Nuts & Seeds in Health and Disease Prevention

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CHAPTER 1

A Primer on Seed and Nut Biology, Improvement, and Use

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CHAPTER OUTLINE

You know that the seed is inside the horse-chestnut tree;
and inside the seed there are the blossoms of the tree, and the chestnuts, and the shade.
So inside the human body there is the seed, and inside the seed there is the human body again.

Kabir

INTRODUCTION

Seeds are the centerpiece of plants' sexual reproductive strategies and a critical stage in their life cycle. Seed traits play a direct role in plants' fitness. The diversity of these traits across species reflects the evolutionary pressures operating on plants. Adaptation to new environments is greatly facilitated by the genetic diversity and distinctiveness of every new seed cohort. Seed-producing plants (i.e., spermatophytes) invest significant resources in seeds, which is why seeds are an invaluable resource for innumerable animal species, including humans. Seeds have played a vital role in human diet and health since prehistory, yet they have numerous other roles in modern society. Science and technology are continuously expanding the range of potential uses for seeds. Substantial contributions to human wellbeing and health could derive from promising applications in emerging fields such as biopharmaceuticals. However, realizing this potential requires that we acknowledge the complexity of the issues involved — a task that entails recourse to the natural sciences as well as the humanities. In this chapter, we

Nuts & Seeds in Health and Disease Prevention. DOI: 10.1016/B978-0-12-375688-6.10001-5

consider some of these issues. We devote the first half of the chapter to definitions and concise descriptions of seed anatomy, development, and ecology. In the second half we turn to the social sciences for a brief outline of the historical interaction of seed plants and humans, and some of the consequences that this has had on both groups.

SEEDS, FRUITS, AND NUTS

Definition and Classification

Seeds can be defined in various ways. In science, they are defined from a developmental perspective: a seed is a mature, fertilized ovule (Copeland & McDonald, 2001). In everyday language, by contrast, seeds are defined by function – i.e., the term "seed" ordinarily refers to various structures involved in plant propagation (Table 1.1). Clearly, common usage of the term is much broader than scientific usage: fruits (containing one or more seeds) and tubers (which do not involve seed at all) often are referred to as "seed." Scientists sometimes speak of "true seed" when referring to structures derived from an ovule in order to distinguish between the scientific and colloquial connotations of the word. Scientific and popular usage of the term "fruit" also differs. From a developmental perspective, a fruit is a mature ovary that contains one or more seeds (Copeland & McDonald, 2001). In this case, therefore, the scientific definition is broader than the colloquial: apples, oranges, and peaches are fruit, as are legume pods, peppers, and cereal grains. False fruits are fruit-like structures not derived from an ovary (Table 1.1). Strictly speaking, a nut is a dry, one-seeded fruit with an extremely hard pericarp.[1] However, the term is commonly used in reference to various edible, oily seeds, often (but not necessarily) derived from a true nut (Copeland & McDonald, 2001).

Seeds and fruits exhibit remarkable diversity in shape, size, color, and chemical composition. There is no pre-eminent way to classify this diversity. Classifications can be based on a functional, morphological, or developmental perspective (Dickie & Stuppy, 2003). Anatomical classifications have the disadvantage that similar structures might have different origins in separate taxa. A classification based on developmental homology is more likely to generate monophyletic groups exhibiting anatomical and physiological similarities, but a rigorous developmental classification can be exceedingly complex. The most useful and widespread

TABLE 1.1 Definitions of Seeds, Fruits, and Nuts*

	Definition	Examples
True seed	A mature, fertilized ovule	Beans, cashew nuts
False seed	Any structure that works as a propagule	Cereal grains, potatoes and other tubers
True fruit	A mature ovary	Apples, legume pods, cereal grains, chestnuts
False fruit	Any fleshy, sweet-tasting, structure; structures involved in zoochorous seed dispersal	Cashew apples (marañón); arils, ovuliferous scales and other appendages of gymnosperm seed
True nut	A dry, one-seeded fruit with an extremely hard pericarp	Acorns, hazels and chestnuts
False nut	Any oily, edible seed, including those derived from true nuts	Peanuts, cashew nuts

*In science, seeds, nuts, and fruits are specific structures associated with sexual reproduction – i.e., the mature ovule and ovary. In everyday language, use of these terms is not as restrictive but applied to a wider set of structures involved in plant propagation.

[1] The pericarp, mesocarp, and endocarp are, respectively, the outer, middle, and inner layers of the ovary wall.

TABLE 1.2 Classification of Seeds

According to:	Types of Seed	Description
Ovule morphology	Anotropous	Ovules and seeds with a raphe (a continuation of the funicle that ends at the chalaza)
	Orthotropous	Ovules in which funicle, chalaza, nucellus, and micropyle are in a direct line
	Campylotropous	Ovules with curved embryo sacs
Embryo morphology	Basal	Small embryo restricted to the lower half of the seed
	Peripheral	Large, elongate, often curved embryo contiguous to the testa
	Axile (or axial)	Small to total embryo, central, straight, curved, coiled, bent, or folded
Origin of the mechanical layer of the seed coat	Exotegmic or exotestal	Inner (tegmen) or outer (testa) derived from outer epidermis
	Mesotegmic or mesotestal	Inner (tegmen) or outer (testa) derived from middle layer
	Endotegmic or endotestal	Inner (tegmen) or outer (testa) derived from inner epidermis
Endosperm	Exalbumious	Seeds with little or no endosperm.
	Albuminous	Seeds with a well-developed endosperm or perisperm

Scientific classifications of seeds are often based on internal morphology (Dickie & Stuppy, 2003).

TABLE 1.3 Classification of Fruits

	Mesocarp Dehiscence	Examples
Dry fruits	Dehiscent	Legume (e.g., bean, soybean), follicle (e.g., milkweed), capsule (e.g., tulip), or silicle
	Indehiscent	Achene (e.g., sunflower), utricle (e.g., Russian thistle), caryopsis (e.g., grasses), samara (e.g., maple), nut (e.g., acorn, hazel, filbert, chestnut), nutlet (e.g., mint family), or a schizocarp (e.g., carrot family)
Fleshy fruits	Dehiscent	Pomegranate, nutmeg
	Indehiscent	Berry (e.g., tomato), pepo (e.g., squash), pome (e.g., apple), drupe (e.g., cherry, coconut, walnut), or hesperidia (e.g., oranges)

Fruits are classified into dry or fleshy, dehiscent or indehiscent types according to the atrophy and dehiscence of the mesocarp (Copeland & McDonald, 2001; Dickie & Stuppy, 2003).

classifications tend to be eclectic (Copeland & McDonald, 2001; Dickie & Stuppy, 2003). Informal seed classifications are often based on external morphology (e.g., winged seeds); scientific classifications tend to rely on internal morphology (Table 1.2). As regards fruit, classifications commonly focus on characteristics of the mesocarp and pericarp (Table 1.3).

Structure and Development

A typical seed consists of three elements: the embryo (or young sporophyte), endosperm, and seed coat (Copeland & McDonald, 2001). Each element has a particular chemical composition (and consequently nutritious value) that depends as much on its genetic identity as on its role

in development. Seed development can be divided into three distinct stages circumscribed by four defining events: ovule fertilization, seed abscission, physiological maturity, and germination. Despite some commonalities, seed development differs considerably across the two groups of spermatophytes (Dickie & Stuppy, 2003). In angiosperms, ovules derive from meristematic tissue on the ovary wall — i.e., they are enclosed within the ovary, where fertilization and development occur. Gymnosperms' ovules, by contrast, are not borne within ovaries, but are naked, or protected by cones or scales.

The embryo, endosperm, and seed coat develop from distinct parts of the ovule. Seed development begins with an archesporial cell in the ovary wall giving rise to a haploid (1N) megaspore through meiosis (Copeland & McDonald, 2001; Werker, 1996). After a series of mitotic divisions, the megaspore is transformed into the mature female gametophyte or embryo sac, which contains a haploid egg and a diploid (2N) nucleus. The embryo sac is enclosed within specialized tissue called the nucellus, and both of them are enveloped by one or more layers of tissue (i.e., the integuments), leaving only a small opening at the apex, the micropyle. The embryo sac, nucellus, and integuments constitute the archetypical angiosperm ovule, which is connected to the ovary wall by the funiculus.

After landing on the flower's stigma, the pollen tube descends through the style and into the embryo sac through the micropyle, delivering two sperm cells (Werker, 1996). One cell fuses with the polar nucleus, forming a triploid (3N) endosperm nucleus; the other fuses with the egg cell to form the diploid fertilized egg (or zygote) that later will become the embryo. This is the characteristic double fertilization of angiosperms. Embryo and endosperm thus have distinct genetic identities because they derive from separate fertilization events. The embryo is diploid: one half maternal, one half paternal. The endosperm is triploid, derived from the fusion of a haploid sperm cell with the diploid polar nucleus.[2] Since the seed coat derives from the integuments of the mature ovule, it is of strictly maternal origin and haploid in nature.

At every stage in the seed's development, nutrients are drawn from several sources and used in the growth process or stored as reserve materials for later use (Werker, 1996). In some species, the nucellus functions as a temporary source of nutrients for the megaspore and then disappears. In other species, it continues to transport and store nutrients for the developing seed and seedling in the form of the pericarp. The embryo derives its nutrients from the nucellus and integuments through the embryo sac, via the endosperm, or directly from the ovary wall through the funiculus or specialized outgrowths called haustoria. In albuminous seeds, which include monocotyledons, endosperm and perisperm act as the main reserve tissue. In exalbuminous seeds, including most dycotyledons, the endosperm is completely consumed during development and absent in the mature seed, so reserve materials accumulate in the seed coat or the embryo itself, particularly the cotyledons. In addition to nutrients for the developing embryo (i.e., carbohydrates, fats, and proteins), seeds contain chemical compounds involved in growth control (e.g., vitamins and plant hormones), as well as reserves to be used by the quiescent embryo after abscission from the parent plant and upon germination (Copeland & McDonald, 2001). Seeds also contain antimetabolites and other compounds whose role requires that we address species-to-species interactions and other issues within the scope of plant ecology.

Ecology and Evolution

Seed traits are best explained from an evolutionary perspective — i.e., in terms of fitness advantages (Fenner & Thompson, 2005; Moles *et al.*, 2005). In many species, successful regeneration depends on traits that improve the sporophyte's survival through unfavorable growing conditions. Alkaloids, glycosides, and a thick seed coat help deter seed predators,

[2] In gymnosperms, the endosperm develops from the female gametophyte and is usually haploid.

while a large seed size increases the sporophyte's survival after germination. Other traits associated with seed dispersal and dormancy improve the sporophyte's chances of reaching a more favorable environment — i.e., a "safe site" (Fenner & Thompson, 2005). Some species depend on wind or water as dispersal vectors; others entice animal dispersers by providing a reward in the form of fruit or nutritious tissue on the seed's exterior, known as the elaiosome. In most cases, a sturdy coat protects seeds as they pass through animals' guts to end in feces and the soil, but sometimes significant numbers of seeds are digested. Sacrificing some seeds so that others might reach a safe site seems to blur the line between dispersion and predation. In fact, some traits play complex roles that defy easy understanding; for example, tannins provide resistance to infections but also influence the uptake of nutrients by predators. Although the advantage of individual traits might seem readily apparent, it still is unclear how different traits combine into a successful reproductive strategy (Moles *et al.*, 2005).[3]

Allocation of resources to seed and other sexual organs necessarily compromises plants' own growth and survival (Fenner & Thompson, 2005). Ovules and seed have a higher nutrient content than other tissues, yet few survive into the next stage in the life cycle. Allocation of resources to different seed traits presents similar compromises — for example, between producing numerous small, easily-dispersed seeds or larger albeit fewer seeds with a greater chance of establishment. According to the principle of allocation, plants distribute resources optimally in order to maximize their fitness given prevalent environmental conditions (Fenner & Thompson, 2005; Moles *et al.*, 2005). Vegetative propagation gives plants a foothold in undisturbed, highly competitive environments where seedling establishment is difficult; seed banks, by contrast, are most advantageous in unstable environments. Explaining a particular reproductive strategy is a more elusive task. Twenty years ago, it was suggested that seed size, dispersal, and dormancy co-evolve to maximize the sporophyte's survival up to the seedling stage. After innumerable studies, it is becoming clear that this explanation is too narrow. Seed traits might not be explicable without accounting for a species' complete life cycle (Moles *et al.*, 2005). In the case of crop species, explaining many seed traits requires addressing their interactions with humans.

A COMPLEX RELATIONSHIP

Ecological interactions between plants and humans going back thousands of years have been unique in many ways. For instance, substances that inhibit other predators — such as caffeine and nicotine — have been readily consumed by humans for their pharmacological effects. However, the relationship between these two groups has been significant in a more profound way, particularly since it developed into a symbiotic relationship where crop plants became a dietary staple for humans but lost the ability to survive in the wild. In this process, humans hijacked crop reproductive strategies, radically altering their ecology and evolution. The consequences for both groups are more readily apparent from a historical vantage point.

Historical perspective

Continuous innovations in dental and masticatory structures (documented in the fossil record) bespeak concomitant changes in the food resources exploited by human ancestors. Anatomical novelties allowed succeeding hominin species to consume previously inaccessible resources, and this had crucial implications. Access to hard nuts and roots as fallback foods opened up new habitats to colonization. Simultaneous increases in body size, robusticity, and encephalization had similar consequences. Increasing brain size presumably facilitated exploitation of an ever-wider resource base, leading to new rounds of changes that ultimately gave rise to modern humans. It has been suggested that the superior intelligence, long lifespan, cooperative

[3] A reproductive strategy is a complex of traits encompassing the spatial and temporal allocation of energy to reproduction.

behavior, and other attributes that characterize humans co-evolved after a dietary shift to high-quality, difficult-to-access food resources. At the same time, our ancestors must have begun cooking food early on, realizing that cooked foods are easier to assimilate — presumably leading to digestive alterations that prevent us from processing raw foods efficiently. A precise account of the forces that shaped humans still might be a long time coming, but there is no doubt that an increasing ability to manipulate food resources had significant consequences on human diet and health. An important milestone in this process was domestication.

Around 10,000 years ago, numerous plant species under cultivation experienced dramatic morphological and physiological changes (Gepts, 2005). A breakdown of seed dispersal and dormancy and other changes affecting the size, shape, and physiology of seeds and fruits (i.e., the so-called domestication syndrome) arose almost simultaneously, yet independently, around the globe. Early scholars described domestication as a natural response to human contact — a biological reaction to an unintentional shift in environmental conditions. More recent discovery of single-gene mutations with large-step effects of agronomic value seems to suggest a conscious effort (on the part of humans) to improve crop qualities. In either view, selection is considered the main force in crop evolution, and diversity a result of crops' adaptive radiation across environments and cultures (Gepts, 2005). Yet such a perspective neglects the larger social context of crop domestication and evolution. Although increases in agronomic value were an undoubtedly important goal for early farmers, since its beginning the relationship between crops and humans has been equally imbued with social, political, and cultural values. Such values constitute powerful forces with important implications for both seed plants and humans.

We often conceive of the rise of civilization as a period of constant increases in the amount, quality, and diversity of resources available to society, entailing a continuous improvement in human nutrition and health. Scholars argue, nevertheless, that the transition from hunting-gathering to farming and contemporary society has been marked by the upsurge of chronic health problems (Cordain *et al.*, 2005). Unable to adapt to continuous changes in diet, our digestive system still resembles that of ancestors whose diet was much richer in vegetable protein and fiber. Unlike pre-agricultural foragers, whose diverse diet included a variety of wild seeds and nuts, agricultural societies came to depend increasingly on domesticated grains. A cereal-based, carbohydrate-rich diet resulted in a sharp increase in dental decay, metabolic acidosis, and other afflictions among farmers. Dietary breadth was further reduced as humans transited from a rural to an urban lifestyle, gradually leading to a deficiency of essential vitamins, minerals, and other nutrients. The result was a long decline in human health extending up to the modern age, particularly in the western hemisphere (Steckel & Rose, 2002). Of course, this was not a result of crops' nutritional qualities alone, but ultimately the product of an inequitable society. Civilization was founded as much on agriculture as on a hierarchical social order where resources are distributed through complex rules (Newman, 1990). In recent years, malnutrition has come to be defined not only by undernourishment but also by overeating. While millions of people in the developing world remain undernourished, seemingly unlimited availability (and consumption) of energy-rich food in developed countries has led to the spread of high blood pressure, heart disease, insulin resistance, and other "diseases of affluence" (Cordain *et al.*, 2005).

Crop improvement today

Continuous advances in crop improvement since the early 20th century have fostered a steady growth of food supplies in industrialized countries (Jauhar, 2006). Introduced to the developing world later in the century, heterosis breeding and cytogenetics have achieved impressive results around the world; however, modern breeding has not reached everyone. Many farmers continue to grow landraces — i.e., low-yielding, traditional crop varieties. Ironically, the genetic diversity embodied in landrace seeds constitutes an invaluable resource for modern breeders, who continuously pore over seed stocks for traits that might help

sustain yields in the future. In the short term, nevertheless, diversity can be an obstacle to breeding. Isolating highly desirable traits from unfavorable ones is a painstaking task. In out-crossing species, including most perennial fruit and nut trees, crosses between attractive parents can yield economically worthless progeny. Since trees must be cultivated for several years before seed and fruit qualities are expressed, cross-pollination can be a serious setback. Early farmers solved this problem by simply cloning individuals of exceptional quality, avoiding sexual reproduction altogether; however, this practice greatly limited the genetic diversity of tree crops. Added to their long juvenile period, asexual reproduction has largely left these crops outside the purview of classical crop breeding. Substituting new clones for old ones has been the main way to improve stocks, but, given the noticeably low rates of turnover, most fruit and nut cultivars are not markedly different from their wild progenitors (Gepts, 2005).

In the past few years, nevertheless, crop improvement has been transformed profoundly by biotechnology.[4] Application of marker-assisted selection, for instance, has shortened the time requirements of conventional breeding in various crops, including tomatoes (Jauhar, 2006). Although advances in perennial fruit and nut crops have been relatively modest, many expect genetic engineering to have its most profound impact on these and other asexually reproduced crops (Litz, 2005). Genetic engineering constitutes a novel approach to breeding because it allows the transfer of genes (and potentially useful traits) across taxonomic groups (Jauhar, 2006). Although four annual species represent the bulk of its commercial applications to date, biotechnology has been (or soon will be) applied to numerous perennial crops, including some profiled in other chapters in this volume — for example, walnuts, olives, cashews, pistachios, chestnuts, almonds, coconuts, and cocoa (Litz, 2005).

It was widely anticipated that genetically modified (GM) crops would lower the price of food and improve the quality of crops, among other benefits, but controversy has notoriously hindered the industry's development (Qaim, 2009). The advent of nutritionally enhanced crops has been further delayed by technical and institutional problems. Most GM varieties released to date exhibit some type of agronomic advantage — for example, herbicide tolerance or insect resistance. Advocates claim that these "first generation" crops have already raised agricultural outputs while reducing environmental impacts, but debate persists on both counts (Gurian-Sherman, 2009; Qaim, 2009). Widespread adoption of GM crops in developed countries has resulted mostly in changes in pest-management strategies, with few yield increases.[5] In developing countries, where biotechnology has the potential to increase yields because pest control is lacking, evidence of actual gains is still limited (Smale *et al.*, 2006). The implications of GM crops for the environment are also controversial. Although insect-resistant and herbicide-tolerant varieties have been linked to multiple environmental gains, they have also raised numerous concerns (Cedreira & Duke, 2006; Wolfenbarger *et al.*, 2008).[6] A major unresolved controversy involves the risk of transgene flow into non-GM cultivars and their wild relatives (Gepts & Papa, 2003). Widely underplayed until recently, the likelihood of transgenes spreading into landraces in centres of crop diversity is significant, while the implications for the (generally poor) farmers who grow them are completely unknown (Dyer *et al.*, 2009).

A new generation of GM plants under development — so-called pharma crops — has exacerbated the complexity of these issues. Intended as a vehicle for the production of

[4] Biotechnology consists of an assortment of molecular methods, including genetic engineering, marker-assisted selection, somatic embryogenesis, and *in vitro* mutagenesis.
[5] Current GM crops have the same intrinsic yields (i.e., maximum yields) as their non-GM isolines. Yields under field conditions (i.e., operational yields) might be higher in GM crops or not, depending on which pest-management practices are in place.
[6] Adoption of GM crops has resulted in a reduction of toxic agrochemicals and promoted soil-conserving practices, but it has also favored herbicide tolerance in weeds, and its effects on non-target organisms have been mixed.

pharmaceutical and industrial proteins, pharma crops could have substantial advantages over alternative systems, which some believe would make medicines affordable to the world's poor (Ma *et al.*, 2005).[7] Scientists working in this field advocate the use of staple crops as hosts, highlighting the potential benefits of producing, storing, distributing, and even delivering pharmaceuticals within seeds; however, the possibility of admixtures with food and feed supplies has raised concerns. Although the likelihood of commingling could be reduced through various containment strategies, all measures are susceptible to human error, particularly wherever institutional oversight is lacking (Ma *et al.*, 2005; Spok *et al.*, 2008). Containment strategies have been designed and tested under conditions prevalent in developed countries, but a substantial gap exists in our knowledge of conditions in developing countries, where seeds are saved across cycles and the risks of containment failures could be much higher (Dyer *et al.*, 2009).

As documented throughout this volume, seed and nut cultivars possess countless nutritional, medical, and pharmacological attributes that have, or could have, application in different areas of human endeavor. Converting potential applications into actual improvements in wellbeing and health, particularly for those in greatest need, will require deliberate effort. Advances in science and technology rarely translate directly or immediately into improvements for most of the human population, particularly when the market is the main means of achieving it. If human wellbeing is the ultimate purpose of science, new approaches that bridge the gap between scientific research and downstream applications are needed. Such approaches will require that we earnestly reassess the role of public institutions in society. Acknowledging the complex social processes that separate scientists' laboratories from the realities of the human condition is a step forward.

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[7] These proteins include monoclonal antibodies, enzymes, blood proteins, hormones, growth regulators, and vaccines.

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CHAPTER 2

Seeds as Herbal Drugs

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CHAPTER OUTLINE

INTRODUCTION

The seed is the transportation stage in the plant's life. Seed is derived from the fertilized ovule; it contains an embryo, and is constructed so as to facilitate its transportation and germination in favorable conditions. Seed represents a condensed form of life, and is a characteristic of phenograms. Care must be taken to distinguish seeds from fruits or parts of fruits containing a single seed (for example, cereals and mericarps of the umbelliferous plants).

The use of plants and plant-based products for treatment of diseases is as old as mankind. Likewise, other plant parts as well as seeds have been used as natural drugs for the management of several ailments as advocated by traditional healers since time immemorial. Seeds are still widely used as herbal drugs, either in their crude form or as preparations thereof, or as sources of medicinally active natural products to be used in traditional or contemporary medicine. In the past few decades there has been an exponential growth in the field of herbal medicine. It is becoming popular in developed and developing countries as people consider herbal remedies to be a better option for their healthcare needs, owing to their natural origin and reduced side effects. Apart from dietary (for edible oils) and industrial (mainly as lubricants) purposes, seeds serve as important herbal drugs intended for several therapeutic indications. Numerous types of seeds have traditionally been used for medicinal purposes in different parts of the world, and among these are certain seeds that are still being used today, with renewed interest in their medicinal properties. The present chapter provides a broad overview of medicinally important seeds of contemporary interest.

MEDICINAL CONSTITUENTS OF SEEDS

The chemical constituents of seeds are responsible for their medicinal properties. Most seeds have a certain general chemical composition. All seeds contain reserve foods for the

Nuts & Seeds in Health and Disease Prevention. DOI: 10.1016/B978-0-12-375688-6.10002-7

nourishment of the embryo during germination. These foods may be present in the endosperm or the perisperm, or in both, or they may be present in the embryo itself either in the cotyledons or in the axis. Sometimes, as in linseed, the food is found in both endosperm and cotyledons. The reserve foods present are usually of two types: carbohydrates and fixed oils, which supply the elements carbon, hydrogen, and oxygen; and proteins, which supply nitrogen and phosphorus in addition to the former three elements. The commonest carbohydrate is starch; others are cellulose, hemicellulose, and sugar. Starches, sugars, fixed oils, and proteins are stored in the cell cavities, whereas celluloses are present as heavily thickened cell walls. These constituents give rise to starchy or farinaceous seeds, such as in the calabar bean; oily seeds, such as in linseed and the umbelliferous seeds; and very hard horny seeds, such as in nux vomica and the ignatius bean. Endosperm, which is present in many seeds, is composed of a cellulose-walled parenchyma containing food reserves. The walls are usually thin, but in some seeds (such as nux vomica and ignatius bean) the walls become very thick, being largely composed of hemi-cellulose. Seeds also contain protein reserves (aleurone grains), sclereids, mucilage, pigments, enzymes, several other nutrients, etc. (Wallis, 1985).

Apart from the general constituents, comprising both primary and secondary plant metabolites, the seeds contain a spectrum of specific secondary metabolites, most of which are very limited in distribution and some of which have complex chemical structures. Examples include alkaloids in nux vomica, calabar bean, and colchicum seeds; essential oils (terpenoids) in cardamom and nutmeg seeds; cardenolides in thevitia and stropanthus seeds; cyanogenetic glycosides in bitter almond seed; isothiocyanate glycosides in mustard seed; flavonolignans in milk thistle seed; bitter principles in neem and karela seeds; saponins in fenugreek seed; resinous matter in croton seed; gum in guar gum seed; liquid wax in jojoba seed, etc. It has been found that all of these secondary metabolites are principally responsible for the medicinal activity of seeds. Different specific secondary metabolites and the general seed constituents like fixed oils, carbohydrates, proteins, mucilage, etc., together contribute to the biological activity of seeds used as herbal drugs (Evans, 2002).

FACTORS INFLUENCING MEDICINAL PROPERTIES OF SEEDS

The seeds that reach the herbal market pass through various stages, all of which influence the nature and quantity of chemical constituents, and hence their therapeutic activity.

1. *Cultivation*. Environmental factors, including temperature, rainfall, length of daylight, radiation characteristics, altitude, soil and fertilizer/pesticide use, etc., may influence the chemical constituents.
2. *Collection*. To ensure maximum therapeutic potential, the seeds must be collected when perfectly ripe. Cleaning or washing is an important step, as the seeds must be free from fruit pulp before drying. Seeds which are obtained from mucilaginous fruits, such as nux vomica and cocoa, should be washed well to ensure that they are free from pulp. Some seeds, such as linseed, are separated from their husks by threshing and winnowing the fruits. Other factors include the nature of the plant (wild or cultivated, and its age), the season and time of collection, the personnel involved, etc.
3. *Drying*. Immediately after collection the seeds should be dried in the open air; generally, they should not be kiln-dried. Moist seeds are liable to develop microbial growth if not dried immediately. In some instances, however, drying by artificial heat becomes necessary, especially in tropical countries. Cocoa seeds are fermented by slow drying at a moderate temperature prior to sun drying. Some seeds, such as those of guarana, cola, and nutmeg, are decorticated during or after drying and their kernels are removed. These seeds require special drying techniques.
4. *Storage*. The dry seeds are normally stored in a cool, dry place. Microbial contamination must be avoided. Properly stored seeds retain their medicinal values for several years.

SEEDS AS A SOURCE OF MEDICINALLY IMPORTANT FIXED OILS

As agricultural crops, seeds used for the extraction of fixed oils are rated second in importance to cereals. Over the past six decades the production of oils for the food industry has increased enormously, whereas consumption by industrial and other users has remained relatively static although there have been interesting developments in the pharmaceutical industry.

Fixed oils or fatty oils are the reserve food materials of plant and animals. Those that are liquid at 15.5—16.5°C are termed fixed oils, while those that are solid or semi-solid at that temperature are termed fats. Fixed oils derived from plant sources generally occur in seeds (Kokate *et al.*, 2008).

Chemically fixed oils are esters of glycerol and various straight-chain monocarboxylic acids, known as fatty acids, which may be saturated, monounsaturated, or polyunsaturated. They contain various minor components (i.e., vitamins, sterols, antioxidants, phospholipids, pigments, and traces of hydrocarbons and ketones), some of which contribute to the medicinal properties.

Physiologically fixed oils are emollients and demulcents, and have nutritional value. The unsaturated fatty acids, namely linoleic, linolenic, and arachidonic acids (present in several seed fixed oils), are termed essential fatty acids, as they are not produced in the human body and must be provided in the diet. Fixed oils containing essential fatty acids (such as evening primrose oil) serve as nutritive and dietary supplements (nutraceuticals). Polyunsaturated fatty acids including omega-3 fatty acids are present in various seed fixed oils, namely safflower, flaxseed, mustard, and soya oils, etc. They help to reduce cholesterol formation and/or deposition, and hence to decrease the risk of atherosclerosis and ischemic heart disease. Fixed oils containing mainly unsaturated fatty acids are used as nutraceuticals in the prophylaxis of hypercholesteremia and atherosclerosis. Phospholipids present in fixed oils are hepatoprotective (Awang, 2009). Apart from these specific therapeutic uses, fixed oils are also utilized in the preparation of ointment, suppository, and cream bases; in dermatological preparations; as oily vehicles for oil-soluble injectable drugs; and as demulcents, emollients, and lubricants, etc. (Duke *et al.*, 2002). Table 2.1 provides a comprehensive list of the names, sources, constituents, and pharmacological (or therapeutic) activities and uses of medicinally important fixed seed oils.

Fixed oils are generally obtained from seeds by expression, centrifugation, and/or solvent extraction, depending on the product. Some fixed oils, such as arachis or linseed oil, are obtained by hot expression (or pressing); others, such as castor oil and chaulmoogra oil, are obtained by cold pressing. In some cases — for example, chaulmoogra oil — the seeds are decorticated and the kernels are pressed. Some oils, such as theobroma oil, are obtained from roasted seeds. The crude oil obtained by pressing then requires appropriate refining. Cold-pressed oils usually require nothing further than filtration, although sometimes the crude oil obtained is treated to get rid of unwanted or toxic constituents — for example, crude castor oil is steamed at 80°C to destroy the enzyme lipase and ricin (a toxic protein) to render it suitable for medicinal use, and linseed oil is left to settle so as to precipitate mucilage and coloring matter, before being treated with alkali to remove free fatty acids. Some of the fixed oils are bleached (for example, linseed oil and arachis oil), while others may be refined industrially according to their specific medicinal purposes (for example, as a vehicle for intramuscular oily injections) (DerMarderosian, 2001).

SEEDS AS HERBAL DRUGS AND A SOURCE OF MEDICINALLY ACTIVE COMPOUNDS

This section deals with the seeds used, either in crude form or as preparations, or as a source of medicinally active natural products other than fixed oils and fatty acids, in traditional and contemporary medicine. These seeds show diverse medicinal actions due mainly to the

TABLE 2.1 Seeds as a Source of Medicinally Important Fixed Oils

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
1	Arachis or earthnut or groundnut or peanut oil	*Arachis hypogaea* (Leguminosae)	Glycerides of fatty acids, *viz*. oleic, linoleic, palmitic, stearic, arachidic and other acids	Vehicle for intramuscular injections, preparation of liniments, plasters, soap, margarine.
2	Castor or ricinus oil	*Ricinus communis* (Euphorbiaceae)	Glycerides of ricinoleic, isoricinoleic, linoleic, and other acids	Purgative, lubricant
3	Coconut oil	*Cocos nucifera* (Palmae)	Glycerides of mainly lauric, myristic, and other acids	Non-aqueous medium for oral administration of drugs
4	Hydnocarpus or chaulmoogra oil	*Hydnocarpus wightiana*, *Taraktogenous kurzii* (Flacourtriaceae)	Glycerides of hydnocarpic, chaulmoogric, and palmitic acids	Antibacterial, antileprotic, antitubercular, in psoriasis and rheumatism
5	Linseed (Flax seed) oil	*Linum usitatissimum* (Linaceae)	Glycerides of fatty acids, sterols, tocopherol, pectin, mucilage, phenylpropanoids, flavonoids, linamarin (glycoside)	Demulcent, as poultice, lotions, and liniments in skin diseases
6	Palm kernel oil	*Elaeis guineensis* (Palmae)	Glycerides of mainly lauric and other acids	As a suppository base
7	Sesame oil (Gingelly oil, Teel oil)	*Sesamum indicum* (Pedaliaceae)	Glycerides of higher fatty acids, olein, sesamol, lignans (sesamin, sesamolin)	Nutritive, laxative, demulcent, emollient; in preparation of liniments and ointments; vehicle for intramuscular oily injections
8	Mangosteen oil (Kokum oil)	*Garcinia indica* (Guttiferae)	Glycerides of stearic, oleic, hydroxycapric, palmitic, and linoleic acids	Nutritive, demulcent, astringent, emollient; as ointment and suppository bases
9	Safflower oil	*Carthamus tinctorius* (Compositae)	Glycerides of palmitic, stearic, arachidic, oleic, linoleic, and linolenic acids	In preparation of oleomargarine, as dietary supplement in hypercholesteremia and atherosclerosis
10	Black mustard oil	*Brassica nigra*, or *Brassica juncea* (Brassicaceae)	Glycerides of fatty acids, sinigrin (glycoside), myrosin (enzyme)	Local irritant, externally as rubefacient and vesicant; internally as emetic
11	White mustard oil	*Brassica alba* (Brassicaceae)	Glycerides of fatty acids, mucilage, sinalbin (glycoside), myrosin (enzyme)	Used externally as rubefacient and vesicant

Continued

TABLE 2.1 Seeds as a Source of Medicinally Important Fixed Oils—continued

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
12	Poppy seed oil or poppy oil	*Papaver somniferum* (Papaveraceae)	Glycerides of mainly linoleic, palmitic, arachidic, and oleic acids; no narcotic principles	In preparation of iodized oils, soaps
13	Karanja oil	*Pongamia glabra* (Papilionaceae)	Glycerides of different fatty acids, β-sitosterol, karanjin, pongapin, pongamol	In scabies, herpes, leukoderma, and other cutaneous diseases; in rheumatism
14	Neem oil	*Azadirachta indica* (Meliaceae)	Glycerides of mainly oleic and stearic acids, sulfur-containing bitters, nimbidin, nimbin, nimbinin, nimbidiol, and nimbosterol	Antiviral, insect repellent, spermicide, pesticide; in rheumatism.
15	Soya oil	*Glycine max* or *G. soja* (Leguminosae)	Glycerides of linoleic, oleic, palmitic, linolenic, and stearic acids, phosphatidylcholine, proteins, isoflavones	Nutritive, hepatoprotective, lipotropic agent, hypolipidemic, antitumor
16	Croton oil	*Croton tinglum* (Euphorbiaceae)	Glycerides of different fatty acids, croton resin, protein (crotin), phorbol esters	Local irritant, counter-irritant, internally drastic purgative
17	Cotton seed oil	*Gossypium herbaceum* (Malvaceae)	Glycerides of mainly palmitic, oleic, and linoleic acids	Pediculicide, acaricide, and laxative in veterinary medicine
18	Theobroma oil	*Theobroma caco* (Sterculiaceae)	Glycerides of mainly stearic, palmitic, and oleic acids	Base for suppositories, ointments, creams
19	Jojoba oil	*Simmondsia chinensis, S. californica* (Buxaceae)	Liquid wax, i.e. esters of monounsaturated acids and alcohols, tocopherols, and phytosterols	Cosmetics, vehicle of dermatological preparations, lubricant
20	Evening primrose oil	*Oenothera biennis* (Onagraceae)	Glycerides of mainly linoleic acid and γ-linolenic acid	Dietary supplement for essential fatty acids, antithrombotic; in premenopausal syndrome, atopic eczema, rheumatic arthritis, diabetic neuropathy; hepatoprotective
21	Rapeseed oil	*Brassica napus, B. campestris* (Brassicaceae)	Glycerides of mainly omega-3 fatty acids	Nutritive, antiplatelet; as dietary supplement in hypercholesteremia
22	Sweet almond oil	*Prunus communis* (Rosaceae)	Glycerides of different fatty acids, proteins, emulsion (enzyme),	Demulcent, nutritive

Continued

TABLE 2.1 Seeds as a Source of Medicinally Important Fixed Oils—continued

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
			gum, sucrose, asparagin	
23	Tung oil	*Aleurites moluccna* (Euphorbiaceae)	Glycerides of fatty acids, *viz*. eleostearic, linolenic, linoleic, and oleic acids, proteins	Wood preservative; in varnishes, paints; toxic to humans
24	Sunflower oil	*Helianthus annus* (Asteraceae)	Glycerides of unsaturated fatty acids, stigmasterol, β-sitosterol, tocopherol	Dietary supplement

presence of different secondary plant metabolites as the pharmacologically active chemical constituents, thus demonstrating therapeutic activities for a multitude of diseases. They may also contain fixed oils. Some of them are toxic in nature, especially when consumed in appreciable amounts (Barceloux, 2008). Most of their chemical constituents have been isolated and pharmacologically evaluated, and some of the active constituents are commercially extracted and isolated from the seeds for prophylactic and therapeutic uses in medicine. Table 2.2 provides a detailed list of the names, sources, active constituents, and pharmacological (or therapeutic) activities and uses of such important seeds. However, numerous references to the multiple biological activities of various seeds and their constituents can be found in the literature, and new reports of pharmacological screening continually appear.

TABLE 2.2 Seeds as Herbal Drugs and a Source of Medicinally Active Compounds

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
1	Nux vomica	*Strychnos nuxvomica* (Loganiaceae)	Alkaloids, *viz*. strychnine, brucine, vomicine, α-colubrine, pseudostrychnine, chlorogenic acid	Bitter stomachic, tonic, central nervous system stimulant; in cardiac failure, as rodenticide
2	Ignatius beans	*Strychnos ignatti* (Loganiaceae)	Alkaloids, *viz*. strychnine, brucine	Similar to those of nux vomica
3	Physostigma or Calabar bean	*Physostigma venenosum* (Leguminosae)	Alkaloids, *viz*. physostigmine, eseramine, physovenine, 8-norphysostigmine, isophysostigmine	Parasympathomimetic, anticholinesterase; as miotic for contraction of eye pupils, antidote for belladonna poisoning
4	Belladonna	*Atropa belladonna* (Solanaceae)	Alkaloids, *viz*. atropine, L-hyoscyamine, hyoscine, belladonine, scopoletin	Parasympatholytic; as antisecretory in peptic ulcer, mydriatic (pupil dilatation), antispasmodic, antidote of chloral hydrate poisoning
5	Datura	*Datura metel* (Solanaceae)	Alkaloids, *viz*. hyoscine, atropine, l-hyoscyamine	Parasympatholytic, CNS depressant; in bronchial asthma, cough, cerebral excitement, preoperative medication

Continued

TABLE 2.2 Seeds as Herbal Drugs and a Source of Medicinally Active Compounds—continued

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
6	Stramonium	*Datura stramonium* (Solanaceae)	Alkaloids, *viz*. L-hyoscyamine, hyoscine, proteins, fixed oil	Similar to those of belladonna; in bronchial asthma, parkinsonism
7	Lobelia, Asthma weed	*Lobelia nicotianaefolia* (Campanulaceae)	Alkaloids, *viz*. lobeline, lobelidine, lobelanine, isolobelanine	In bronchial asthma, chronic bronchitis, as respiratory stimulant
8	Areca	*Areca catechu* (Palmae)	Alkaloids, *viz*. arecoline, arecaidine, guvacine, guvacoline	Parasympathomimetic, sialogogue; as anthelmintic (vermicide and taenifuge) in veterinary practice
9	Coffee	*Coffea arabica* (Rubiaceae)	Caffeine (alkaloid), tannin, fixed oil, protein, chlorogenic acid, sugars	Stimulant, diuretic; to antagonize toxic effects of CNS-depressant drugs
10	Cocoa	*Theobroma cocoa* (Sterculiaceae)	Alkaloids, mainly theobromine, caffeine, polyphenols	Stimulant, diuretic, nutritive
11	Kola (Bissy or Gooroo)	*Cola nitida* (Sterculiaceae)	Alkaloids, *viz*. caffeine, theobromine, kolacatechin (tannin)	Stimulant, diuretic; in aerated beverages
12	Colchicum	*Colchicum leutum*, *C. autumnale.*	Alkaloids, *viz*. colchicines, demecolcine	Antitumor; in gout and rheumatism; colchicine causes polyploidy
13	Cardamom	*Elettaria cardamomum* (Zingiberaceae)	Essential oil comprising cineole, terpineol, borneol, terpinene; fixed oil, protein	Aromatic, carminative, stimulant, flavoring agent, condiment
14	Carrot	*Dacuus carota* (Umbelliferae)	Essential oil comprising α-pinene, β-pinene, limonene, carotol, daucol, β-elemene, geraniol, etc.	Carminative, aromatic, diuretic, smooth-muscle relaxant, vasodilator, antidysenteric, aphrodisiac
15	Nutmeg	*Myristica fragrans* (Myristicaceae)	Essential oil comprising myristicin, elimicin, saffrole; fixed oil comprising glycerides of mainly myristic and other fatty acids	Aromatic, carminative, stimulant, flavoring agent, condiment; poisonous and narcotic in large amounts
16	Annatto	*Bixa orellana* (Bixaceae)	Annatto oleo-resin, bixin (carotenoid pigment)	Coloring agent for food, cosmetics and pharmaceuticals
17	Guar gum	*Cyamposis tetragonolobus* (Leguminosae)	Gum comprising guaran (hydrocolloid polysaccharide), sugars, proteins	Laxative, anoretic, anti-ulcer, hypocholesterolemic, oral hypoglycemic, emulsifying agent
18	Isapgol	*Plantago ovata* (Plantaginaceae)	Mucilage, fixed oils, protein	Demulcent, laxative, emollient, antidysenteric
19	Psyllium, Flea seed	*Plantago psyllium* (Plantaginaceae)	Mucilage, aromatic. carboxylic acids, alkaloids, amino acids, flavonoids, fats, iridoids, sugars	Laxative, demulcent, antihyperlipidemic, antitumor
20	Thevetia	*Thevetia peruviana* (Apocynaceae)	Cardenolides, *viz*. thevetin A and B, peruvoside, neriifolin, thevenarin, peruvosidic acid	Cardiotonic, emetic febrifuge, abortifacient, purgative; in rheumatism and dropsy; poisonous in large amounts

Continued

TABLE 2.2 Seeds as Herbal Drugs and a Source of Medicinally Active Compounds—continued

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
21	Stropanthus	*Stropanthus kombe* (Apocynaceae)	Cardenolides, *viz*. K-stropanthoside, K-stropanthoside β-cymarin, cymarol; mucilage, resin, trigonelline, fixed oil	Cardiotonic in congestive heart failure; poisonous in large amounts
22	Stropanthus	*Stropanthus gratus* (Apocynaceae)	Cardenolide: G-stropanthidin (ouabain)	Emergency cardiotonic in acute cardiac failure; poisonous in large amounts
23	Bitter almond	*Prunus amygdalus* (Rosaceae)	Fixed oil, proteins, enzymes, amygdalin (cyanogenetic glycoside)	Sedative, demulcent, flavoring agent; poisonous in large amounts
24	Milk-thistle	*Silybum marianum* (Asteraceae)	Silymarin (a mixture of three isomeric flavonolignans), and other flavonolignans, betaine, silybonol	Hepatoprotective in hepatitis, cirrhosis and fatty liver; antihepatotoxic; as bitter tonic
25	Tonka-bean	*Dipteryx odorata* (Leguminosae)	Coumarin, fats	Flavoring agent, fixative in perfumery
26	Visnaga	*Ammi visnaga* (Umbelliferae)	Furanocoumarins, *viz*. khellin, visnagin, khelloside, fixed oil comprising samidine, dihydrosamidine visnadine, etc.	Smooth-muscle relaxant, coronary vasodilator in angina pectoris; in uterine and renal colic, bronchial asthma, and whooping cough
27	Ammi	*Ammi majas* (Umbelliferae)	Furanocoumarins, *viz*. xanthotoxin, bergapten, isopimpilin, imperatorin	Melanizing agent, in idiopathic vitiligo, leukoderma
28	Psoralea	*Psoralea corylifolia* (Leguminosae)	Essential oil, fixed oil, resin, pigments; furanocoumarins, *viz*. psoralen, psoralidin, isopsoralidin	In leukoderma, leprosy, psoriasis, skin inflammations
29	Ambrette	*Abelmoschus moschatus* (Malvaceae)	β-sitosterol, cholesterol, stigmasterol, ergosterol, campesterol; fixed oil comprising higher fatty acids; ambrettolide (lactone)	Aphrodisiac, antispasmodic, carminative, demulcent, diuretic, stomachic, tonic, flavoring agent; dermal irritant in large amounts
30	Asparagus	*Asparagus officinale* (Liliaceae)	Polysaccharides, proteins	As coffee substitute, diuretic, laxative, analgesic, antirheumatic
31	Bitter melon, Karela	*Momordica charantia* (Cucurbitaceae)	Fixed oil comprising triglycerides of stearic, linoleic, oleic acids; vicine (nucleoside), α and β-momorcharin (glycoproteins), lectins, amino acids	Oral hypoglycemic, antimicrobial; not recommended in pregnancy
32	Celery	*Apium graveolens* (Umbelliferae)	d-limonene, selinene, 3-*n*-butylpthalide, sedanenolide, sedanonic anhydride	Sedative, anti-arthritic, antirheumatic, analgesic, diuretic, nervine tonic, hypoglycemic, antifungal
33	Corn cockle	*Agrostema githago* (Caryophyllaceae)	Aromatic amino acids, L (+) citrullin, fixed oil, sugars, starch, saponins	Diuretic, expectorant, vermifuge, emmenagogue; in jaundice, gastritis; fatally poisonous in large amounts
34	Cucurbita	*Cucurbita pepo* (Cucurbitaceae)	Fixed oil, carotenoids, cucurbitin	Anthelmintic; in prostate disorders

Continued

TABLE 2.2 Seeds as Herbal Drugs and a Source of Medicinally Active Compounds—continued

Sl No.	Common Name	Biological Source	Chemical Constituents of Seeds	Actions and Uses
			(carboxypyrrolidine), flavonols, sterols, amino acids, tocopherols	
35	Guarana	*Paullinia cupana* (Sapindaceae)	Caffeine, guaranatin, catechutannic acid, fat, starch	Nervine stimulant, diuretic, anoretic
36	Quince seeds	*Cydonia oblongata* (Rosaceae)	Mucilage, fixed oil, proteins, amygdalin	Demulcent, suspending agent, emulsifying agent
37	Stavesacre	*Delphinium stapisagria* (Ranunculaceae)	Alkaloids, *viz*. delphinine, delphisine, delphinoidine, staphisagroine; fixed oil	Antiparasitic; in neuralgia; extremely poisonous in large amounts
38	Cevadilla or Sabadilla	*Schoenocaulon officinale* (Liliaceae)	Alkaloids, *viz*. cevadine, verarine, sebadilline, sebadine, sebadinine; fats	Antiparasitic, insecticide, counter-irritant, local anesthetic; in neuralgia
39	Grains of paradise	*Aframomum melegueta* (Zingiberaceae)	Essential oil, paradol (pungent principle)	Stimulant, analgesic, condiment
40	Fenugreek	*Trigonella foenum-graecum* (Leguminosae)	Coumarin derivatives, alkaloids (trigonelline, gentianine, carpaine), fixed oil, saponins (smilagenin, sarasapogenin, yuccagenin), mucilage	Oral hypoglycemic, hypocholesterolemic, anti-inflammatory, diuretic, condiment
41	Grape seed	*Vitis vinifera* (Vitaceae)	Essential fatty acids, tocopherols, oligostilbenes, procyanidines, and other polyphenols	Nutritive, hepatoprotective, antitumor
42	Horse chestnut	*Aesculus sp.* (Sapindaceae)	Fixed oil comprising glycerides of mainly oleic acids; proteins, sugars, triterpene, coumarin and saponin glycosides	Circulatory tonic in chronic venous insufficiency, anti-inflammatory, antipyretic; poisonous in large amounts
43	Black cumin	*Nigella sativa* (Ranunculaceae)	Fixed oils, proteins, nigellone, thymoquinone, essential oil, saponins, alkaloids, ascorbic acid	Anti-inflammatory, antimicrobial, anthelmintic; in respiratory diseases (cough, bronchitis, asthma, flu); not recommended in pregnancy
44	Custard apple, Pawpaw	*Asimia triloba* (Annonaceae)	Acetogenins	Antitumor, antimicrobial, pesticide
45	Jequirty seed	*Abrus precatorius* (Fabaceae)	Alkaloids, *viz*. abrine, hyaphorine, precatorine, lectins; proteins, *viz*. abrin A, B and C	Analgesic, neuromuscular blocker; fatally poisonous, especially in children
46	Kavach, Cowharge	*Mucuna pruriens or M. prurita* (Fabaceae)	Amino acids, mainly L 3,4-dihydroxy phenyl alanine (L-dopa); lecithin, alkaloids, fat	Antiparkinsonian; in manufacture of antiparkinsonian drug levodopa

SUMMARY POINTS

- Seeds have been traditionally used as herbal drugs, and are still being used in this way.
- Seeds contain several constituents, including certain secondary plant metabolites in specific seeds which are mainly responsible for their medicinal values.
- Environmental and pre- and post-harvesting factors can influence the medicinal properties of seeds.

- Seeds serve as sources of different medicinally important fixed oils.
- Seeds serve as herbal drugs for various therapeutic indications, and as source of medicinally active compounds, most of which are putative secondary metabolites.

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CHAPTER 3

Seeds, Nuts, and Vector-Borne Diseases

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

DDT, dichlorodiphenyltrichloroethane
DEET, *N*,*N*-diethyl-*meta*-toluamide
IGR, insect growth regulator
LC_{50} , lethal concentration for 50% of the target population
LD_{50}, lethal dose for 50% of the target population

INTRODUCTION

Many arthropods, such as mosquitoes, sand flies, black flies, fleas, lice, and ticks, can vector serious human diseases. Vector control has relied upon synthetic insecticides since the discovery of DDT. However, owing to their adverse effects on humans and the environment, interest in other methods has increased. Chemicals of botanical origin, called phytochemicals, have displayed a range of acute and chronic insecticidal effects against a variety of insects, and are promising candidates as future synthetic insecticides. Almost all research on the activity of seed extracts against disease vectors has focused on mosquitoes. This is because mosquitoes carry vector diseases such as malaria, filariasis, and dengue, and cause most arthropod-borne disease. The World Health Organization has indicated that malaria infects 500 million people annually, and kills more than 1.5 million annually – particularly African children. Thus, the focus on other arthropod disease vectors has been minimal. While mosquito control relies extensively on synthetic insecticides, mosquitoes have also been the primary focus for seed-based phytochemical and bioinsecticide investigations.

Nuts & Seeds in Health and Disease Prevention. DOI: 10.1016/B978-0-12-375688-6.10003-9

Research has focused on testing plants that have previously demonstrated some degree of activity. Since there are thousands of plants, and bioinsecticide research is underfunded, a broadside biodiscovery approach has never been possible. Thus, prior plant activity as determined through non-scientific anecdotal "herb lore" has tended to provide the first clues. Not surprisingly, active phytochemical components have been extracted from a broad selection of plant types and species. Phytochemicals have been extracted from medicinal plants, citrus plants, some leguminous plants, and some marine weeds. They also come from different parts of plants, such as the leaves, stems, roots, tubers, fruits, seeds and seeds kernels, and shoot systems (Shaalan *et al.*, 2005). They have been found to be toxic against different developmental mosquito stages, such as eggs (ovicide), larvae (larvicide), pupae (pupicide), and adults (adulticides and repellents), and some have shown growth-regulating activity (insect growth regulators, IGR). Furthermore, some have displayed considerable synergistic activity with current synthetic chemical insecticides, which is an advantage because it may prolong the usability of some synthetic insecticides when resistance builds up in pest populations. In fact, in original articles and a few reviews, the literature shows hundreds of plant species to have demonstrated mosquitocidal activity, but none of the work specifically reviewed seeds and nuts. Hence, this chapter highlights the mosquitocidal activity that can be ascribed to phytochemicals of seed and nut origin.

MOSQUITOCIDAL ACTIVITY OF SEEDS

The seeds of over 40 plant species have been found to display some mosquitocidal activity (Table 3.1). Seed extracts and some of their constituent compounds have been observed to possess larvicidal, growth regulating, and repellent activity against different mosquito vector species. Since adulticides often only act to reduce the adult mosquito population temporarily, most mosquito control programs are based on the judicious and evidence-based use of larvicides. Larvicides are considered to be more effective, since they provide longer-term control of larval stages in their breeding habitats. However, identifying plants with these qualities can be difficult, because results can be unexpected. When looking at neem-producing trees, for instance, an acetone seed extract of *Melia volkensii* was equally toxic to both larvae and pupae with a LD_{50} of 30 μg/ml, while an extract of a closely related species, *M. azedarach*, was exclusively larvicidal with a LD_{50} of 40 μg/ml and had no inhibitory effect on the pupal stage (Al-Sharook *et al.*, 2009). Similarly, when looking at some spices, the array of effects can be broad but very selective. For instance, a seed extract of anise (*Pimpinella anisum*) was better as a larvicide and an ovicide than in other activities; a seed extract of cumin (*Cuminum cyminum*) proved to be more effective as an adulticide, an oviposition deterrent, and a repellent than as a larvicide and an ovicide; and a seed extract of black cumin (*Nigella sativa*) was more effective as an oviposition deterrent and larvicide against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Prajapati *et al.*, 2005).

Mosquitocidal activity, whether ovicidal, larvicidal, growth regulating, adulticidal, oviposition deterrent, or repellent, is basically a toxic effect that is governed by the familiar pharmaceutical dose—response curve. Thus, different effects can be produced at different concentrations of the same extract. For instance, a petroleum ether extract of *Argemone mexicana* seeds exhibited larvicidal and growth inhibiting activity against the second instar *Ae. aegypti* larvae at concentrations between 25 and 200 ppm, while chemosterilant activity (reduction in blood meal utilization, reduction in fecundity, adult mortality, and sterility of first-generation eggs) occurred at a lower concentration of 10 ppm (Sakthivadivel & Thilagavathy, 2003).

It may be assumed that seeds are the most potent part of a plant; however, the mosquitocidal activity of seed extracts is only occasionally better than that of other plant parts. For instance, a seed acetone extract from *Tribulus terrestris* produced 100% larval mortality against four species of mosquito larvae (*An. Culicifacies*, *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*) at 100 ppm, while a leaf acetone extract only killed all larvae at 200 ppm (Singh *et al.*, 2008).

TABLE 3.1 Mosquitocidal Activity of Seeds

Scientific Name	Activity Type	Mosquito Species	Reference
Agave americana	Larvicide	*Aedes aegypti* *Anopheles stephensi* *Culex quinquefasciatus*	Dharmshaktu *et al.*, 1987
Annona crassiflora *A. glabra* *A. squamosa* *Pterodon polygalaeflorus* *Senna occidentalis* *Dorstenia sp.*	Larvicide	*Ae. aegypti*	De Omena *et al.*, 2007
Ammi visnaga	Larvicide	*Cx. quinquefasciatus*	Pavela, 2008
Annona squamosa	Larvicide, adulticide, IGR	*An. stephensi*	Senthilkumar *et al.*, 2009
Apium graveolens	Larvicide, adulticide, repellent	*Ae. aegypti*	Choochote *et al.*, 2007
Apium graveolens *Carum carvi*	Adulticide	*Ae. aegypti*	Chaiyasit *et al.*, 2006
Carum carvi	Repellent	*Ae. aegypti*	Choochote *et al.*, 2007
Apium graveolens *Pimpinella anisum*	Larvicide	*Ae. aegypti*	Shaalan & Canyon 2008
Argemone mexicana	Larvicide, adulticide, IGR, chemosterilant	*Ae. aegypti*	Sakthivadivel & Thilagavathy 2003
Azadiracta indica	Repellent	*Ae. aegypti*	Hati *et al.*, 1995
Azadiracta indica (neem seed kernel)	Larvicide, IGR	*Ae. aegypti* *Ae. togoi* *An. stephensi* *Cx. quinquefasciatus*	Zebitz, 2009
Azadiracta indica *Melia azedarach*	Larvicide	*Ae. aegypti*	Wandscheer *et al.*, 2004
Azadiracta indica *Momordica charantia* *Ricinus communis*	Larvicide	*An. stephensi*	Batabyal *et al.*, 2007
Azadiracta indica *Pongamia glabra*	Larvicide, IGR	*Cx. quinquefasciatus*	Sagar & Sehgal 1996
Carica papaya	Larvicide	*Cx. quinquefasciatus*	Rawani *et al.*, 2009
Chenopodium spp.	Repellent	*Ae. albopictus*	Chio & Yang 2008
Cinnamomum camphora	Larvicide	*Cx. pipines pallens*	Zhou *et al.*, 2000
Citrus reticulate	Larvicide	*Ae. aegypti* *Cx. quinquefasciatus*	Sumroiphon *et al.*, 2006
Clitoria ternatea	Larvicide	*Ae. aegypti,* *An. stephensi* *Cx. quinquefasciatus*	Mathew *et al.*, 2009
Coriander sativum *Petroselinum crispum* *Pimpinella anisum*	Larvicide	*Ochlerotatus caspius*	Knio *et al.*, 2008
Cuminum cyminum *Nigella sativa* *Pimpinella anisum*	Adulticide, larvicide, ovicide, oviposition deterrent, repellent	*Ae. aegypti, An. stephensi* *Cx. quinquefasciatus*	Prajapati *et al.*, 2005
Daucus carota *Khaya senegalensis*	Larvicide	*Cx. annulirostris*	Shaalan *et al.*, 2006
Delonix regia *Raphia vinifera*	Larvicide	*An. gambiae*	Aina *et al.*, 2009

Continued

TABLE 3.1 Mosquitocidal Activity of Seeds—continued

Scientific Name	Activity Type	Mosquito Species	Reference
Eucalyptus globulus	Larvicide	*Cx. pipiens*	Elbanna, 2006
Gloriosa superba *Solannum trilobatum*	Larvicide	*An. subpictus* *Cx. tritaeniorhynchus*	Abduz Zahir *et al.*, 2009
Jatropha curcas *Datura innoxia*	Larvicide	*Och. triseriatus*	Georges *et al.*, 2008
Litsea cubeba	Repellent, oviposition deterrent	*Ae. aegypti* *Ae. albopictus* *An. dirus* *Cx. quinquefasciatus* *Ae. aegypti*	Tawatsin *et al.*, 2006
Melia volkensii *M. azedarach*	Larvicide, IGR	*Cx. pipiens molestus*	Al-Sharook *et al.*, 2009
Millettia dura	Larvicide	*Ae. aegypti*	Yenesew *et al.*, 2003
Ocimum basilicum	Larvicide, repellent	*Anopheles sp.*	Nour *et al.*, 2009
Pimpinella anisum	Repellent	*Cx. pipiens*	Erler *et al.*, 2006
Piper guineense	Larvicide	*Ae. aegypti*	Oke *et al.*, 2001
Piper guineense *Jatropha curcas*	Larvicide	*An. gambiae*	Aina *et al.*, 2009
Polylophium involvucratum	Larvicide	*An. stephensi* *Cx. pipiens*	Reza & Abbas 2007
Pongamia glabra	Larvicide, IGR	*Ae. aegypti* *Cx. quinquefasciatus*	Sagar *et al.*, 1999
Rumex obtusifolius	Larvicide	*Ae. aegypti* *Cx. pipiens pallens*	Kim *et al.*, 2002
Solanum torvum	Larvicide	*An. subpictus* *Cx. tritaeniorhynchus*	Kamaraj *et al.*, 2009
Sterculia guttata	Larvicide	*Ae. aegypti* *Cx. quinquefasciatus*	Katade *et al.*, 2006
Tribulus terrestris	Larvicide, repellent	*Ae. aegypti* *An. culicifacies* *An. stephensi* *Cx. quinquefasciatus*	Singh *et al.*, 2008
Trachyspermum ammi	Larvicide, repellent, oviposition deterrent, vapor toxic	*An. stephensi*	Pandey *et al.*, 2009

See Shaalan and Canyon (2010) for references cited in this Table.

Likewise, a seed extract from *Eucalyptus globulus* was more effective as a larvicide than was a leaf extract against *Cx. pipiens* (Elbanna, 2006). The lowest LC_{50} values discovered for a seed extract, thus indicating the most effective toxicity, were 2.6 mg/l recorded for *Annona squamosa* (custard apple) seeds against *An. stephensi* (Senthilkumar *et al.*, 2009), and 0.06 mg/l recorded against *Ae. aegypti* (De Omena *et al.*, 2007). The abnormal movements observed in larvae exposed to *Annona graveolens* indicate that the active phytochemicals most likely affected the nervous system (Choochote *et al.*, 2007).

Earlier research on *An. squamosa* seeds found that they were effective against head lice. While head lice are not vectors of disease, they are a disease in themselves — pediculosis. The earliest study on the pediculicidal efficacy of *An. squamosa* seed extracts was carried out in 1980 in Thailand (Intaranongpai *et al.*, 2006). This clinical head-lice study used a seed extract diluted in coconut oil (1:2), which was found to cause 98% mortality of head lice within 2 hours of application to the heads of participants. A leaf extract showed less potency. Two other studies on petroleum ether extract of custard apple seeds that used oil diluents reported 90–93% mortality in *in vitro* tests after 1–3 hours (Intaranongpai *et al.*, 2006). In a clinical study on

schoolgirls, Gritsanapan *et al.* (1996) found that 20 g of a 20% cream caused 95 ± 9% mortality of head lice 3 hours after application.

A recent study focused on the separation and identification of the active compounds from a hexane extract of the seeds (Intaranongpai *et al.*, 2006). Two primary compounds were identified: oleic acid, and a triglyceride with one oleate ester. In *in vitro* tests against head lice where all test substances were diluted with coconut oil (1 : 1), crude hexane extract killed all the lice after 31 minutes, oleic acid caused 100% mortality after 50 minutes, and the triglyceride with one oleate ester was 100% effective after only 11 minutes. Since head-lice preparations are usually applied to the head for up to 20 minutes, this finding has commercial potential.

Although there have only been a few field studies on the evaluation of seed extracts for mosquito control, they have shown excellent results. Essential oils derived from caraway and celery seeds were tested as adulticides in the laboratory and then in natural field conditions against laboratory and wild strains of the dengue vector, *Ae. aegypti*, in Chiang Mai Province, Thailand (Chaiyasit *et al.*, 2006). It was suggested that these bioinsecticides had potential for field control and eradication of mosquito vectors.

An African village study evaluated the efficacy of neem seed extract for sustainable malaria vector control (Gianotti *et al.*, 2008). The study was conducted in Banizoumbou village, western Niger. Neem seeds, collected around the village, were dried, ground into a coarse powder, then sprinkled onto known *Anopheles* breeding habitats twice weekly during the 2007 rainy season. Weekly adult mosquito captures were compared to those from 2005 and 2006 during the same seasonal period. Adult mosquitoes were captured in a nearby village, Zindarou, as a control, and were compared to those from Banizoumbou. Results revealed that twice-weekly applications of the powder to breeding habitats of *Anopheles* larvae in 2007 resulted in 49% fewer adult female *An. gambiae* s.l. mosquitoes in Banizoumbou, compared with previous captures under similar environmental conditions in 2005 and 2006. Results of this study suggest that larval control using neem seed powder offers a sustainable additional tool for vector control that can be employed by local people at minimal cost.

Some seed extracts have demonstrated strong repellent activity against mosquito vectors. For instance, a 10% seed acetone extract of *Tribulus terrestris* was 100% effective in repelling adult *An. culicifacies* species A and *An. stephensi* mosquitoes for up to 6 hours, and *Cx. quinquefasciatus* mosquitoes for up to 4 hours (Singh *et al.*, 2008). A survey of the knowledge and usage of traditional insect mosquito-repellent plants in Addis Zemen Town, South Gonder, North Western Ethiopia, revealed that less than 1% (0.17%) of local residents used seeds of plants as repellents against mosquitoes and other insects compared to the other parts of plants, including leaves, flowers, roots, stems, and bark (Karunamoorthi *et al.*, 2009). These parts are underutilized either due to processing issues, or because they are less effective.

In another repellent study, a gel formulation of anise seed hexane extract was found to provide remarkable repellency with a median protection time of 4.5 hours (4.5− 5 h) (Tuetun *et al.*, 2008). This was greater than that of ethanolic DEET solution (25% DEET, 3.5 h), and comparable to that of the best commercial repellent, Insect Block 28 (28.5% DEET, 4.5 h). This laboratory investigation against female *Ae. aegypti* found that the gel formulation caused no irritation in volunteers. Such promising results, including high repellent activity and no skin irritation, are significant, because they provide proof that plant-based alternatives for synthetic mosquito repellents are a reality.

The formulation of commercial products may take many forms. In one example in Thailand, the dried root powder of a local plant, *Rhinacanthus nasutus*, was packaged as 10% tablets. These were tested against *Ae. aegypti* and *Cx. Quinquefasciatus*, for which the LC_{50s} were 14.2 mg/l and 17.3 mg/l, respectively (Rongsriyam *et al.*, 2006). Furthermore, acute-toxicity bioassays with fish (*Poecilia reticulata*) showed that these prepared tablets could be used to

TABLE 3.2 Mosquitocidal Activity of Nuts

Scientific Name	Activity Type	Mosquito Species	References*
Anacardium occidentale (shell)	Larvicide	*Ae. aegypti*	Laurens *et al.*, 1997
Anacardium occidentale	Ovicide, larvicide pupicide	*Ae. aegypti*	Farias *et al.*, 2009
Atriplex canescens (saltbrush nut)	Larvicide, adulticide, ovicide, oviposition detterent	*Cx. quinquefasciatus*	Ouda *et al.*, 1998
Myristica fragrans	Larvicide, adulticide, IGR	*An. stephensi*	Senthilkumar *et al.*, 2009

*See Shaalan and Canyon (2010) for these citation references.

TABLE 3.3 Safety of Seed and Nut Extracts

Scientific Name	Effect on Non-Target Organisms	References*
Anacardium occidentale	Safe for mice at high dose (0.3 g/kg).	Farias *et al.*, 2009
Apium graveolens	No adverse effects on human skin observed.	Choochote *et al.*, 2004
Azadiracta indica	More toxic against *Aphanius dispar* (killifish) than *An. stephensi* larvae	Khan *et al.*, 2000
Citrus reticulate	Safe for *Oreochromis niloticus* Nile Tilapia fishes at 2.3 ppm	Sumroiphon *et al.*, 2006
Carica papaya	Safe for the bug *Diplonychus annulatum* and the midge *Chironomus circumdatus*. No change in behavior and survival noticed at LC_{50}	Rawani *et al.*, 2009

*See Shaalan and Canyon (2010) for these citation references.

control mosquito vectors, and should be considered for inclusion in mosquito control programs. The remaining obstacle is how to make production acceptable, efficient, and affordable in order for local affected populations to prevent mosquito bites and interrupt mosquito-borne disease transmission.

MOSQUITOCIDAL ACTIVITY OF NUTS

By comparison, only three plants – cashew, saltbrush, and nutmeg – have nuts that contain mosquitocidal phytochemicals (Table 3.2). The lowest LC_{50} published was 2.22 ppm, recorded for a *Myristica fragrans* nut extract against *An. stephensi* larvae (Senthilkumar *et al.*, 2009). This is an excellent result, and merits further research.

SAFETY

There have been only a few safety studies, but these provide an indication of the safety of essential oils extracted from seeds, for aquatic organisms, predaceous insects, mammals, and humans, when applied topically to the skin for protection against mosquito bites (Table 3.3). Despite the eco-friendly advantages of seed extracts, research is required into the non-target effects of inert surfactants used in extract formulation for those extracts that are destined to be used as insecticides (Kumar *et al.*, 2000).

CONCLUSIONS

In comparison to other plant parts, relatively few seeds and nuts have been evaluated and used in the control of arthropod disease vectors. A moderate number of seeds display some

mosquitocidal activity, while very few nuts have been tested or show any promise (Table 3.1). A wide variety of larvicidal, adulticidal, repellent, and oviposition deterrent effects were observed against serious mosquito vectors. However, these effects are not easily transferred into practical application because the mosquitocidal efficacy of extracts from seeds or nuts is influenced by the mosquito species, plant species, and extractive solvent (Shaalan *et al.*, 2005). The thermostability and water solubility of some extracts, such as bioactive compounds from *M. volkensii*, give them another advantage over synthetic insecticides (Al-Sharook *et al.*, 2009). Better formulation technology is needed for topical repellents. The provision of more effective fixation for the essential oil content and incorporation of strategies for controlled release of essential oil vapors, whilst also providing solutions for the problem of potential dermal irritancy, would all be beneficial.

SUMMARY POINTS

- Seeds and nuts of different plant species have shown different mosquitocidal activity against both nuisance and mosquito vector-borne diseases.
- The number of types of seeds that produce such activity is larger than that of nuts (40 plant species and 3 plant species, respectively).
- Such mosquitocidal activities are not only against mosquitoes, adult stage, but also against immature stages (eggs, larvae and pupae).
- Experiments and observations have indicated that seed and nut extracts are safe for humans, fish, predaceous insects, and mammals.
- Capacity, eco-friendliness, and other physical factors, such as thermostability and water solubility, will give seeds and nuts advantages over synthetic insecticides for both commercialization and mosquito control.

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CHAPTER 4

Fatty Acid Content of Commonly Available Nuts and Seeds

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

α-ESA, α-eleostearic acid 18: 3(9*c*,11*t*,13*c*)
β-ESA, β-eleostearic acid 18: 3(9*t*,11*t*,13*t*)
CA, catalpic acid 18: 3(9*t*,11*t*,13*c*)
CLNA, conjugated linolenic acid
DAG, diacylglycerol
FFA, free fatty acid
MUFA, monounsaturated fatty acid
PA, punicic acid 18: 3(9*c*,11*t*,13*t*)
PUFA, polyunsaturated fatty acid
S, sterol
SE, sterol esters
SFA, saturated fatty acid
TAG, triacylglycerol

INTRODUCTION

Nuts and seeds are good dietary sources of unsaturated fatty acids. Linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3) are two essential fatty acids in humans and are precursors of C20 and C22 polyunsaturated fatty acids (PUFAs). Other uncommon fatty acids, such as

Nuts & Seeds in Health and Disease Prevention. DOI: 10.1016/B978-0-12-375688-6.10004-0

stearidonic acid (18:4n-3), conjugated linolenic acids (CLNAs), and ximenynic acid (a triple bond fatty acid), are also presented in certain seeds and nuts. The substantial epidemiological evidence shows that fatty acids from seeds and nuts are associated with different health effects (Li *et al.*, 2002). The aim of this chapter is to summarize the composition and content of lipids and fatty acids in commonly available nuts and seeds worldwide.

FATTY ACIDS IN VARIOUS NUTS AND SEEDS

The predominant lipid in all nuts and seeds investigated was triacylglycerol (TAG), which was found at levels above 90%, reaching 98.4% in macadamia nuts. The total lipid concentration in the samples ranged from 2.2 g/100 g in *ginkgo biloba* to 75.4 g/100 g in walnut. Apart from peanut and *T. kirilowii* Maxim. seed, most of the analyzed samples contained phytosterols; pistachio contained the highest amount, at 5.0%. Some seeds contained diacylglycerol and free fatty acids. For instance, diacyglycerol comprised 4.8% of the total lipids in *Cannabis sativa*, and free fatty acids comprised 1.7% of the total lipids in *Ginkgo biloba*. Phytosterol ester ranged from 0.2% of total lipids in peanut seed to 7.1% in grand torreya seed; however, it was not detected in walnut, pistachio, almond, and black melon seed (Table 4.1).

The primary saturated fatty acids (SFAs) identified in the 20 nuts and seeds were palmitic acid (16:0) and stearic acid (18:0), with a particularly high content of the former in the fig-leaf gourd seed, and of the latter in the Brazil nut (15.4% and 11.8% of the total, respectively). The proportion of total SFAs ranged from 6.34% to 26.21%, with the Brazil nut yielding the greatest percentage and the pecan nut the least. The most predominant SFA was 16:0, ranging from 4.28% in pecan nut to 15.4% in fig-leaf gourd (Tables 4.2–4.4). The levels of total unsaturated fatty acids ranged from 73% in the Brazil nut to 93% in the pecan. The total proportion of PUFAs was the highest in black melon seed, at 75.8%, and the lowest in macadamia, at 2.8%. Furthermore, PUFAs were predominant over monounsaturated fatty acids (MUFAs) in all nut and seed samples except the pistachio, filbert, almond, macadamia, pumpkin, and cashew nuts, which were found to contain MUFAs ranging from 42.7% in pumpkin to 82.6% in macadamia (Figure 4.1).

There were three main fatty acids in all nuts and seeds: 18:2n-6, 18:1n-9, and 16:0. The former, 18:1n-9, was present in high levels in the macadamia and filbert, at 57.1% and 74.7% of total fatty acids respectively, and black melon seed had the lowest level, at 8.63%. The proportion of

TABLE 4.1 Lipid Content (g/100 g) and Lipid Compositions (% of Total Lipid) of Commonly Consumed Nuts and Seeds

	Lipid Content (g/100g)	Lipid Composition (% of Total Lipid)				
		SE	TAG	DAG	FFA	S
Grand torreya seed	51.2 ± 1.0	7.1 ± 4.5	92.1 ± 4.5	nd	nd	0.8 ± 0.3
Walnut	75.4 ± 2.5	nd	97.0 ± 0.2	nd	nd	3.0 ± 0.4
Pistachio	53.9 ± 0.5	nd	95.0 ± 14.2	nd	nd	5.0 ± 1.0
Filbert	60.0 ± 2.5	3.2 ± 0.5	95.1 ± 0.8	nd	nd	1.7 ± 0.2
Pine nut	66.6 ± 1.2	1.4 ± 0.2	95.9 ± 2.1	nd	nd	2.7 ± 0.3
Almond	53.5 ± 0.8	nd	95.9 ± 0.4	2.0 ± 0.4	nd	2.1 ± 0.5
Macadamia	70.1 ± 0.6	0.7 ± 0.1	98.4 ± 0.3	nd	nd	0.9 ± 0.2
Cannabis sativa	49.5 ± 3.0	1.4 ± 0.8	91.1 ± 4.2	4.8 ± 1.3	nd	2.7 ± 1.2
Peanut	35.4	0.2	97.3	0.6	0.2	nd
Ginkgo biloba	2.2	3.7	85.5	3.6	1.7	1.6
T. kirilowii Maxim. seed	49.4 ± 1.0	1.1 ± 0.8	98.1 ± 0.8	0.2 ± 0.2	0.4 ± 0.2	nd
Black melon seed	34.6	nd	95.5	nd	1.4	3.1

Mean ± SD, $n = 3$, nd = not detected.
SE, sterol esters; TAG, triacylglycerols; DAG, diacylglycerols; FFA, free fatty acids; S, sterol.
Sources: Li *et al.* (2006); Yoshida *et al.* (2005).

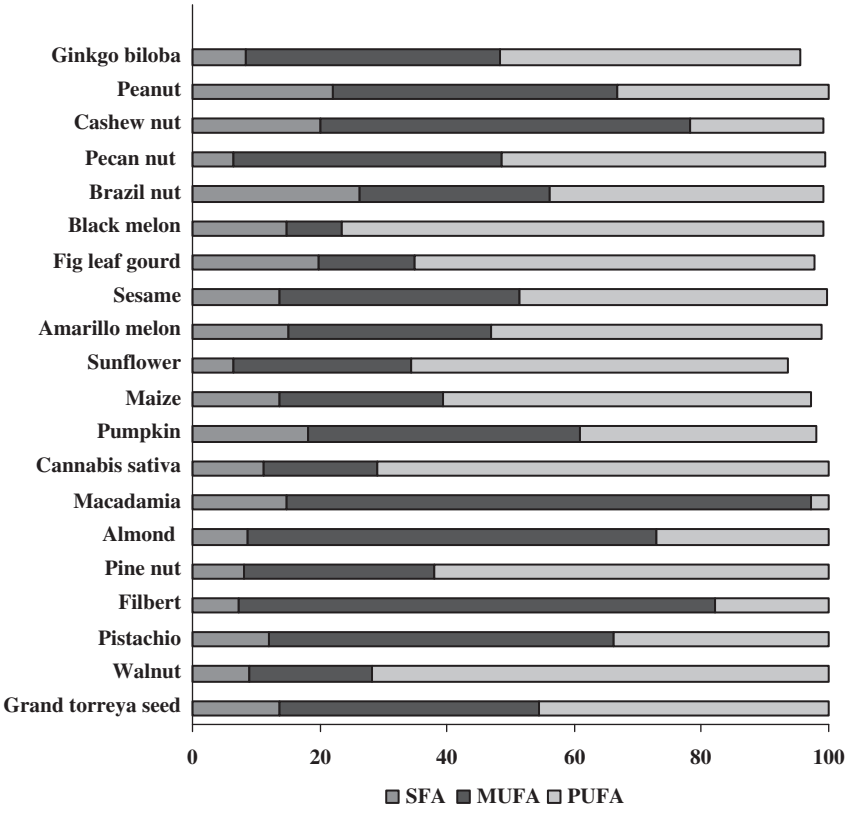

FIGURE 4.1
Proportion of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in oil from 20 edible nuts and seeds. *Sources: Hierro* et al., *1996; Li* et al., *2006; Ryan* et al., *2006; Were* et al., *2006; Sabudak, 2007; Bernardo-Gil* et al., *2009.*

16:1 in analyzed nuts was very low, at less than 1% of the total fatty acids, except in macadamia (25.5%), ginkgo nut (3.43%), and almond (1.1%). The fatty acid 18:2n-6 was the most abundant PUFA, with lesser amounts of 18:3n-3. The content of 18:2n-6 varies remarkably, ranging from 2.5% in macadamia to 75.7% in black melon seed, but most nuts and seeds are rich in this fatty acid – especially walnut "pine nut", *Cannabis sativa*, maize, sunflower seed, "Amarillo melon", fig-leaf gourd seeds, and pecan nut, where the proportion is approximately 50–60%. Similarly, the content of 18:3n-3 also varies greatly, ranging from 0.01% in sunflower seed to 15.2% in *Cannabis sativa*. *Ginkgo biloba* nut contained 20:2n-6 and 20:3n-6 (Tables 4.2–4.4).

The total content of CLNA amounted to 36.9% in *T. kirilowii* Maxim seed and 83.4–87.9% in pomegranate seed. The content of punicic acid, the predominant isomer of CLNA,

TABLE 4.2 Fatty Acid Composition (% of Total Fatty Acid) of Common Consumed Nuts

Fatty Acids	Grand Torreya	Walnut	Pistachio	Filbert	Pine Nut	Almond	Macadamia	Cannabis Sativa	*P*-Value
16:0	9.6 ± 0.3	6.7 ± 0.8	10.5 ± 3.5	4.8 ± 0.2	5.4±0.3	7.1 ± 0.3	10.3±0.0	7.7 ± 0.2	< 0.001
16:1n-7	0.2 ± 0.1	0.2 ± 0.1	0.8 ± 0.3	0.1±0.0	0.2 ± 0.2	1.1±0.4	25.5 ± 0.2	0.3±0.1	< 0.001
17:0	0.1 ± 0.0	nd	0.1 ± 0.0	nd	0.2 ± 0.2	nd	nd	0.1 ± 0.0	
18:0	3.9 ± 0.2	2.1 ± 0.2	1.4 ± 0.1	2.5 ± 0.1	2.6 ± 0.3	1.5 ± 0.1	4.3 ± 0.3	3.4 ± 0.0	< 0.001
18:1	40.7 ± 1.4	19.3 ± 6.8	53.4 ± 1.8	74.7 ± 1.0	29.6 ± 1.0	63.2 ± 1.3	57.1 ± 0.4	17.7 ± 0.5	< 0.001
18:2n-6	44.7 ± 1.4	60.3 ± 6.7	33.1 ± 1.8	16.8 ± 0.1	61.6 ± 1.8	26.9 ± 1.3	2.5 ± 0.2	55.7 ± 0.5	< 0.001
18:3n-3	0.8 ± 0.2	11.4 ± 1.4	0.7 ± 0.1	1.0 ± 0.8	0.5 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	15.2 ± 0.3	< 0.001
SFA	13.7 ± 0.4	8.8 ± 0.7	12.0 ± 3.6	7.3 ± 0.2	8.2 ± 0.7	8.6 ± 0.4	14.7 ± 0.3	11.1 ± 0.2	< 0.001
MUFA	40.9 ± 1.4	19.4 ± 6.8	54.2 ± 2.0	74.8 ± 1.0	29.7 ± 1.1	64.2 ± 1.5	82.6 ± 0.2	18.0 ± 0.6	< 0.001
PUFA	45.4 ± 1.5	71.7 ± 7.5	33.8 ± 1.8	17.8 ± 0.9	62.1 ± 1.7	27.1 ± 1.2	2.8 ± 0.3	70.9 ± 0.7	< 0.001

Mean ± SD, $n = 3$, nd = not detected.
Source: Reproduced from Li *et al.* (2006), with permission.

TABLE 4.3 Fatty Acid Composition (% of Total Fatty Acid) of Commonly Consumed Seeds

Fatty Acids	Pumpkin	Maize	Sunflower	Amarillo Melon	Sesame	Fig Leaf Gourd	Black Melon
14:0	0.12	0.09	0.05	0.07	nd	0.16	nd
16:0	12.26	11.03	4.66	8.51	8.24	15.4	9.31
16:1n-7	trace	0.06	0.02	0.08	nd	0.29	nd
17:0	trace	0.06	0.06	0.08	nd	nd	nd
17:1	0.05	nd	0.05	nd	nd	nd	nd
18:0	5.22	1.7	0.4	6.09	4.89	4.21	5.43
18:1	42.49	25.52	27.73	31.5	37.64	14.7	8.63
18:2n-6	36.99	56.9	59.22	51.6	47.82	61.0	75.70
18:3n-3	0.11	1.04	0.01	0.19	0.45	1.92	0.13
20:0	0.37	0.37	0.26	0.29	0.50	nd	0.16
20:1n-9	0.1	0.05	0.01	0.16	0.24	nd	nd
22:0	0.11	0.19	0.74	nd	nd	nd	nd
22:1	nd	nd	0.01	0.25	nd	nd	nd
24:0	0.07	0.22	0.29	nd	nd	nd	nd
24:1	0.1	nd	0.01	nd	nd	nd	nd
SFA	18.15	13.66	6.46	15.04	13.63	19.77	14.9
MUFA	42.74	25.63	27.83	31.99	37.88	14.99	8.63
PUFA	37.1	57.94	59.23	51.79	48.27	62.9	75.83
Others	2.01	2.77	6.48	1.18	0.22	2.32	0.64

nd = not detected.
Sources: Were *et al.* (2006); Sabudak (2007); Bernardo-Gil *et al.* (2009).

TABLE 4.4 Fatty Acid Composition (% of Total Fatty Acid) of Commonly Consumed Nuts

Fatty Acids	Brazil Nut	Pecan Nut	Cashew Nut	Peanut	Ginkgo Biloba
14:0	0.06	nd	0.07	nd	trace
16:0	13.50	4.28	9.93	11.49	6.62
16:1n-9	nd	nd	nd	nd	0.13
16:1n-7	0.33	0.09	0.36	nd	3.30
17:0	0.22	0.10	0.14	nd	nd
18:0	11.77	1.80	8.70	3.97	0.99
18:1n-9	29.09	40.63	57.24	43.72	13.76
18:1n-7	nd	nd	nd	nd	21.53
18:2n-6	42.80	50.31	20.80	33.30	38.99
18:3n-3	0.20	0.65	0.23	nd	nd
18:3n-6	nd	nd	nd	nd	1.61
20:0	0.54	Tr.	0.97	1.90	0.37
20:1n-9	0.21	1.21	0.25	0.89	0.44
20:1n-7	nd	nd	nd	nd	0.66
20:2n-6	nd	nd	nd	nd	0.90
20:3n-6	nd	nd	nd	nd	5.70
22:0	0.12	0.16	0.39	3.46	0.40
22:1	0.34	0.25	0.28	nd	nd
24:0	nd	nd	nd	1.26	nd
SFA	26.21	6.34	20.20	22.08	8.38
MUFA	29.97	42.18	58.13	44.61	39.82
PUFA	43.00	50.96	21.03	33.30	47.2
Others	0.82	0.52	0.64	0.01	4.6

nd = not detected.
Sources: Hierro *et al.* (1996); Ryan *et al.* (2006).

reached 32.6% in *T. kirilowii* Maxim seed, while it rose to 73.4–77.5% in pomegranate seed (Table 4.5).

All *Santalum* species contained significant amounts of ximenynic acid, especially *S. obtusifolium*, *S. insulare*, and *S. album*; the latter had the greatest quantity, at 82.8% (Table 4.6).

Regarding the five families of *Ribes* berries, Boraginaceae, Scrophulariaceae, Onagraceae, and Ranunculaceae, 18:3n-3 was detected in all the samples, and variable contents of γ-linolenic acid (18:3n-6) and 18:4n-3 were found in seeds of the *Ribes* berries and Boraginaceae species (Table 4.7).

CONCLUSIONS

All the nuts, seeds, currants, and *Santalum* kernels contained a low level of saturated fatty acids and a high level of unsaturated fatty acids. A high content of 18:3n-3 was found in *sativa*, walnut, *Ribes* berries, and Ranunculaceae; variable levels of 18:3n-3 were also detected in the Scrophulariaceae, Onagraceae, and Boraginaceae plant families; and 18:4n-3 was detected in *Ribes* berries and Boraginaceae species. Meanwhile, *T. kirilowii* Maxim and pomegranate seeds were rich in CLNA, while *Santalum* kernels contained ximenynic acid.

TABLE 4.5 Fatty Acid Composition (% of Total Fatty Acids) of Some Seeds from China

Fatty Acids	*T. Kirilowii* Maxim	Pomegranate		
		San bai yu	*Qing pi ruan zi*	*Tian lv zi*
16:0	5.8 ± 0.3	2.2 ± 0.2	2.3 ± 0.0	2.4 ± 0.0
17:0	nd	trace	trace	trace
18:0	2.4 ± 0.2	1.3 ± 0.1	1.8 ± 0.0	1.6 ± 0.3
18:1n-9	22.6 ± 1.1	2.5 ± 0.1a	5.2 ± 0.0b	4.4 ± 0.9b
18:2(iso)	nd	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
18:2n-6	32.6 ± 0.6	5.4 ± 0.2	6.4 ± 0.0	5.6 ± 0.9
18:3n-3	nd	0.1 ± 0.0	trace	trace
20:0	nd	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
21:1n-9	nd	0.5 ± 0.0	0.5 ± 0.2	0.6 ± 0.1
PA	32.6 ± 0.8	77.5 ± 1.3a	73.4 ± 1.4b	75.5 ± 1.6ab
α-ESA	3.0 ± 0.0	nd	nd	nd
CA	0.9 ± 0.1	8.4 ± 0.8	7.8 ± 0.7	8.3 ± 0.5
β-ESA	nd	1.4 ± 0.2	2.2 ± 0.5	1.1 ± 0.1

Mean ± SD, $n = 3$, nd = not detected.
PA, punicic acid 18:3(9*c*,11*t*,13*t*); CA, catalpic acid 18:3(9*t*,11*t*,13*c*); α-ESA, α-eleostearic acid 18:3(9*c*,11*t*,13*c*); β-ESA, β-eleostearic acid 18:3(9*t*,11*t*,13*t*).
Sources: Yuan, *et al.* (2009); Zhou (2010).

TABLE 4.6 Fatty Acid Composition (% of Total Fatty Acid) of Various *Santalum* Species Kernel Oils

Species	Album	Acuminatum	Murrayanum	Obtusifolium	Spicatum	Insulare
16:0	0.8	2-2.9	2.4	0.6	3.5	1.0
16:1n-7	0.5	0.3–2.7	0.3	0.4	0.7	0.6
18:0	1	1.1–2.3	2.1	1.2	1.9	1.0
18:1n-9	12.3	43.8–57.7	54.8	14.3	54.4	18.1
18:2n-6	nd	0.3–1.4	1.4	0.7	0.6	0.5
18:3n-3	0.8	0–2.5	2.3	3.2	nd	1.0
Ximenynic	82.8	32.2–46.2	35.5	71.5	33.4	74.5
Others	1.8	2.3	1.2	8.1	5.5	3.3

nd = not detected,
Sources: Liu *et al.* (1996); Butaud *et al.* (2008).

TABLE 4.7 Fatty Acid Composition (% of Total Fatty Acid) of Seed Oils from Five Plant Families

Species	14:0	16:0	16: 1n-7	18:0	18: 1n-9	18:1n-7	18: 2n-6	18:3n-6	18:3n-3	18: 4n-3	20:0	20: 1n-9	20: 2n-6	22:0	22: 1n-9	24:0	24: 1n-9
Ribes																	
nigrum	0.1(2.7)	5.2 (0.9)	–	1.8 (0.2)	10.3 (0.2)	0.5(1.0)	48.2 (0.3)	11.3 (0.3)	17.5 (0.1)	3.0 (0.2)	0.2 (7.6)	0.9 (1.0)	0	0	0	0	0
spicatum	–	3.8 (7.4)	–	1.2 (0.8)	13.5 (5.2)	0.5(4.2)	38.5 (1.5)	16.1 (3.5)	19.1 (7.0)	6.0 (4.5)	0.2 (2.4)	0.1 (6.2)	0	0	0	0	0
alpinum	0.1 (44.2)	5.2 (0.9)	0.1 (1.8)	1.1 (1.0)	18.6 (0.1)	0.8(1.1)	36.1 (0.6)	13.3 (1.6)	18.2 (1.0)	5.1 (0.4)	0.1 (1.2)	0.1 (5.0)	0	0	0	0	0
Boraginaceae																	
Anchusa azurea	0.09	8.63	0.35	2.19	24.1	0.43	41.78	11.11	0.43	0.08	0.23	3.57	0.18	0.37	0	0	0
Anchusa undulata	0.16	8.76	0.54	2.15	24.4	0	25.37	8.35	17.99	3.55	0.24	4.21	0.15	0.32	0	0	0
Asperugo procumbens	0.12	8.07	0.16	1.85	15.48	0.62	15.2	5.35	36.46	11.75	0.19	2.03	0.12	0.24	0	0	0
Borago officinalis	0.10	9.57	0.18	6.18	20.92	0.46	33.21	19.2	1.00	0.49	0.43	3.95	0.15	0.27	0.05	0	0
Buglosoides arvensis	0.23	9.41	0.15	2.81	6.83	0.61	14.8	6.44	39.68	14.08	1.63	0.98	0	0.28	0	0	0
Cynoglossum cheirifolium	1.56	17.34	0	3.41	7.93	0	13.81	1.52	39.35	3.24	2.03	0.54	0.02	1.27	0.08	0.05	0
Cynoglossum creticum	1.55	16.25	0.35	2.92	8.57	0.35	18.27	0.66	44.75	1.16	1.88	0.42	0	1.93	0	0	0
Cynoglossum nebrodense	0.19	6.01	0.11	1.61	46.42	0	6.79	1.42	16.53	2.83	0.68	5.31	0	0.68	0	0	0
Cynoglossum officinale	0.21	7.01	0.15	1.4	42.6	0	9.02	1.68	16.14	2.48	0.68	5.14	0	0.73	0	0	0
Echium asperrimum	0.08	7.7	0.13	2.77	14.68	0	16.33	9.62	35.3	21.06	0.09	0.98	0.05	0.08	0.34	0	0.07
Echium boissieri	0	5.48	0.09	2.28	14.7	0	8.64	5.52	47.14	14.31	0.1	0.74	0.04	0.06	0	0	0
Echium creticum	0.05	5.58	0.06	2.98	8.18	0.35	14.31	9.70	42.68	14.73	0.11	0.58	0.06	0.06	0	0	0
Echium flavum	0	6.29	0.07	2.12	21.05	0.52	24.16	8.38	32.23	3.14	0.09	1.01	0.06	0.07	0	0	0
Echium humile	6.69	7.28	0.43	3.95	17.18	0.45	24.43	7.95	31.21	5.88	0.44	0.52	0.22	0	0	0	0
Echium sabulicola	0.06	5.51	0.08	2.42	8.03	0.36	16.31	10.94	40.39	14.72	0.08	0.65	0.10	0.06	0	0	0
Echium vulgare	0.14	7.38	0.06	2.52	11.14	0.44	21.18	11.74	34.14	9.68	0.1	0.74	0.07	0.08	0	0	0.04
Myosotis alpina	0.04	8.12	0.02	2.3	24.92	0.45	27.02	4.38	18.03	8.38	0.45	3.65	0.14	0.33	1.03	0	0.05
Myosotis nemorosa	0.16	13.15	0.35	3.89	20.79	0	30.76	20.25	4.69	1.56	0.27	2.57	0.10	0.16	1.23	0	0.02
Myosotis secunda	0.08	8.22	0.16	4.08	25.22	0	23.07	12.17	15.62	4.29	0.36	3.44	0.13	0.29	2.89	0	0
Nonea vesicaria	0.21	9.49	0.17	2.57	26.23	0.4	26.52	9.39	13.88	4.90	0.29	3.40	0.13	0.23	0.05	0	0
Ranunculaceae																	
Delphinium gracile	0.40	22.43	0.14	2.25	11.18	0.91	38.44	0	19.87	0	0.67	0.01	0	0.01	0	0	0
Ranunculus repens	0.37	10.46	0.20	2.07	7.36	0.73	36.55	0	39.71	0	0.27	0.13	0	0.31	0	0	0
Ranunculus peltatus	0.37	13.35	1.69	1.50	8.49	0.94	28.44	0	37.89	0	0.41	0.01	0	0.38	0	0	0

Onagraceae																	
Epilobium hirsutum	0.04	10.93	0.12	3.84	9.25	0.49	71.55	0.02	1.84	0	0.66	0.10	0.08	0.17	0	0	0
Epilobium lanceolatum	0.05	10.56	0.14	2.60	6.47	0.21	76.75	0.08	1.71	0	0.56	0.13	0.11	0.18	0	0	0
Scrophulariaceae																	
Antirrhinum barrelieri	0.04	5.13	0.17	2.58	18.04	0.81	71.77	0	0.34	0	0.13	0.11	0.04	0.02	0	0	0
Antirrhinum charidemi	0.04	6.40	0.21	1.54	15.67	0.87	73.20	0	0.53	0	0.12	0.12	0	0.08	0	0	0
Antirrhinum hispanicum	0.04	5.48	0.15	1.8	16.15	1.79	72.73	0.07	0.42	0	0.15	0.13	0.06	0.14	0	0	0
Antirrhinum majus	2.15	16.14	0.78	7.61	3.90	1.04	31.41	0	25.89	0	3.09	2.63	0.3	1.48	0	0	0
Antirrhinum molle	0.12	6.23	0.29	1.89	16.76	1.94	70.43	0.06	0.59	0	0.21	0.13	0.11	0.18	0.02	0	0
Bellardia trixago	0.05	8.71	0.17	1.76	18.37	1.06	43.56	0	24.8	0	0.36	0.16	0	0.16	0	0	0
Chaenorhinum macropodum	0.15	7.55	0	1.83	10.84	0.98	75.73	0	2.22	0	0.01	0.01	0	0.01	0	0	0
Chaenorhinum origanifolium	0.10	6.00	0.22	2.07	12.75	1.12	73.34	0	3.42	0	0.17	0.09	0.11	0.10	0	0.02	0
Chanaenorhinum villosum	0.23	5.71	0	3.87	2.03	0	73.14	0.53	2.02	0	0.21	0.12	0	0.23	0.19	0.18	0.2
Cymbalaria muralis	0.14	5.12	0	2.56	22.66	0	63.08	1.33	3.05	0	0.14	0.38	0.42	0.09	0.15	0.02	0
Digitalis obscura	0.15	8.04	0.15	2.69	18.97	2.11	60.57	0.06	4.44	0	0.42	0.07	0.04	0.21	0	0	0
Lafuentea rotundifolia	1.16	14.85	0.46	2.94	7.32	0.39	24.04	0	19.89	0	1.51	0.01	0	0.64	0.07	0.03	0
Linaria aeruginea	0.12	26.4	0	9.35	6.66	0	26.38	0	11.23	0	0.69	0.32	0	0.12	0.21	0.11	0.29
Linaria amoi	0.08	6.29	0.12	1.96	19.49	0.12	67.43	0.46	2.04	0	0.18	0.14	0	0.01	0	0	0
Misopates orontium	0.04	6.90	0.11	2.34	16.38	0.54	71.45	0	0.38	0	0.37	0.12	0	0.19	0.02	0	0
Odontites longiflora	0.09	8.12	0.84	2.07	32.38	2.97	11.86	0	40.49	0	0.16	0.09	0	0.05	0.04	0	0
Parentucela viscosa	0.21	8.93	0.33	2.63	21.65	0.12	37.28	0.21	28.56	0	0.28	0.16	0.12	0.15	0.18	0	0.12
Scrophularia auriculata	0.06	12.01	0.15	2.48	14.89	2.63	63.45	2.66	0.36	0	0.39	0.13	0.03	0.13	0	0	0
Scrophularia nodosa	0.12	26.4	0	3.6	13.92	0	60.33	2.26	4.39	0	0.12	0.89	0	0.11	0	0.12	0.23
Scrophularia sciophila	0.06	11.05	0.10	2.12	10.82	0.71	63.57	10.17	0.38	0	0.34	0.10	0.05	0.17	0	0	0
Verbascum phlomoides	0.19	6.39	0.17	3.11	17.03	0.59	69.58	0	1.26	0	0.53	0.09	0.05	0.01	0	0	0
Verbascum thapsus	0.04	6.4	0.17	2.61	16.75	0.59	70.84	0	1.03	0	0.47	0.15	0.05	0.01	0.08	0	0
Veronica anagalloides	0.35	10.81	0.25	2.21	19.12	0.42	52.12	0	12.24	0	0.36	0.16	0	0.43	0	0	0
Veronica persica	1.24	15.82	0.68	2.43	28.87	0.54	28.39	0	19.24	0	0.59	0.41	0	0.4	0	0	0[a]

Sources: Horrobin (1992); Johansson (1997); Guerrero *et al.* (2001).

SUMMARY POINTS

1. Nuts, seeds, currants and *Santalum* kernels contained a high proportion of unsaturated fatty acid, of which 18:2n-6 was the most abundant PUFA, with lesser amounts of 18:3n-3.
2. The Boraginaceae, Scrophulariaceae, Onagraceae, Ranunculaceae, and *Ribes* berries families contained relatively high levels of 18:3n-6.
3. *The Ribes* berries and Boraginaceae families contained 18:4n-3.
4. *T. kirilowii* Maxim and pomegranate seed contained relatively high CLNA.
5. *Santalum* kernels contained ximenynic acid.

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CHAPTER 5

Triacylglycerols in Nut and Seed Oils

Detailed Characterization Using High-performance Liquid Chromatography/ Mass Spectrometry

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CHAPTER OUTLINE

LIST OF ABBREVIATIONS

APCI, atmospheric pressure chemical ionization
CN, carbon number
DB, double bond
ECN, equivalent carbon number
FA, fatty acid
HPLC, high-performance liquid chromatography
NARP, non-aqueous reversed-phase
RF, response factor
TG, triacylglycerol

Fatty Acid Abbreviations

Cy, caprylic (CN:DB, C8:0)
C, capric (C10:0)

Nuts & Seeds in Health and Disease Prevention. DOI: 10.1016/B978-0-12-375688-6.10005-2

La, lauric (C12:0)
M, myristic (C14:0)
C15: 0, pentadecanoic
P, palmitic (C16:0)
Po, palmitoleic (Δ9-C16:1)
Ma, margaric (C17:0)
Mo, margaroleic (Δ9-C17:1)
S, stearic (C18:0)
O, oleic (Δ9-C18:1)
L, linoleic (Δ9,12-C18:2)
Ln, *alpha*-linolenic (Δ9,12,15-C18:3)
γLn, *gamma*-linolenic (Δ6,9,12-C18:3)
St, stearidonic (Δ6,9,12,15-C18:4)
C19: 0, nonadecanoic (C19:0)
A, arachidic (C20:0)
G, gadoleic (Δ9-C20:1)
C20: 2, eicosadienoic (Δ11,14-C20:2)
C21: 0, heneicosanoic (C21:0)
B, behenic (C22:0)
C22: 1, erucic (Δ13-C22:1)
C23: 0, tricosanoic (C23:0)
24: 1, nervonic (Δ15-C24:1)
Lg, lignoceric (C24:0)
C25: 0, pentacosanoic (C25:0)
C26: 0, hexacosanoic (C26:0)

INTRODUCTION

Triacylglycerols (TGs) form an important part of the human diet, serve as a source of energy stored in fat tissues, provide a thermal and mechanical protective layer surrounding important organs, and are a source of essential FAs (linoleic and linolenic acids), fat-soluble vitamins and other non-polar compounds. The main sources of TGs in the human diet are oil plants, and, especially, the oils prepared from them (Gunstone, 2006; Leray, 2009). The final use of plant oils depends on their composition, and comprehensive triacylglycerol profiling provides valuable information in this respect. Individual TGs can differ in the number of carbon atoms (CNs), the number of double bonds (DBs), and the *cis*/*trans* configuration of the double bonds. Also, different stereochemical positions *sn*-1, -2, or -3 of FAs on the glycerol backbone (regioisomers), or *R*/*S* optical configuration of TGs esterified in *sn*-1 and *sn*-3 positions by two different FAs (optical isomers), lead to enormous complexity. Two techniques of high-performance liquid chromatography (HPLC) are widely used in the analysis of TG mixtures: silver ion normal-phase HPLC (Ag-HPLC), and non-aqueous reversed-phase HPLC (NARP-HPLC). Ag-HPLC is widely used for the separation of lipids according to the number, position, and *cis*/*trans* configuration of double bonds. TG regioisomers can be also separated under carefully optimized chromatographic conditions (Holčapek *et al.*, 2009; Lísa *et al.*, 2009a). In NARP-HPLC, TGs are separated according to acyl chain lengths and the number of double bonds. The retention of TGs is governed by the equivalent carbon number (ECN), which is defined as ECN = CN − 2DB. The separation of most TGs within one ECN group is feasible under optimized chromatographic conditions (Lísa & Holčapek, 2008; Lísa *et al.*, 2009b). The separation of *cis*/*trans* isomers or double-bond positional isomers is also possible in NARP-HPLC (Lísa *et al.*, 2007; Holčapek *et al.*, 2009). Mass spectrometry (MS) coupled to HPLC is the most powerful tool for the identification of lipids. Atmospheric pressure chemical ionization (APCI) provides the best results for TGs (Holčapek *et al.*, 2005; Lísa *et al.*, 2009c) because of the

full compatibility with common NARP conditions, easy ionization of non-polar TGs, and the presence of both protonated molecules $[M+H]^+$ and fragment ions $[M+H-R_iCOOH]^+$. The APCI quantitation approach is based on response factors (RFs) of 23 single-acid TG standards calculated from calibration parameters of these TGs related to triolein, as one of the most widespread TGs in nature (Holčapek *et al.*, 2005). RFs of mixed-acid TGs are calculated as the arithmetic mean of RFs of individual FAs present in each TG.

METHOD OF TRIACYLGLYCEROL ANALYSIS IN NUTS AND SEEDS

Full details of triacylglycerol analysis can be found in Holčapek *et al.* (2005) and Lísa and Holčapek (2008). Briefly, the process includes the following:

- a liquid chromatograph, Waters 616 (Milford, MA, USA)
- two chromatographic columns, Nova-Pak C_{18}, a total length 45 cm
- an acetonitrile-2-propanol mobile phase gradient
- an Esquire 3000 ion trap mass analyzer (Bruker Daltonics, Germany)
- positive-ion atmospheric pressure chemical ionization (APCI)
- UV detection at 205 nm.

Chromatographic behavior of triacylglycerols

Most of TGs within one ECN group were separated (Figures 5.1–5.3), including the partial separation of critical pairs of TGs – i.e., SLO ($t_R = 85.1$ min) and OOP (85.4) with ECN = 48. The retention behavior of TGs in one ECN group was strongly influenced by the FA composition in individual TGs, mainly by the unsaturation degree and acyl chain lengths. For example, the group of OOO ($t_R = 84.0$ min), OOP (85.4), POP (87.0), and PPP (88.7) with ECN = 48 was well resolved. The retention of TGs within one ECN group increased with decreasing double-bond number in acyl chains – for example, with replacement of oleic acid by palmitic acid, or linoleic acid by palmitoleic acid; i.e., pairs LLL ($t_R = 65.3$ min) and LLPo (65.7) with ECN = 42, OLL (71.8) and OLPo (72.2) with ECN = 44, etc.

Retention times of identified TGs in the wide range of analyzed samples were used for the identification of TGs containing FAs with unusual positions of double bonds (double-bond positional isomers). TG double-bond positional isomers shifted retention times in comparison to common TGs containing FAs with the same number of carbon atoms and the same number of double bonds. Characteristic shifts in their retention factors (Δk) were used for the identification of TGs containing unusual gamma-linolenic acid (Δ6,9,12-18:3, γLn) in blackcurrant and redcurrant (Figure 5.2C) oils. In NARP-HPLC systems, TGs containing γLn had a higher retention in comparison to TGs containing only Ln – for example, pairs of double-bond positional isomers LnLnLn ($t_R = 48.3$ min) and LnLnγLn (49.0), LnLSt (49.4) and γLnLSt (50.1), etc. The differences in retention factors of TGs containing one γLn and TGs containing only Ln were constant, with an average value $\Delta k = 0.22$. Differences in retention factors for TGs containing two and three γLn acids corresponded approximately two times and slightly more than three times with the Δk value of one γLn – i.e., $\Delta k = 0.44$ and $\Delta k = 0.72$, respectively. TGs containing only γLn without any Ln were identified in borage (Figure 5.2D) and evening primrose (Figure 5.3B) oils.

APCI-MS PROFILING OF TRIACYLGLYCEROL COMPOSITION IN NUTS AND SEEDS

TGs were identified using positive-ion APCI-MS based on both protonated molecules $[M+H]^+$ and fragment ions $[M+H\text{-}R_iCOOH]^+$. The standard notation of TGs using initials of FA trivial names (Table 5.1) arranged according to their *sn*-1, *sn*-2, and *sn*-3 positions was used. FAs in *sn*-1 and *sn*-3 positions were not resolved using NARP-HPLC/APCI-MS, and they were considered as equivalent. FAs in these positions were arranged according to their decreasing molecular weights. Unlike in the *sn*-1 and *sn*-3 positions, the FA in the *sn*-2 position was identified

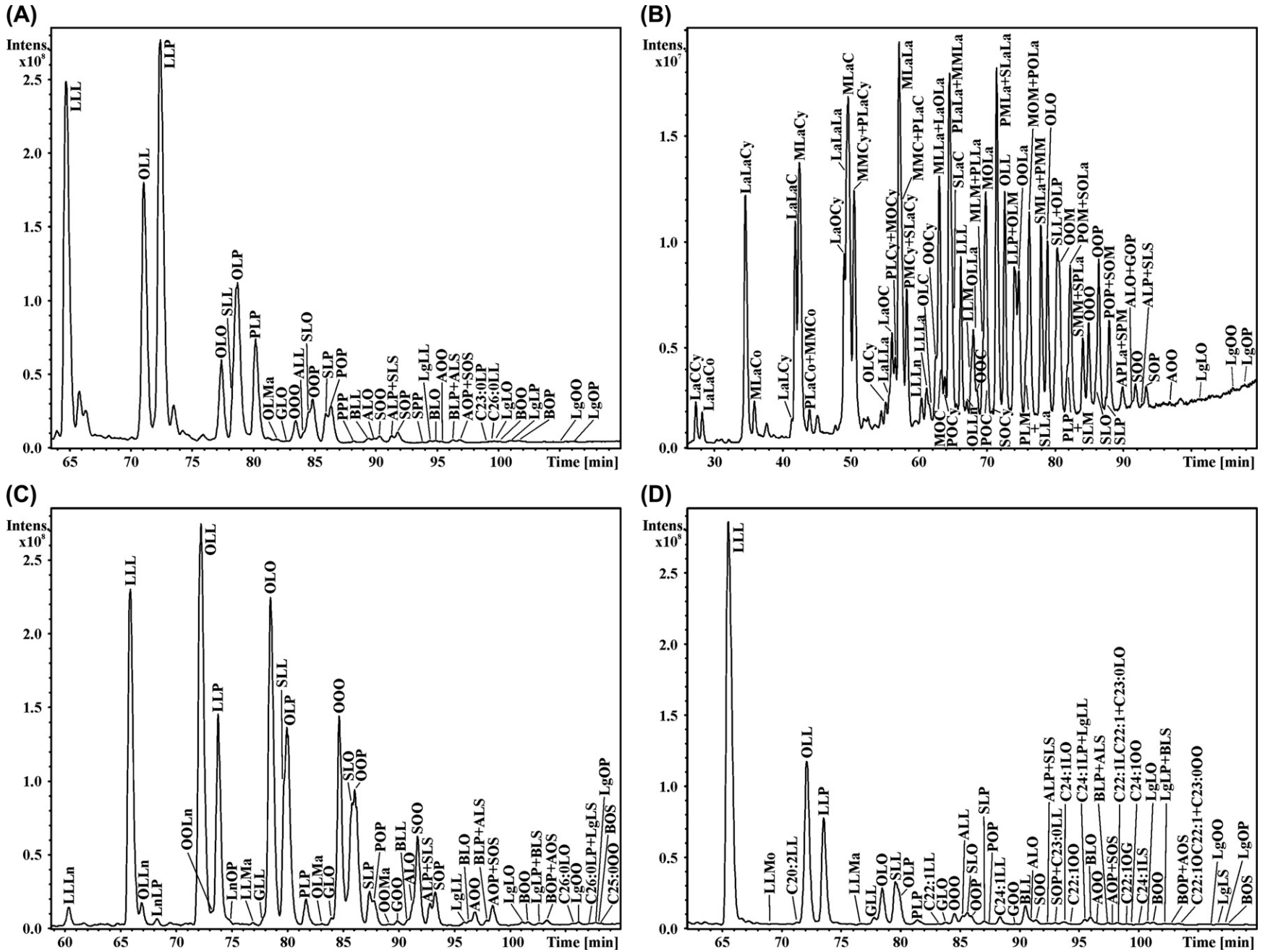

FIGURE 5.1
NARP-HPLC/APCI-MS analysis of plant oils: (A) cottonseed (*Gossypium hirsutum*), (B) coconut (*Cocos nucifera*), (C) sesame (*Sesamum indicum*), and (D) safflower (*Carthamus tinctorius*).

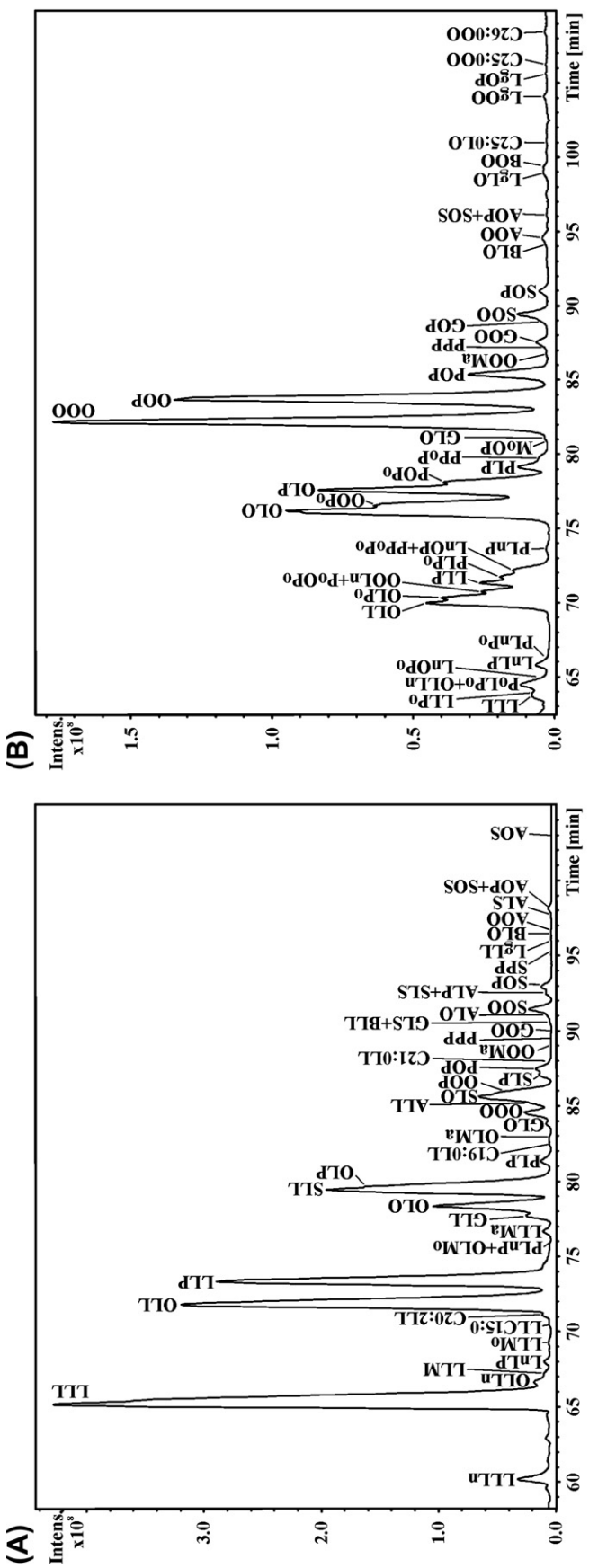

FIGURE 5.2
NARP-HPLC/APCI-MS analysis of plant oils: (A) grape seed — red (*Vitis vinifera*), (B) avocado (*Persea americana*), (C) redcurrant (*Ribes rubrum*), and (D) borage (*Borago officinalis*).

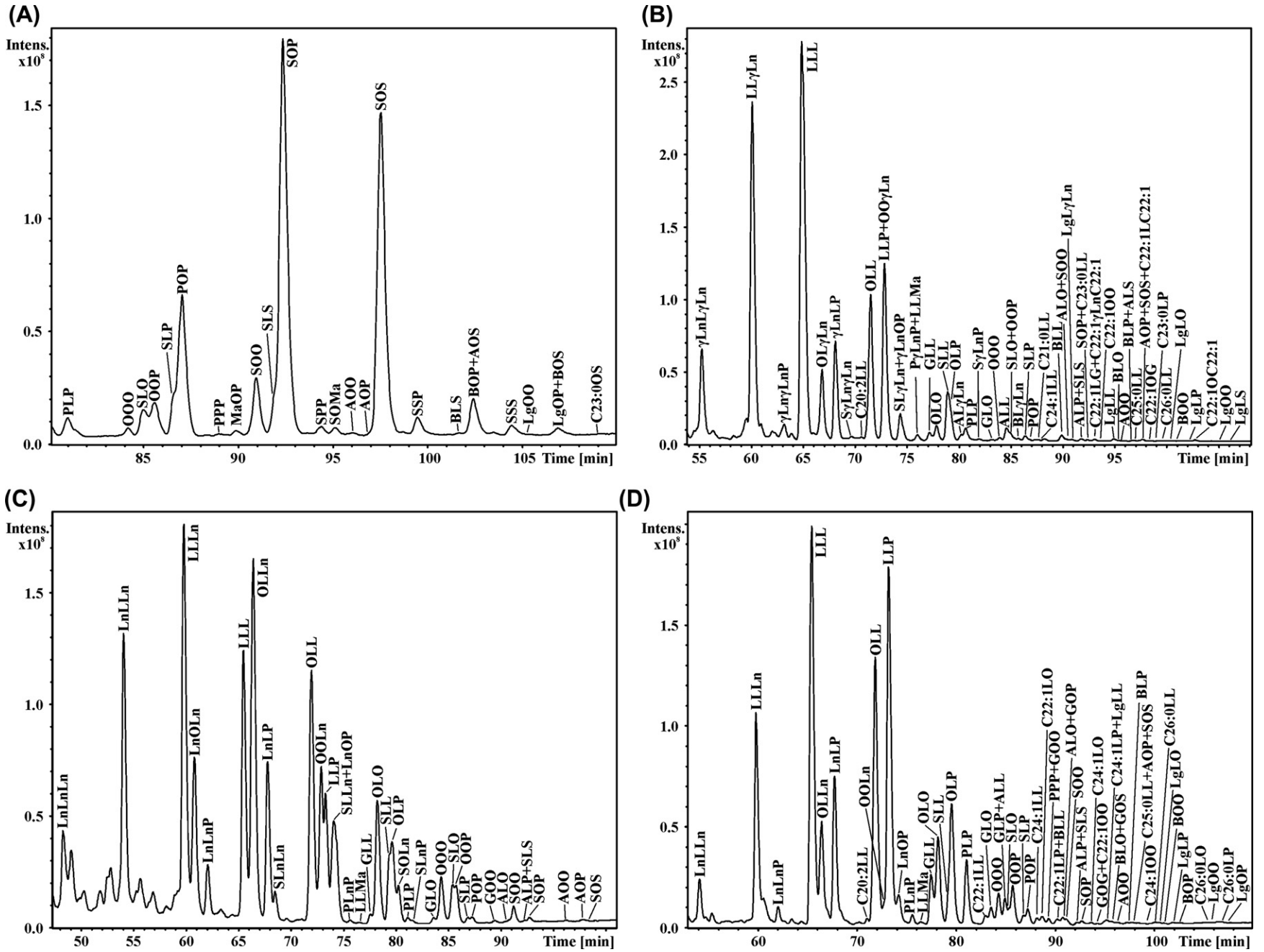

FIGURE 5.3

NARP-HPLC/APCI-MS analysis of plant oils: (A) cocoa butter (*Theobroma cacao*), (B) evening primrose (*Oenothera biennis*), (C) kukui (*Aleurites moluccana*), and (D) wheat germ (*Triticum vulgare*).

TABLE 5.1 Relative Concentrations [%] of Individual Fatty Acids in Analyzed Plant Oils Calculated From NARP-HPLC/APCI-MS of Triacylglycerols With Their Response Factors (RF)

Fatty Acid	Symbol	CN:DB	RF	Palm	Rape	Soybean	Sunflower	Peanut	Cotton	Coconut	Maize	Olive	Sesame	Almond	Safflower	Grape Seed – White	Grape Seed – Red	Hazelnut
Caproic	Co	6:0	134.76							1.37								
Caprylic	Cy	8:0	74.44							15.60								
Capric	C	10:0	17.62							3.70								
Lauric	La	12:0	6.04							37.05								
Myristic	M	14:0	2.77	2.36			0.18			18.80						0.08	0.13	
	C15:0	15:0	1.75													0.03	0.03	
Palmitoleic	Po	Δ9-16:1	1.33		0.13							1.10						0.20
Palmitic	P	16:0	1.32	40.57	6.51	11.66	7.69	9.47	22.12	7.33	11.95	11.75	10.86	9.47	6.60	9.40	10.50	10.60
Margaroleic	Mo	Δ9-17:1	0.81			0.12	0.06	0.12			0.04	0.08		0.23	0.02	0.05	0.03	0.18
Margaric	Ma	17:0	0.81	0.03	0.09	0.13	0.11	0.08	0.02		0.04		0.05	0.09	0.03	0.12	0.10	0.10
Stearidonic	St	Δ6,9,12, 15-18:4	0.23															
alpha-Linolenic	Ln	Δ9,12, 15-18:3	0.40	0.23	12.87	12.52	0.14	0.27		0.01	1.73	0.77	0.63			0.60	0.61	0.19
gamma-Linolenic	γLn	Δ6,9, 12-18:3	0.29															
Linoleic	L	Δ9,12-C18:2	0.57	10.26	19.21	51.76	61.52	35.63	57.27	2.20	55.99	8.53	41.52	27.03	73.96	63.20	65.07	17.61
Oleic	O	Δ9-C18:1	1.00	41.36	57.67	19.18	22.94	43.50	18.15	10.73	27.41	73.85	40.91	61.65	15.15	22.21	19.36	67.83
Stearic	S	18:0	0.61	4.59	1.46	3.41	5.15	1.94	1.93	3.18	1.44	2.57	4.95	1.43	1.86	3.69	3.63	2.91
	C19:0	19:0	0.49				< 0.01									0.01	0.01	
	C20:2	Δ11, 14-20:2	0.36			0.05	0.03	0.04			0.02				0.04	0.04	0.05	
Gadoleic	G	Δ9-20:1	0.36	0.05	0.97	0.16	0.15	1.90	0.01	< 0.01	0.34	0.34	0.16	0.04	0.25	0.28	0.24	0.18
Arachidic	A	20:0	0.40	0.38	0.45	0.32	0.49	0.92	0.25	0.03	0.65	0.49	0.59	0.06	0.40	0.25	0.21	0.20
	C21:0	21:0	0.39					0.02				0.01				0.01	0.01	
Erucic	C22:1	Δ13-22:1	0.42					0.22							0.12			
Behenic	B	22:0	0.46	0.07	0.40	0.42	1.14	3.49	0.15		0.16	0.28	0.19		0.95	0.02	0.02	
	C23:0	23:0	0.40		0.02	0.08	0.06	0.03	0.01			0.04			0.05	< 0.01		
Nervonic	C24:1	Δ15-24:1	0.40		0.08										0.33			
	C25:0	25:0	0.39									0.01	< 0.01					
Cerotic	C26:0	26:0	0.39			0.01	0.01	0.38	0.01			0.02	0.01					

Continued

TABLE 5.1 Relative Concentrations [%] of Individual Fatty Acids in Analyzed Plant Oils Calculated From NARP-HPLC/APCI-MS of Triacylglycerols With Their Response Factors (RF)—continued

Fatty acid	Symbol	CN:DB	RF	Linseed	Poppy Seed	Walnut	Avocado Pear	Blackcurrant	Redcurrant	Borage	Cocoa Butter	Evening Primrose	Kukui Oil	Wheat Germ
Caproic	Co	6:0	134.76											
Caprylic	Cy	8:0	74.44											
Capric	C	10:0	17.62											
Lauric	La	12:0	6.04											
Myristic	M	14:0	2.77											
	C15:0	15:0	1.75	0.08					0.03					
Palmitoleic	Po	Δ9-16:1	1.33				6.52							
Palmitic	P	16:0	1.32	6.90	10.95	8.67	16.75	9.23	5.76	10.97	27.03	9.02	7.29	16.52
Margaroleic	Mo	Δ9-17:1	0.81		0.16	0.04	0.01							
Margaric	Ma	17:0	0.81	0.04	0.12	0.07	0.01	0.03			0.32	0.06	0.01	0.02
Stearidonic	St	Δ6,9,12, 15-18:4	0.23					3.54	5.32					
alpha-Linolenic	Ln	Δ9,12, 15-18:3	0.40	52.32	1.68	16.59	1.32	15.80	20.10				25.56	8.06
gamma-Linolenic	γLn	Δ6,9, 12-18:3	0.29					13.73	9.20	18.41		13.04		
Linoleic	L	Δ9,12-C18:2	0.57	15.89	66.05	52.69	13.48	37.86	36.34	35.42	1.89	67.49	39.36	54.85
Oleic	O	Δ9-C18:1	1.00	20.82	18.58	19.34	60.98	17.29	21.20	22.88	34.58	7.66	24.95	17.81
Stearic	S	18:0	0.61	3.65	2.10	2.19	0.46	1.52	1.86	4.18	34.51	1.71	2.66	0.76
	C19:0	19:0	0.49											
	C20:2	Δ11, 14-20:2	0.36		0.04	0.02		0.07	0.02			0.04		0.05
Gadoleic	G	Δ9-20:1	0.36	0.11	0.12	0.15	0.20	0.73	0.09	3.37		0.17	0.13	1.11
Arachidic	A	20:0	0.40	0.06	0.17	0.24	0.07	0.16	0.05	0.28	1.05	0.36	0.04	0.21
	C21:0	21:0	0.39									0.01		
Erucic	C22:1	Δ13-22:1	0.42							2.53		0.15		0.21
Behenic	B	22:0	0.46	0.09	0.01	< 0.01	0.06	0.03		0.35	0.45	0.16		0.12
	C23:0	23:0	0.40	0.01				< 0.01			0.01	0.02		
Nervonic	C24:1	Δ15-24:1	0.40							1.49		0.01		0.13
	C25:0	25:0	0.39	0.03	0.02		0.10	0.01		0.12	0.16	0.09		0.10
Cerotic	C26:0	26:0	0.39	< 0.01			0.02					< 0.01		0.01
							0.02					0.01		0.04

according to the ratio of fragment ions $[M+H\text{-}R_iCOOH]^+$, because the neutral loss of FA from the middle *sn*-2 position was less favored in comparison to *sn*-1 and *sn*-3 positions, and therefore it provided the fragment ion with lower relative abundance than statistically expected. In nature, regioisomers were present in mixtures, and relative abundances of fragment ions were composed from fragment ions of all isomers. Relative abundances of $[M+H-R_iCOOH]^+$ fragment ions also depended on the number of double bonds and carbon atoms; thus, the precise determination of the FA in the *sn*-2 position was achieved only by using regioisomeric standards, but not all of them were commercially available. Therefore, only predominant FAs in the *sn*-2 position were designated. In agreement with previously published data, the *sn*-2 position in plant oils was preferentially occupied by unsaturated FAs, mainly linoleic acid.

APCI-MS QUANTITATION OF TRIACYLGLYCEROLS

The previously developed HPLC/APCI-MS method of quantitative analysis of TGs using RFs (Holčapek *et al.*, 2005) was applied for the quantitation of TGs in natural samples. Briefly, the RFs of individual FAs (Table 5.1) were calculated from calibration parameters of 23 commercially available single-acid TG standards of $R_1R_1R_1$ type (i.e., single-acid saturated TGs from C7:0 to C22:0 and unsaturated single-acid TGs C16:1, C18:1, C18:2, C18:3, γC18:3, C20:1, and C22:1) using the ratios of individual TG calibration slopes to the calibration slope of triolein − for example, $RF(LLL) = a_{LLL}/a_{OOO}$. RFs of mixed-acid TGs were calculated as the arithmetic mean of RFs of FAs present in TGs; for example, RF(OLP) = (RF(OOO) + RF(LLL) + RF(PPP))/3. Single-acid TG standards from 9 identified trace FAs were not available, and therefore their RFs were approximated by the following way. RFs of saturated FAs − i.e., hexanoic (C6:0), tricosanoic (C23:0), tetracosanoic (C24:0), pentacosanoic (C25:0), and hexacosanoic (C26:0) acids − were calculated from the equation $y = 2959\ H\ \exp(-0.5134x) + 0.3824$ ($R^2 = 0.999$) corresponding to the dependence of RFs of 16 saturated TGs on their carbon atoms, where y was the RF and x was the number of carbon atoms. The RF of stearidonic acid (C18:4) was calculated from the equation corresponding to the dependence of RFs of unsaturated TGs with 18 carbon atoms and 1 (OOO), 2 (LLL), and 3 (LnLnLn) double bonds on the number of double bonds, i.e., $y = 1.615 \exp(-0.4904x)$ ($R^2 = 0.988$), where y was the RF and x was the number of double bonds. The RF values of monounsaturated palmitoleic (C16:1, RF = 1.33) and saturated palmitic (C16:0, 1.32) acids with the same number of carbon atoms, gadoleic (C20:1, 0.36) and arachidic (C20:0, 0.40) acids, or erucic(C22:1, 0.42) and behenic (C22:0, 0.46) acids, were very similar, therefore RFs of margaroleic (C17:1) and nervonic (C24:1) acids were considered the same as their saturated analogs, i.e. RF(C17:1) = F(C17:0) and RF(C24:1) = RF(C24:0). Similarly, the RF of diunsaturated C20:2 acid was considered the same as its monounsaturated analog, i.e. RF(C20:2) = RF(C20:1). TGs containing these FAs were present in analyzed samples only at trace levels, at a maximum of a few tenths of a percent, with the exception of 1.49% nervonic acid in borage oil (Table 5.1). In all cases, FAs with approximated RFs formed only one third of mixed-acid TGs.

TRIACYLGLYCEROL AND FATTY ACID COMPOSITION IN NUTS AND SEEDS

In total, 264 TG species were identified and quantified using HPLC/APCI-MS in 26 plant oils important in different branches of the nutrition and cosmetic industries. Identified TGs were composed of 28 FAs with 6−26 carbon atoms and 0−4 double bonds (Table 5.1). ECNs of all identified TGs ranged from 32 to 58. Only six TGs (PLP, OOO, OOP, POP, SOO and SOP) were detected in all samples. The number of TGs (Table 5.2) ranged from simple almond oil and cacao butter containing only 25 TGs to very complex blackcurrant oil with 77 TGs, redcurrant oil with 78 TGs, and borage oil with 88 TGs. Generally, about five to six TGs formed the main constituents in each sample at a relative concentration level > 5% (Table 5.2).