, reniform seeds.

Biology of Alfalfa

One of the distinctive features of alfalfa among forage crops is its symbiotic relationship with nitrogen-fixing bacteria. This form of symbiosis arose in the course of evolution independently several times, both within legumes and among other families. In the specialized root nodules, free atmospheric nitrogen is reduced to ammonium and is then assimilated into organic compounds including amino acids (protein monomers), nucleotides (DNA and RNA monomers), the energy storage molecule adenosine triphosphate (ATP), vitamins, flavones, and phytohormones. Because of this unique ability to obtain nitrogen, alfalfa can colonize steep slopes with minimal soil fertility in many mountain regions of Central Asia.

Alfalfa has evolved in a continental climate characterized by cold winters and hot, dry summers. In the natural environment, the soil (*Calcisols*, *Gypsisols*, *Aridisols*, *Kasztanozems*, *Mollisols*, *Chernozems*, *Cambisols*, *Inceptisols*) on which alfalfa grows is often near neutral with subsoils containing significant amounts of calcium carbonate. Optimal soil pH ranges from 6.5 to 7.8. Alfalfa has evolved deep-growing root systems in order to grow in dry and semi-dry climates such as that of non-irrigated areas (bogara) in Central Asia. Although alfalfa can grow in areas with reduced fertility and rainfall, in Central Asia and surrounding regions, alfalfa cultivation typically requires irrigation to increase productivity. With irrigation, 3-year-old alfalfa accumulates 300–400 kg/ha or more of nitrogen in the soil from atmospheric nitrogen fixation (Shatilov, 1986). Alfalfa thrives in full sun exposure, and the growing season lasts a very long time from early spring to late autumn. In some areas of Central Asia, with irrigation, alfalfa can produce up to seven harvests in a single season. Alfalfa is a cold-resistant species and can tolerate frosts up to −6°C. Spring re-growth begins between 7°C and 9°C.

Alfalfa as a Fodder Plant

Alfalfa is grown on ~45 million ha worldwide (Mielmann, 2013). As fodder, it is valued as a good source of slow-release carbohydrates, proteins, minerals, and vitamins (Tharanathan and Mahadevamma, 2003; Hao et al., 2008). It contains between 15% and 22% crude protein on a dry matter basis, as well as macro and trace elements and all fat and water-soluble vitamins (Adapa et al., 2007). Alfalfa is a valuable source of vitamins A and E. It contains beta-carotene, thiamine, riboflavin, niacin, pantothenic acid, biotin, folic acid, choline, inositol, pyridoxine, vitamin B12, and vitamin K (Aganga and Tshwenyane, 2003). Furthermore, alfalfa hay has a higher mineral content than grains like maize and wheat (Morrison, 1961).

In Central Asia, alfalfa is irrigated and fertilized to increase the quality of forage or silage and in some areas may be harvested three or four times per season. The number of harvests can influence chemical composition and yield. In high-mountain areas without irrigation, plants are harvested only once per season. After that, the area is used as pastureland for livestock. Alfalfa may be harvested up to seven times per year in some prime growing areas of the world with optimal control of fertilization and irrigation.

Alfalfa is mainly used to make hay and silage but can also be used for grazing purposes because of its high yield, quality, and wide adaptability to different climates and soil types. In the last few decades, alfalfa's popularity and potential for human consumption for specific health conditions have increased (Mielmann, 2013). Because of its high nutritive value, it is cultivated widely for livestock production to promote weight gain and for wool production (Douglas et al., 1995).

Use of Alfalfa in Folk Medicine

In folk medicine, alfalfa has been used as an antidiabetic (hypoglycemic), a diuretic, and an antitumor agent. The seeds and leaves have been used as an abortifacient, as a sedative, and to reduce fatigue. In medieval medicine in Armenia, plant seeds were used to increase male potency (Druzhinina and Novikova, 2010). In Buryatia (Russia), the above-ground plant material of *M. falcata* is collected during the flowering period and a water decoction is used to treat nervous disorders. In Tibetan medicine, the decoction is also used in traditional medicine (Alekseeva et al., 1974). In Chinese and Indian traditional medical systems, doctors used young leaves of alfalfa to treat disorders related to the digestive tract, arthritis, and water retention. A cooling poultice prepared from seeds has been used to treat boils. In the Caucasus, powdered, dried alfalfa has been used as a wound-healing treatment, especially for cuts (Khalmatov, 1964). Extracts of alfalfa have been used as an ingredient in cosmetics. Due to the content of phytoestrogens (isoflavones and coumarins), alfalfa has been thought to regulate menstrual cycles and stimulate milk flow in breastfeeding women. In addition, traditional medicinal use of alfalfa sprouts or leaves includes treatment of arthritis, kidney problems, boils, cancer, and as an anti-rheumatic, cardiotonic, depurative, lactagogue, antipyretic, emmenagogue, and antiscorbutic (Foster and Duke, 1990; Barnes et al., 2002). Leaves of alfalfa are used traditionally as a tea to treat diabetes in South Africa.

Documented Biological Effects of Alfalfa

The extracts from alfalfa sprouts, leaves, and roots have been indicated to be helpful in lowering cholesterol levels in animal and human studies (Story et al., 1984). Eating a diet containing alfalfa decreased blood-cholesterol levels and helped to protect monkeys from atherosclerosis that were on a high-cholesterol diet (Mielmann, 2013). Consuming alfalfa seeds helped normalize serum cholesterol concentrations and decreased low-density lipoprotein cholesterol and apolipoprotein B concentrations in patients with type II hyperlipoproteinemia (Mölgaard et al., 1987). A diet containing alfalfa decreased hyperglycemia in streptozotocin-induced diabetic mice. The results demonstrated the presence of anti-hyperglycemic, insulin-releasing, and insulin-like activities in alfalfa (Gray and Flatt, 1997). Wang et al. (2012) investigated the effects of alfalfa saponins on cholesterol metabolism and the gene expression in the liver of hyperlipidemic rats. Alfalfa saponins prevented and treated hyperlipidemia by increasing the expression of CYP7Al and LDL-R in the liver and by promoting the excretion of liver cholesterol. Shi et al. (2014) assessed the cholesterol-lowering effects of alfalfa saponin extract and observed an increase in cholesterol excretion and a down-regulation of the Hmgcr and Acat2 genes, as well as up-regulation of Cyp7a1 and Ldlr in the liver of hyperlipidemic rats. In an *in vitro* analysis assessing antibacterial activity, alfalfa leaves extracts exhibited activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Chavan et al., 2015). In an experiment to assess the effects of a saponin-rich alfalfa extract on the pathogenic fungi *Candida albicans*, results revealed a significant reduction in germ tube formation, reduced hyphal growth, reduced yeast adherence and biofilm formation, and eradication of a mature biofilm (Sadowska et al., 2014). As the content of the phytoestrogen coumestrol increases in pasture feed, there is a correlated reduction in animal fecundity (Khalmatov, 1964). In an experiment with streptozotocin-diabetic mice, a diet containing alfalfa had antihyperglycemic, insulin-releasing, and insulin-like effects and reduced hyperglycemia (Gray and Flatt, 1997).

Alfalfa for Human Consumption

Although alfalfa is generally known as a feed source for livestock, it is becoming more popular in many parts of the world for human consumption as it is a valuable source of protein which could contribute to sustainable food development in developed countries (Mielmann, 2013). Alfalfa sprouts are widely consumed by humans as a garnish. Concentrates of proteins from leaves and the dehydrated plant are components of many nutritional supplement products (Hatfield, 1990). Alfalfa has been used as food in parts of Russia, China, America, and South Africa. In the past, alfalfa meal was incorporated into a cereal mixture and used to nourish small children (Levy and Fox, 1935). Chinese farmers have also consumed it as a vegetable, and it has been utilized to increase the protein, dietary fiber, mineral, and vitamin content of wheat flour (Hao et al., 2008). Alfalfa is one of the most popular sprouts available and is often consumed raw or slightly cooked in salads and sandwiches or as decorative appetizers (Peñas et al., 2009). Alfalfa sometimes has a bitter taste due to its saponin content (Sen et al., 1998). Recent sensory tests conducted with human volunteers using saponins isolated from above-ground parts of alfalfa have shown that zahnic acid tredismoside is responsible for the taste (Oleszek, 2002). L-canavanine is a potentially toxic non-protein amino acid, antimetabolite of L-arginine that is stored by many leguminous plants. This compound has shown anticancer activity against a number of carcinomas and cancer cell lines. The occurrence of canavanine in alfalfa products has also stimulated considerable interest due to the correlation between high amounts of canavanine consumption and the onset of a systemic lupus erythematosus-like syndrome (Rosenthal and Nkomo, 2000). An investigation was conducted to determine the canavanine content in commercially available sprouts and in the seed of ten alfalfa cultivars. The sprouts contained canavanine ranging from 1.3% to 2.4% of the dry matter, depending on the source. Alfalfa seeds were also rich in canavanine with contents varying from 1.4% to 1.8% of the dry matter. On average, the tested seeds contained 1.5%±0.03% canavanine. Breeding cultivars of alfalfa with high protein and nutrient content and low canavanine will be important if human consumption of alfalfa is going to increase substantially.

Phytochemistry of Alfalfa

Alfalfa contains many secondary metabolites including saponins, tannins, coumarins, carotenoids, tocols, flavonoids, steroids and alkaloids (Alekseeva et al., 1974; Knuckles et al., 1976; Livingston et al., 1980; Hernández et al., 1991; Bisby et al., 1994; Thring et al., 2009). The above-ground portions contain vitamin C (110%–304.4 mg%), vitamin D, carotene, phytoestrogenic compounds, including formononetin glycosides and coumestrol (Figure 6.6), saponins with a hemolytic index of 1:100 and 5%–7% ash, in which there are up to 40% calcium salts. As the content of coumestrol increases in pasture feed, there is a correlated reduction in animal fecundity (Khalmatov, 1964). Alkaloids contained in the plant include trigonelline, stachydrine, and homostachydrine (Duke, 1985; Mills, 1994; Dixon, 2004; Bora and Sharma, 2011).

Stochmal et al (2001) identified nine flavones and adenosine in the aerial parts of alfalfa including apigenin 7-*O*-[β-D-glucuronopyranosyl(1→2)-*O*-β-D-glucuronopyranosyl]-4′-*O*-β-D-glucuronopyranoside, apigenin 7-*O*-[2-*O*-feruloyl-β-D-glucuronopyranosyl(1 → 2)-*O*-β-D-glucuronopyranosyl]-*4*β-*O*-β-D-glucuronopyranoside, apigenin 7-O-{2-*O*-feruloyl-[β-D-glucuronopyranosyl(1→3)]-*O*-β-D-glucuronopyranosyl(1→2)-*O*-β-D-glucuronopyranoside}, apigenin 7-*O*-{2-*O*-p-coumaroyl-[β-D-glucuronopyranosyl(1 → 3)]-*O*-β-D-glucuronopyranosyl(1 → 2)-*O*-β-D-glucuronopyranoside}, and luteolin 7-*O*-[2-*O*-feruloyl-β-D-glucuronopyranosyl(1 → 2)-*O*-β-D-glucuronopyranosyl]-4′-*O*-β-D-glucuronopyranoside.

The chemical composition of the forage crop was presented by Bickoff et al. (1972) and Mustafa et al. (2001). In the above-ground parts of alfalfa, measurements of crude protein, crude fat, nitrogen-containing compounds, proteins, non-protein nitrogen, amide and amino acid nitrogen, lipids, plant sterols, triterpenoid, carbohydrates, vitamins, water-soluble vitamins, micro-macro elements, and others were taken. The individual tested elements or organic compounds were found in varying concentrations in different parts of the plant (leaves, stem, seeds, and roots). Higher concentrations of protein, vitamin, and macroelements occurred in the leaves and young stems than in main stalk (Bickoff et al., 1972; Mustafa et al., 2001). Nitrogen-containing compounds and carbohydrates occur mainly in stems and seed. *M. sativa* contained the highest amount of polyphenol compounds and exhibited the greatest antioxidant activity through the scavenging of free radicals (Rana et al., 2010).

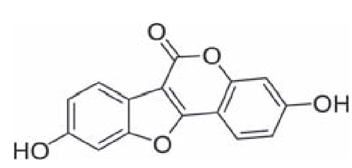

FIGURE 6.6 Molecular structure of the phytoestrogenic compound coumestrol.

Summary

Alfalfa (*M. sativa* L.) is likely to have been the first plant species grown exclusively for forage. It has become one of the most important forage crops for animals and is now cultivated worldwide. Having high nutritional value, alfalfa served as the perfect fodder for the beasts of burden transporting goods along the silk route.

The uniqueness of alfalfa for agriculture lies in its biological and ecological properties. Despite the lack of scientific knowledge, people from earlier millennia recognized these beneficial characteristics which led to the domestication and cultivation of alfalfa. The greatest diversity of genetic material is concentrated in centers of origin which are Central Asia, South Asia, and Siberia. While the Mediterranean and North American genetic centers are secondary, they have played an important role in the evolution, selection, and distribution of new alfalfa cultivars around the globe.

The largest genetic collections of alfalfa plants are maintained by the South Australian Research and Development Institute (SARDI), the United States Department of Agriculture and Germplasm Resources Information Network (USDA-GRIN), the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria, and the French Institute National de la Recherche Agronomique (INRA). While alfalfa is a valuable and essential forage plant, there is no doubt that a focused breeding program could provide a healthy and nutritious crop for human consumption.

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