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Nuts and Nut Products in Human Health and Nutrition

Edited by Venketeshwer Rao, Leticia Rao, Md Ahiduzzaman and A. K. M. Aminul Islam

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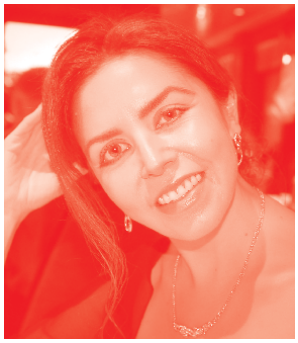

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Meet the editors

Dr. Rao, Professor Emeritus, Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, has established a major focus on diet and health. His research focuses on the role of oxidative stress and antioxidant phytochemicals in the causation and prevention of chronic diseases, with particular emphasis on the role of carotenoids and polyphenols. His research interests also include the role of prebiotics and probiotics in human health. He has more than 100 publications in scientific journals and several books and book chapters to his credit. He has a distinguished academic career spanning more than forty-five years. He is popularly sought by the international media to express his opinions about nutrition and health.

Dr. Leticia Rao is Professor Emeritus at the University of Toronto, former director of the Calcium Research Laboratory, Department of Medicine at the same university, and former staff scientist at St. Michael's Hospital, Toronto, Ontario. She has a BSc in Chemistry from the University of the Philippines, an MSc in Food Science from Oregon State University, and a Ph.D. in Biochemistry from the University of Toronto. Her expertise is in bone cell biology with a focus on preventing osteoporosis by studying bone cells in the laboratory and carrying out basic and clinical studies of drugs, nutritional supplements, and phytonutrients including carotenoids and polyphenols in postmenopausal women. Her research has been presented at national and international conferences and symposia and she has published extensively in peer-reviewed scientific journals. She co-authored The Bone-Building Solution and co-edited several books on phytochemicals and probiotics in human nutrition and health.

Professor Md. Ahiduzzaman, Ph.D., was born in 1972 in Bangladesh. He obtained a bachelor's degree in Agricultural Engineering from Bangladesh Agricultural University, Bangladesh, in 1993, an MSc in Energy Systems and Management from the University of Flensburg, Germany, in 2006, and a Ph.D. in Mechanical Engineering from Islamic University of Technology, Bangladesh, in 2011. He completed his postdoctoral studies at the University of Alberta, Canada. He was awarded a DAAD Scholarship (Germany) and NSERC Postdoctoral Fellowship (Canada). Dr. Ahiduzzaman has been researching and teaching in the field of agricultural engineering, focusing on processing of field crops, processing technology of fruits and vegetables, drying technology, renewable energy, and framework development for greenhouse gas mitigation policies in agriculture and other sectors. He has experience working in multi-organizational and interdisciplinary teams, evaluating research, and developing projects in the agricultural engineering sector.

Prof. Dr. A. K. M. Aminul Islam received a Ph.D. in Chemical and Process Engineering from Universiti Kebangsaan Malaysia. He is currently Director (Research) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh. Dr. Islam is the author of 120 journal articles, 20 book chapters, and 4 books. He is an editorial board member and referee for several national and international journals. He is General Secretary of the Plant Breeding and Genetics Society of Bangladesh, Seminar & Research Secretary of JICA Alumni Association of Bangladesh, and a member of several professional societies. Dr. Islam developed nineteen varieties of different crops cultivated in Bangladesh. He supervised fifty MS and three Ph.D. students. His research focuses on the development of hybrid vegetables, hybrid *Brassica napus* using a Cytoplasmic Male Sterility system, stress-tolerant rice varieties, and renewable energy research with *Jatropha curcas*. He received the BSMRAU Integrity Award for 2020–2021.

Contents

Preface

Nuts, also referred to as "tree nuts," and peanuts are associated with good health and have always been an important part of the human diet. There has been renewed interest in understanding the reasons why nuts and peanuts, when consumed as part of a healthy diet, are beneficial in the prevention of several chronic diseases such as cancer, cardiovascular diseases, diabetes mellitus, obesity, and high blood pressure. Over the past few decades, extensive research has been undertaken to study the nutritional and phytochemical composition of nuts, their mechanism of action, and the need for dietary guidelines for the consumption of nuts. At the same time, research is also being directed at the issues of fungal contamination of nuts and the associated toxicity and risk to human health. In recognition of the important role that nuts and nut products play in human health and nutrition, several original research and review articles have now been published. This book, *Nuts and Nut Products in Human Health and Nutrition*, contains chapters that address some important aspects of consuming nuts as part of a healthy diet. It is organized into four sections. Chapter 1 in Section 1, by AV Rao and LG Rao, is an introduction to the important role that nuts and nut products play in human health, their composition, including health-friendly lipids and beneficial phytochemicals, and their micronutrient composition. It points out the nature of basic, clinical, and epidemiological research that still needs to be undertaken in the future for a better understanding of the role of nuts, including peanuts, and formulation of dietary guidelines based on such research outcomes. Section 2, "Nutrient and Phytonutrient Composition of Nuts", includes two chapters: Chapter 2, "Nut Phytonutrients for Healthy Gut: Probiotic Potential," by Jinu Medhi and Mohan Chandra Kalita, and Chapter 3, "Nuts as Dietary Source of Fatty Acids and Micro Nutrients in Human Health" by Chiranjiv Pradhan, Nikhila Peter and Namitha Dileep. Together, these two chapters address the reasons why nuts and peanuts are health-friendly components of a good diet. Section 3, "Fungal Contamination of Nuts," contains Chapter 4 "Fungal Contaminants and Mycotoxins in Nuts" by Giulia Mirabile, Patrizia Bella, Antonio Vella, Vincenzo Ferrantelli and Livio Torta, and Chapter 5 "Nutrient Composition and Aflatoxin Contamination of African Sourced Peanuts and Cashew Nuts: Its Implications on Health" by Modupeade C. Adetunji, Stephen A. Akinola, Nancy Nleya and Mwanza Mulunda. Fungal contamination and production of toxic byproducts are important since they can mitigate the beneficial role that nuts play in human health. These two chapters balance out the pros and cons that need to be considered with respect to nuts. Finally, Section 4, "Genetic Improvements Towards Human Health," includes Chapter 6, "Genetic Potential and Possible Improvement of Sesamum indicum L." by Muthulakshmi Chellamuthu, Selvi Subramanian and Manonmani Swaminathan, which looks at the genetic potential for possible improvement of nuts. This chapter uses sesame seeds, though not nuts, to highlight the need for genetic research that needs to be done to improve nutritional quality and the health beneficial properties of nuts.

Overall, this book provides important information, authored by international authors, to health professionals, researchers, and other scientists that will be very useful in understanding the mechanisms by which nuts provide health benefits, the concern of fungal infestation of nuts with the resultant production of toxic

metabolites and how to prevent such undesirable effects, and formulation of dietary guidelines for the consumption of nuts and nut products. It also provides a guide to future research directions in the important area of human health.

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Section 1

Introduction

Chapter 1

Introductory Chapter: The Role of Nuts and Nut Products in Human Health and Disease

A. Venketeshwer Rao and Leticia Rao

1. Introduction

Consumption of nuts dates back to the prehistoric times. Nuts were an important part of the diet of early humans. Since then, humans have shown continued interest in the consumption of nuts and more recently in nut products as well, both for their taste and for their nutritional quality and health benefits. Mediterranean diets that are associated with lower incidence of several chronic diseases in particular, contain significant amounts of nuts [1–3]. Several epidemiological and human intervention studies since then have provided evidence for the association between the consumption of nuts and reduced risk in the incidence of several chronic diseases such as cancer, cardiovascular diseases, diabetes mellitus, obesity and high blood pressure just to name a few [4–6]. These observations encouraged scientific search to investigate the reasons as to why nuts are considered to promote health. This introductory chapter will look into the diversity of nuts that are consumed by humans and their nutritional and phytochemical composition. It will also discuss briefly the scientific evidence supporting the health beneficial properties of nuts. Chapters that follow this introductory one are authored by internationally known researchers and will cover various aspects of tree nuts and peanuts.

Common use of the term nuts is somewhat confusing. Nuts are in reality fruits. A common definition is that 'they are single-seeded fruits that have high oil content'. Wikipedia defines a nut 'as a fruit composed of an inedible hard shell and a seed which is generally edible'. However, in its general usage a wide variety of dried seeds are referred to as tree nuts. In a botanical context, nuts are strictly a particular kind of dry fruit that has a single seed, a hard shell, and a protective husk [2]. In the context of this chapter, a more general term 'Tree nuts and peanuts' will be used, although peanuts are strictly not nuts but are legumes.

In general nuts have a similar macronutrient content with an average energy content of 2,900 kJ. However, their micronutrient content including vitamins and mineral may differ to some extent.

Table 1 provides a list of tree nuts and peanuts that are commonly consumed by humans and their macronutrient nutrient content.

Fatty acid composition of the commonly consumed nuts is shown in **Table 2**.

As shown in **Tables 1** and **2**, nuts in general are high fat, energy dense and fiber rich food products. Their fats are composed mainly of monounsaturated and polyunsaturated fatty acids with very low saturated fats and no cholesterol. They are also good sources of linoleic and alpha linoleic fatty acids [7, 8]. In addition to the heart and health friendly lipid profiles of nuts, they are also good sources of plant sterols and other beneficial phytochemicals with significant antioxidant properties [9]. They are rich in vitamins such as pyridoxine and folate. With respect to minerals, they are

Nuts	Energy (kJ)	Fat (g)	Protein (g)	Fiber (g)
Almonds	2418	50.6	21.3	8.8
Brazil nuts (dried)	2743	66.4	14.3	8.5
Cashews	2314	46.4	18.2	5.9
Hazelnuts	2629	60.8	15.0	10.4
Macadamia nuts	3004	75.8	7.9	6.0
Peanuts	2220	49.2	25.8	8.5
Pecans	2889	72.0	9.2	8.4
Pine nuts (dried)	2816	68.4	13.7	3.7
Pistachios	2332	44.4	20.6	9.0
Walnuts	2738	65.2	15.2	6.4

Source: US Department of Agriculture Nutrient Data base at: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl [7].
Ros E. 2010.Health Benefits of Nut Consumption. Nutrients: 2, 652–682 [8].

Table 1.
Macronutrient composition of some common nuts and peanuts (per 100 grams).

Nuts	Short chain fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids	Linoleic acid	Alpha linoleic acid	Plant sterols
Almonds	3.9	32.2	12.2	12.2	0.00	120
Brazil nuts (dried)	15.1	24.5	20.6	20.5	0.05	—
Cashews	9.2	27.3	7.8	7.7	0.15	158
Hazelnuts	4.5	45.7	7.9	7.8	0.09	96
Macadamia nuts	12.1	58.9	1.5	1.3	0.21	116
Peanuts	6.8	24.4	15.6	15.6	0.00	220
Pecans	6.2	40.8	21.6	20.6	1.00	102
Pine nuts (dried)	4.9	18.8	34.1	33.2	0.16	141
Pistachios	5.4	23.3	13.5	13.2	0.25	214
Walnuts	6.1	8.9	47.2	38.1	9.08	72

Source: US Department of Agriculture Nutrient Data base at: http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl [7].
Ros E. 2010.Health Benefits of Nut Consumption. Nutrients: 2, 652–682 [8].

Table 2.
Fatty acid and plant sterol content of common nuts and peanuts (per 100 grams).

significant dietary sources of calcium, iron, phosphorus, zinc, copper and selenium. In addition to the lipids, nuts are also a good source of proteins and essential amino acids such as arginine that promote good health [3]. Overall, when consumed on a regular basis they provide significant amount to the daily requirements of nutrients essential to lower the risk of chronic diseases and maintain good health.

In view of the potential positive nutritional characteristics of nuts, they have been studied extensively over the years for their role in preventing several human chronic diseases and maintaining good health [10]. **Table 3** shows some of the

Atherosclerosis and other endothelial disorders
Brian health – Alzheimer
Coronary arterial diseases (CAD)
Diverticulitis
Hypertension
Inflammation
Kidney disorders – Gallstone related diseases
Type 2 diabetes and other glycemic related disorders

Table 3.
Examples of health benefits of consuming nuts as part of a healthy diet and life style.

health benefits, based on research conducted over the years, of consuming nuts as part of a healthy diet and a life style. It should be pointed out that the health benefits shown in the table are only a few examples. However, the research to evaluate the health benefits of consuming nuts continues and new information as to the prevention of diseases, their mechanism of action and recommendation are published regularly.

It should be pointed out that there are a few concerns of consuming nuts and nut products such as bodyweight, allergic reactions for some individuals and the possibility of adverse effects associated with fungal contamination of nuts, have to be kept in mind.

2. Conclusion

In conclusion it should be pointed out that consuming nuts including peanuts can play a significant role in the prevention and treatment of human diseases and benefiting human health. Following chapters, authored by well-known international researchers will contribute significantly to our understanding of the role of nuts and nut products in human health.

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Section 2

Nutrient and Phytonutrient Composition of Nuts

Chapter 2

Nut Phytonutrients for Healthy Gut: Prebiotic Potential

Jinu Medhi and Mohan Chandra Kalita

Abstract

Nuts are a combination of prebiotic fiber and phytonutrients and have antioxidant, anti-inflammatory effects. According to 2005 "My Pyramid" it has been grouped with the meat and bean group. Bioactive compounds of nuts such as resveratrol, phytosterols, phenolic acids, flavonoids, and carotenoids display synergistic effects on preventing many age related pathologies. Resveratrol has been reported to extend the lifespan in model organisms such as yeast, *Drosophila* and mouse. Reports propose nuts as the best substitute for red meat to reduce mortality risk. Macadamia nuts with a rich source of monounsaturated fats (oleic and palmitoleic acids) imparts cholesterol lowering effects thereby preventing coronary artery disease. Anacardic acid, a phenolic lipid found in cashew nut shells, is specifically enriched in metastatic melanoma patients in response to immunotherapy. The non-bio-accessible materials of nuts serve as a substrate for human gut microbiota. Regular Walnut enriched diet improves lipid content and enhances probiotic and butyrate producing bacteria composition in healthy individuals. This also reduces cardiovascular risk factors by promoting beneficial bacteria. Gut microbiota diversity studies report an enrichment with genera capable of producing short chain fatty acids (SCFA) following consumption of nuts. The prebiotic effect of nuts can be partly from refining butyrate producing bacteria composition. Hence an optimized diet rich with nuts can be an intervention for promoting a healthy microbiota population and thereby improving overall physiology.

Keywords: lifespan, yeast, Macademia nut, immunotherapy, SCFA, prebiotic, microbiota

1. Introduction

The human gut microbiome consists of many microorganisms constituting bacteria corresponding to different species involving the collective genome consisting 100 times the genes present in the human genomes [1]. The existing microbial communities contribute towards the host health through different functions involving the probiotic properties along with the synthesis of the vitamins and important amino acids. Various changes have been observed in the constituents of the gut microbiota among all healthy individuals which has evolved correlation between the health, disease and diversity of the human gut microbiome. Also, the gut microbiota has been linked with pathogenesis involving intestinal and extra-intestinal disorders [2]. Hence, diet has been considered as the major determinants for the microbial composition in the gut that further influences the diversity, distribution and more of microbial populations from the early life stages [3]. The diet variations

Sl No.	Aim	Mediation	Study type	Study design	Findings	Reference
1.	Investigation of prebiotic effects of almonds using mixed fecal bacterial culture	Fine and defatted almonds	*In vitro*	In vitro gastric and duodenal digestion of the samples of almond has been used in the form of substrates for colonic model for evaluation of population, composition and metabolic activity of gut bacteria	For whole almond: *Bifidobacteri, Eubacterium rectale* and butyrate production increases	Mandalari et al. [17]
2.	Evaluation of the components of chestnut in the form of probiotic carriers by examining the effect on the viability of selected lactic acid bacteria (LAB)	Extracts of chestnut and its fiber	*In vitro*	Simulated gastric (with pepsin) and bile (with pancreatin) juices have been prepared for adding into the cultured LAB cells with chestnut fibers and extracts	*Lactobacillus paracasei GG*, *Lactobacillus rhamnosus* and *Lactobacillus casei* increases. *Streptococcus macedonicus* and *Streptococcus thermophilus* decreases.	Blaiotta et al. [20]
3.	Comparing the fermentation properties of raw and roasted almonds	Raw and roasted almonds	*In vitro*	Hydrolyzed raw and roasted almonds under simulated gastric and duodenal digestion have been added into cultured Lactobacillus acidophilus, Bifidobacterium breve, and *Escherichia coli* and incubation at 37°C for 48 h in anaerobic condition is done	*Lactobacillus acidophilus* and *Bifidobacterium breve* increases. *Escherichia coli* decreases.	Liu et al. [16]
4.	Analyzing prebiotic effects of raw and roasted almonds on fecal and caecal bacteria	Raw and roasted almonds	Animal model	Male specific-pathogen-free (SPF) Wistar rats of 10 weeks older are divided into three groups as per the feeding regime of raw and roasted almonds	*Lactobacillus spp.* and *Bifidobacterium ssp.* increases. *Enterococcus spp.* and *Escherichia coli* decreases.	Liu et al. [16]
5.	Investigating the walnuts modulation to be effective for gut microbiome and promoting health benefits	Walnuts	Animal model	Male Fischer rats divide into two groups as control diets and walnut diet. Fecal samples collected descending colon at the time of sacrifice	*Lactobacillus*, *Ruminococcaceae* and *Roseburia* increases. *Bacteroides*, *Anaerotruncus* and *Alphaproteobacteria* decreases.	Byerley et al. [26]

Sl No.	Aim	Mediation	Study type	Study design	Findings	Reference
6.	Evaluating the effects of bacterial or fungal microbiota composition while consumption of nuts	Almond and Pistachio	Randomized, controlled, crossover trial	Healthy adults with 18 days feeding time interval being separated by a washout period of 2 weeks. Low-fiber American diet has been provided during these three treatment periods.	*Lactobacillus* and *Bifidobacteria* is maintained	Ukhanova et al. [14]
7.	Investigating the prebiotic effects of almond and almond skin intake in healthy humans	Roasted almond and almond skin	Randomized, controlled trial	Healthy adult volunteers consuming almonds and almonds skin, the diet being provided by the school canteens	*Lactobacillus spp.* and *Bifidobacterium spp.* increases. *Clostridium perfringens* decreases. *Escherichia coli* is maintained.	Liu et al. [18]
8.	Assessing the interrelationship of almond consumption and processing on the gastrointestinal microbiota(bacterial genera)	Whole almonds, whole, roasted almonds, roasted, chopped almonds and almond butter	Randomized, controlled, crossover trial	Healthy adults with controlled feeding	*Lachnospira*, *Roseburia*, *Oscillospira*, and *Dialister* increases.	Holscher et al. [33]
9.	Investigating the effect of walnut intake on the gut microbiome composition and microbial diversity	Walnut	Randomized, controlled, crossover trial	Healthy nonsmoking men and women of age above 50 years having different diet phases of walnut-enriched diet and nut-free control diet	Butyrate-producing bacteria, *Ruminococcaceae* and *Bifidobacteria* increase. *Clostridium spp.* decreases.	Bamberger et al. [34, 35]

Table 1.
Prebiotic prospects of consuming nuts phytonutrients for maintaining a healthy gut.

also evolve 57% of the total structural variations in the gut microbiota [4]. The acute alteration in the diet has shown variations in the microbial composition. All these variations contribute towards the modification of the gut microbiota for longer durability of health benefits.

Nut consumption has been found to be effective on the metabolic risk factors [5–7]. Nuts are a combination of prebiotic fiber and phytonutrients and have antioxidant, anti-inflammatory effects. Nuts also consist of high content of monosaturated fatty acids, mainly in the hazelnuts along with consistency of lipophilic compounds such as tocopherols (almonds and hazelnuts) and high amount of phytosterols and carotenoids (pistachio) [8–10]. Phenolic compounds have been considered to be found in abundance in the form of phytochemicals in the nuts, mainly involving flavonoids and tannins that are basically found in walnuts and pecans. These diversity of compounds constituting antioxidant and anti-inflammatory effects have been proved to be beneficial concerning the health benefits. It further shows effects on the remodeling of the gut microbiota [11, 12]. The fibers along with the contents of the polyphenols plays an important role in mediating the profile of the gut microbiota resulting into the mechanism of health benefits such as generation of anti-inflammatory effects, maintaining the intestinal mechanisms and enteric barrier integrity. Hence, it has been revealed that the consumption of nut phytonutrients has been assisting the gut microbiome for the management of the inflammatory diseases (**Table 1**) [13, 14].

The potential prebiotic properties of the nut phytonutrients have been considered important for analyzing the mechanism of healthy gut. The nut phytonutrients have been effective in maintaining the health by activating the mechanisms in the gut due to the consistency of high fiber levels, antioxidants and anti-inflammatory properties. All the nut phytonutrients involving macadamias also contain fibers. These phytonutrients nuts are capable of feeding the gut bacteria as these nuts consist of prebiotic that are mainly involved in feeding the probiotic bacteria dwelling in the gut [15–17]. One of the studies has revealed that 56 g of almonds and 10 g of almond skins in a single day for continuity of six weeks have been shown to increase the growth of the important bacterial strains in the gut [18]. The similar results have been seen while using the pistachios [19]. The nut phytonutrients may also protect the proteobacteria present in the gut. Another major study in Food Microbiology has revealed that the extracts of the chestnuts and its flour has helped the different strains of lactobacilli bacteria in survival of the acids and bile present in the stomach [20]. Hence, this effectively proves that these nuts actively show mechanism in the large intestine and results in maintenance of a healthy gut. Also, the healthy bacteria present in our gut feed on the fiber of the nut phytonutrients resulting in the fermentation into the product of short-chain fatty acids. Hence, weight management is effectively maintained by these compounds.

The prebiotic potential has been referred to as the growth of the selective microbial species found in the gut microbiota that provides benefits to the health in any individual with the mechanism of selective stimulation [21]. Limited research has been conducted on the prebiotic effects of nut phytonutrients and its impact on the gut microflora. But most of the studies have shown positive impact of nut phytonutrients for maintaining a healthy gut considering the prebiotic assistance.

2. Effects of nut phytonutrients on human gut microbiota

Natural fibers and phytochemicals are present in various nuts, and these components reach the proximal colon, providing substrates for the healthy maintenance of diverse microbiota. Nuts are food components rich in prebiotic fiber and

polyphenols, and have proven benefits on human gut health and gut microbiota [22]. Specific nuts are rich in fiber and other phytonutrients, however, the effect of the increased consumption of nuts on human gut microbiota is yet to be investigated [23]. There has been a history of epidemiological studies and clinical trials, suggesting the metabolic and gut health benefits of nut consumption. In comparison to other nuts, pistachios have proven to have a balanced nutrition profile, with lower levels of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). Pistachios have higher levels of protein, soluble and insoluble fiber, potassium, vitamin K, phytosterols, xanthophyll carotenoids, γ-tocopherol, along with high antioxidant potential [24].

Nuts also play a role in reducing postprandial glucose fluctuations. Along with maintaining the gut health, nuts also help in potentially improving glucose homeostasis in patients suffering from gestational diabetes mellitus. GLP-1 and GIP are gut incretin hormones that possess strong glucose-dependent insulin regulatory properties and are released to lower blood sugar levels after having a meal. These hormones augment the glucose-dependent insulin secretion which plays a crucial role in controlling postprandial glucose excursions [24].

According to the reports by Emily et al. [22], there is increasing evidence showing the association of gut microbiota with different aspects of human health. Eight weeks of walnut consumption, approximately 43 g/day, has a significant improvement on lipid levels, and regular walnut consumption is linked with better gut health [25]. In a randomized, controlled, prospective, cross-over study by Charlotte et al. [25], 194 healthy individuals with 134 females aged 63 ± 7 years, and with BMI 25.1 ± 4.0 kg/m^2 were included to evaluate the gut microbiome. The results showed a significant decrease in *Clostridium sp.* cluster XIVa species with walnut consumption, proving the beneficial effect of nuts on the human gut. As reported in a study by Maria et al. [23], the effect of pistachio consumption on gut microbiota composition was much higher than that of almond consumption. The results also showed an increase in the number of potentially beneficial butyrate-producing bacteria. Nuts have proven efficacy to increase the good bacteria *Clostridium*, *Roseburia*, *Lachnospira* and *Dialister*, paving a way to yield a modulatory effect on the human gut [22]. Walnut consumption has evidently shown the enhancement in the probiotic- and butyric acid-producing species in healthy individuals [25]. *Lactobacillus, Ruminococcaceae,* and *Roseburia,* the probiotic-type bacteria significantly increased with walnut consumption, whereas Bacteroides, *Anaerotruncus*, and *Alphaproteobacteria* significantly decreased. **Figure 1** showing a schematic diagram showing prebiotic effects on host gastro-intestinal (GI) tract. Regular walnut consumption brought a drastic change in the gut microbiota, thereby suggesting a new mechanism that will further prove the beneficial health effects of walnut consumption [26].

2.1 Potential health benefits of nuts

Since the ancient times, nuts and dried fruits have been an important part of the human diet. Nuts are nutrient-rich foods and consist of excellent health-promoting and beneficial bioactive compounds [27]. According to a study by Rune et al. [28], nuts contain dietary antioxidants which are said to have a protective effect in chronic degenerative disease. Nuts possess antioxidants that reduce the oxidative stress which is common in chronic degenerative disease. Considering all the tree nuts, the highest amounts of antioxidants is present in walnuts, chestnuts, and pecans. Walnuts have a walnut pellicle, that contains more than 20 mmol antioxidants per 100 g, whereas peanuts contribute a significant number of antioxidants to the daily dietary intake. Nuts have shown strong and consistent reductions in

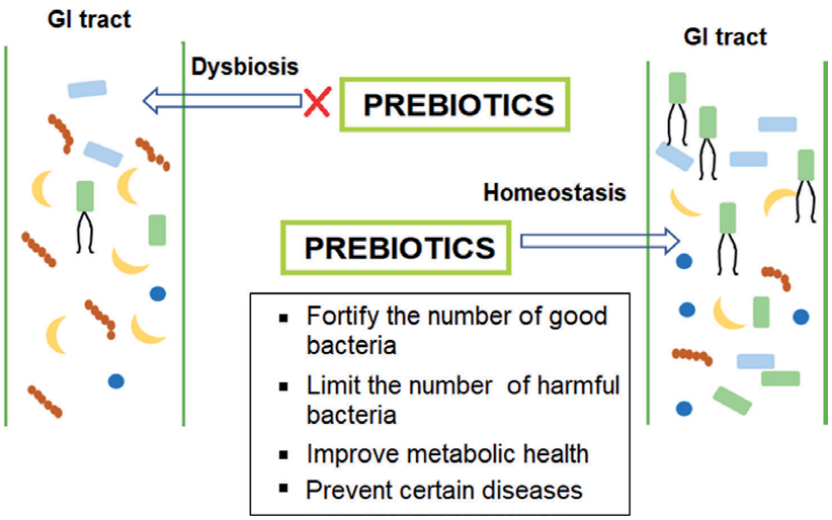

Figure 1.
Schematic diagram showing prebiotic effects on host gastro-intestinal (GI) tract.

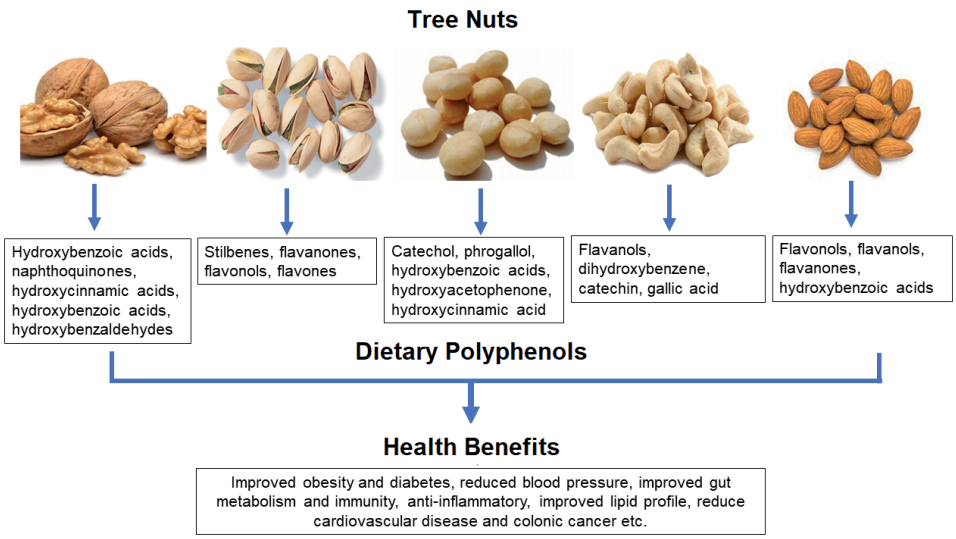

Figure 2.
The dietary polyphenols of different tree nuts and their health benefits.

the number of cardiovascular and coronary heart diseases death attributes. Nuts have played a major role in regulating the lipid and cholesterol level and maintain a healthy heart [28]. According to the German Nutrition Society, daily consumption of 25 g nuts is recommended as nuts are a rich source of nutrients for the healthy functioning of the heart and other organs. Nuts are a good source of all the important nutrients such as monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), dietary fiber, vitamins, antioxidants, and minerals. They have positive health effects even when consumed on a regular basis. Evidence states that regular nut consumption seems to have no negative impact on the body, heart, weight, healthy or obese patients. Nuts have shown proven benefits in the prevention of metabolic disorders, hyperlipidemia, atherosclerotic diseases, hyperglycemia, heart ailments, myocardial and coronary artery disease. Moreover, daily nut

consumption has shown to reduce inflammatory processes in the body and increase the antioxidant capacity of the body [29]. The dietary polyphenols of different tree nuts and their health benefits are shown in **Figure 2**.

To obtain the full benefit of nutrients, bioactive compounds, and antioxidants present in nuts, frequent consumption of the same is highly recommended. Nuts are very flavourful, so consuming them on a daily basis will only give benefits. The risk of cardiometabolic and other non-communicable diseases can be modulated by the synergistical contribution of nuts and dried fruits that contain tremendous amounts of macronutrients, micronutrients, bioactive compounds, other health-promoting nutrients and flavor. The beneficial effects of nut consumption on various health outcomes have been reported by experimental research, prospective studies, and human clinical trials [27]. The dietary polyphenols of tree nut for health benefits.

3. *In-vitro* studies of prebiotic effects of nuts

The Dietary Guidelines for Americans has revealed that the intake of nut phytonutrients has given positive outcomes with effective health benefits. The health benefits of intaking these nuts have been found due to the presence of fatty acid, vegetable proteins, phenolics and vitamin phytosterols. Nut the most important attribute of the nut phytonutrients have been found due to the high content of dietary fiber and polymerized polyphenols that contribute towards the prebiotic properties. These components are further metabolized by the gut microbiota in the presence of the bioactive molecules that have been helpful in benefiting the health of the individuals. The *in vitro* studies by Blaiotta et al. [20] and Mandalari et al. [17] have shown the prebiotic effects of chestnuts and almonds. Liu et al. [18] and Ukhanova et al. [19] have also shown similar prebiotic effects concerning the various interventions of human clinical trials. The studies have proved that the butyrate production has been observed in both the groups revealing the effects of almonds and pistachios on the fecal bacterial and fungal microbiota. Also, the conclusion has been derived that neither almond nor pistachio intake have been efficient in increasing the *Lactobacillus* or *Bifidobacterium* strains. But the study by Liu et al. [18] has been capable of showing efficient increase of *Bifidobacterium* spp. and *Lactobacillus* spp. Among the almond and its skin along with the little variations in the population of *Escherichia coli* and *Clostridium perfringens*. Also, some bacterial enzymes have shown significant variations such as β-galactosidase activity has been observed to increase and fecal β-glucuronidase, nitroreductase and azoreductase activities have been found to show reduced effect.

The nut phytonutrients have been found to be rich in complex polyphenols, tannins to be the major one and other dietary fibers that have shown prebiotic effects in the gut of the individuals. The studies have shown that the dietary polyphenols have been found to be partially absorbed in the small intestine for carrying out an effective digestion process. The complex polyphenols have been seen to be unabsorbed in the gut and later they get bioactivated in the colon by the microbiota. Hence, these microbiota metabolites derived from the complex polyphenols are smaller molecules which are easily absorbed through the colon barrier [30]. Ellagitannins (hydrolysable tannins) and proanthocyanins (condensed tannins) have been known to be the major constituents as phenolic compounds of the nut phytonutrients [31, 32]. These have been mainly found in the blood of the individual that shows potential prebiotic effects on the gut while maintaining the human metabolism and health.

The prebiotic effects have been observed in the gut mechanism due to the presence of the prebiotic compounds in the nuts that stimulate the growth of non-pathogenic gut bacterial species along with the inhibition of growth of the pathogenic ones. The in vitro studies have revealed that the whole and defatted almonds, raw and roasted almonds and fiber and extracts of chestnut have shown prebiotic effects on the human gut. One of the recent studies have revealed that the almonds processing has been affecting the composition of the gastrointestinal microbiota when the treatment of intake of 42 g per day of chopped almond or almond butter for 21 days is carried on that results in enhancing the beneficial bacterial genera [33]. Similar results have been seen in a study that involves the daily intake of walnuts of 43 g for 56 days, resulting in affecting the gut microbiome by enhancement of the probiotic butyric acid-producing bacteria in healthy individuals [34, 35].

4. Bioactive compounds and nutritional composition of nuts

Nut phytonutrients have been found to have a high energy density and high nutrient content along with the healthy profiles. Almond, cashew, pistachio, baru almond, and peanuts have been found to have lowest amounts while Brazil nut, hazelnut, pecan, and walnut have been found to have highest lipid concentrations. The major components of the fatty acids of the almond, cashew, pecan, edible seed and hazelnuts consist of monounsaturated fat acids (MUFA). The Brazil nut, pistachio and walnut consist of polyunsaturated fat acids (PUFA). The Brazil nut and cashew consist of considerable content of saturated fatty acids (SFA). Also, almond, hazelnut, and pecan have been observed to have the highest MUFA:SFA ratios. Hence consumption of these phytonutritional nuts provides health benefits of lower risk for cardiometabolic disorders, dyslipidemia, obesity, and insulin resistance [36].

The lipophilic compounds have been also found in the nut phytonutrients involving higher concentration of tocopherols in almond, hazelnut, baru almond and peanuts. It consists of nutritional attributes of vitamin E efficiency, antioxidative, anti-inflammatory, and antiobesity properties [37]. Phytosterols components have also been found in the nuts, mainly in pistachio. These components help in inhibiting the intestinal absorption of cholesterol and reduce the risk of hyperlipidemia [38, 39]. Carotenoids are another lipophilic compound that have been found in lower amounts in nuts. Lutein is another bioactive compound which shows antioxidant activity [40].

Phenolic compounds are majorly found in the nut phytonutrients. The polyphenolic compound resveratrol has been shown to extend lifespan in different organisms. Wang et al. (2013) investigated the effect of resveratrol on lifespan on both gender and dietary nutrient composition in *Drosophila melanogaster*. The lifespan extension by resveratrol was found to be associated with downregulation of genes in aging-related pathways, including antioxidant peroxiredoxins, insulin-like peptides involved in insulin-like signaling and several downstream genes in Jun-kinase signaling involved in oxidative stress response. Pecan, pistachio, walnut, and baru almond have been found to have the highest values among the nuts. Some of the oilseeds have been propounded to have high concentrations of flavonoids and tannin (pecan), flavonoids (walnut), and tannins (baru almond). Many other nuts have been found to have more concentration of tannins in them. Flavonoids and tannins show reduced pro-oxidant and proinflammatory conditions and hence decrease the risk of obesity and inflammatory diseases.

5. Conclusion

Nuts are an important part of our diet. The enriching bioactive compounds of nuts have profound influence on human health. An optimum intake of these nut phytonutrients have prebiotic effect on our health. Different studies reveals the promoting effect of these nutrients on healthy gut microbiota population. These nut based phytonutrients acting as prebiotic to fortify the host probiotic bacteria and also limit the pathogenic bacteria maintaining a homeostasis condition in the host. With the advancing studies of prebiotics and probiotics on model organisms including *Drosophila*, mice new avenues are open to explore a beneficial diet plan with nut based prebiotics. In this new era of personalized medicine these prebiotic and probiotic supplements can provide a therapeutic target for different pathological condition. This will provide a basic understanding of the trilogue of diet, host and gut microbiota interactions.

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Chapter 3

Nuts as Dietary Source of Fatty Acids and Micro Nutrients in Human Health

Chiranjiv Pradhan, Nikhila Peter and Namitha Dileep

Abstract

In recent times, the Mediterranean diet plans are very popular because it has a lot of advantage in protecting from chronic health problems. Nuts are the integral part of the Mediterranean diet and advised to be incorporated in diet for health benefits. Both tree nuts and pea nut are good source of unsaturated fatty acids, soluble and insoluble fibers, good quantity of vitamins, minerals and phytochemicals with recognized benefits to human health. Due to life style disorders many chronic diseases are increasing in human beings. There are many epidemiological studies and research conducted on the relationship between consumption of nuts and chronic disease risks. This book chapter elaborately discusses about the nutritional composition of the nuts and their effect on cardiovascular disease, obesity, diabetes and cancer.

Keywords: nuts, chemical composition, cardiovascular disease, obesity, diabetes, cancer

1. Introduction

Nuts are excellent source of nutrients and bio active compounds. It is consumed by majority of the populations of the world in some form or other depending upon the availability in the geographical area. Nuts can be divided in to two types such as tree nuts and legumes. The important tree nuts are viz., almonds, walnuts, pistachios, cashew nuts, Brazil nuts, hazelnuts, macadamia, and pine nut. Peanut is a legume commonly consumed as roasted or as pea nut butter. Pea nut is more economical than other nuts and popularly called as 'poor man's almond'. These nuts due to their unique nutritional properties are very much appreciated in vegan diets, gluten free diets and paleo diets [1]. Nut consumption and associated health benefits gained much interest in recent times and even considered as alternative to milk and meat [2, 3]. Nuts are the part of traditional Mediterranean diet and now the nutrition research community advocates its consumption in a regular basis [4].

There is a gradual increase in non-communicable diseases (NCDs) such as cardiovascular, obesity, diabetes and cancer in the population. The major reason for the NCDs are basically due to life style disorders, poor feeding habit, work related stress, and less physical activity. Cardiovascular diseases (CVDs) are a leading cause of mortality globally and in 2010 around 13 million people died due to heart related diseases [5]. Obesity constitutes a worldwide epidemic and is the major cause of type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease,

non-alcoholic fatty liver disease, reproductive dysfunction, respiratory abnormalities and psychiatric conditions [6]. According to the International Diabetes Federation (IDF) data there are 382 million diabetic people worldwide in 2013, and by 2035 the number will grow to 592 million. Diabetes brings huge economic burden to the individuals, family as well as the health care system of a country. Similarly, as per WHO reports the global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018. These diseases are the serious concerns in the society for future developments and progress.

Nut consumption has proven role in reducing cardiovascular diseases [7], obesity [8], hypertension [9], diabetes mellitus [10], and cancer [11]. Dietary nuts reduces the mediators of chronic diseases such as oxidative stress, inflammation, visceral adiposity, hyperglycemia, insulin resistance, endothelial dysfunction, and metabolic syndrome with the unique bioactive compounds what they possess [12].

This chapter will review the nutritional composition of tree nuts and pea nut. The consumption of nut and health aspects related to cardiovascular disease, obesity, diabetes and cancer are also the major highlights of this chapter.

2. Nutrient composition of nuts

Nuts are good source of nutrients such as fats with good quantity of mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA), soluble and insoluble fibers, vitamins E and K, folate, thiamine and minerals such as magnesium, copper, potassium, and selenium [13]. The phytochemicals such as phenolic compounds, zanthophyll carotenoids, alkaloids, and phytosterols compounds, with recognized benefits to human health are also the major constituents of nuts.

Nuts are very good source of fats after vegetable oil seeds and are calorie-dense (∼500–700 kcal/100 g edible portion). The lipid quantity in the nuts varies in a range of 40 to 75% (**Table 1**). Typically, walnut, macadamia, pine nut, Brazil nut and pecans contain higher lipid (∼70%) as compared to cashew, almond, pistachio and hazelnut which contains lipid between 45 and 62%. The lipid content of all the nuts varies according to the agro-climatic condition, maturity of kernels and varieties. Barreca et al. [14] observed lipid content 42.4–56% in 19 varieties of almond grown in different agro-climatic conditions. Similarly, Venkatachalam et al. [15] indicated a range in the lipid content 67–78.1% for 27 pecan cultivars grown in different regions of the United States. The availability of phytosterols and sphingolipids are also common in tree nuts. The sterol content in tree nuts ranges from 0.16 (pine nut) to 0.28 g/100 g (pecans) of the oil content. In the phospholipid fraction, all most all the tree nuts contain more amount of phosphotidylcholine in a range of 0.37 g/100 g oil (pine nut) to 0.78 g/100 g oil (Brazil nut) followed by phosphotididylserine in range of 0.32 g/100 g oil (almond and Brazil nut) to 0.59 g/100 g oil (pistachio) and phosphatidylinositol in a range of 0.08 g/100 g oil (hazel nut) to 0.31 g/100 g/100 g oil (walnut).

All most all the edible nuts contain good amount mono unsaturated fatty acids (MUFA) followed by poly unsaturated fatty acids (PUFAs) (**Table 2**). The MUFA content of nuts are basically in the form of oleic acid (18:1 n-9). The oleic acid content of almond varies in a range of 50 to 80% of the total MUFA content. Similarly, the oleic acid is 76 to 86% of the total MUFA content of hazelnut [16]. The PUFA content of almond is in the form of linoleic acid (LA, 18:2 n-6) which is around 24% of the total fat content. Nuts basically contain LA and alpha linolenic acid (ALA, 18:3 n-3) as PUFAs. However, LA is the dominant PUFA in nuts.

Micronutrients such as vitamins and minerals are the major groups of nutrients that the body needs for several physiological functions. Nuts such as almond,

Nuts	Oil content	TG	Sterol	Sterol ester	PS	PI	PC	PA	SL	Energy kcal
Almonds	53	98	0.25	0.05	0.32	0.17	0.56	ND	0.63	581
Walnuts	72.5	97.1	0.28	0.09	0.46	0.31	0.52	ND	0.68	618
Pistachio	54.1	95.8	0.21	0.03	0.59	0.28	0.68	ND	0.82	557
Cashew	45	96	—	—	—	—	0.54	—	—	553
Brazil nuts	68.9	96.6	0.19	0.05	0.32	0.10	0.78	ND	0.91	656
Pine nut	75.1	97.1	0.16	0.05	0.33	0.19	0.37	ND	0.57	629
Pecans	73.4	96.3	0.28	0.07	0.47	0.18	0.52	ND	0.55	691
Macadamia nuts	71.0	—	—	—	—	—	—	—	—	718
Hazelnuts	61.9	97.6	0.22	0.04	0.36	0.08	0.48	0.05	0.32	629

TG, triacylglycerol.
Source: Miraliakbari and Shahidi [17]; US Department of Agriculture Nutrient Data Base at http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl
Note: The oils of tree nuts were extracted using chloroform/methanol system; ND, Not Detected.

Table 1.
Lipid Classes (g/100 g oil) and energy content of nuts.

Nuts	Total fat	SFA	MUFA	PUFA	18:2n-6	18:3n-3
Almonds	50.6	3.9	32.2	12.2	12.2	0.00
Walnuts	65.2	6.1	8.9	47.2	38.1	9.08
Pistachio	44.4	5.4	23.3	13.5	13.2	0.25
Cashew	46.4	9.2	27.3	7.8	7.7	0.15
Brazil nuts	66.4	15.1	24.5	20.6	20.5	0.05
Pine nut	68.4	4.9	18.8	34.1	33.2	0.16
Pecans	72.0	6.2	40.8	21.6	20.6	1.00
Macadamia nuts	75.8	12.1	58.9	1.5	1.3	0.21
Hazelnuts	60.8	4.5	45.7	7.9	7.8	0.09
Peanuts	49.2	6.8	24.4	15.6	15.6	0.00

Source: Kim et al. [18], modified.

Table 2.
Fatty acid profile of nuts (g/100 g).

cashew nuts, pistachios, walnut and peanuts are very good source of B-vitamins, folate and vitamin E (**Table 3**). Nuts are also rich source of minerals such as magnesium and potassium (**Table 4**). Selenium is found particularly in Brazil nuts in greater concentrations.

There are varieties of phytochemicals present in nuts (**Table 5**) and they are well known in health and disease of humans [20]. Tree nut phytochemicals such as total phenols, flavonoids, proantocyanidins (PAC), stilbenes, phytosterols, carotenoids have been associated with many bioactivities such as antioxidant, antiviral, antiproliferative, hypocholesterolemic, and anti-inflammatory actions [21, 22]. The poly phenolic compounds are the major phytochemical class. All most all the tree nut are very good source of total phenolic compounds. However, walnuts and pecans are considered as the richest source of the total phenolic compounds

Nuts (mg/100 g)		Riboflavin (B_2) (mg/100 g)	Niacin (B_3) (mg/100 g)	Pantothenic acid (B_5) (mg/100 g)	Pyridoxine (B_6) (mg/100 g)	Cobalamin (B_{12}) (mg/100 g)	Folate (mg/100 g)	Vitamin C (total ascorbic acid) (mg/100 g)	Vitamin E (α-tocopherol) (mg/100 g)	Vitamin A (retinol activity equivalents) (mg/100 g)	Vitamin K (phylloquinone) (mg/100 g)
Almond	0.2	0.8	3.9	0.3	0.1	0.0001	0.05	0.0	25.9	0.0	0.0
Walnut	0.3	0.2	1.1	0.6	0.5	0.0005	0.098	1.3	13.0	0.001	0.0003
Pistachio	0.9	0.2	1.3	0.5	1.7	0.0017	0.051	5.0	2.3	0.028	0.0
Cashew nut	0.4	0.1	1.1	0.9	0.4	0.0004	0.025	0.5	0.9	0.0	0.0341
Brazil nut	0.1	0.04	0.3	0.2	0.1	0.0001	0.022	0.7	5.7	0.0	0.0
Hazelnuts	0.6	0.1	1.8	0.9	0.6	0.0006	0.133	6.3	15.0	0.001	0.0142
Pine nut	0.4	0.2	4.4	0.3	0.1	0.0001	0.034	0.8	9.3	0.0	0.0539
Pecan	0.7	0.1	1.2	0.9	0.2	0.0002	0.022	1.1	1.4	0.0	0.0035
Macademia	1.2	0.2	2.5	0.8	0.3	0.0003	0.011	1.2	0.5	0.0	0.0
Peanuts	0.18	0.04	5.75	0.59	0.11	-	0.062	0	0	0	0.00295

Source: Sathe et al. [19], modified.

Table 3.
Vitamin content of tree nuts and pea nut.

Nuts (mg/100 g)	Calcium	Magnesium	Sodium	Potassium	Copper	Iron	Zinc	Selenium
Almonds	248	275	1	728	1.03	3.71	3.12	0.004
Walnuts	98	158	2	441	1585	2.91	3.09	0.005
Pistachio	107	121	1	1025	1.3	3.92	2.2	0.007
Cashew	37	292	12	660	2195	6.68	5.78	0.019
Brazil nut	160	376	3	659	1.74	2.43	4.06	1.917
Hazelnut	114	163	0	680	1725	4.7	2.45	0.002
Pine nut	16	251	2	597	1.32	5.53	6.45	0.0007
Pecans	70	121	0	410	1.2	2.53	4.53	0.004
Macadamia	85	130	5	368	0.75	3.69	1.3	0.004
Peanut	92	168	18	705	1.14	4.58	3.27	0.007

Source: United States Department of Agriculture Nutrient Database for Standard Reference.

Table 4.
Mineral composition of nuts.

containing around 1600 mg GAE/100 g. Almond, Brazil nuts, cashews, macadamias and pine nuts contains almost similar amount of total phenol between 200 to 300 mg GAE/100 g. Flavonoids are a subclass of phenolics and mainly are available in three forms such as flavan-3-ols, flavonols and anthocyanins. In tree nut, the flavonoid content varies from 0.03 mg/100 g in pine nuts to 2700 mg/100 g in pecans. Hazel nuts and pecans are the major source of Proanthocyanidins (PAC) and contains around 500 mg/100 g. Stilbenes are the plant secondary metabolites derived from the phenylpropanoid pathway [23]. Among nuts, pistachio (803.22 μg/100 g) is the only nut has been reported to contain stilbenes. The sterol content in tree nuts ranges between 105 to 233 mg/100 g. Carotenoids are the color pigments which is ubiquitous in the plant kingdom. There are more than 750 carotenoids have been reported in nature, out of which 500 have been properly characterized. Brazil nut and Macadamias are reported to have no carotenoids however; pistachios are very rich in carotenoid content (22,832 μg/100 g). Cashew nut kernel contains carotenoids such asβ carotene (9.57 μg/100 g), lutein (30.29 μg/100 g) and zeaxanthin (0.56 μg/100 g) [24]. Hazel nut contains appreciable level of carotenoids (106 μg/100 g). Carotenoid content in the hazelnut oil was estimated as 5.51 mg/kg oil while lutein and zeaxanthin were determined as main carotenoids with the concentration of 0.19 and 0.85 mg/kg oil, respectively [25]. Alkaloids are widely distributed and occurring in approximately 20% of plant species. Alkaloids are not common in nuts only negligible amount was detected in walnuts. Tree nuts contain a significant amount of phytates from 100 to 2500 mg/100 g. Almonds have the highest reported phytate content, while pine nuts and Brazil nuts having the lowest content.

3. Nut consumption and relationship between disease conditions

3.1 Almond

Almonds are rich in macro and micro nutrients and upon consumption in adequate quantity impart a lot of health benefits. Traditionally, the ancient Greeks, Persians, Chinese and Indians use almonds for medical purposes as a health-promoting food [26]. There are also several epidemiological studies and clinical trials

Tree nut	Total phenols (mg GAE/100 g)	Flavonoids (mg/100 g)	Proanthocyanidins (mg/100 g)	Stilbenes (mg/100 g)	Sterol (mg/100 g)	Carotenoids (mg/100 g)	Alkaloids (mg/100 g)	Phytates (mg/100 g)
Almond	261	25.01	184.10	ND	192.37	0.002	ND	2542.11
Walnuts	1602	0.54	67.25	ND	197.89	0.021	0.0004	2070.00
Pistachios	703	136.45	252.71	0.803	189.43	22.83	ND	1562.50
Cashews	242	1.12	8.70	ND	154.00	0.031	ND	697.73
Brazil nuts	197	0.85	0–10	ND	160.19	—	ND	190.00
Hazelnuts	447	13.21	500.66	ND	132.47	0.106	ND	1285.00
Macadamias	233	137.9	0–10	ND	105.70	ND	ND	470.85
Pecans	1588	2713.49	494.05	ND	233.52	0.055	ND	851.60
Pine nuts	206	0.03	0–1	ND	190.75	0.026	ND	200.00

Source: Kim et al. [18].

Table 5.
The phytochemical content of the nuts.

which reports positive effects of nut consumption against a different diseases conditions like cardiovascular conditions, inflammation and oxidative stress, obesity, hypertension, diabetes mellitus and metabolic syndrome [14]. The consumption of almond and relationship between different disease conditions is presented in **Table 6**.

3.1.1 Almond and cardiovascular disease

The role of dietary almonds against cardiovascular disease (CVD) has been shown in several studies. The high carbohydrate or saturated fatty acid diet is often associated with impaired glucose and lipid homeostasis which alters blood lipid profile in terms of elevated low density lipoprotein cholesterol (LDL-C), total cholesterol, apolipoprotein B (Apo B), and increases body weight which leads to cardiovascular risks. A recent study conducted by Wang et al. [27] reported that 42.5 g almond consumption per day changes LDL-C which is also cost effective to prevent cardiovascular disease in the short term and potentially in the long term. Furthermore, studies conducted by Gulati et al.[28] and Jalali-Khanabadi [29] reported that dietary almond can improve blood lipid profile by lowering total cholesterol (TC) and triglyceride, respectively in cardiovascular patients. In addition to their cholesterol-lowering effects, the ability of almonds to minimize the risk of heart disease may also be attributed to the antioxidant activity of vitamin E found in them. A meta-analysis of randomized controlled trial conducted by Lee-Bravatti et al. [30] also found that >42.5 g daily consumption of almond reduced TC, LDL cholesterol, and also significantly decreased other CVD risk factors such as body weight. Almond consumption, other than cholesterol reduction also associated with control of hyperlipidemia, inflammation, blood pressure, blood glucose and insulin concentrations, metabolic syndrome, and body weight/fat/composition which also plays key roles in managing cardiovascular risk factors [31]. The lower blood HDL-C is the marker of cardiovascular risk [32]. Drug therapies to increase the HDL-C level are not very much successful. Some combination of medicine such as high doses of niacin, fibric acid or bile acid sequestrants can improve HDL-C minimally [33]. According to the Indian Heart Association 2015, risk of heart disease comes to half with every 10 fold increase in HDL-C [34].

The phytochemicals such as phytates and phenolics compounds in almonds confers antioxidant, anti-inflammatory and lipid-lowering properties [35]. Almond also contains good quantity of magnesium and potassium. Magnesium deficiency is not only associated with heart disease, but also the lack of adequate magnesium causes free radical damage to the heart [36]. Potassium is an effective electrolyte necessary to maintain normal blood pressure.

3.1.2 Almond and obesity

Obesity is a very serious problem in the affluent nations which is also the basic reason of type 2 diabetes (T2D). There are studies which demonstrate the role of almond consumption on overweight and obesity in humans. A 24 week clinical trial was conducted with 65 overweight and obese (body mass index (BMI): 27–55 kg/m^2) adults of age group between 27 to 79 y [37]. In the weight reduction program, the subjects were received almond enriched and complex carbohydrate enriched low calorie diet (LCD) with similar protein and calories. The main outcome of the study was based on the anthropometric, body composition and metabolic parameters which showed that almond-LCD group experienced a sustained and greater weight reduction than the complex carbohydrate fed group. The authors concluded that the results could be due to high MUFA content in almonds which have high oxidation rate than get stored as fat. In another 12 week clinical trial with 86 healthy subjects with a BMI

Design and study population	Intervention	Duration	Main outcomes	Reference
Cardiovascular disease				
US adults of mean age approximately 30 years (150 participants)	42.5 g almond/day	12 months	42.5 g almond/day is a cost-effective approach to prevent CVD. Decrease in LDL-C	[27]
Asian Indians	Raw almonds (20% of energy intake)	24 weeks	Lowered waist circumference, waist-to-height ratio, TC, serum triglycerides and LDL-C	[28]
Thirty healthy volunteer men (age 45.57 $\pm$ 7.14 years and body-mass index 24.29 $\pm$ 2.15 kg/m^2)	60 g almond/day	4 weeks	Decreased LDL-C, total cholesterol (TC), and apolipoprotein B100 (apo-B100)	[29]
Meta-analysis (534 subjects)	>42.5 g almond/day	15 eligible trials	Decreased TC and LDL cholesterol, and body weight.	[30]
Obesity				
A randomized trial (65 overweight and obese adults)	A formula-based low calorie diet enriched with 84 g/day of almonds	24 weeks	Weight reduction	[37]
A randomized controlled clinical trial 86 healthy adults [body mass index (in kg/m^2): 25–40]	Almond-enriched diet (AED) (15% energy from almonds)	12 weeks	Reduced truncal and total body fat. Reduced diastolic blood pressure	[38]
A randomized study (Overweight and obese individuals [n = 123; age = 46.8 y, BMI (in kg/m^2) = 34.0])	Hypocaloric almond-enriched diet	18 months	Reduced weight. Improved lipid profile	[39]
Diabetes				
Asian Indians	Raw almonds (20% of energy intake)	24 weeks	Lowered glycosylated hemoglobin and improvements in sensitivity C-reactive protein (hs-CRP)	[28]
A randomized, 5-arm, crossover design study (14 impaired glucose tolerant (IGT) adults)	Whole almonds (WA), almond butter (AB), defatted almond flour (AF), almond oil (AO) or no almonds (vehicle - V) were incorporated into a 75 g available carbohydrate-matched breakfast meal		Reduced blood glucose	[41]

Design and study population	Intervention	Duration	Main outcomes	Reference
Randomized, crossover and controlled feeding trial (33 Chinese patients)	60 d almond/day	12 weeks	Decreased post-interventional fasting serum glucose	[42]
15 healthy subjects	Almond co consumed with bread	4 hours	Decreased glycemic excursion. Increased Serum protein thiol	[43]
A randomized crossover trial [19 adults (including 7 adults with type 2 diabetes mellitus)]	28 g/day	12 weeks	Reduced hemoglobin A (1c). Reduced postprandial glycemia	[40]
Cancer				
A case control study (923 colorectal cancer patients and 1846 controls, Korea)	Nut consumption was categorized as none, < 1 serving per week, 1–3 servings per week, and ≥ 3 servings per week	Dietary intake information collected using a semi-quantitative food frequency questionnaire	≥3 servings per week reduced risk of colorectal cancer	[45]
Six-week-old male F344 rats (Treated with azoxymethane)	Whole almond, almond meal or almond oil containing diet	26 weeks	Reduced colon cancer risk	[51]

Table 6.
Effect of almond consumption on different disease conditions.

25 to 40 kg/m^2 when treated in two diet intervention groups: an almond-enriched hypocaloric diet (AED, 15% of total kcal from almonds) and a nut-free hypocaloric diet (NFD) for 12 weeks showed AED followed group had significant weight loss with reduced truncal fat mass. Similarly, the authors concluded that the results might be due to the unsaturated fatty content in almonds which is more oxidation prone than storage [38]. A study conducted by Foster et al. [39] on 123 overweight and obese adults found that a daily dose of 56 g almond for 6 months is effective in body weight loss. Taking the above results in to consideration it is understood that the MUFA, fiber and protein of almond have a satiating effect and benefit individuals during weight loss program.

3.1.3 Almond and diabetes

The role of almond supplementation in glucose homeostasis has been studied by several researchers [28, 40]. In 2017, Gulati and colleagues [28] reported the health benefits of almond in terms of measures of glycemia through the assessment of glycosylated hemoglobin (HbA1c) in the type 2 diabetic (T2D) patients when provided 20% of the total energy intake for 24 weeks along with diet and physical activity counseling. The HbA1c levels in subjects after almond supplementation declined significantly compared with their levels on the control diet which is clinically accepted as the indicator of reduction in the diabetic complications. Overall, the study suggested that dietary almond controls glycemic condition through insulin management rather than in reduction of glucose absorption or increased clearance. Similarly, Mori and colleagues [41] reported that almond consumption

improved the metabolic profile by decreasing blood glucose concentrations when included in the breakfast meal of 14 impaired glucose tolerant (IGT) adults. It is suggested that the high content of fiber, high unsaturated fatty acid and low carbohydrate which makes almond as low glycemic index food played a role in the reduction of glucose. Under nutrition therapy Chen et al. [42] made 40 T2D patients receive almond for 12 weeks after a 2 weeks run in period among 27 of 33 patients with the baseline HbA1c ≤8, almond receiving groups decreased post-interventional fasting serum glucose and HbA1c by 5.9% and 3.0% as compared to that of control, respectively. It is concluded from the study that almonds incorporated into healthful diets can improve glycemic status in diabetic patients with a better glycemic control. Many clinical interventions suggested that dietary almond through multiple mechanisms of actions, i.e., reducing glycemic index value of co-consumed food, increasing insulin secretion, and alleviating insulin resistance controls diabetes [40, 43]. It is also suggested that the polyphenols mainly flavonoids present in almond also controls blood glucose levels by the action of inhibiting amylase which is a carbohydrate digestive enzyme [40, 44].

3.1.4 Almond and cancer

In 2018, Lee and colleagues [45] conducted a case study among 923 colorectal cancer patients and 1846 controls recruited from the National Cancer Center in Korea. They collected the dietary intake information of food items including nuts such as peanuts, pine nuts, and almonds (as 1 food item). The results of the study showed that high consumption of nuts (>45 g/week, in three servings) was strongly associated with reduced risk of colorectal cancer. Authors concluded that the relationship between nut consumption and reduction of colorectal cancer risk could be due to the presence of fiber, resveratrol, selenium, flavonoids (quercetin), polyphenols (ellagic acid), and folic acid. These bioactive compounds have strong antioxidant properties which regulate cell proliferation, reduce DNA damage, inflammatory response and immunological activity as indicated by Gonzalez and Salas-Salvado [46]. The triterpenoids present in almond, including betulinic, oleanolic, and ursolic acids, have also been reported earlier as antitumor agents [47, 48]. There are studies which depicts about the link between the consumption of roasted almond and development human cancer because of the presence of acrylamide in it. Almonds contain free asparagine and reducing sugars and acrylamide is formed when undergo roasting above 154°C temperature [49]. Acrylamide can be found in roasted almonds, but is not found in raw almonds. As per National Health and Nutrition Examination Survey (NHANES) (2001–2010), daily almond consumption for a habitual American consumer is 29.5 g [50]. A consumer of body weight of 65 kg the daily acrylamide exposure from almonds would be 0.08 μg/kg body weight. The unroasted almond consumption is more a common practice therefore; the actual acrylamide exposure from almonds would be lower. Anticancer property of almond and its extracts has been also been reported. Davis and Iwahashi [51] reported that whole almond and almond fractions can reduce aberrant crypt foci (ACF) in a rat model of colon carcinogenesis. According to Heasman and Mellentin [52] the anticancer properties of almond is mainly attributed to the phytochemicals such as, quercetin and kaempferol, which suppresses lung and prostate tumor cell growth. Almond consumption and the overall health benefits are presented in **Figure 1**.

3.2 Walnuts

Walnut is one of the world's most popular temperate grown nuts. Walnut kernel is a rich source of proteins, fats, minerals, vitamins, and polyphenols that render the

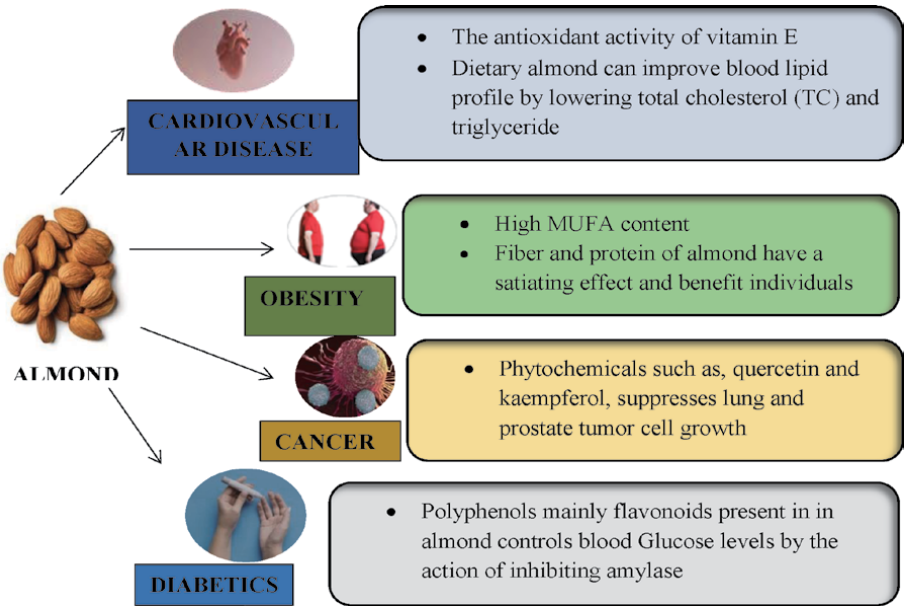

Figure 1.
Almond consumption and health benefits.

fruit indispensable for human nutrition. These also have a good source of flavonoids, sterols, pectic compounds and phenolic acids. Worldwide 3.7 tonnes of walnut is produced per year and china contributes approximately 50 per cent of the total world walnut production (atlasbig.com/en-in/countries-by-walnut-production). The consumption of walnut and relationship between different disease conditions is presented in **Table 7**.

3.2.1 Walnut and cardiovascular disease

Walnuts differ from other nuts in terms high LA and ALA content which has known anti atherogenic properties [53]. It is also recommended that 84 g of walnut on a daily basis for four weeks has a potential to decrease serum levels of total cholesterol by 12% [54]. The beneficial effect of walnut consumption in reducing the risk of CVD is not limited to reducing blood cholesterol level only but also attributed to the lowering LDL-C, vascular inflammation, improving endothelial dysfunction and enhancing antioxidant activity [55–57]. The cholesterol lowering effect of walnut could be due to the presence of phytosterols in it. According to Gylling et al. [58] intake of phytosterols (2 g/day) is associated with a significant reduction (8–10%) in LDL-cholesterol because phytosterol mostly interfere in the intestinal absorption of cholesterol. In 2014, Wu and colleagues [59] assessed the effect of walnuts on lipid and glucose metabolism, adipokines, inflammation and endothelial function in 40 healthy subjects, 10 men and 30 postmenopausal women ≥50 years old with BMI 24.9 ± 0.6 kg/m^2. The subjects first received a walnut enriched (43 g/d) and then a Western-type (control) diet or vice-versa, with each lasting 8 weeks and separated by a 2-week wash-out. The study reported that walnut diet significantly reduced non-HDL cholesterol (walnut vs. control: −10 ± 3 vs. −3 ± 2 mg/dL; p = 0.025) and apolipoprotein-B (−5.0 ± 1.3 vs. −0.2 ± 1.1 mg/dL; p = 0.009) in comparison to control diet after adjusting for age, gender, BMI and diet sequence. Total cholesterol showed a trend toward reduction. However, fasting adipokines, C-reactive protein, biomarkers of endothelial dysfunction, postprandial

Design and study population	Intervention	Duration	Main outcomes	Reference
Cardiovascular disease				
A randomized trial (18 healthy men)	20% calorie from walnut	4 weeks	Reduced total cholesterol, lowered LDL-C/HDL-C ratio	[54]
Five controlled clinical trial (200 subjects)	Walnut consumption varied from 28 g/day to 78 g/day	3–6 weeks	Reduced the TC, LDL-C, and HDL-C values. Decreased ratios of TC:HDL-C and LDL-C:HDL-C. Reduced apo B levels	[55]
A randomized 2-period, crossover and controlled intervention study (27 overweight volunteers)	23.1% energy from walnut	2–6 weeks	Improved flow mediated dilation and biochemically by sVCAM (soluble vascular cell adhesion molecules)	[57]
A randomized cross over study (40 subjects)	43 g/day	8 weeks	Reduced total cholesterol, non-HDL-cholesterol and apolipoprotein-B	[59]
Obesity				
Overweight and obese subjects (n = 100)	walnut-enriched (15% of energy) reduced-energy diet	3–6 months	Weight loss, favorable effects on LDL-C and systolic blood pressure	[61]
A randomized, controlled, crossover trial (46 overweight adults)	Walnut enriched *ad libitum* diet	8 weeks	Improved flow-mediated vasodilation (FMD), no weight gain	[62]
A double-blinded, randomized, placebo-controlled study (15 obese subjects)	48 g/day	4 days	Increases apolipoprotein A concentrations	[65]
Diabetes				
Dietary interventions and cohort studies	Walnut consumption from dietary recall	Over a period of 15 years	Reduced risk of diabetes, reduced blood glucose and HbA1c	[66]
A randomized control clinical trial (100 patients)	Walnut oil 15 g/day	3 months	Decreased HbA1c level, reduced fasting blood sugar	[69]
Diabetic male rats (200 g)	Walnut oil gavage with β-sitosterol0.5 ml/kg	4 weeks	Decreased inflammation of lymphocytes, improved blood parameters	[71]
Cancer				
Human esophageal adenocarcinoma cell line	20 mg/mL walnut oil		Induced necrosis and accumulation of cells in G0/G1 phase. Down regulated NFkB expression	[78]
Effect of walnut methanolic extracts on human renal cancer cell lines A-498 and 769-P and the colon cancer cell line Caco-2	0.226 and 0.291 mg/mL walnut extract		Inhibited growth of human kidney and colon cancer cells	[79]
Human cancer cell line	Chloroform and ethyl acetate extract of walnut		Reduced proliferation of HepG-2, liver cancer cell line	[80]
Cancer stem cells (CSCs)	Walnut phenolic extract (WPE)		Inhibited cell differentiation, down regulated CSCs markers	[81]

Table 7.
Effect of walnut consumption on different disease conditions.

lipid and glucose metabolism and endothelial function were unaffected. It was concluded from the study that daily consumption of 43 g of walnuts for 8 weeks could be beneficial for the reduction of non-HDL-cholesterol and apolipoprotein-B, beneficial in lowering CHD risk. It is suggested that the increased PUFA intake through walnut could be responsible for the lowered cholesterol in the subjects. Walnut not only a good source of PUFA but also contains a lower ratio n6/n3 ratio. It is suggested that the lower n-6/n-3 ratio is desirable in reducing the risk of many of the chronic diseases including lowering blood cholesterol, reducing vascular inflammation and improving endothelial function [60].

3.2.2 Walnut and obesity

Better weight management and less adiposity can be maintained with regular nut consumption. Rock and colleagues [61] conducted a study where overweight and obese men and women (n = 100) were randomly assigned to a standard reduced energy- density diet or a walnut-enriched (15% of energy) reduced-energy diet in the context of a behavioral weight loss intervention. Both study groups reduced weight, body mass index and waist circumference. Change in weight was $9.4 \pm 0.9\%$ vs. $8.9 \pm 0.7\%$ for the standard vs. walnut-enriched diet groups, respectively. The results of the study demonstrated that a walnut-enriched reduced-energy diet can promote weight loss that is comparable to a standard reduced-energy-density diet in the context of a behavioral weight loss intervention. It is suggested that despite walnut is very high in energy density, but when consumed as a component of a reduced-energy diet the total energy intake get reduced and also give a satiety response. The study conducted by Katz et al. [62] on effects of walnuts on endothelial function in overweight adults with visceral obesity found that daily ingestion of 56 g of walnuts with *ad libitum* diet in comparison ad libitum diet without walnut did not lead to weight gain however, improved endothelial function. Adiponectin is a peptide secreted from adipocytes, whose low concentration is an indicator of overweight, visceral fat accumulations and related diseases such as insulin resistance/T2D and cardiovascular disease [63]. The production of adiponectin and its role is presented in **Figure 2**. Cardona-Alvarado et al. [64] found that adiponectin concentration increased by 30% in obese subjects supplemented with a daily intake of approximately 15 g of walnuts. Similarly, Aronis et al. [65] reported that after short-term (four days) consumption of 48 g/d walnut, 6.4% adiponectin increased in the circulation. The results suggested that the improvement observed in the metabolic state may be due to the activation of peroxisome proliferator-activated receptor alpha (PPARγ) and adiponectin expression, promoted by the increase in circulating lipocalin2 (Lcn2) concentrations which is a novel regulator of brown adipose tissue.

3.2.3 Walnut and diabetes

Arab and colleagues [66] examined the associations between walnut consumption and diabetes risk using data from the National Health and Nutrition Examination Survey. Diabetes status or risk was assessed on self-report, medication use, fasting plasma glucose levels, and haemoglobinA1c (HbA1c) levels. The results demonstrated that walnut consumers had lower risk for diabetes compared with non-nut consumers and prevalence of diabetes dropped 47%. It is suggested from the study that walnut possibly impact hunger. The decreased feelings of hunger and appetite and increased activation of the right insula indirectly reduces risk of diabetes. In the diet of diabetic patients, carbohydrate is replaced with MUFAs and PUFAs under therapeutic strategy [67]. The intake of MUFAs by diabetic patients

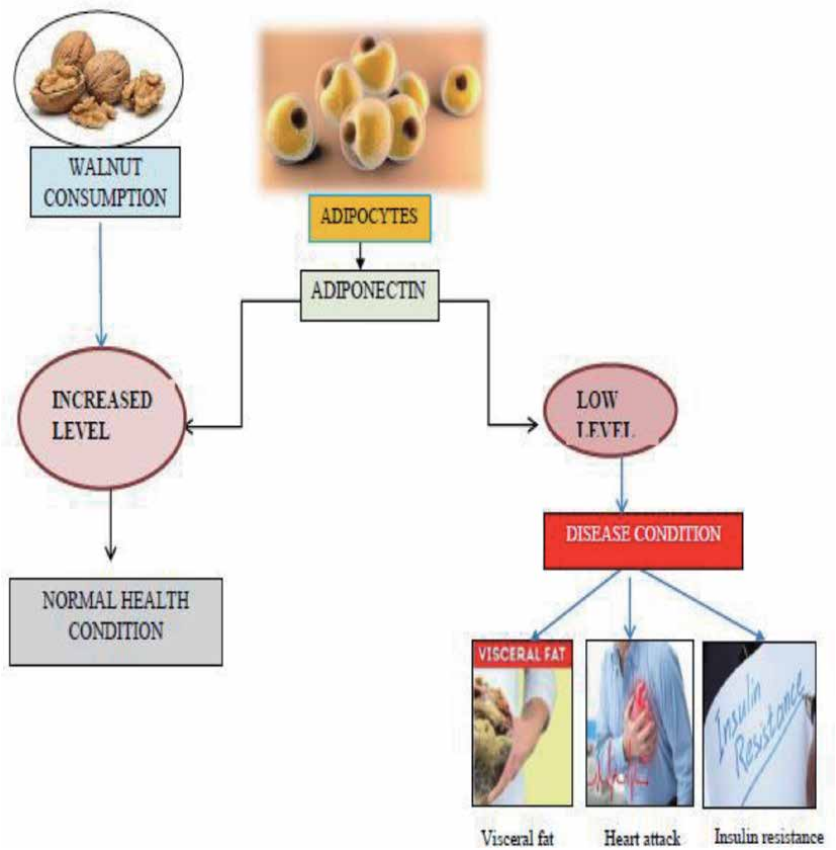

Figure 2.
Walnut intake increases circulating adiponectin levels.

has also been seen with elevated high density lipoprotein (HDL)-cholesterol levels and improved blood sugar changes [68]. In this regard, Nezhad et al. [69] reported that consumption of walnut oil (15 g/day for three months) can improve blood glucose level. It is also suggested that oils containing PUFA could exert their antidiabetic effect by reducing resistance and enhancing sensitivity to insulin via the mechanism of over expression of glucose transporter GLUT4 and insulin receptors on the adipocyte membrane [70]. A study conducted by Ghorbani et al. [71] found that walnut oil led to reduction in blood glucose, cholesterol, triglyceride, ASP, ALT, ALP, and bilirubin in diabetic rat. It is suggested that the better blood profile was primary because of the β-sitosterol in the walnut oil. β-Sitosterol is considered as an important potential factor in diabetes mellitus due to its effect on regulation of glucose adsorption, adipogenesis, and lipolysis in adipocytes. It is also presumed that like insulin, β-sitosterol down regulates GLUT4 expression however, not fully confirmed yet [72]. Recent studies have reported that the composition and the balance of gut microbiota are involved in the obesity and obesity-related complications such as nonalcoholic fatty liver disease (NAFLD), insulin resistance and T2D [73]. In this regard, Li et al. [74] observed that polyphenol rich walnut meal (WMP) impeded the changes of intestinal flora in feces of rats, the gut microbiota mainly included Firmicutes, Bacteroidetes, and Proteobacteria, the symbol of healthy gut micro biota composition. This is the indication of promising effects of WMP on T2DM through the change in gut microbiota composition. Authors of the study highlighted that WMP decreased the levels of TNF-α and IL-6 in serum. TNF-

α is the first pro-inflammatory cytokine recognized for its involvement in pathogenesis of insulin resistance and T2DM. TNF-α reduces the expression of insulin-regulated glucose transporter type 4 (GLUT4) and increases the diabetic risk [75].

3.2.4 Walnut and cancer

There are several studies which highlights the anticancer properties of walnuts in mice. Hardman [76] summarized the potential of walnut in cancer prevention and treatment in mice. The points what he put forth was as follows: (1) the walnut-containing diet inhibited the growth rate of human breast cancers implanted in nude mice by 80%, (2) the walnut-containing diet reduced the number of mammary gland tumors by 60% in a transgenic mouse model, (3) the reduction in mammary gland tumors was greater with whole walnuts than with a diet containing the same amount of n-3 fatty acids, supporting the idea that multiple components in walnuts additively or synergistically contribute to cancer suppression and (4) walnuts slowed the growth of prostate, colon, and renal cancers by anti-proliferative and antiangiogenic mechanisms. The author explained that the n-3 fatty acids, tocopherols, b-sitosterol, and pedunculagin, present in walnuts all have cancer-prevention properties. Induja et al. [77] reported the cytotoxic effects of walnut oil on oral cancer cell line by DNA fragmentation. They suggested that other than perfect balance of n-6 and n-3 fatty acids, the walnut residue is rich in protein with anticancer peptides. In 2018, Batirel and colleagues [78] investigated the effect of walnut oil on tumor growth and metastatic potential in esophageal cancer cells. The results of the study confirmed that 20 mg/mL walnut oil reduced cell viability of esophageal cancer cells by ~50% when compared with control. Walnut oil exhibited anti-carcinogenic effect by inducing necrosis and cell cycle arrest at the G0/G1 phase. It down regulated the NFkB pathway. It is suggested from the study that walnut oil, and walnut consumption, may have beneficial effects in esophageal cancer in humans. Similarly, the anti-carcinogenic effect of walnut extract has been seen due to the antioxidant and anti-proliferative activity in many different cell lines such as MCF-7 (estrogen receptor positive breast adenocarcinoma), HepG-2 (liver), WRL-68 (liver), A-498 (renal), 769-P (renal), KB (oral and mouth), and Caco2 (colon) [79, 80]. In another study, walnut phenolic extract (WPE) and its bioactive compounds suppressed colon cancer cell growth by regulating colon cancer stem cells (CSC) [81]. This study evaluated the anti-CSCs potential of walnut phenolic extract (WPE) and its bioactive compounds, including (+)-catechin, chlorogenic acid, ellagic acid, and gallic acid. WPE and its bioactive compounds to mediate an inhibition of colon CSCs by inducing cell differentiation, down-regulation in the expression of the CSC markers, CD133, CD44, DLK1, Notch1, and β-catenin/p-GSK3 signaling pathways and suppress CSC self-renewal capacity. These results suggest that WPE has the potential to prevent and treat human malignant colorectal cancer by regulating CSCs. In another study, it was found that telomere length in WPE-treated cells were significantly decreased in a dose dependent manner which could be a mechanistic link to the effect of walnuts on the viability of colon CSCs. Telomere maintenance is a prerequisite for indefinite proliferation in cancer. Therefore, inhibiting the maintenance of telomeres may prove to be a useful strategy for cancer therapy [82]. Walnut consumption and the overall health benefits are presented in **Figure 3**.

3.3 Pistachio

The Islamic Republic of Iran and United States of America are the world leaders in pistachio production and cultivation followed by Turkey and China. Pistachio is

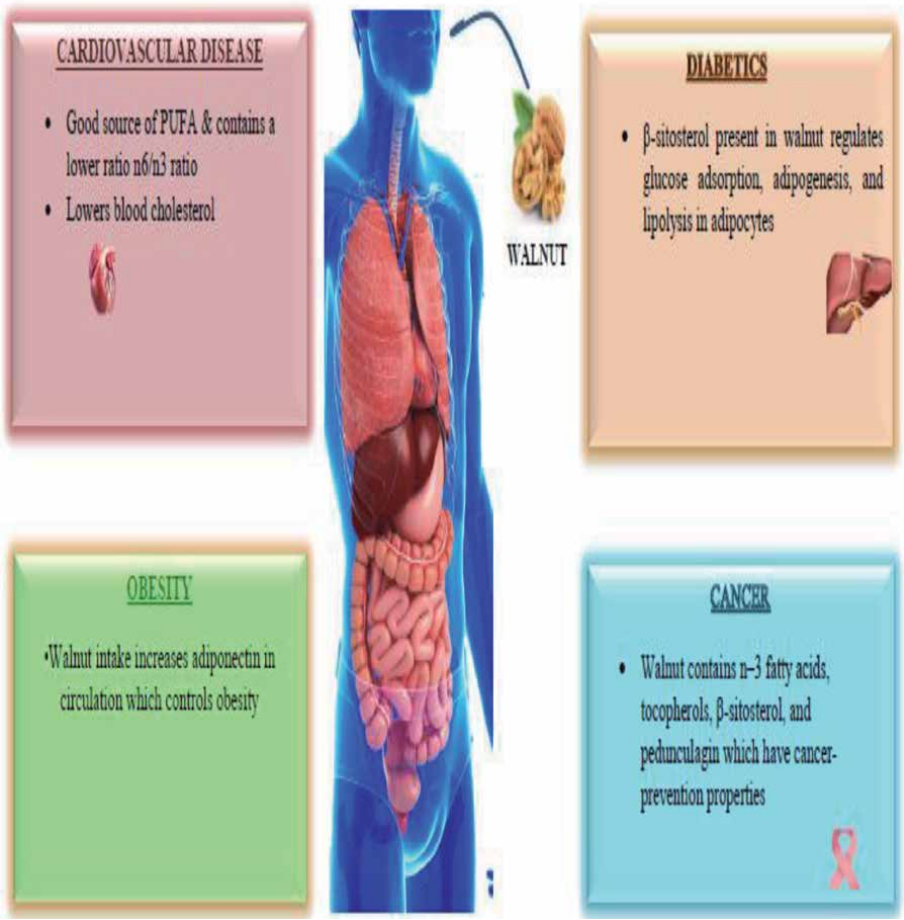

Figure 3.
Walnut consumption and health benefits.

known as the "green nut" and has been associated with human activities since centuries [83]. Pistachios are good source of unsaturated fatty acids (linoleic acid, linolenic acid, and oleic acid). They also contain a high amount of monounsaturated fatty acids (MUFA) (>55%) [84]. Pistachios contain the highest levels of potassium, g-tocopherol, vitamin K, phytosterols, folate, and xanthophyll carotenoids in comparison to other nuts. The diverse set up of nutrients makes pistachio a very good health promoting food. The consumption of pistachio and relationship between different disease conditions is presented in **Table 8**.

3.3.1 Pistachio and cardiovascular disease

Pistachios are rich source of numerous antioxidants, including tocopherols, carotenes, lutein, selenium, flavonoids, and phytoestrogens. The high rate of tocopherol in pistachio prevents heart disease, LDL oxidation, diabetes, and cancer and promotes the immune system. Pistachios can enhance blood lipid profiles in subjects with moderate hypercholesterolemia, and can in turn, reduce the risk of Chronic Vascular Diseases [85]. The beneficial effect of pistachios on lipid profiles may be due to essential fatty acids (EFAs) in its composition. In a study, when pistachios were given to healthy young men for 4 weeks, significant decreases in

blood glucose, total cholesterol, and serum interleukin-6 were observed with improved endothelium vasodilation and total antioxidant status [86]. The intake of pistachios has been shown to significantly decrease oxidative stress as it contains the highest amount of antioxidants. In another study, when twenty-eight hypercholesterolaemic adults were intervened with a diet containing 10 and 20% of energy from pistachios (32–63 and 63–126 g/d, respectively) showed increased antioxidant concentrations in serum, such as g-tocopherol, lutein and b-carotene, whereas it decreased oxidized LDL concentrations comparison to a control diet without pistachios [87]. In 2007, Sheridan and colleagues [88] conducted a randomized cross over trial where 15 patients with moderate hypercholesterolemia intervened with 4 weeks of dietary modification with 15% caloric intake (about 2–3 ounces per day), from pistachio nuts. Results of the study showed significant changes in some blood parameters such as 12% increase HDL-C and 9% decrease in TC/HDL-C, 14% decrease in LDL-C/HDL-C, 13% decrease in B-100/A-1 and 9% decrease in LDL-C. The results elucidate the effect of pistachio in reducing risk of coronary disease by improvement of the lipid profiles. It has been reported that TC/HDL-C and LDL-C/HDL-C are better predictors of CHD risk reduction than changes in levels [89]. London et al. [90] in a rat model found the health benefits of dietary pistachio in terms of lowered total cholesterol, LDL-C fractions, a beneficial change in TC/HDL-C and LDL-C/HDL-C. Regarding the ideal doses, 40 g or 1.5 oz./day of pistachio intake found suitable for reduced fasting glucose and LDL-C concentrations and increased HDL-C with improvements in vascular function in a clinical trial with mild dyslipidemic adults [91]. The pistachio (42 and 84 g/day) nut intervention during three weeks also lowered LDL-c concentrations (6%) in healthy volunteers [92]. Pistachio is a magnesium rich nut (121 mg/100 g). Magnesium is associated with lower the risk of abnormal cardiac excitation, atherosclerosis, ischemic heart disease, and congestive heart failure [93]. Considering all these studies, the beneficial effects of pistachios on lowering the risk of CVD could be through lipid-lowering, imparting many antioxidant properties and magnesium content in it.

3.3.2 Pistachio and obesity

Obesity is associated with chronic low-grade inflammation which eventually leads to abnormal metabolisms. A recent study conducted on mice demonstrated that chronic intake of pistachio exerts beneficial effects in obese mice by alleviating inflammation in adipose tissues and liver, and impacting the gut microbiome composition [94]. Nuts are very energy dense food item and there are reports of weight gain with increased consumption of nuts without energy balance. However, the daily ingestion of either 42 g or 70 g/day of pistachios for 12 weeks did not contribute to change in BMI or waist-to-hip ratio in Chinese subjects with metabolic syndrome [95]. In a recent randomized controlled intervention, sixty healthy premenopausal women in a group of 30 each consumed 44 g (250 kcal) pistachios midmorning while controls (n = 30) maintained their current eating habits for 12 weeks. Pre- and post-intervention tests showed that *ad libitum* intake adjusted to the pistachio portion, mostly via reduced intakes of carbohydrates and starch, in parallel with decreased hunger and increased satiety following the morning snack. It was concluded from the study that daily intake of 44 g pistachios improved nutrient intake without affecting body weight or composition in healthy women. It was also found that Intakes of MUFA, PUFA, linoleic acid, thiamin, pyridoxine, copper, manganese, and zinc were significantly higher among women consuming the pistachio snack, in spite of compensatory adjustments in intake [96]. Similarly, in another study, in healthy French women, in which a daily portion of pistachios was compared to an isoenergetic load of a comparator food (cheese biscuits) ingested in

Design and study population	Intervention	Duration	Main outcomes	Reference
Cardiovascular disease				
A randomized, crossover controlled-feeding study	Pistachio amounts ranged from 32 to 63 g/d for the 1 PD and 63 to 126 g/d for the 2 PD, depending on energy level	4 weeks	Increased plasma leutin, α-carotene, and β-carotene. Lowered serum oxidized-LDL concentrations, lowered cholesterol	[87]
Randomized crossover trial (15 subjects)	15% caloric intake from pistachio nuts	4 weeks	Reduced TC/HDL-C, LDL-C/HDL-C andB-100/A-1. Increased HDL-C	[88]
Open label, randomized parallel-group study (60 adults with mild dyslipidemia)	40 g/day	3 months	Increased HDL-C, reduced LDL-C, total cholesterol and fasting blood sugar. Improved brachial artery flow-mediated vasodilation (BAFMD)	[91]
Obesity				
90 subjects with metabolic syndrome	42 g/day normal dose 70 g/day high dose	12 weeks	No change in body weight, lowered serum triglyceride level in normal dose	[95]
A randomized, controlled intervention (n = 30)	44 g/day	12 weeks	No change in body weight and composition. Improved nutrient (MUFA, PUFA, linoleic acid, thiamin, pyridoxine, copper, manganese, and zinc) intake	[96]
A randomized controlled pilot study (n = 30, French women)	56 g/day	1 month	No change in body weight. Improved nutrient (thiamin, vitamin B6, copper, and potassium) intake	[97]
31 obese subjects	53 g/day	12 weeks	Lowered weight and serum triglyceride	[98]
Diabetes				
A randomized crossover study (20 subjects with metabolic syndrome)	Carbohydrate meal with pistachio	5–10 weeks	Reduced postprandial glycemia, increased glucagon-like-peptide levels	[104]
A randomized control trial (60 Asian Indian)	Pistachio (20% of energy)	24 weeks	Reduced waste circumference, fasting blood sugar, total cholesterol, LDL-C, high-sensitivity C-reactive protein. Improvement in mean values of free fatty acids, TNFα, TBARS	[105]
A randomized, controlled, crossover Study (gestational diabetes mellitus (GDM) Chinese women patients)	42 g/day	90 and 120 min after intake	Improve postprandial glucose, insulin, and glucagon-like peptide-1response	[106]

Design and study population	Intervention	Duration	Main outcomes	Reference
Cancer				
MCF-7 Human Breast Cancer Cells	Pistachio (*Pistaciavera* L.) hulls extract		Induced apoptosis and inhibited angiogenesis	[112]
A549, MCF-7, and HeLa human cancer cells	Extracts of red hulls, kernels and oleo-gum resins of Pistachio	100 to 1000 μg mL^{-1} concentrations	Showed neuro-protective potentials, inhibited AChE and BChE enzymes	[113]

Table 8.
Effect of pistachio consumption on different disease conditions.

the afternoon, reported no change in body weight after four weeks but it did improve micronutrient intake [97]. It had also been seen that pistachio did not contribute to weight gain in patients with obesity, prediabetic or diabetic conditions [98–100]. It is suggested that the main mode of weight control of pistachio is through increased satiation, satiety signals and lower metabolizable energy [92, 101, 102].

3.3.3 Pistachio and diabetes

Studies have shown that pistachios promote a healthier metabolic profile by lowering glucose level [92, 103]. Among all nuts, pistachios have a low glycemic index. In a randomized cross-over study conducted on 20 subjects with the metabolic syndrome, 85.04 g of pistachios consumed along with bread reduced postprandial glycaemia levels and increased glucagon-like peptide levels compared with bread alone therefore, contribute to reducing the T2DM risk [104]. Jenkins et al. [68] reported the reduction of HbA1c in T2DM subjects consumed mixed nuts (including pistachios) for 3 months in a randomized controlled study as a replacement for carbohydrate-containing foods compared with a half-nut and control-muffin doses. In 2014, Gulati and colleagues [105] conducted a 24-wk randomized control trial, 60 individuals with the metabolic syndrome were randomized to either pistachio (20% energy) (intervention group) or control group. At the end of the study, statistically significant improvement was seen in levels of fasting blood sugar (FBG) however, no significant effect was seen on HbA1c and insulin levels. In a recent study conducted to evaluate the acute effects of two isocaloric test meals, 42 g pistachios and 100 g whole-wheat bread (WWB) on postprandial glucose, insulin, and gut derived incretin levels in Chinese women with gestational impaired glucose tolerance (GIGT) or gestational diabetes mellitus (GDM) suggested that pistachios are effective alternative to a low-fat, high-carbohydrate food to improve postprandial glucose, insulin, and GLP-1 response in women with GDM and GIGT [106]. The fiber, healthy fats, low available carbohydrate and carotenoid content of pistachios are the important nutrients involved in glucose metabolism as suggested by Bullo et al. [107]. Akbaraly et al. [108] reported that the higher plasma carotenoid level was associated with 58% lower risk of T2DM mellitus. Similarly, other phytonutrients in pistachios, such as ellagitannins, can also possibly affect gastrointestinal sugar absorption and thus influence postprandial blood glucose levels [109]. Pistachio consumption and effects on insulin resistance, secretion or diabetes control are less so, more studies are required to clarify the long term effects.

3.3.4 Pistachio and cancer

Pistachios are rich sources of phenolic compounds such as epicatechin, quercetin, kaempferol, cyaniding-3-O-galactoside, cyanindin-3-O-glucoside, among other polyphenol [110]. These poly phenolic compounds have strong antioxidant properties for which pistachio is ranked among 50 highest antioxidant food products [111]. Phenolic compounds play protective roles against free radical production and controls disease like CVD and cancer. Seifaddinipour et al. [112] assessed the cytotoxic effects of hexane, ethyl acetate, methanol, and water extract of pistachio hull against human colon cancer (HT-29 and HCT-116), breast adenocarcinoma (MCF-7), lung adenocarcinoma (H23), liver hepatocellular carcinoma (HepG2), cervical cancer (Ca Ski), and normal fibroblast (BJ-5ta) cells using a MTT cell viability assay and reported that pistachio hull extract has anti-tumor and anti-angiogenic potentials. It is also suggested that the apoptosis induction and angiogenesis potential of pistachio hull extract makes it a suitable product for further cancer research. In a similar kind of study, pistachio extracts exhibited noteworthy cytotoxic potentials against adenocarcinomic human alveolar basal epithelial cells (A-549), MCF-7, and HeLa human cancer cells, compared to HUVEC control cells [113]. It is also reported that the parts of pistachio known as waste parts such as hulls and oleo-gum resins were found to possess higher cancer prevention potentials, compared to those of the part consumed as food such kernels. The antioxidant properties of pistachio on reduction of precancerous colon cancer lesion in rats were evaluated and it was found that pistachio enhanced activities endogenous antioxidants such as glutathione-s-transferase (GST), glutathione (GSH), glutathione peroxidase (GPx) super oxide dismutase (SOD) and catalase. Glutathione is a very important non enzymatic antioxidant which offers protection against reactive oxygen species (ROS) as well as exogenous carcinogens [114]. This study also showed reduced incidence of Aberrant Crypt Foci (ACF) and crypt multiplicity which are the earliest identifiable neoplastic lesions in the colon carcinogenetic model. There are several studies on the anticancer properties of pistachio products and they all indicate toward the presence of phytochemicals such as flavonoids, quercetin and kaempferol and their antioxidant, antimicrobial, enzyme inhibitory and radical scavenging effects to control cancer [115–117]. Pistachio consumption and the overall health benefits are presented in **Figure 4**.

3.4 Cashew nuts

Cashew (*Anacardium occidentale*) is an evergreen tree native to Central and South America. However, now a days, cultivated widely in Africa, India, Vietnam and Sri Lanka. India is a major producer, processor, consumer and exporter of cashew in the world. Nutritional composition of cashew nuts includes good quantity of MUFA, squalenes, phytosterols, β-carotene, lutein, zeaxanthin, α-tocopherol, γ-tocopherol and thiamin [24, 118]. Cashews nut is also very good source of copper and zinc. The consumption of cashew nut and relationship between different disease conditions is presented in **Table 9**.

3.4.1 Cashew nut and cardiovascular disease

The micronutrients such as folate and tocopherols what found in cashew nut are very important in terms of protecting against atherosclerosis and other chronic non-communicable diseases (CNCD) [119]. Various epidemiological studies have drawn a link between folate status and atherosclerotic vascular disease. The relationship between low serum folate levels and increased cardiovascular disease risk has also

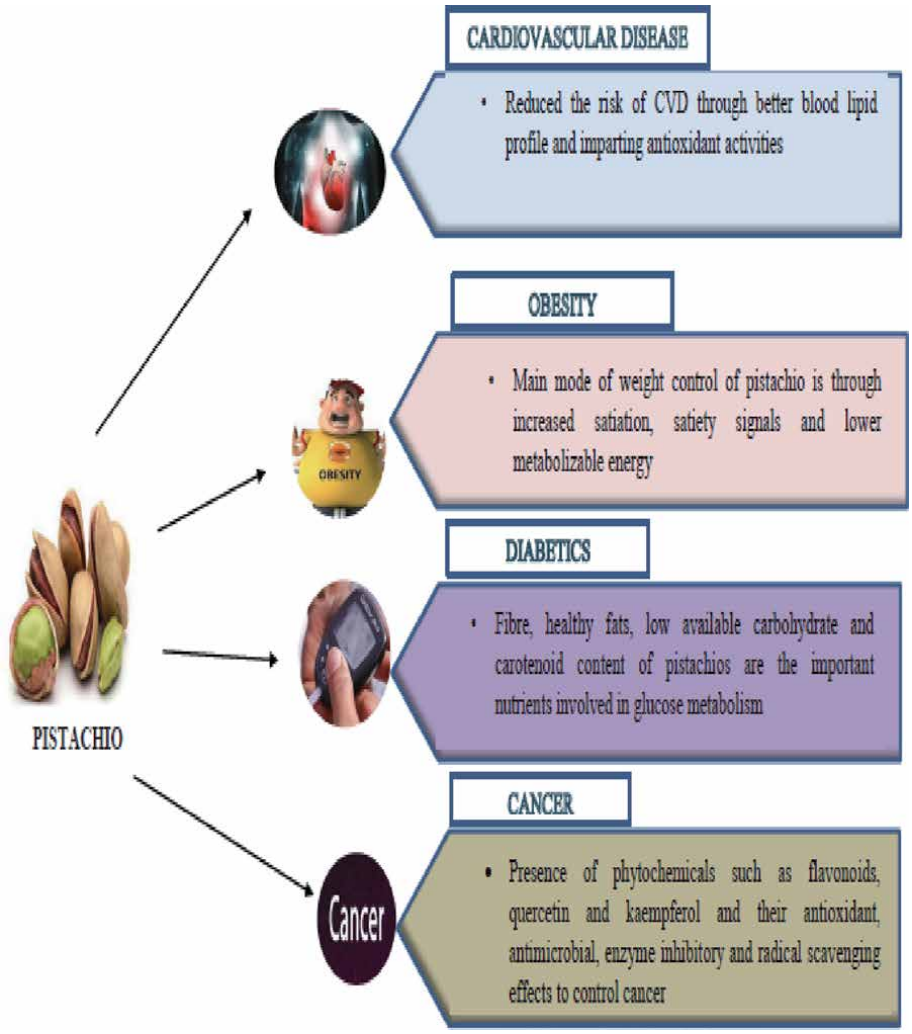

Figure 4.
Pistachio consumption and health benefits.

been seen in many studies [120, 121]. Dietary factors such as single high fat meal, oral fat load, animal protein and less fruit and vegetable consumption causes temporary endothelial dysfunction and increases cardiovascular disease risk [122, 123]. Reports claim that the supplementation of folic acid completely prevented the observed diet-induced impairment in endothelial function [124]. The mechanistic principle behind the action of folic acid is that, it reduces the plasma homocysteine levels which happen to be a causal factor in cardiovascular disease [125]. Tocopherols are strong antioxidants which exert cardiovascular (CV) benefit, including inhibition of oxidation of low-density lipoprotein (LDL) cholesterol in plasma [126]. Cashew nuts because of their high saturated fat content were exempted from "heart healthy" health claim by Federal Drug Administration in the year 2003. However, many recent studies claim the ability of cashew nuts to lower total and LDL-C [127]. In 2018, a 12-week randomized controlled study conducted by Mohan V and colleagues [128] reported that cashew nut supplementation reduced systolic blood pressure and increased HDL-C in Asian Indians with type-2 diabetes mellitus with no deleterious effects on body weight, glycemia, or other lipid variables.

Design and study population	Intervention	Duration	Main outcomes	Reference
Cardiovascular disease				
A randomized controlled trial (300 Asian Indians with T2DM)	30 g/day	12 weeks	Decreased systolic blood pressure and increased plasma HDL-C	[128]
Meta-analysis (five studies with 246 participants)	Increased cashew nut intake		Improved TG levels as well as systolic and diastolic blood pressure	[129]
Obesity				
Male Swiss albino mice (25–30 g) and Female Sprague Dawley rats (150–200 g)	200 mg/kg/day	40 days	Decreased body weight, LDL, VLDL, TG, TC and increased HDL level. Decreased fat-pad weights like Kidney fat, Mesenteric fat and Uterine fat	[130]
Diet-induced obesity (DIO) mouse model	Cashew apple extract 200 mg/kg	8 weeks	Reduced body-weight gain and fat storage. Lowered glycaemia, insulinaemia and insulin resistance	[131]
A randomized controlled trial (300 Asian Indians with T2DM)	30 g/day	12 weeks	No weight gain	[128]
Diabetes				
A randomized, controlled-feeding trial (50 patients with type 2 diabetes mellitus)	10% of total calorie from cashews	8 weeks	Decreased serum insulin and LDL-C/HDL-C ratio. Decreased HOMA-IR (homeostatic model assessment of insulin resistance). No change in body weight, BMI and waist circumference (WC)	[132]
Fructose-fed (diabetic) rats	Cashew plant stem-bark methanol extract 200.0 mg/kg body weight	21 days	Prevented changes in plasma glucose, triglyceride, total cholesterol/HDL-cholesterol ratio, malonyldialdehyde, urea, and creatinine	[133]
Cancer				
Six- to eight-week-old male BALB/c mice (20–25g)	50 mg/kg Anacardic acids from cashew nut shell liquid	30 days	Decreased levels of neutrophils and tumor necrosis factor in the lungs	[135]
HeLa cells	Cashew nut shell liquid (CNSL)		Inhibited cell proliferation and growth of *Bacillus subtilis*	[140]

Table 9.
Effect of Cashew nut consumption on different disease conditions.

According to the authors the MUFA content of cashew nut increased the level of apolipoprotein A-I (apoA-I) which is capable to increase the endogenous synthesis of functional HDL particles. However, the meta-analysis data of Mahboobi [129] showed that cashew can improve triglyceride levels as well as maintain systolic and diastolic blood pressure with no significant effects on other cardiometabolic factors such as total cholesterol, HDL-C, and LDL-C.

3.4.2 Cashew nut and obesity

Cashew nuts are rich in energy and dietary fiber which exerts beneficial effect on weight management. Dietary fiber can produce a feeling of fullness in the stomach, controls appetite suggested as an aid in weight loss diets. A study conducted by Asdaq and Malsawmtluangi [130] on the anti-obesity effect of cashew nut in rats fed on cafeteria and atherogenic diet reported that cashew high dose (200 mg/kg) is effective in decreasing body weight, lipid parameters like LDL, VLDL, TG, TC and increased HDL level. They also observed from the study that there was decreased fat-pad weights like Kidney fat, Mesenteric fat, and Uterine fat which showed the anti-obesity potential of cashew nut. The authors suggested the anti-obesity activity of cashew nut might be due to the presence of soluble fibers and flavonoids, namely, catechin, epicatechin, and epigallocatechin, which inhibit lipoxygenase. In another study, Beejmohun et al. [131] evaluated the effect of cashew nut apple extract on obesity and diabetes using the diet-induced obesity (DIO) mouse model. Two different designs: a 'prevention' design and curative design was adopted to evaluate the ability of the extract to prevent the development of obesity, hyperglycaemia and insulin resistance, and capacity to reverse an established disease state, respectively. In both the designs, cashew apple extract of 200 mg/kg body weight significantly reduced body-weight gain, fat storage, hyperglycaemia, hyperinsulinaemia and insulin resistance in DIO mice. It was suggested from the study that reduction in body-weight gain was at least partly due to a decrease in the peri-epididymal (perivisceral) adipose tissue mass. It has been seen that cashew nut supplementation in Asian Indians with type-2 diabetes had no deleterious effects on body weight [128].

3.4.3 Cashew nut and diabetes

An eight-week, randomized, isocaloric, controlled-feeding study was conducted by Darvish Damavandi et al. [132] on 50 patients with type 2 diabetes mellitus (T2DM) randomly assigned to either the control or intervention group with 10% of total calorie from cashews. The results demonstrated that replacing 10% of daily total energy intake with unsalted cashews reduced serum insulin and LDL-C/HDL-C ratio (as an atherogenic index) in patients with T2D. Authors suggested that the decrease could be due to the bioactive compounds, as well as unsaturated fatty acids (MUFAs and PUFAs) present in cashews, which may play an important role in insulin and lipid profile control. Also, fiber and polyphenols may have anti-diabetic effects by regulating microbiome and lipid profile ratio. In an animal model study, the cashew plant bark extract showed a potential antidiabetic activity. Methanol extract of cashew plant stem-bark at a dose of 200.0 mg/kg body weight improved plasma glucose and lipids in fructose-induced diabetic rats, which was associated with a reduced lipid peroxidation [133]. Viguiliouk et al. [10] through a meta-analysis reported that diet supplemented with tree nuts (almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios and walnuts) at a median dose of 56 g/day improved glycemic control in subjects with T2DM, showing significantly decreased HbA1c levels and fasting glucose with no effect on fasting insulin or homeostasis model assessment of insulin resistance index (HOMA-IR).

3.4.4 Cashew nut and cancer

The cashew nuts contain phenolic compounds which are strong antioxidants and capable of scavenging free super oxide radicals, reducing the risk of cancer [134].

However, there are many reports on the use of cashew nut shell liquid (CNSL) from cashew nut as a potent anti- cancer agent. Anacardic acids are the main constituents of natural CNSL. In a study, 50 mg/kg of anacardic acids ameliorated tumor necrosis factor in lungs induced by exposure to diesel exhaust particles in mice [135]. Anacardic acid was the first natural product inhibitor of histone acetyltransferase (HAT) activities reported [136]. HATs are the critical regulators of cell development and carcinogenesis [137]. It is also reported that anacardic acid presents anti-inflammatory and anti-invasive properties by suppressing tumor necrosis factor (TNF)-α-induced overexpression of anti-apoptotic proteins (e.g., Bcl-2, Bcl-xl, and survivin) and UV-induced tumorigenesis [138, 139]. In a study conducted by Ashraf and Rathinasamy [140] to check the antibacterial and anticancer activity of the purified CNSL, it inhibited the proliferation of HeLa cells with an IC50 of 0.004% (v/v) and inhibited the growth of *Bacillus subtilis* with an IC50 of 0.35% (v/v). It induced apoptosis in HeLa cells and accelerated wound closure in L929 cells. Authors concluded that CNSL have the potential to be used as anticancer and antibacterial drug development. These data suggests the anticancer role of anacardic acid. Anacardic acids (AAs) are alkyl phenols. Higher amounts of AAs have been detected in CNSL (353.6 g/kg) followed by cashew fiber (6.1 g/kg), while the lowest (0.65 g/kg) amounts were found in roasted cashew nut [141]. Cashew nut consumption and the overall health benefits are presented in **Figure 5**.

3.5 Other nuts

The tree nuts such as Brazil nut, hazel nut, macadamia nut, pine nut, pecans and legume, pea nut possess good quantity of protein, unsaturated fatty acids, dietary fibers, vitamins, minerals and different bioactive compounds, such as phytosterols, phenolic

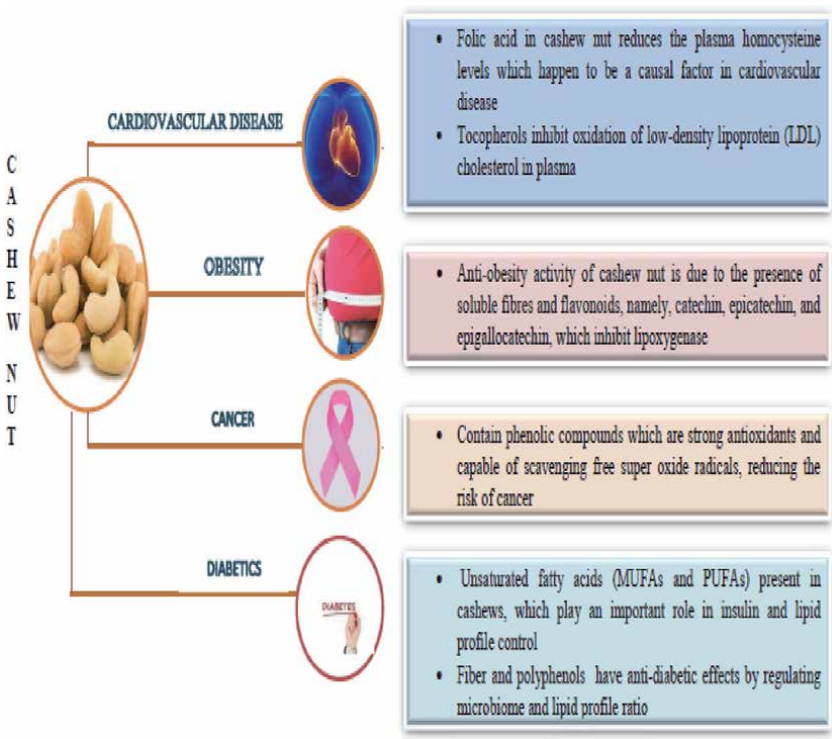

Figure 5.
Cashew nut consumption and health benefits.

compounds and carotenoids. These compounds are able to reduce cholesterol levels and promote antioxidant and anti-inflammatory effects. The consumption of some other nuts and relationship between different disease conditions is presented in **Table 10**.

3.5.1 Cardiovascular diseases

In a study, it was observed that 9 hours after the ingestion of 20 or 50 g of Brazil nut serum LDL-c level significantly reduced and HDL-c significantly improved. Interestingly, significant increase of the plasma selenium levels was observed at 6 hours within the groups receiving the nuts [142]. It was suggested from the study

Design and study population	Intervention	Duration	Main outcomes	Reference
Cardiovascular disease				
10 healthy subjects	20 or 50 g Brazil nut	9 hrs	Decreased LDL-C and increased HDL-C. Increased plasma selenium	[142]
A randomized crossover trial (30 volunteers aged 18 to 53 years)	Macadamia nut-based monounsaturated fat diet (37% energy from fat)	30 days	Decreased cholesterol and LDL-C	[146]
Obesity				
Obese female adolescents (n = 17)	15–25 g/day Brazil nut	16 weeks	Reduced total cholesterol and LDL-C. Improved microvascular function	[150]
A randomized, controlled trial (107 overweight and obese individuals)	60 g/day hazelnut	12 weeks	Improved blood cholesterol and no weight gain	[153]
A pilot clinical trial (24 healthy volunteers)	40 g/day hazelnut	6 weeks	No weight gain, improved antioxidant capacity. Up regulated of genes implied in oxidant reactions and inflammation	[154]
Cross-over, intervention study (15 healthy individuals)	66% of the energy provided by the peanuts	30 weeks	No change in body weight. No decline in pleasantness or hunger ratings	[155]
Diabetes				
Sixty type 2 diabetic patients (aged 43–81 years)	1 Brazil nut/day	6 months	Reduced oxidative DNA damage	[157]
A randomized, parallel-group (50 patients with metabolic syndrome)	30 g/day of raw nuts (15 g walnuts, 7.5 g almonds and 7.5 g hazelnuts)	12 weeks	Decreased lipid responsiveness but improved insulin sensitivity	[160]
Cancer				
A375 and HeLa cells	A methanol hazelnut shells extract (526 μg/mL)		A pro-apoptotic effect	[165]

Table 10.
Effect of some other nuts on different disease conditions.

that the change in lipid level could be due to the presence of selenium which mediates selenoproteins. Some selenoproteins such as glutathione peroxidase (GPx) and thioredoxinreductase (TrxR) are important antioxidant enzymes and can provide cardiovascular benefits [143]. Similarly, it was observed that hazelnut-enriched diets exerted antiatherogenic effect by improving endothelial function, preventing LDL oxidation, and inflammatory markers beyond only lipid lowering effect [144]. However, after 4 weeks on a hazel nut free diet, the beneficial effects got changed. Hazel nut is a good source of vitamin E, which protects low-density lipoprotein (LDL) against oxidation and a high level of L-arginine, which is precursor of nitric oxide and other bioactives, could be the reason of antiatherogenic effect. It is suggested from the study that hazelnut may be incorporated into daily diet without change in total caloric intake for sustained health benefit. It has also been seen in women with type 2 diabetes that frequent nut and peanut butter consumption (serving size, 28 g (1 ounce) for nuts and 16 g (1 tablespoon) for pea nut butter) was associated with a significantly lower CVD risk [145]. Macadamia nuts are good source of MUFA and have been tried in the diets to reduce the TC and LDL-cholesterol with positive results [146, 147]. Improvements of vascular function reduce CVD risk. It is indicated from many epidemiological studies that nuts have positive effects on vascular endothelial function [148, 149].

3.5.2 Obesity

Obesity is one of the reasons of morpho-functional microvascular damage. The intake of 15–25 g/day Brazil nuts improved the microvascular function in obese female adolescents BMI of 35.6 ± 3.3 kg/m^2. It is suggested that the bioactive substances of Brazil nut like selenium, α- e γ- tocopherol, folate and polyunsaturated fatty acids improved the microcirculation with their antioxidant capacity [150]. It is also observed that a high intake of Se from Brazil nut for long periods by obese women was harmful to health which was associated with the gene expression of some inflammatory markers [151]. The tolerable upper intake level (UL) of Se is around 400 mg/day, for both males and females [152] could be the reason with high intake of Brazil nut and associated health problems. Experimental evidences suggest that consumption of hazel nut up to 60 g per day did not affect weight and improve blood cholesterol levels [153]. Renzo et al. [154] observed that when 24 healthy volunteers consumed 40 g of hazelnuts (261.99 kcal/1096.17 kJ) daily as a snack for six weeks, did not gain weight but a significant up regulation was detected for SOD1, CAT, macrophage migration inhibitory factor (MIF), PPARγ, vitamin D receptor (VDR), methyl enetetra hydrofolate reductase (MTHFR) and angiotensin I-converting enzyme (ACE) which was an indication of modulation in oxidative stress and inflammation gene expression. Alper and Mattes [155] reported that peanut consumption has little effect on energy balance when peanuts are added to, or incorporated into, an energy-controlled diet. Little change in body weight over 3 weeks with peanuts added to habitual diets. In a study when obese mice were supplemented with macadamia nut oil, hypertrophy of adipocytes, inflammation in the adipose tissue and macrophages were attenuated [156]. It is suggested from the study that the reduced/controlled growth of adipocytes brings more cellular homeostasis and decreases leptin production and secretion. Otherwise, leptin is associated with an elevation in low grade inflammation.

3.5.3 Diabetes

Diabetes elicits oxidative stress and causes DNA damage. Prevention of DNA oxidation is extremely important and through dietary interventions, T2D-related

complications and DNA damage can be prevented. In this regard, Mecan et al. [157] investigated the correlation of Brazil nut supplementation and DNA damage in T2D patients and found significant increase in fasting blood glucose after six months of consuming Brazil nuts; however, no significant effect was observed on the levels of HbA1c level. The cells were more resistant to H_2O_2-induced DNA damage after six months of supplementation with Brazil nut. It was concluded from the study that consumption of Brazil nuts could decrease oxidative DNA damage in T2D patients, probably through the antioxidative effects of Se. An 8-week controlled randomized parallel study in patients with T2D indicated that incorporation of hazelnuts into diet (10% of total daily calorie intake was replaced with hazelnuts) in intervention group can prevent reduction of HDL-C concentrations in patients with type 2 diabetes, but had no effect on FBS or other lipid profile indices [158]. However, it is suggested from the studies that the Mediterranean-style diet with 30 g of mixed nuts (15 g/d walnuts, 7.5 g/d hazelnuts and 7.5 g/d almonds) could improve FBS concentration after 3 months in patients at high-risk for CVD [159]. In contrast, Casas-Agustench et al. [160] demonstrated that incorporating 30 g of mixed nuts (15 g/d walnuts, 7.5 g/d hazelnuts, and 7.5 g/d almonds) into a healthy diet for 12 weeks did not affect FBS. Study conducted by Jiang et al. [161] very elaborately discussed about the nut and pea nut butter consumption on the diabetes outcomes. The authors reported that nut and peanut butter consumption was inversely associated with risk of type 2 diabetes. Diabetes reduced 27% in those who ate nuts 5 or more times per week compared with those who rarely or never ate nuts.

3.5.4 Cancer

Ip and Lisk [162] reported the relationship between Brazil nut and prevention of mammary cancer. They compared the selenium in Brazil nut with selenite selenium and found both were equally bioactive. Their study demonstrated a dose-dependent inhibitory response at dietary Se concentrations of 1–3 mg/g in dimethylbenz[a] anthracene-treated rat model. The anti-carcinogenic activity of Se has also been tried in other animal models with positive response. Selenium is an important component of antioxidant enzymes and the beneficial effects in terms of inhibition of cell proliferation, triggering apoptosis and repairing DNA activating p53 is mainly because of the antioxidant properties [163]. Antimutagenecity and anticancer activities has also been reported from fresh hazelnuts whereas dried hazelnut shows moderate activity [164]. Hazenut shell extract contains phenolic compounds, including neolignans, and a diarylheptanoid which has strong antioxidant properties. It is a potent anticancer agent and showed an inhibitory effect on the growth of human cancer cell lines A375, SK-Mel-28 and HeLa [165]. Macadamia nut is a good source of tocopherols, tocotrienols, and squalene which can confer antioxidant and anticancer properties to consumers. Nieuwenhuisa and Brandt [166] conducted a large prospective cohort study and reported that total nut, tree nut, and peanut intake reduced the risk of small cell carcinoma in men.

4. Conclusion

All nuts contain good quantity of MUFAs, PUFAs, fibers, vitamins, minerals and bioactive compounds with antioxidant potential which makes them a good candidate in human diets. Almond consumption reduces serum triglyceride, total cholesterol and LDL-C in the subjects mainly due to the presence of vitamin E and phenolic compounds in it. Similarly, almond consumption also helps in weight loss, lowered glycosylated hemoglobin, and reduced blood glucose when tried in patients

with obesity and diabetes. The benefit of almond for cancer prevention is mainly due to the phytochemicals such as quercentin and kampferol which is known for reducing prostrate and lungs cancer cell growth. Walnut consumption is also very useful for reducing cardiovascular disease risk. Walnut intake not only improves blood lipid profile by reducing triglyceride and total cholesterol but also improves flow mediated dialation, HDL-C level and apolipoproten B status which is very useful for heart health. Despite an energy dese nut, walnut did not contribute to weight gain in any of the trials with obese patients but helped in controlling systolic blood pressure. In diabetic patients walnut intake reduces fasting blood sugar and HBA1C. Walnut contains tocopherol, βsitosterol, and peduncalgin which have anti-cancer properties. In human cell line studies for role of walnut in cancer prevention found walnut oil is capable of inducing necrosis and down regulated NFkB. Pistachio intake increases serum antioxidants and reduces oxidized LDL-C through which imparted beneficial effect on cardiovascular health status. It also controls obesity and diabetes through increasing satiation and regulating glucose metabolism. The hull extract of pistachio shows anticancer properties and radical scavenging activities. Almond, walnut and pistachio consumption in a range of 42 to 85 g/day was found to be beneficial for human health. Cashew nut is very common and popular among nuts. The intake of 30 g/day cashew nut is beneficial for heart health and also does not contribute to weight gain. In an animal model study, the cashew plant bark extract showed a potential antidiabetic activity. Cashew nut shell liquid (CNSL) from cashew nut is a potent anti- cancer agent. The phenolic compounds present in cashew nut are also strong antioxidants and capable of scavenging free super oxide radicals, reducing the risk of cancer. Similarly, intake of Brazil nut and hazel nut in a range of 25–50 g/day imparted beneficial effects on antioxidant status and reduced risk of diabetes and cancer. Moreover, daily nut intake of tree nut or pea nut has demonstrated an active role in controlling inflammation, dyslipidemia and oxidative stress.

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Section 3

Fungal Contamination of Nuts

Chapter 4

Fungal Contaminants and Mycotoxins in Nuts

Giulia Mirabile, Patrizia Bella, Antonio Vella, Vincenzo Ferrantelli and Livio Torta

Abstract

Contamination by fungi and mycotoxins in nuts has achieved much attention in recent years. In fact, the fungal metabolites produced by the species of *Aspergillus*, *Penicillium* (aflatoxins and ochratoxins), *Fusarium* (trichothecenes, zearalenones and fumonisins) and *Alternaria* (alternariotoxins) with toxic and/or carcinogenic effects are considered a threat to human and animal health. In this chapter we will discuss the main fungal *taxa* and related mycotoxins most frequently associated with these materials. In this regard, the first results on the level of contamination by fungi and mycotoxins in samples of almonds and pistachios of different origins will be reported. The main strategies to reduce the risk of contamination will also be recommended.

Keywords: nuts, contaminating fungi, mycotoxins, *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*

1. Introduction

Due to high nutritional value, pleasant taste and importance also in the pharmaceutical and cosmetic fields, nuts have been consumed by humans for thousands of years all over the world [1, 2]. Furthermore, some nuts are included in feed for several animal species. However, both the allergic forms to these products [3] and the health risks for prolonged and massive ingestion of mycotoxins present in these foods [4–6] are widely documented. Nut allergy is a hypersensitivity to some organic substances of this food caused by the consumer's immune system, whereas the risk of mycotoxicosis is related to the health of the nut. In particular, contaminating mycotoxins are produced by various saprophytic or phytopathogenic fungal microorganisms that can infect nuts and other foodstuffs throughout the whole production chain, from the farm to the plate [7].

Mycotoxins are a heterogeneous group of organic substances, generally characterized by low molecular weight, active at extremely low concentration (parts per billion, ppb, or parts per trillion, ppt; μg/L and ng/L, respectively), very resistant to degradation, produced by the secondary metabolism of numerous species of filamentous fungi (molds). When these microorganisms infect and develop on food and feed, they release the toxic molecules which, once ingested by the consumer (man or animal), can cause various acute (rash, vomiting, diarrhea, headache, etc.) or chronic (nephropathies, immunosuppression, carcinogenic effects, etc.) damages [8].

The health risks may arise from their carcinogenicity, so much so that the International Agency for Research on Cancer (IARC), based on epidemiological data, studies on cancer in experimental animals and mechanistic studies, has evaluated the carcinogenic risk of some mycotoxins in humans [9]. In order to protect the consumer from excessive exposure to the most dangerous mycotoxins, several Countries and federations of States have issued regulations that limit the maximum permissible content in a wide variety of foods for human and zootechnical use (for the EU, Commission Regulation (EC) No 1881/2006, Official Journal of the European Union, L. 364/5, 20.12.2006; Commission Regulation (EC) No 401/2006, Official Journal of the European Union, L70/12, 9.3.2006 and subsequent updates).

From the first report of a turkey disease ("turkey X disease") [10] associated with feeding these poultry with mycotoxin-contaminated peanuts (groundnuts), a large number of publications concerning the presence of fungi and their mycotoxins contaminating nuts have been spread all over the world. It is now known that among the mycotoxins most harmful to the consumer, listed by the IARC, are indicated aflatoxins, produced by fungi of the genus *Aspergillus*, ochratoxins produced by fungi of the genus *Aspergillus* and *Penicillium* and numerous others produced by different species of *Fusarium*, all capable of infecting nuts [7, 11]. Furthermore, several other mycotoxins, the "emerging mycotoxins" (alternariotoxins, enniatins, sterigmatocystein, etc.), produced by fungal species belonging to the aforementioned or to others genera (*Alternaria*), found in different nut samples, are currently being studied for their presumed dangerousness [12, 13].

2. Mycotoxins and associated risk to human health

The acute and chronic damages from massive and prolonged ingestion, inhalation or cutaneous absorption of mycotoxins are well documented and reported from all over the world.

From a historical point of view, the best known and described mycotoxicosis is ergotism (ergot of rye), widespread in some European regions since the Middle Ages. The disease is due to the ingestion of *Claviceps purpurea* sclerotia (rich in clavine alkaloids, lysergic acids, simple lysergic acid amides and peptide alkaloids), ground up with grains of various infected plants of cultivated and spontaneous species of the *Pooideae* subfamily (rye, millet, barley, oats, triticale, wheat, etc.). Two types of ergotism have been documented: convulsive and gangrenous. The symptoms included convulsions, hallucinations, skin burning and gangrene due to the constriction of the blood vessels, loss of hands and feets [14].

Several clinical studies have highlighted the correlation of mycotoxin effects with various pathological states in consumers, even if in most cases a "cause–effect" relationship is not clearly demonstrated [15]. Only for aflatoxins (in particular aflatoxin B1, AFB1) produced by some *Aspergillus* and *Penicillium* species, have been ascertained carcinogenic, hepatotoxic, teratogenic, and mutagenic effects on human health [16]. For this reason, IARC included the aflatoxins in the Group 1B: "Carcinogenic for humans" [17]. The risk of liver cirrhosis and immune effects, acute diseases such as hemorrhagic liver necrosis, edema and lethargy, up to death, have also been reported in frail individuals, children and in high-risk areas in developing countries [18–20]. Moreover, AFB1, once ingested by animals, is metabolized in aflatoxin M1 (AFM1) and excreted in the milk. Hence AFM1, included by IARC in the Group 2B, "Possibly carcinogenic to humans" [17], can be found in milk or milk products.

As regards ochratoxins, and in particular, ocrhatoxin A (OTA), carcinogenicity on laboratory animals is known, as well as damage to the kidneys, heart and liver injuries in humans. To date, there is no evidence of direct effects on the

appearance of carcinogenic phenomena on humans [21]. According to the IARC, OTA is included in the Group 2B [17]. OTA has been also associated with the Balkan endemic nephrophaty (BEN), disease affecting the rural populations of the Balkans and resulting in a high incidence of chronic kidney problems and cancers of the organs of the excretory system [22].

With reference to mycotoxins produced by fungi belonging to the genus *Fusarium*, the toxicological data indicate that fumonisins, trichothecenes (deoxynivalenol, T2 toxin, HT2 toxin) and zearalenone caused evident and known effects on humans, while others are considered a potential risk for consumer health. In particular, the fumonisins produced mainly by *F. verticillioides* and related species are suspected to cause esophageal cancer [23–26]. Fumonisin B1 is included in the IARC Group 2B [17].

Among the trichothecenes, deoxynivalenol (DON, known also as vomitoxin) produced by *F. graminearum* and related species, shows acute toxicity in animals (gastroenteritis, immunotoxicity, cardiotoxicity, refusal of feed, etc.) and poisoning in humans, with various symptoms such as nausea, vomiting, diarrhea and fever, as reported in China in the second half of the last century [27]. Because of the lack of data on its carcinogenicity in humans and only limited evidence on its carcinogenicity in experimental animals, DON is not classified by IARC [17]. T2 and HT2 (metabolized from the T-2 toxin after ingestion) toxins are considered agents of cytotoxic and immunosuppressive effects, which can cause acute intoxication and chronic diseases in both humans and animals. The fungal species most involved in the production of these secondary metabolites are *F. langsethiae*, *F. poae* and *F. sporotrichioides* [28]. The toxins derived from *F. sporotrichioides* are classified by IARC into Group 3 of carcinogenic substances [17]. Trichothecenes have been identified as the toxic agent in cases of Alimentary Toxic Aleukia (ATA, septic angina) associated with the consumption of moldy grain by both animals and humans in the USA, Japan, the former Soviet Union and elsewhere. The symptoms of the disease are characterized initially by general toxic stage (headache, weakness, fever, nausea, vomiting, gastroenteritis, etc.), followed by leukopenic stage manifested by changes in blood and, finally the angina-hemorrhagic stage [14].

Zearalenone (ZEA), mainly produced by *F. graminearum* and related species, does not cause acute poisoning in humans, but no studies on carcinogenic effects have been reported. ZEA caused an increased incidence of tumors in liver and pituitary cells in mice, but no carcinogenic effect was seen in rats, therefore considering limited carcinogenic effects in animals [17].

Aflatoxin, trichothecenes, ochratoxin A, fumonisins, zearalenone, fusarochromanone, have been shown to cause also immunosuppression and increase the susceptibility of animals to infectious disease [29].

Mycotoxins can pose several risks to human and animal health. The risks associated with health have often been characterized, however the mechanisms by which these toxins cause such damages have not yet well defined. Anyhow, the quantity and duration of exposure to mycotoxins, the synergy between the various fungal metabolites, the genetic predisposition and physical conditions of the consumer and other factors, can ultimately play a fundamental role in the manifestation of their toxic activity.

3. Main mycotoxigenic fungal genera in nuts

3.1 The genus Aspergillus

Aspergillus, a widespread Ascomycota genus belonging to the *Aspergillaceae* family, is divided in 6 subgenera and 27 sections with more than 400 species [30]. It

received its name from Michieli in 1729 that, viewing the microscopic structure of its conidiophore, was reminded of the device called in Latin "aspergillum", used to sprinkle holy water during the liturgy [31]. *Aspergillus* species grow most as saprophytes on decaying vegetable organic matter, but they can also colonize human tissues, home and hospital environments. Furthermore, *Aspergillus* is one of the most important genera with high economic and social impact due to its ability to produce mycotoxins, dangerous secondary metabolites with potential carcinogenic activity against humans and animals [32]. On the other hand, some species are employed in several industrial and food production processes (production of citric acid, gluconic acid, kojic acid, amylases, cellulases, hemicellulases, soy sauce and miso) [33].

In this fungal *Taxon*, conidiophore is the most important microscopic structure for its identification. During mycelial growth, hyphae called "foot cells" form a single conidiophore perpendicular to cell axis. The conidiophore presents a large apex that form a rounded or elliptical vesicle (columella). In uniseriate species (*A. fumigatus*), the fertile area of the vesicle is surrounded by a layer of cell called phialides that produce long chains of conidia or conidiospores (**Figure 1a**). In biseriate *Aspergillus* species (*A. niger*) another layer of hyphae called metulae exist between the vesicle and the phialides. In some *Aspergillus* species (*A. flavus*), conidiophores can be uni- or biseriate. The vesicle with phialides, metulae if are present, and conidiospores form the "conidial heads" [32]. The size of conidiophore and conidial head, the presence of metulae, the shape and the color of conidiospore are important identifying features. All these structures are typical of asexual reproduction, but in *Aspergillus* sexual reproduction also occurs and its microscopic features (cleistothecia, asci, ascospores, Hülle-cells) are also important for identification. Macroscopically, colonies are granular or with a suede-like surface consisting of dens felt of conidiophores. Growth is usually very fast and mature colonies are white, yellow, yellow-brown, dark brown-black, green, gray or light blue in color (**Figure 2a** and **b**) [34].

Aspergillus species are thermophilic, preferring hot humid climates but can grow in a wide range of temperature (7–42°C). Spores are usually present in aerosol and can be easily dispersed by air for long distances. When they find optimal conditions of temperature and humidity, they germinate starting the colonization of the substrate. Most of *Aspergillus* species are saprophytes and soil inhabitants. Human and animal foods are ideal organic substrates in which, thanks to their enzymatic activity, they can grow degrading chemical components like hemicellulose, celluloses, pectins, but also fats and oils. They can contaminate major agricultural commodities before or after harvest, causing decay in storage or disease in plants. Although acid pH and low amounts of water normally do not support fungal growth, most of *Aspergillus* species are able to grow at these conditions, colonizing foods and nuts [35].

During the last decades of '900 the discovery of toxins produced by *Aspergillus* associated with poultry and other domesticated animals' deaths all over the world, raised new awareness that *Aspergillus* were very dangerous for both human and animal health [36, 37].

There are 4 groups of aflatoxins (AFs): aflatoxins B1 and B2, aflatoxins G1 and G2. Other mycotoxins, as M1 and M2, originating from their metabolism in humans and animals, can be found in milk and dairy products [38]. Aflatoxins are the most important and dangeroustoxins produced by *Aspergillus* in food, feed and nuts, especially in peanuts. They are produced mostly by some strains of *A. flavus* and *A. parasiticus*. In recent years other species were classified as aflatoxigenic, like *A. bombycis*, *A. ochraceoroseus*, *A. nomius* and *A. pseudotamari*, but compared to the first two mentioned, they are found less frequently in foodstuffs [39].

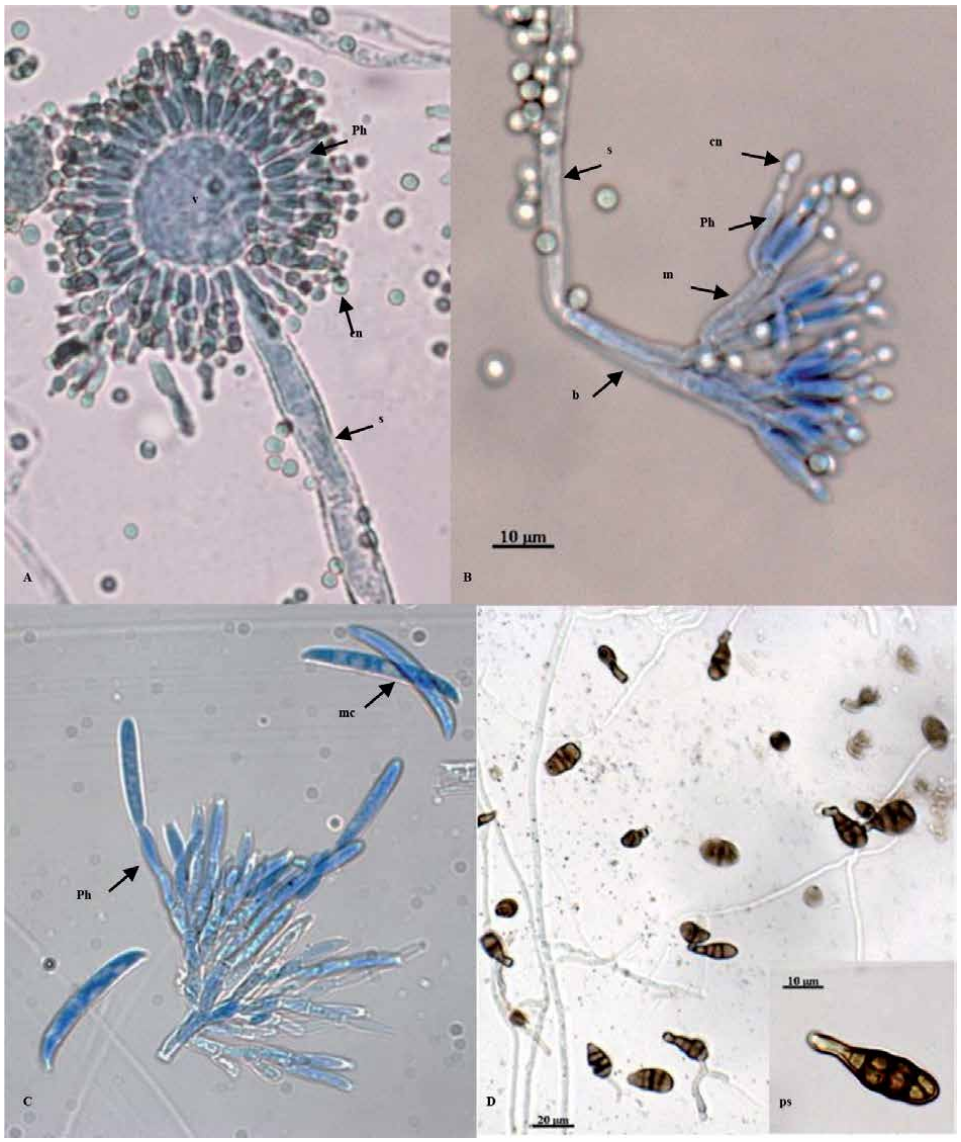

Figure 1.
Microscopic characteristics of Aspergillus *sp. (A),* Penicillium *sp. (B),* Fusarium *sp. (C),* Alternaria *sp. (D). cn = conidia; Ph = phialides; v = vesicle; m = metulae, s = stipe; b = branch; mc = macroconidia; ps = pluriseptate conidium of* Alternaria *sp. Scale bar: A-C = 10 µm; D = 20 µm.*

Other secondary toxic metabolites produced by *Aspergillus* species and in particular by *A. niger* and *A. ochraceus*, are ochratoxins. This group includes ochratoxin A (OTA), ochratoxin B (dechlorinated OTA) and ochratoxin C (ethylated OTA). Among them the most studied for its high diffusion and its toxicological importance is OTA. This toxin, has nephrotoxic effects in humans and animals and has carcinogenic action [38, 40].

3.2 The genus *Penicillium*

Penicillium is one of the most common fungi occurring in a wide range of habitats with worldwide distribution and high impact on economy, human and

Figure 2.
Nuts contaminated with Aspergillus flavus *(a),* A. niger *(b),* Penicillium *sp (c),* Fusarium *sp (d).*

animal life. Its name derives from its conidiophore shape, with a brush-like appearance (a penicillus). It belongs to *Aspergillaceae* family and it's divided in 32 sections with almost 500 species; the teleomophs are ascribed in ascomycetes genera, as *Eupenicillium* and *Talaromyces* [30]. In nature, Penicillium species live decomposing organic materials. On the contrary, other species of *Penicillium* are widely used in the food industry for the production of cheeses such as Roquefort and fermented sausages [41].

For *Penicillium* identification the conidiophores are of great taxonomic importance, resulting from the different branching of the stipe. Depending on the species, in fact, there may be only phialides at apex of stipe, producing conidia, metulae and phialidia, or different order of metulae and phialides. In particular, conidiophore shapes range from being simple to quarter-verticillate depending on the branching levels between the stipe and metulae and phialides. Conidia, typically globose, are produced in long chains by phialides and, when mature, show different colors (**Figure 1b**). Macroscopically, colonies appear of velvety consistency, they are fast in growth and with color varying from green to light blue (**Figure 2c**) [41, 42].

Ecologically, *Penicillium* species live as saprophytes showing optimal growth at low and moderate temperature ranging from 5 and 37°C. Some species colonize decaying vegetation, other are specialized in infecting food commodities. *Penicillium* spores are consistently spread by airborne dispersion and contaminate a large variety of organic substrates. Rapid fungal colonization and intense enzymatic activity cause the spoilage of infected materials. *Penicillium* can invade plants before harvest or during storage. They are also capable to grow during drying process, colonizing substrate poor in water, like nuts [43, 44].

In addition to damages caused in fruits and vegetables by *Penicillium* colonization, some species are able to produce a great variety of mycotoxins. These include ochratoxin A, patulin, citrinin, penicillic acid, cyclopiazonic acid, citreoviridin, roquefortine C and other secondary metabolites. Ochratoxin A and patulin are the most studied due to their worldwide diffusion and their implication in animal and human diseases [45]. In particular, exposure to these two toxins results in mutagenic, teratogenic, neurotoxic, genotoxic and nephrotoxic effects or acute effects like nausea and gastrointestinal damages. The major producers of ochratoxin A in nature are *P. verrucosum* and *P. nordicum*, while *P. expansum* is the major producer of patulin [46]. While patulin is commonly detected in fresh fruits like apple and its derivates, ochratoxin A can be found in a large number of food products including nuts [47].

3.3 The genus *Fusarium*

The anamorphic fungi belonging to genus *Fusarium* are ubiquitous, growing in soils but also in living or dead plants and in several commodities. These ascomycetes, ascribed to *Hypocreaceae* family and mainly included in the genus *Giberella*, currently contains several complexes species. Some of them, in particular those contaminating commodities, produce several mycotoxins with acute and chronic effects on human and animal health [48, 49].

Microscopic characteristics such as macro-, microconidia and chlamydospores of *Fusarium* are distinctive for genus identification. Macroconidia, produced from phialides sometimes gathered in sporodochia, are long, slender, dorso-ventrally curved (spindle-shaped), pluricellular and with a basal foot cell near the attachment point to the phialide (**Figure 1c**). Is this particular shape of macroconidia that defines the genus. Microconidia, formed from aerial mycelium are little propagule, mono or bicellular, smooth, hyaline and ovoid or cylindrical in shapes. Chlamydospores are overwintering structures, rounded, produced vegetatively from mycelium, able to resist to unfavorable conditions and germinate under the onset of suitable conditions. Macroscopically, colonies are wooly or cottony, flat and presents several colors such as white, cream, tan, salmon, cinnamon, violet, purple (**Figure 2d**) [50, 51].

Fungi of the genus *Fusarium* generally prefer cool climates and have a complex ecology. As saprotrophs, they live in the soil, on crops or on decaying organic material. Many species are primary pathogens in field, capable of causing vascular diseases. The most affected crops in the field are wheat and corn, but infection by these fungi can also occur in vegetables, nuts, ornamental plants, trees and foodstuffs during post-harvest storage [7, 52, 53]. Some *Fusarium* species produce dangerous mycotoxins that cause cellular toxicity, effects on animal growth and development and human cancer [54]. The main toxic classes produced by *Fusarium* include trichothecenes such as T-2 toxin, deoxynivalenol (DON) and nivalenol (NIV) produced mainly from *F. sporotrichoioides*, *F. graminearum* and *F. culmorum*; fumonisins B and B2 produced mainly from *F. fujikuroi* and *F. proliferatum*; zearalenone produced from *F. culmorum*, *F. graminearum* and *F. crookwellense* [55].

3.4 The genus *Alternaria*

The genus *Alternaria* belongs to the Ascomycota family of *Pleosporaceae* and it is divided into 26 section and approximately 300 species (*Lewia* is the most known sexual stage), characterized by saprophytic and pathogenic species causing plants diseases, postharvest damages and humans' allergies [56]. Some species of *Alternaria* also produce several mycotoxins in infected plants and foods [57].

Macroscopically, the colonies are flat, downy to wooly and the surface is grayish-with at the beginning and greenish-black or olive-brown at maturity [58].

Characteristics of *Alternaria* is the production in chains of dark-colored and multi-celled conidia ovoid to oblcavate, with longitudinal and transverse septa and a beak tapering apical cells (**Figure 1d**) [59, 60].

Mycotoxigenic fungi	Nuts	References
Aflatoxigenic fungi		
Aspergillus flavus	Almonds	[65–69]
	Hazelnut	[65, 66, 70, 71]
	Peanuts	[65, 66, 69, 72–74]
	Pistachios	[65, 66, 72, 74, 75]
	Walnuts	[11, 65, 66, 69, 72, 76–78]
A. parasiticus	Hazelnut	[70]
	Walnuts	[78]
Ochratoxigenic fungi		
A. ochraceus/*A niger*	Almond	[79]
	Chestnut	[80]
	Hazelnut	[74]
	Peanuts	[72, 73]
	Pistachio	[69, 72, 74]
	Walnuts	[69, 74–77]
Fusarium		
Fusarium spp.	Almonds	[65, 81, 82]
Fusarium spp.	Chestnuts	[81]
Fusarium spp.	Hazelnuts	[65, 74, 79, 83, 84]
F. sporotrchioides		
Fusarium spp.	Peanuts	[65, 66, 73, 83]
F. reticulatum		
F. sambucinum		
Fusarium spp.	Pistachio	[83]
Fusarium spp.	Walnuts	[65, 75, 78]
F. solani		
F. culmorum		
F.oxysporum		
Alternaria		
Alternaria spp.	Almonds	[81]
Alternaria spp.	Chestnuts	[81]
A. alternata	Walnuts	[75, 76]
A. atrans		
A. quercus		
A. tenuissima		

Table 1.
Most recurrent toxigenic fungi isolated from some nuts reported in recent studies.

Alternaria spp. colonize a wide range of plants and growth as saprophytes in plant residues, in soil or as fungal pathogens, colonizing mostly fruits and herbaceous plants and it is diffused in humid environment characterized by temperature ranging from 18 and 32°C [61]. *Alternaria* spp. can produce toxic metabolites that play a fundamental role in fungal pathogenicity and food safety. Today are known about 70 alternaria-toxins, some of them very dangerous for humans and animals [62], including alternaiol, alternariol monomethyl ether and tentoxin commonly founded in substrates like tomato, oil seeds, wheat, blueberries and walnuts. Toxicological data about mycotoxins produced by *Alternaria* are very limited but they have been shown to have cytotoxic, fetotoxic and teratogenic effect on animals [63]. Recent studies focused on emerging groups of mycotoxins produced by *Alternaria* species, described as potentially hazardous [64].

All the fungi mentioned above were frequently isolated from nuts all over the world (**Table 1**).

4. Detection methods of contaminating fungi and mycotoxins in nuts

In the last decades several laboratory methodologies were defined both to detect, enumerate and isolate fungi from nuts and to evaluate the presence of single specific mycotoxins and their respective concentrations. However, so far, only few methods can be considerate "official" and validate by Public Authority.

To apply isolation techniques, is necessary to take a representative sample to submit to the laboratory test and, also for this, the official regulations provide the correct methods of sampling, handling and pretreatment of the samples.

To detect, enumerate and isolate contaminating fungi from sampled nuts two cultural-based methods are most frequently applied: the dilution plating [85] and the direct plating [86]. In the first case, unshelled or shelled nuts are finely chopped or homogenized in a mixer and suspended in water or water containing 0.1% peptone. Serial decimal dilutions are spread on agarized nutrient medium in Petri dishes (plates). In the second case, nuts particles (or whole seeds) are placed directly on media, eventually after surface sterilization in sodium hypochlorite.

The agarized artificial nutrient media most employed are:

PDA (Potato Dextrose Agar), also known as the "universal substrate", it is mainly used to stimulate the growth and sporulation of filamentous fungi;

SDA (Sabouraud Dextrose Agar) is the medium normally used for the primary isolation of fungi, often with the addition of antibiotics. This medium allows the detection of the "standard" morphology, limiting the development and sporulation of fungal colonies;

DG18 (dichloran-glycerol agar), recommended for the evaluation of CFU and the isolation of yeasts and molds from dry and semi-dried foods, including fruits, spices, grains, nuts, meat and fish products [85];

DRBC (Agar Dichloran Rosa Bengal Chloramphenicol), promotes the selective growth of molds and yeasts present in food [87];

PCA (Agar Plate Count) is an agar medium that allows the non-selective growth of molds, yeasts and bacteria;

OGYE AGAR BASE, contains yeast extract, useful for the growth and UFC count of molds in clinical, food and dairy product samples.

Media recommended for *Aspergillus* and *Penicillium* identification include Czapek Yeast Autolysate agar (CYA) and Malt Extract agar [34, 41]. More characters useful for other taxonomic characters can be obtained by using other media such as

Czapek's agar (CZ), Yeast Extract Sucrose agar (YES), Oatmeal agar (OA), Creatine Sucrose agar (CREA), Dichloran 18% Glycerol agar (DG18), Blakeslee's MEA and CYA with 5% NaCl [34, 41].

Specifikke nutrient-arme agar (SNA), potato dextrose agar (PDA) and YES agar can be used for *Fusarium* identification while dichloran rose Bengal yeast extract sucrose (DRYES) agar and potato carrot agar (PCA) are suggested for *Alternaria* species [88, 89].

All plates are incubated at temperatures ranging from 25 and 30°C for up to 7–9 days and, every 3 days, observations are made on the number and type of fungal colonies grown. Higher incubation temperatures are useful to distinguish between species [34, 41].

In the methodology of the dilution plating the total number of fungal colonies (colony-forming units, CFU), referred to the relative decimal dilution, is used to calculate the level of fungal contamination of the analyzed sample. Moreover, observations under the stereoscopic microscope and the optical microscope allow to identify the genus of belonging of the colonies. It is therefore possible, on the total of the CFUs detected, to determine the percentage of the different mycotoxinogenic genera.

In direct plating analyses, results are usually expressed as percentage of infected particles/nuts.

Among the grown fungal colonies, some of the most representative, because probably belonging to mycotoxigenic species (yellow and black aspergillia, green and blue penicilli, *Fusarium* spp., etc.) are first transferred into plate containing agarized medium and purified to be submitted to identification tests and other analysis.

Although culture-based methods for detection and identification of fungal toxin producing fungi are widely used, they showed some limitation due to time-consuming and labour-intensive aspects. It also requires facilities and mycological expertise.

For this reason polyphasic approach based on morphological, physiological and molecular methodologies is suggested for a more accurate identification of mycotoxin fungi producer [89]. Morphological analysis, macro and microscopic observations are made, also growing the isolate on suitable media and at different temperatures. Macroscopic features such as shape, color, texture and speedy growth of the colonies, among others, and microscopic characteristics as mycelial structures, spores, conidia are often fundamental taxonomic characters to identify the species [31, 34, 41, 90–92].

It is not uncommon, however, that these characters are not sufficient for exact identification. In order to avoid mistakes it is possible to consider some physiological parameters (enzymatic activities, secondary metabolites production, etc.) that can provide further data for better identification [93]. It is always good practice, in any case, to support and confirm the identification of the fungal isolate with appropriate DNA based molecular analyzes. A DNA marker for reliable species identification is the internal transcribed spacer rDNA area (ITS) now accepted as the official barcode for fungi [94]. However, this locus is insufficient for correctly identifying some species and other possible secondary markers include 'nuclear large ribosomal subunit' (LSU rDNA) [95], the 'nuclear small ribosomal subunit' (SSU rDNA) [96], 'β-tubulin (BenA)' [97], 'elongation factor 1-α (EF-1-α)' [98] and the 'second largest subunit of RNA polymerase II (RPB2)' [99].

The identification of fungi using molecular markers is improved after DNA isolation from mycelium of pure and axenic cultures [100]. A great variety of DNA extraction methods are available [101]. Commercial kits and customized methods are typically employed. Critical to the successful isolation of nucleic

acids is the cell disruption step with an appropriate buffer (typically CTAB) and techniques that assure high quantity and quality of nucleic acids and no release of potential PCR inhibitors. After this step, nucleic acids can be purified to eliminate impurities [102]. Species-specific PCR methodologies targeting conserved genes or regions of taxonomical interest or by focusing on the mycotoxigenic genes have been extensively applied for identification of mycotoxigenic fungal contaminants [103–106].

In order to detect and quantify mycotoxins in nuts there are several analytical techniques, but official controls are carried out with screening methods, using immunoenzymatic techniques (ELISA) and with confirmatory methods as high-performance liquid chromatography with fluorescence detection (HPLC-FLD) coupled to tandem mass spectrometry (LC–MS/MS). In most cases, methods are validated on a single matrix and do not meet the supervisory bodies' needs that require different mycotoxin limits for different matrices. Regarding extraction method, solid-phase extraction (SPE), solid–liquid extraction (SLE), and liquid–liquid extraction (LLE) are common techniques used for LC–MS/MS analysis for mycotoxin. The challenge of mycotoxin analyses is that they have different proprieties and polarity. Therefore, the right choice of the extraction method can be difficult.

More recently new techniques were developed to detect low-level mycotoxin contamination, to reveal the presence of "masked mycotoxins", complex food matrices in which the mycotoxin contamination occurs or to evaluate the co-occurrence of more mycotoxins in the same sample [6].

5. Strategies to limit contaminating fungi and mycotoxins in nuts industrial chain

Fungal and mycotoxins contamination occurs both in field (pre-harvest), during harvesting and post-harvest management [107]. In particular, aflatoxigenic aspergilli are of significant concern in terms of consumer health.

Generally, in field, several factors not easily controllable, can have a significant impact on fungal infection, including: crops damages due to drought or to insect, delay in harvesting, extreme weather events such as heavy rains, and or sudden frosts. Moreover, it is well known that any management practice to maximize plant performance and decrease plant stresses will decrease fungal and AFs contamination. Proper agronomic practices (tillage, fertilization regimes, right plant density and irrigation), guaranteeing the best vegetative development of dried fruit plants, can represent a valid means of prevention. Different cultivars of nuts show different susceptibility to *A. flavus* and AF accumulation. The conversion of orchards into more resistant cultivars is one possible measure of control [108–111].

Moreover, control of parasitic insects or other biotic adversities can limit the development of contaminating fungi, able to settle in the fruits through the lesions they cause [112].

During the harvesting process, great care must be taken to maintain the integrity of the product, avoiding contact with the ground or with other materials with a high risk of contamination. The damaged products are certainly those most susceptible to contamination during the subsequent phases.

Transport and storage are two important stages to be monitored [68]. Drying should take place soon after harvest and as rapidly as possible, because the prolongation of temperature, humidity and ventilation conditions favorable to the development of microorganisms, can lead to irreversible contamination of the product by both fungi and mycotoxins.

Nuts differ in their storage requirements in function of oil and fatty acid compositions. Temperatures ranging from 4 to 15°C, kernel moisture content around 2.5%, a_w of about 0.7, or relative humidity below 80%, oxygen concentration below 2.5%, and dark conditions are ideal storage conditions for most tree nuts [107, 113].

In post-harvest some physical and chemical methods aimed at decontamination from aflatoxins can be effective for their control. Removal of visibly damaged nuts by manual or mechanical sorting prior to processing significantly reduces AF contamination, limiting the number of potentially contaminated nuts in subsequent processing steps [114]. Other processes, although not very convenient from a practical point of view, can allow to reduce the concentration of AF, such as chemical (ammonia) or thermal (peanut roasting) treatments. Some other technologies, such as irradiation and improved packaging materials, can also minimize post-harvest aflatoxin contamination [115].

However, the best solutions to reduce aflatoxin contamination and improve both economic sustainability and food safety, are the integration of pre- and post-harvest technologies.

In order to protect the health of consumers and ensure the fairness of international trade, FAO and WHO have developed a code of good practices to help contain the phenomenon (www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B59-2005%252FCXP_059e.pdf).

These practices are also applied to all nuts and are summarized in the following points:

- early harvest of the fruit, preferably manual in order to reduce mechanical damage and avoid contact with the ground;
- in the post-harvest period, select the product and dry it quickly, reducing the level of humidity and the possibility of fungal growth;
- maintain proper ventilation during product storage, so that the fungal population and the concentration of aflatoxins are as low as possible or better still non-existent;
- thoroughly clean and sanitize the premises to reduce the risk of contamination.

In addition to these practices, some studies have highlighted the possibility of using biological control strategies for the control of mycotoxinogenic fungi [112].

The definition of programs to monitor fungal and mycotoxin contamination in processed nuts are essential to ensure the food safety for the consumers and an effective scientific system for the control of mycotoxin levels is represented by the HACCP method (Hazard Analysis and Critical Control Point).

This method is based on the preventive analysis of hazards and on the control of so-called critical points detected during the production and manufacturing process [116].

The HACCP strategy is quite simple, at least in theory, as it considers all the steps of the supply chain, from the choice of the cultivars to the finished product ready for marketing and defines the control protocols on each phase of the process.

6. A study case: fungal contaminants and mycotoxins in almonds and pistachios in Sicily (Italy)

In last years (2016–2021) at the Experimental Zooprophylactic Institute of Sicily (Palermo, Italy), analyzes on contamination by total aflatoxins or AF B1 have been

carried out on 618 samples of pistachios, from both Sicilian and foreign origin, using the validate HPLC method coupled with fluorescence detection (FLD). Out of this large numbers of controls, only 7 samples were positive for total aflatoxins, whose contamination ranges were included between the values 0.2 and 1.7 micrograms per kg, lower than those required by European regulations.

In order to acquire information also on the state of contamination by mycotoxinogenic fungi, an investigation aimed at the isolation and identification of any fungal microorganisms present in both pistachio and almond samples was recently carried out. The samples were analyzed at the laboratories of the Department of Agricultural, Food and Forestry Sciences of the University of Palermo, for mycological tests and at the Experimental Zooprophylactic Institute of Sicily, for toxicological tests.

Shelled almond and pistachio samples were collected from different warehouses located in Southern Italy and analyzed in order to determinate total fungal contamination and the frequencies of toxigenic genera (*Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*). Isolation and enumeration of fungal colonies were carried out employing the dilution plating methodology [85] on PDA. All plates were incubated at 25±1°C for 9 days and the total fungal contamination was evaluated every 3 days. *Aspergillus*-like colonies found growing in the isolation plates were subcultured into PDA and monoconidial pure cultures were assigned to their corresponding section or species based on macroscopic and microscopic characteristics.

The macro-morphological features recorded were colonies diameter, color, shape and texture. Microscopic observations were performed using an Axioskop (Zeiss, Oberkochen, Germany) microscope coupled to an AxioCam MRc5 (Zeiss, Oberkochen, Germany) digital camera. The microscopic characteristics recorded were dimension of conidial heads, vesicle shape and diameter, presence of metulae, size and shape of phialides and conidia. Finally, morphological identification was carried out using taxonomic keys.

Although the toxicological tests on the samples of nine walnuts excluded the presence of aflatoxins, first results showed that fungal contamination in almond and pistachio samples ranged from 2.5x10 to $2.6x10^4$ and 2.5x10 to $1.5x10^3$ CFU/g respectively. The genera *Aspergillus* and *Penicillium* were recovered at high frequencies both in the pistachio and almond samples with significantly differences within the samples. Occasionally, other fungal genera were isolates (*Mucor*, *Rhizopus*, *Paecilomyces*, etc.), while no *Alternaria* or *Fusarium* colonies were observed. *Aspergillus* was the most predominant fungal genus both in almond (77%) and pistachio (89.8%) samples. *Penicillium* spp. frequencies ranged from <1 to 15.9%. These data are comparable to those reported in similar studies both in relation to the level of total fungal contamination an as regard the diffusion of the two considered genera [72, 74].

According to morphological characteristics, *A. flavus* and *A. niger* were identified in pistachio samples, whereas no *A. flavus* was isolated from almonds samples.

7. Conclusion

The problem of mycotoxins has become one of the aspects that most affect the nuts market, even if the real problem is represented by mycotoxinogenic fungi that can contaminate these commodities. Fungi infecting nuts in field can rapidly grow and produce mycotoxins during storage when conditions are suitable. Preventing AFs and other mycotoxins accumulation in the finished product can be achieved by either controlling the contaminating fungi or mycotoxins production in pre- or

post-harvest stages of production, by using any of several measures alone or in combination [108–111].

However, the more articulated and complex the production chain is, the greater is the risks and possibilities of contamination by fungi and their mycotoxins. Furthermore, although the concentration of the most dangerous aflatoxins may be very low or completely absent, some mycotoxigenic fungi may be present and in favorable condition can produce these secondary metabolites making the food no longer safe for the consumer.

In order to guarantee, products healthiness, mycotoxins detection should be supported by mycological analysis throughout the entire supply chain. Another appropriate practice should be to investigate the presence of a greater number of mycotoxins, given the great biodiversity of fungi potentially capable of contaminating nuts.

Several guidelines, therefore, have been developed to identify critical points in the production process and to define strategies aimed at directly and indirectly reducing the production and spread of mycotoxins.

Contamination prevention is necessary because most detoxification methods require testing and are not completely approved by the industry or follow all safety requirements. Quality assurance systems for tree nut industries, including supplier qualification, are essential to prevent the presence of toxigenic fungi and prevent the consequent production of toxins along the production chain.

Moreover, there is a significant interest in considering the impact of climate changes on mycotoxin-producing fungi during pre- and post-harvesting [117].

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Chapter 5

Nutrient Composition and Aflatoxin Contamination of African Sourced Peanuts and Cashew Nuts: Its Implications on Health

Modupeade C. Adetunji, Stephen A. Akinola, Nancy Nleya and Mwanza Mulunda

Abstract

Edible nuts are popular worldwide based on their varied attributes such as desirable taste, high nutritional value as well as some health benefits. Globally, the most popular edible nuts are groundnuts or peanuts, almond, cashew nut among others. Due to the rich nutritional composition of nuts, they tend to be prone to contamination by toxigenic fungi which could ultimately results in the release of fungal metabolites known as mycotoxins into nuts. In view of the nutritional composition of nut and its high susceptibility to fungal attack, this chapter looks at the nutritional profile, mycotoxigenic fungi and aflatoxins contamination of peanuts, cashew nuts and their products with a central focus on Africa where the effect of aflatoxin contaminations is more prominent.

Keywords: public health, peanut, cashew nut, aflatoxigenic fungi, aflatoxin, mycobiota diversity, food safety, Africa

1. Introduction

Edible nuts are popular worldwide based on their varied attributes such as desirable taste, high nutritional value as well as some health benefits. Moreover, production of edible nuts can be done under various growing conditions and climates [1], however edible nut production is mainly in the tropical and subtropical regions of the world.

Globally, the most popular edible nuts are groundnuts or peanuts (*Arachis hypogaea*) and are classified as legumes [2] with several other tree nuts such as almond (*Prunus dulcis*), cashew (*Anacardium occidentale*), Brazil nut (*Bertholetia excelssa*), hazelnut (*Corylus avellana*), macadamia (*Macadamia integrifolia*), pecan (*Carya illinoinensis*), pine nut (*Pinus pinea*), pistachio (*Pistachia Vera*), and walnut (*Juglansregia*) [1, 3]. Although nuts are considered as food with numerous health benefits, they are prone to contamination by toxigenic fungi which could ultimately results in the release of fungal metabolites known as mycotoxins into nuts [4].

Contamination of food commodities by mycotoxins has become a global food safety concern [5, 6]. Aflatoxins are secondary metabolites produced by members of the genus *Aspergillus* mainly *A. flavus*, *A. parasiticus* and *A. nomius* [7]. This genus is very ubiquitous and is known as a primary inhabitant of soil that contaminate a variety of agricultural commodities especially cereal grains and oil seeds including nuts [8]. Consumption of aflatoxin contaminated food results in a condition known as aflatoxicosis. The severity of aflatoxicosis symptoms ranges from vomiting, abdominal pains and liver damage in acute aflatoxicosis as a result of ingesting large doses of the toxin. Whereas ingestion of smaller doses leads to chronic aflatoxicosis which is asymptomatic and may result in hepatocellular carcinoma [9]. There over 20 aflatoxins but only four of them occur naturally i.e. aflatoxins (B_1, B_2, G_1, G_2). These aflatoxins are identified based on their fluorescence (B-blue or G-green) under ultraviolet light [10].

Aflatoxin contamination has led to reduced international markets especially in the developed nations, thereby advocating for stringent measures requiring import products to have very low concentrations of aflatoxins. Since nuts are used as food as well as food ingredients, regulatory limits have been established and set at 4 μg/kg for total aflatoxin ($B_1 + B_2 + G_1 + G_2$) and ≤ 2 μg/kg for aflatoxin B_1 by the European Commission [11, 12]. Implementation of good manufacturing practices in the nut production chain is very important so that the nuts comply with limits of the importers [13]. As a result, many countries have conducted research on the diversity of aflatoxigenic fungi as well as the extent of aflatoxin contamination in edible nuts as well as their products to ensure that their produce meets the required standards [14]. This chapter looks at the nutritional profile, mycotoxigenic fungi and aflatoxins contamination of peanuts, cashew nuts and their products with a central focus on Africa where the effect of aflatoxin contaminations is more prominent.

2. Nutritional profile of peanut

Peanut (*Arachis hypogaea* L.), groundnut or monkey nut (**Figure 1**) is the fourth oilseeds crop and 13th food crop grown worldwide because of its nutritional, medicinal and economic values [17, 18]. Several delicacies could be prepared from peanut ranging from products like roasted peanuts, peanut butter, peanut oil, peanut paste, peanut sauce, peanut flour, peanut milk, peanut beverage, peanut snacks (salted and sweet bars) and peanut cheese analog (**Figure 2**). Raw peanut is subjected to different processing which could alter or determine the nutritional composition of the end products. Processing such as roasting of peanut enhance its colour, flavour, taste, aroma and crunchy texture [20]. It also reduces the bacteria load and aflatoxin-producing fungi in raw peanut [21].

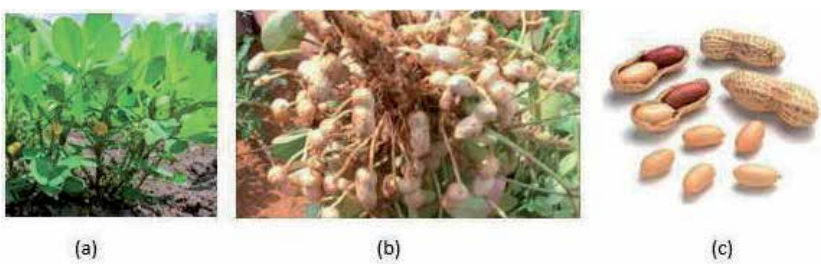

Figure 1.
Showing (a) the groundnut plant in the field, (b) harvested plant and (c) unshelled and shelled groundnuts [15, 16].

Figure 2.
Various forms in which ground nut can be consumed, adopted from market insider with modifications [19].

3. Proximate composition of peanut

Peanut is an excellent source of nutrients similar to other nuts with a substantial amount of lipid, protein and fibre content along with some amount of carbohydrate, vitamins, and minerals. The nutrient constituents and content of peanut have been previously reported; protein 20.7%–25.3%, crude fat 31–46%, ash 1.2%–2.3%, crude fibre 1.4%–3.9%, carbohydrate 21–37%, and moisture 4.9%–6.8%) [22]. Nuts including peanuts provide over 10% of the recommended dietary allowance of nutrients (protein, iron, thiamine and vitamin E) for adult males [23]. The nutritional composition of peanut is shown in **Table 1**.

Principle	Nutrients	Nutrient value	Percentage of RDA
Macro nutrients	Energy	567 Kcal	29
	Carbohydrates	16.13 g	12
	Protein	25.80 g	46
	Total Fat	49.24 g	165
	Cholesterol	0 mg	0
	Dietary Fibre	8.5 g	22
Vitamins	Folates	240 µg	60
	Niacin	12.066 mg	75
	Pantothenic acid	1.767 mg	35
	Pyridoxine	0.348 mg	27
	Riboflavin	0.135 mg	10
	Thiamin	0.640 mg	53
	Vitamin A	0 IU	0
	Vitamin C	0	0
	Vitamin E	8.33 mg	55.5
Minerals	Sodium	18 mg	1
	Potassium	705 mg	15
	Calcium	92 mg	9
	Copper	1.144 mg	127
	Iron	4.58 mg	57
	Magnesium	168 mg	42
	Manganese	1.934 mg	84
	Phosphorus	76 mg	54

Principle	Nutrients	Nutrient value	Percentage of RDA
	Selenium	7.2 µg	13
	Zinc	3.27 mg	30

Source: USDA 2014.

Table 1.
Nutritional composition of groundnut.

4. Nutritional benefits of peanut on human health

The major components of peanut are Protein, fats, and fibre (**Table 1**), and are present in their most beneficial forms. The protein is plant-based, while the fat is unsaturated, and the fibre is composed of a complex carbohydrate that are beneficial for human nutrition.

4.1 Peanut protein

The nutritional value of a food protein is determined by its essential amino acid contents and its digestibility. The protein content in the cake could reach 50% after the peanut oil has been extracted [24]. Peanuts contain all the 20 amino acids in variable proportions [25]. According to its Protein Digestibility Corrected Amino Acid Score (PDCAAS) peanut proteins and other legume proteins such as soy proteins are nutritionally equivalent to meat and eggs and ideal for human growth and health [26]. The true protein digestibility of peanuts is comparable with that of animal protein [27].

4.2 Fatty acid composition of peanut oil

Although peanuts and tree nuts have high lipid contents, peanut oil is rich in unsaturated fat, predominantly, monounsaturated fats (MUFA which have been associated with lower cardio-vascular risk) [28]. The MUFA of the regular US peanuts is 49–57% while a medium (66–69%) and high oleic (78–80%) rich peanuts have been reported [29]. The consumption of MUFA promotes artery-clearing which keeps the flow of blood and lowers the risk of atherosclerosis, heart attack or stroke [30]. Clinical studies demonstrated that intakes of MUFAs and PUFAs are associated with low risk of cardiovascular diseases (CVD) and death, whereas saturated fat and trans-fat intakes are associated with high risk of CVD [28].

4.3 Dietary Fibre

There are soluble and insoluble dietary fibres which have health benefits such as lowering the risk of heart diseases, diabetes and maintenance of a healthy weight [31]. Other health benefits includes, the lowering of blood cholesterol, improvement of bowel movement and reduced risk of metabolic syndrome [32]. The dietary fibre content of dry roasted peanut was reported as 8.4 g per 100 g of peanut similar to that in soybean (9.3 g per 100 g) while the total dietary fibre of defatted peanut flour (15.8%) was comparable to that of defatted soybean flour (17.5%) [33]. This substantial amount of dietary fibre could help individuals reach their recommended daily allowance of 38 g for men and 25 g for women.

4.4 Vitamins and minerals

Peanut has been recognised as a great source of niacin, which is important for the proper functioning of the digestive systems, skin and the nerves. It helps in the conversion of food to energy and supposed to protect against Alzheimers disease and cognitive decline [34]. Peanut is an excellent source of vitamin E whose consumption in good quantities could lead to benefits against coronary heart disease [35]. Peanut also contains good amounts of folates which are important during infancy and pregnancy for the production and maintenance of cells. It was reported that a 100 g of peanut can provides the Recommended Dietary Allowance [36] levels of copper (127%), manganese (84%), iron (57%), phosphorus (54%) and magnesium (42%) intake which is associated with reduced inflammation [37, 38] decreased risk of metabolic syndrome [38] and type II diabetes [39]. They are also referred to as a nutrient dense food, rich in multiple natural micronutrients (**Table 1**) including bioactive compounds such as resveratrol, phytosterols, phenolic acids and flavonoids that are beneficial to health. This makes them a viable option for improving the nutrition status of the malnourished, neonates, growing, or those in need of critical nutrients [40].

5. Health benefits of peanuts

Peanuts lipid profile is high in unsaturated fatty acids than the saturated fatty acids, trans- fat-free, cholesterol-free. Its low saturated fats thus qualify it as safe and desirable. Apart from basic benefits of daily nutrition, peanut consumption leads to long term health benefits such as the management of cancer [41], effective weight management [42] and lower body Mass Index [43] and management of hunger [44].

6. Cashew nut

Cashew (*Anacardium occidentale* L.) is one of the most traded processed tree nuts on the global market [45] and has become a source of income to people in the producing countries through export markets [13]. Cashew nuts are among tree nuts which are known to possess many health benefits such as reduced chances of cardiovascular diseases, diabetes, metabolic syndrome, weight gain and obesity as well as mental instability [46, 47]. This is mainly because tree nuts contain several nutrients needed for the proper functioning of the human body such as unsaturated fatty acids, proteins, mineral, vitamins, phenolic compounds and fibre [47, 48].

Following an analysis of raw cashew nuts from Brazil, India, Vietnam, East and West Africa, Rico, Bulló [47] highlighted fat as the major constituents of cashew nuts with an average of 48.3% of its total weight. However, in another study, Abubakar, Abubakar [46] reported a much higher percentage of total fats (56.4%) in raw cashew nuts from Nigeria. Nevertheless their findings were within the range (40–57%) reported in literature [49]. Fats are known to play several roles in the diet such as provision of energy, essential fatty acids as well as fat soluble vitamins [50].

The cashew tree produces the nut (kidney shaped) as the main fruit and an accessory fruit known as the cashew apple (**Figure 3**) [52, 53]. The main traded cashew products are the raw nut, the seed and the cashew nut shell liquid (CNSL) whereas the apple is converted into various beverages [54].

Figure 3.
Showing (a) cashew tree, (b) developing fruit, (c) cashew nut and apple, (d) roasting of shelled nuts, (e) raw shell nuts [51].

7. Contamination of peanuts and cashew nuts by toxigenic *Aspergillus* species

Despite the fact that developing countries cover the world's greatest peanut producing area, yield is very low especially in Africa because of poor socio-economic factors as well as the prevailing weather conditions [55]. The climatic conditions in the tropics and subtropics promote the proliferation of diverse pathogenic fungi capable of producing aflatoxins [56–58]. Extensive research on aflatoxins began in the 1960s with analysis on peanuts after poultry deaths in the United Kingdom, Kenya and India. This death was linked to the consumption of contaminated peanut meal [59]. The analysis of mould contaminants in peanut meals from various countries, implicated *Aspergillus flavus* [58] as the chief mould contaminant. *Aspergillus* species are ubiquitous group of soil borne fungi that can be found in agricultural soils, storage and food processing facilities as well as in the distribution systems [7, 60]. *Aspergillus parasiticus* is a soil inhabitant normally associated with pod and seed contamination whereas *A. flavus* thrives well in aerial environment and are therefore more likely to contaminate tree nuts [61]. Nuts can be colonised by fungi before harvest (**Figure 4**). Several authors have reported the presence of diverse *Aspergillus* species in the groundnuts and cashew nuts (**Table 2**).

Initially aflatoxin production was linked to *A. flavus* [85], therefore most researchers use *A. flavus* as an indicator of possible aflatoxin contamination in food commodities. For example, Sultan and Magan, [11] isolated several *Aspergillus* species from *Flavi, Circumdati* and *Nigri* sections, but performed aflatoxin producing potential tests only on the isolates from section *Flavi*. Similarly, Oyedele and co-workers [77] also isolated three *Aspergillus* species (*A. flavus, A. parasiticus* and *A. tamarii*) from peanut samples. However, determination of the toxigenicity of the isolates was carried out on those identified as *A. flavus* only. In another survey by Riba and colleagues [64] isolated species belonging to *Flavi, Circumdati, Terrei* and *Nigri* sections from shelled peanuts. Despite the fact that species from section *Nigri* had the highest incidence of occurrence, toxigenicity tests were done only for isolates from *Flavi* section. However some authors have reported aflatoxin production by species outside the section *Flavi* [86–89]. Therefore, it is important for researchers to determine the aflatoxigenicity of all isolates even if they do not belong to the *Flavi* section.

The high prevalence of the black *Aspergillus* i.e. section *Nigri* has been reported in many peanuts samples. Mohammed and Chali [55] reported the prevalence rates of *A. niger* ranging from 35 to 66% in peanut samples from the fields, storages and

Figure 4.
Colonisation of peanuts by Aspergillus at different production stages, (a) showing pods and kernels already contaminated before harvesting, (b) contaminated unshelled peanuts and (c) contaminated shelled peanuts in storage [36, 62, 63].

Country	Product	Sample size	Number of strains	Isolates' Identity	Aflatoxigenic (%)	Reference
Algeria	Shelled peanuts	8	55	*Aspergillus* section *Flavi*	40	[64]
	Cashew nuts	84	—	*Apergillus* section *Flavi* and *Nigri*	n.c	[65]
Benin	Cashew nuts	—	150	*A. tubingensis, A. niger, A. brasiliensis, A. carbonarius, A. luchuensis, A. aculeatus, A. aculeatinus.*	0	[66]
Botswana	Peanuts	120		*A flavus* and *A. parasiticus*	69	[67]
Egypt	Peanuts (raw, roasted and roasted with salt)	60	170	*A. niger, A. flavus, A. fucuum, A. oryzae and A parasiticus*	23	[68]
	Peanuts	45	88	*Aspergillus* section *Flavi*	90	[11]
	Peanuts	10	15	*A. flavus, A. niger, A. terreus* and *A.candidus*	80	[69]
	Peanuts	8	—	*A. flavus* and *A. niger*	—	[70]
	Peanuts	40	17	*A flavus* and *A. parasiticus*	29	[71]
Kenya	Peanuts and peanut butter	82	—	*A. flavus S strain, A. flavus L strain, A. parasiticus, A. niger, A. tamarii, A. alliaceus and A. caeletus*	n.c	[2]
	Peanuts (raw, roasted coated and roasted decoated)	228	—	*Aspergillus flavus L strain, A. flavus S strain, A. parasiticus, A. tamarii, A. caelatus, A. alliaceus* and *A.niger*	n.c	[72]

Country	Product	Sample size	Number of strains	Isolates' Identity	Aflatoxigenic (%)	Reference
	Peanuts (roasted, shelled raw, spoilt, fried, boiled podded, spoiled podded, peanut butter and peanut flour.	705	1027	*A. flavus S-strain, A. flavus L-strain, A. niger, A. tamari, A. alliaceus, A. parasiticus* and *A. caelatus*	73	[73]
Nigeria	Cashew nuts	32	14	*A. niger, A. restrictus, A. flavus, A. fumigatus* and *Aspergillus sp.*	n.c	[74]
	Cashew nuts	10	8	*A. flavus, A. glaucus, A. niger*	30	[75]
	Peanut kernels, Peanut cake, Peanut oil	189		*A. niger, A. flavus* and *A. fumigatus*	n.c	[76]
	Peanuts	84	140	*A. flavus*	64–88	[77]
	Peanuts cake	48	48	*A. flavus, A. parasiticus, A. niger, A. tamarii, A. fumigatus*	n.c	[78]
	Cashew nuts Peanuts	15 9	9 7	*A. carbonarius, A. niger* and *A. flavus.*	56 -	[79]
	Cashew nuts	—	—	*A. niger, A. flavus, A. tereus*	—	[80]
South Africa	Cashew nuts	36		*A. flavus, A. fumigatus, A. oryzae, A. niger* and other *Aspergillus spp*	15	[81]
Uganda	Peanuts (clean) Peanuts (rejects)	- -	45	*A flavus, A. parasiticus, A. niger, A. ochraceus, A. tamarii.*	24	[82]
	Peanuts	240	96	*A flavus, A. parasiticus* and section *Nigri*	34	[83]
Zambia	Soils cropped to peanuts	499	91	*A. flavus (S) and (L) strains), A. parasiticus, A. nomius.*	56–100	[84]

Keys: n.c – not counted, 0 = none was aflatoxin positive.

Table 2.
Incidence of Aflatoxigenic Aspergillus spp. in peanuts and cashew nuts from Africa.

in market places. According to Riba and colleagues [64], *A. niger* is mainly used in fermentations. Riba and associates [64] also reported high incidence of *A. niger* in analysed peanut samples. Although there are no reports on aflatoxin production by *A. niger,* there have been reports on ochratoxin A production by some *A. niger*

strains [90, 91]. On the other hand, Akinola and co-workers [92] highlighted the possibility of gene transfer between toxigenic and atoxigenic strains in feedlots resulting in strains previously known as non aflatoxigenic becoming toxigenic. Acquisition of aflatoxin producing genes by *A. niger* may become a threat to the fermentation industry where it is mainly used.

The quality of the edible part of cashew nut is of great importance as it is used as either as food or ingredients in processed products. However, like others cashew nuts are also prone to fungal contamination with consequent mycotoxin production. **Table 2** shows the presence of *Aspergillus* species especially the section *Nigri* being present in most of the samples. Lamboni and others [65] highlights the implication of this section in food deterioration. The same author reported production of mycotoxins by some isolates from cashew nuts in Benin belonging to the section *Nigri* such as *Aspergillus tubingensis, A. niger, A. brasiliensis, A. carbonarius, A. luchuensis, A. aculeatus, A. aculeatinus*, however none of the isolates were able to produce aflatoxins in culture. A similar observation was noted by El-Samawaty and colleagues [93] where the majority of the *Aspergillus* species in cashew nuts from Saudi Arabia belonged to section *Nigri*. Adeniyi and Adedeji [94], emphasised the importance of moisture in the kernels during storage as it may contribute to the colonisation of the nuts by mycotoxigenic fungi.

8. Aflatoxin contamination of groundnut and cashew nut

Aflatoxin B_1 is the most potent and commonly produced naturally occurring aflatoxin [95], hence its presence in most of the samples and the most reported by researchers (**Table 3**). African countries especially those in Southern Africa namely Botswana [98], Democratic Republic of Congo [100, 101], Malawi, Mozambique [56], South Africa [101], Zambia [123, 124] and Zimbabwe [125–128] had the highest incidence of aflatoxins in the samples analysed. Most batches had more than 50% of the samples testing positive to one or more of the naturally occurring aflatoxins (**Table 3**).

In samples from Northern Africa, aflatoxins were detected though incidences of contamination were less than those reported in Southern Africa. Most samples from the batches analysed had less than 50% aflatoxin contamination occurrence rate with a few exceptions like Sudan in peanut butter. Weather conditions in Northern African countries are characterised by high temperature and humidity [129], due to being surrounded by the Mediterranean Sea and the Atlantic Ocean which promotes growth, occurrence of toxigenic moulds and subsequent aflatoxin production [130].

From West African States, Nigeria and Benin had majority of their peanuts and cashew nuts contaminated with aflatoxins and with almost 100% incidence rates. Total aflatoxin contamination in peanut cake from Benin exceeded the stipulated EC regulatory limit (4 μg/kg) whereas in cashew nuts the concentration was below the limit of detection (LOD). Studies have shown that peanut production in Africa is faced with so many challenges such as poor resources and field management which resulted to the nut being neglected. Peanuts harvest and marketing are often delayed thereby exposing the peanuts to high levels of aflatoxin contamination [95]. Contamination of nuts by aflatoxin can either be before or after harvest and a gradual increase in aflatoxin content with prolonged storage [131] could be expected. In an analysis of aflatoxin levels in peanuts at farms and markets in Uganda, Kaya and co-workers [132] reported the presence of aflatoxins at farm level in ≥60% of the peanuts. Results from analysis of raw peanuts, peanut flour, roasted peanuts and peanut butter [73, 109] showed a

Country	Product	Sample size	Positive (%)	Total Aflatoxin (range μg/kg)	Aflatoxins Detected (μg/kg)				Analysis Method	Reference
					B_1	B_2	G_1	G_2		
Algeria	Shelled peanuts	37	49	0.34–26	LOD-175	10–193	n.d	n.d	HPLC	[96]
	Unshelled peanuts	12	83		0.53–47	-	n.d	n.d		[64]
	Shelled nuts	8	100		0.2–21					
Benin	Peanut cake	15	100	33–346	<LOQ282	<LOD-31	<LOD-79	6–96	LC–MS/MS	[97]
	Cashew nuts	84	—	<LOD	<LOD	<LOD	<LOD	<LOD	UHPLC–MS/MS	[65]
Botswana	Peanuts	120	78	12.0–329	1.3–223	0.5–203	0.6–259	1.0–164	HPLC	[67]
	Peanuts	29	52	3.2–48	0.8–16	1.6–16	1.6–8	1.6–16	TLC & HPLC	[98]
	Peanut Butter	21	71	1.6–64	3.2–16	0 1.6–20	3.2–20	1.6–20		
Cameroon	Peanuts	90	29	n.d	6–125	n.d	n.d	n.d	LC–MS/MS	[99]
DRC	Peanuts	60	72	n.d	1.5–937	n.d	n.d	n.d	TLC	[100]
	Peanuts	20	100	2.19–1258	2.19–543	0–211	0–310	0–193	HPLC	[101]
Egypt	Peanuts (roasted)	36	75	0–39	0–33	0–4	0–3	0–0.2	HPLC	[102]
	Peanuts	8	100	n.d	210–600	—	250–400	n.d	TLC	[70]
Ethiopia	Peanuts	120	76	15–11,900	n.d	n.d	n.d	n.d	ELISA	[103]
	Peanut cake	50	50	n.d	0–158	0–158	—	—	UPLC	[104]
Gambia	Peanuts	3	67	n.d	2–31	n.d	n.d	n.d	HPLC	[105]
Ghana	Raw peanuts	240	—	0–1546	n.d	n.d	n.d	n.d	ELISA	[106]

Country	Product	Sample size	Positive (%)	Total Aflatoxin (range μg/kg)	Aflatoxins Detected (μg/kg)				Analysis Method	Reference
					B_1	B_2	G_1	G_2		
Kenya	Peanuts [121]	63	74	0–365	n.d	n.d	n.d	n.d	ELISA	[2]
	Peanuts (roasted)	8	50	2–298	n.d	n.d	n.d	n.d		
	Peanut butter	11	73	0–2377	n.d	n.d	n.d	n.d		
	Peanuts (raw, roasted coated and roasted decoated)	228	81	0–2345	n.d	n.d	n.d	n.d	ELISA	[72]
	Peanuts	769	36	0–7525	n.d	n.d	n.d	n.d	ELISA	[107]
	Shelledraw peanuts	705	41	0–820	n.d	n.d	n.d	n.d	ELISA	[73]
	Spoilt peanuts			2.2–1628	n.d	n.d	n.d	n.d		
	Roasted peanuts			0–757	n.d	n.d	n.d	n.d		
	Fried peanuts			0–22	n.d	n.d	n.d	n.d		
	Peanut butter			0–582	n.d	n.d	n.d	n.d		
	Peanut flour			0–820	n.d	n.d	n.d	n.d		
	Peanuts	204	99	0.1–591	0.0–510	0–48	0–44	0.1–26	HPLC	[108]
Malawi	Raw peanuts	28		LOD-1200	-	-	-	-	Vicam-	[109]
	Peanut flour	26		LOD-820	-	-	-	-	Fluorometer	
	Peanut butter	13		LOD-180	-	-	-	-		
Mozambique	—	57	100	n.d	0–73	n.d	n.d	n.d	ELISA	[56]
Morocco	Peanuts	20	5	0.3	0.17	n.d	n.d	n.d	LC	[110]

Country	Product	Sample size	Positive (%)	Total Aflatoxin (range μg/kg)	Aflatoxins Detected (μg/kg)				Analysis Method	Reference
					B_1	B_2	G_1	G_2		
Nigeria	Peanut kernels (A*)	-	100	Max 600	+	+	+	+	TLC	[111]
	Peanut kernels(B*)	-	100	Max 450	+	+	-	-		
	Peanut pellets	-	100	Max 860	+	+	+	+		
	Peanut oil (crude)	-	100	Max 98	+	+	+	+		
	Peanut oil (refined)	-	100	Max 9	±	±	±	±		
	Peanut cake	—	—	—	20–455	—	—	—	TLC	[112]
	Peanut kernels	-	-	-	281–680	135–782	182–502	217–590	TLC	[113]
	Peanut pellets	-	-	-	389–793	391–513	218–530	196–320		
	Peanut oil (crude)	-	-	-	16–26	14–18	14–19	11–13		
	Peanut oil (refined)	-	-	-	0–7	0	0	0–5		
	Dry roasted peanut	106	64		5–165	6–26	5–20	7–10	TLC	[114]
	Cashew nuts (roasted)	10		0–0.4	n.d	n.d	n.d	n.d	ELISA	[115]
	Peanut (roasted)	10		0-˃20	n.d	n.d	n.d	n.d		
	Peanut (hulled)	7		0–0.2	n.d	n.d	n.d	n.d		
	Peanut (dehulled)	7		0-˃20	n.d	n.d	n.d	n.d		
	Peanut	84	100	0.4–2076	0.9–710	0.4–129	0.4–1202	18.3–123	LC–MS/MS	[77]
	Peanut	9	—	29–34	n.d	n.d	n.d	n.d	ELISA	[79]
	Cashew nuts	15		0.1–6.8						
	Cashew nuts	39	—	0.01–0.28	n.d	n.d	n.d	n.d	HPLC	[81]
South Africa	Peanut	20	90	0–73	0–35	0–16	0–10	0–8	HPLC	[101]
	Cashew nuts	36	—	0.03–0.77	n.d	n.d	n.d	n.d	HPLC	[81]

Country	Product	Sample size	Positive (%)	Total Aflatoxin (range μg/kg)	Aflatoxins Detected (μg/kg)				Analysis Method	Reference
					B_1	B_2	G_1	G_2		
Sudan	Peanut butter	120	100	n.d	17–170	n.d	n.d	n.d	Vicam fluorometer	[116]
	Peanut	60	58	n.d	17.57–404	n.d	n.d	n.d	TLC	[117]
	Peanut oil (unrefined)	8	13	n.d	0.2	-	-	-	HPLC	[118]
	Peanut oil (semi-refined)	18	0	n.d	-	-	-	-		
	Peanut oil (refined)	2	0	n.d	-	-	-	-		
	Peanut	400	2		3–8	-	-	-	TLC & HPLC	[119]
	Peanut (roasted)	400	11		4–12	-	+	-		
	Peanut butter	400	64		32–54	+	+	+		
	Peanut cake	400	14		7–10	+	+	-		
Tunisia	Peanut	—	42	5	—	—	—	—	HPLC	[120]
Uganda	Peanut	152	57	n.d	0.3–11	n.d	n.d	n.d	TLC	[121]
	Peanut	152	18	1–1000	+	+	+	+	TLC	[122]
Zambia	Peanut butter	24	100	n.d	≤20–10,740	n.d	n.d	n.d	ELISA	[123]
	Raw peanuts	92	55	0.014–48.67	0.015–46.60	0.006–13	0.005–0.5	0.006–0.04	HPLC	[124]
Zimbabwe	Peanut	441	79	n.d	0–25	n.d	0–25	n.d	TLC	[125]
	Peanut	18	17	6.6–622	6.3–528	n.d	n.d	n.d	HPLC	[126]
	Peanut butter	11	91	0–247	0–186	0–25	0–47	0–9		
	Peanut	202	13	9–698	0.7–176	1.3 to 320	21--272	29–378	HPLC	[127]

Keys: - = absent, + = present, n.d = not determined, LOD = limit of detection, LOQ = limit of quantification, Max = maximum, A = kernels from government supply, B* = kernels from miscellaneous supply.*

Table 3.
Aflatoxin contamination of groundnuts, cashew nut and their products in African countries.

decrease in aflatoxin concentration in the order raw peanuts > peanut flour > roasted peanuts > peanut butter. These results are in agreement with those of Siwela and colleagues [133] who reported a 51% reduction in aflatoxin after roasting of peanuts during large scale peanut production.

Peanut oil is the most utilised oil by people in the tropics due to its affordability [134, 135]. Analyses of peanut oil samples have shown the presence of aflatoxins especially in the unrefined oils. It has also been reported that oils extracted from peanuts often have higher aflatoxin contamination [136]. Abalaka [113], highlighted the use of crude oils by the majority of the Nigerian population hence their exposure to aflatoxins [113]. Most of the analyses in oils were from Nigeria and Sudan. Sudan is known as a major vegetable oil producer.

Cashew nut production in Africa is dominated by West African countries [137] hence the high number of reports from this quarters. Most of the studies in Africa were from Nigeria as it is one of the leading producers of cashew nuts worldwide. The result of analyses on aflatoxins in cashew nuts showed that they were within the EU and FDA regulatory limit of 15 μg/kg for total aflatoxin in nuts intended for further processing. However Milhome and associates [14] highlighted some samples from Brazil having total aflatoxins greater than this limit.

9. Impact of Aflatoxin on health

Mycotoxins finds their way into human and animal body through the consumption of mycotoxin contaminated foods [138]. Mycotoxins causes significant decline in animal productivity and general health performance. Out of the over 400 mycotoxins identified in food and animal feeds, those capable of causing significant health effect in humans and animals includes aflatoxin (AFs), fumonisins (FUM), zearalenone (ZEA), T-2/HT-2, deoxynivalenol [139] and ochratoxin A [15], they are of great concern for their effects on animal and human health [140]. Aflatoxins are naturally occurring chemical contaminants of foods such as cereals, legumes and nuts; groundnuts and cashew nuts [141], *Aspergillus parasiticus* and *Aspergillus flavus* are the primary producers of aflatoxin in crops [142]. The consumption of contaminated peanuts and cashew nuts which serves the function of food ingredients and as snacks could results in mycotoxicosis in humans and animals. Similarly, the detection of aflatoxin in animals carcases have been related to the ingestion of aflatoxin contaminated feed ingredients such as peanut [143]. Aside food substrates, mycotoxin occurrence have been reported in animal feed, animal feedlots and animal derived food products [92, 144, 145]. Aflatoxin is regarded as the chief of mycotoxins based on their degree of toxicities [146]. They have been classified as class 1 human carcinogens based on their deleterious effect on the health of both humans and animals [147, 148].

Human exposure to aflatoxin occurs due to the consumption of contaminated agricultural produce and animal derived food product [143]. Aflatoxin ingestion have been reported to cause teratogenic, mutagenic, carcinogenic immunosuppressive, hepatotoxic, nephrotoxic, and genotoxicity effect in humans and animals [144]. The degree of toxicity of mycotoxins on health of animals or humans is a function of the aflatoxin type, species and sex [149]. The liver is the major target organs for aflatoxin toxicity and could show symptoms such as liver lesions and tumour upon exposure to low and moderate doses of aflatoxin [146, 150]. The consumption of aflatoxin contaminated nuts could impair the immunity, feed efficiency and cause a teratogenic and mutagenic effect in animals [151–153]. The consumption of this nuts could pose a threat to consumer's health causing ill-health,

immunosuppression, cancer and liver and kidney damage in humans and animals [150]. The consequences of aflatoxicosis accounts for more than 40% of the diseases in developing countries [9]. In tropical and subtropical countries with less or lack of regulatory activities governing the acceptable level of aflatoxin in food and feeds, the risk of human aflatoxicosis is huge. [154, 155]. The study of Ibeh *et al.* [156] reported the effect of aflatoxin on male fertility. In their study, males with high aflatoxin levels in their serum had abnormal sperm morphology, motility and sperm count. There are also evidence to the transfer of aflatoxin from mothers to babies. Authors [157–159] have reported the detection of aflatoxin M1 in breast milk of mothers exposed to aflatoxin contaminated foods in Gambian and United Arab Emirates respectively. Neonatal jaundice was reported in foetus exposed to aflatoxin in Nigeria and Iran [160, 161]. Studies have also shown the negative effect of aflatoxin on birth weight, gestational age, birth height, in blood samples obtained from mothers [158, 159].

Yousef and Lamplugh [162] have also reported mobility and mortality cases as a result of aflatoxin ingestion in humans. Chronic aflatoxicosis can results from the continuous exposure to aflatoxin contaminated foods and could cause reduction in life expectancy, cancer, immunosuppression and stunting in children [154].

10. Aflatoxin regulation

After the discovery of aflatoxins and their effects on health of both humans and livestock, regulatory limits were set in the late 1960s [163]. The United States of America was the first country to set the aflatoxin limit of 20 μg/kg [164] and the EU limit (4 μg/kg) for total aflatoxin and 2 μg/kg for aflatoxin B1. However there was harmonisation of aflatoxin standards for the EU countries which took place in 1997 and implemented in 1998. Total aflatoxin for peanuts needing further processing which was previously set at 10 μg/kg was changed to 15 μg/kg and 4 μg/kg for nuts intended for human consumption. Aflatoxin B1 was set at 8 μg/kg and 2 μg/kg for nuts that required further processing and direct human consumption respectively [165]. Not all countries adopted the harmonised standards, for examples in Asia, China and the Philippines limit of aflatoxins set by these countries were 20 μg/kg while Indonesia set 15 μg/kg for aflatoxin B1 [166].

11. Conclusion

Nuts and nut products and specially peanuts and cashew nuts have long been recognised for their nutritional content and contribution to good health. One of the limitations to the role of these nuts in human nutrition and health is their susceptibility to *Aspergillus* species and related aflatoxins. Aflatoxins have continued to be a problem from the time of discovery as their presence in nuts especially in African countries is still above the limits. Nuts are sources of livelihood to most people in developing countries as they can be used in nutrition as well as for income generation but its high susceptibility to aflatoxin contamination poses a huge threat to the consumers as well as reducing its value economically especially at the International market [139]. As aflatoxins are not really a threat to the developed countries, because of their stringent rules on acceptable limits in foods meant for human consumption.

This chapter revealed that a larger percentage of the nuts produced in Africa are contaminated with aflatoxin concentration above the regulated permissible level, hence consumers of nuts especially peanuts in Africa are at risk of aflatoxicosis

despite the nutritional importance of the nut. Hence, strategies to reduce the proliferation of aflatoxigenic fungi in nuts while on the field and at post-harvest level should be harnessed. Some suggested strategies to achieve this includes:

- Good Agricultural practices such as planting of improved varieties of nuts that are resistant to drought and stress, good storage practices, proper drying of produce before storage, prevention of kernel damage during harvesting etc. should be encouraged.

- Appropriate controls of storage parameters that could aid impedes *Aspergillus* spp. growth, contamination and aflatoxin production.

- Application of biological techniques such as use of atoxigenic strains of *Aspergillus* to control the toxigenic strains.

It is recommended that future research should focus on the nutritional advantages of peanuts and cashew nuts and their related health benefits beyond the ones that have been identified in this chapter.

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Section 4

Genetic Improvements Towards Human Health

Chapter 6

Genetic Potential and Possible Improvement of *Sesamum indicum* L.

Muthulakshmi Chellamuthu, Selvi Subramanian and Manonmani Swaminathan

Abstract

Sesame (*Sesamum indicum* L.) is one of the traditional oil seed crop widely cultivated in many countries. The top producers of sesame seeds are mainly Tanzania, Myanmar, India, China and Japan. Sesame oil contains high level of unsaturated fatty acids (80%) and low levels of saturated fatty acids (20%). The main fatty acids are palmitic, stearic, oleic, linoleic and trace amounts of linolenic fatty acids. Sesame seed contains 50–60% of high-quality oil rich in natural antioxidants such as sesamin, sesamolin, sesaminol and sesamol it enhances the stability and keeping quality of sesame oil. Sesame seeds have good sources of dietary fibre, fats, vitamins, minerals, proteins and rich in anti-oxidants. Polyunsaturated fatty acids in sesame will reduce the risk of high blood pressure, cardiac disorders and blood sugar levels. Sesame is believed to have been originated in India where maximum variability of genetic resources is available. High yielding varieties available to date have reached the yield plateau even with the advanced cultivation practices. The area under oilseed crops cultivation also reducing every year. Hence, there is an urgent need to increase the oil content and yield of Indian sesame varieties. Understanding the available germplasm and novel interventions to develop high yielding varieties warrant both molecular and phenotypic data which is meagre in case of sesame.

Keywords: sesame, germplasm, genetic diversity, improvement, oil content

1. Introduction

Sesame is one of the imperative, oldest and underexploited oilseed crops in the world. Sesame seeds have different names in diverse locations such as ellu in Tamil, nuvvu in Telugu, til in Hindi, gingelly in English, and also with some other names such as sim sim, ajonjoli, benniseed and gergelim. In India, sesame is placed fifth in the list of edible oil crops after groundnut, rapeseed, mustard, sunflower and soybean [1]. Sesame is considered as a chief oilseed crop in the world due to its extraction process, good stability, and drought resistance [2]. Origin of *Sesamum indicum* is established by the existence of archaeological remnants seeing back to 5500 BC in the Harappa Valley of Indian subcontinent [3, 4].

2. Botany of sesame

Sesemum belongs to Pedaliaceae family, which comprises 16 genus and 60 species. The number of species in sesame is not clear, though 40 species have been identified and 36 were mentioned in the Index Kewensis. In Africa, 18 species were available, 8 species were available in Indian-Srilanka region. All the wild species are prevalent in Africa. *Sesamum indicum*, *S. capense* Burm. (*S. alatum* Thonn.) and *S. schenkii* Aschers have a same somatic number 2n = 26. Other wild species such as *S. occidenale*, *S. radiatum* Schm & Thonn. has 2n = 64, *S. angolens* and *S. prostratum* 2n = 32, *S. laciniatum* 2n = 28. Nowadays, *Sesamum indicum* is cultivated mainly however, a few other species: *S. angustifolium, S. calycinum*, ssp. Baumii, *S. malabaricum*, and *S. radiatum* are harvested and eaten rarely during food scarcity [4].

2.1 Origin and Distribution

Sesame has a wide range of diversity and it was originated in Africa and spread early through West Asia, China and Japan. With the exclusion of *Sesamum prostratum* Retz, all the wild species are establish in Africa [5]. The inconsistency and the location of sesame in the economies of numerous African countries could further justify the African continent to be the ultimate centre of origin. However, Bedigian [6] established that the crop was first domesticated in India, citing morphological and cytogenetic affinities between sesame and the south Indian native *S. mulayanum* Nair, as well as archaeological evidence showed that it was refined at Harrapa in the Indus Valley between 2250 and 1750 BC. All these statements make it difficult to say with inevitability the precise origin of the crop. Due to its moderately low productivity sesame ranks only ninth among the top thirteen oilseed crops, which make up 90% of the world production of edible oil.

2.2 Health benefits of sesame

Sesame seed oil is the most economical important product which is very stable in nature with good antioxidant properties and high PUFA content (**Table 1**) [7, 8]. Besides oil, seeds are also used in various culinary preparations. Sesame seed contains sesamin and sesamolin two lignans with medicinal properties. The term

Components of sesame seed	Quantity		References
	Sesame seed (mg g^{-1} seed)	Sesame oil (mg g^{-1})	
Palmitic acid (16:1)	9.4%	14.45%	Hemalatha and Ghafoorunissa [7]
Oleic acid (18:1)	39.1%	50.54%	
Linoleic acid (18:2)	40%	45.50%	
Linolenic acid (18:3)	0.46%	0.85%	
Sesamin	8.80	6.20	Uzun et al. [8]
Sesamolin	4.50	2.45	
Sesamol	1.20	—	
Sesaminol	1.40	0.01	

Table 1.
Bioactive components present in sesame seed and oil.

'Lignan' was coined by Howarth in 1948. It describes the group of dimeric phenyl propanoids that have therapeutic value. Sesamolin is converted to sesamol on roasting the seeds. Roasting is preferred in confectionary. The molecular structure of sesamol has phenolic and a benzodioxide group. It possesses antioxidant property and confers apoptotic effect in cancer cells. The pharmacological and health promoting effects of sesame seeds are anti-oxidant, anti- proliferative, anti-inflammatory, anti cholestrolemic, anti-hypertensive, lowering LDL, and guarding DNA mutants [9–12]. Sesame lignans also found to increase Vitamin E content in tissues which is also associated with aging process [13]. Besides seed oil and seeds, young leaves also found to have nutritional benefits and used in soup preparations in Africa [6].

3. Sesame Production World Scenario

Sesame is an ancient oilseed crop valuable for export commodity in India. The major sesame producing countries are Myanmar, India, China, Tanzania, Ethiopia, Uganda, Nigeria and others (**Figure 1**). In 2018, 6,016,000 metric tonnes of sesame were grown world-wide on 11,743,000 hectares (ha) with an average harvest of 512 kg ha^{-1}. Asia and Africa produced almost 97% of the world's source of sesame [14]. Globally sesame consumption is progressively raised due to consuming patterns and increasing health awareness of consumers. Consequently, the requirement of sesame seeds is higher at present. Sesame seed has numerous nutritional benefits such as minerals, fibre, protein and vitamins [15]. Tanzania is the highest sesame seed consuming country of about 21% (based on tonnes) followed by China (19%), Sudan (9%), Ethiopia, India, Myanmar and Nigeria (6% each) with approximately 74% of world's consumption [16].

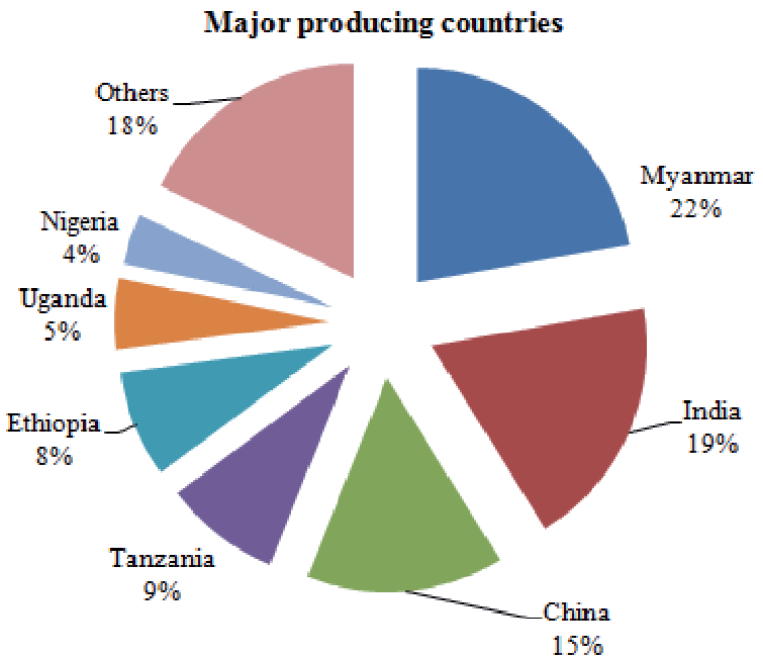

Figure 1.
Major sesame producing countries and its percentage of production. Source: FAOSTAT.

3.1 Sesame Production and export in India

In India, sesame is mostly cultivated in two seasons kharif (June–November) and rabi(November–April) and nearly 75% of annual production comes from kharif season. The major sesame growing states in India are Gujarat (1,16,200 ha),Uttar Pradesh (4,17,435 ha),Rajasthan (2,70,191 ha)and Madhya Pradesh (3,14,300 ha) together accounted for 85% of total acreage and other states served the remaining 15% percent [17]. The average productivity of sesame in India for the last years (2008–2018) are shown in **Figure 2**. In 2008, the production of sesame was around 640300 tonnes and it was increased in 2010 (893,000 tonnes) and it was gradually decreased in 2018 (746,000 tonnes). Global requirement for sesame has increased by nearly 80 percent. Sudan, India, Nigeria, Myanmar, Tanzania and China are the major exporters of sesame seeds and its products. China, Japan, South Korea, Turkey, Iran, Egypt, Germany and USA are the world's largest importers of sesame seed. Area covered, yield and production of sesame in world level was summarized in **Table 2**. India exported 312.62 lakh tonnes of 3920 crores value of sesame seed and oil in the year 2018–2019 [18]. The quantity, value and the share of sesame export for the year 2013 to 2019 was shown in **Table 3**.

3.2 Production Technology

Fertile land with good irrigation and drainage facility is the most suitable land for sesame since it is sensitive to water stagnation. Fine tilth is suitable for sesame seed germination which can be obtained by couple of ploughings and few harrowing activities in any type of soil. A good field suitable for sesame cultivation should be free from weeds and levelled enough to avoid water stagnation. Seed rate used for a good crop stand is 4–5 kg/ha. Seed treatment with Thiram 3 g/kg or Thiram (1.5 g) + Bavistin (1.5 g) is prescribed to avoid seedborne pathogens. Line sowing is preferred for inter culture practices and high yield, when seed drills are used for sowing the seed rate can be reduced to 2.5 to 3 kg/ha. To avoid leaf spot diseases seed pre-treatment with 0.025% solution of Agrimycin-100 is suggested. The fertilizer recommendation for sesame is Sulphur 30 kg /ha in the form of gypsum+60:40:20 (N.P.K.) kg/ha. Sesame responds well to inorganic fertilizers and record higher

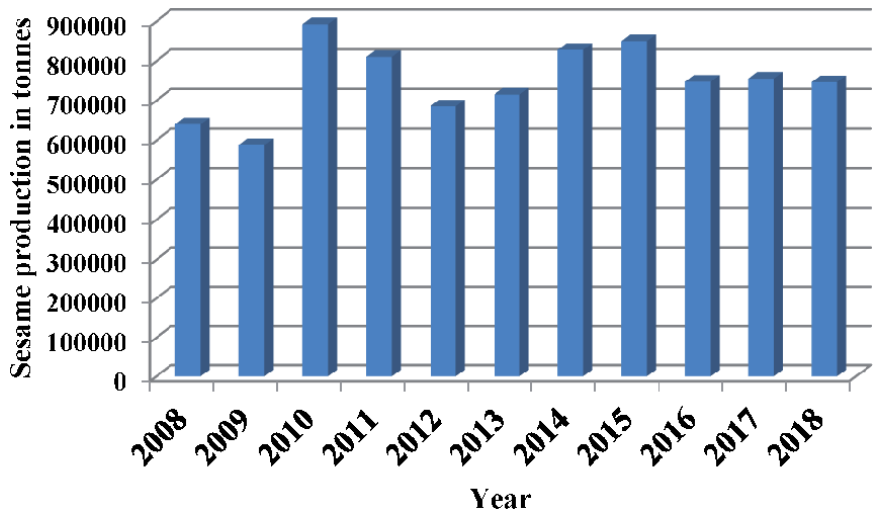

Figure 2.
Sesame productivity in India for the last ten years(2008–2018). Source: Food and Agriculture Organization Statistical Databases (FAOSTAT, 2019).

Sesame producing countries	Area ('000 ha)	Yield (kg ha^{-1})	Production ('000 $MTha^{-1}$)	Percentage of World Production
India	1730	431	746	12.40
China	311	1393	433	7.20
Myanmar	1463	525	769	12.78
Sudan	3480	282	981	9.33
Tanzania	800	701	561	14.56
Nigeria	539	1063	573	9.52
Ethiopia	415	726	301	5.01
Uganda	210	667	140	2.33

Source: Food and Agriculture Organization Statistical Databases (FAOSTAT), 2020.

Table 2.
World status of sesame area, yield and production in 2018.

Year	Seed		Oil and fractions		Total (value)
	Export Quantity (tonnes)	Rupees (crores)	Export Quantity (tonnes)	Rupees (crores)	
2013–2014	2574.4109	3583.46	6.48973	87.45	3670.92
2014–2015	3756.5607	4717.77	7.07017	98.54	4816.30
2015–2016	3284.5572	3012.31	11.17834	77.63	3089.94
2016–2017	3073.2856	2695.84	12.59895	96.61	2792.45
2017–2018	3368.5038	2990.93	9.45222	140.22	3131.14
2018–2019	3119.8706	3761.93	9.22864	165.53	3927.46

Source: Directorate General of Commercial Intelligence and Statistics, (Kolkata).

Table 3.
Quantity of sesame seed and oil exported from India for the period 2013–2014 to 2018–2019.

seed yield. At the time of sowing half of the recommended nitrogen and full of P and K are used. Rest of the nitrogen is applied during flowering stage. Biofertilizer applications such as Azotobactor and phosphorus solubilizing bacteria have resulted in higher yield. In addition foliar spray of urea 2% at flowering and capsule formation resulted in higher yield. In addition to the recommended fertilizer dose, micronutrients zinc 20 kg/ha, iron 25 kg/ha and Farm Yard Manure (FYM) 2.5 t/ha has resulted in maximum yield of sesame [19]. In situ moisture conservation can be accomplished by stirring the soil after each rain and soaking seeds for 8 h in thiourea (500 ppm). Kharif crop requires protective irrigation of 4–5 times depending on the soil type to overcome the moisture stress. Winter crop needs scheduled irrigation for 2–3 times. A good germination and crop establishment is observed when seeds are soaked prior to sowing named as seed priming [20].

4. Sesame crop improvement in Tamil Nadu

Tamil Nadu, the South most state of India harbours several land races and wild sesame varieties. Tamil Nadu Agricultural University is actively involved in genetic improvement of sesame. **Table 4** summarises the varieties released by this University. The following section deals with a recent morphological evaluation of its germplasm for selection for further use. Sesame crop improvement by crossing

Crop/ Variety	Year ofrelease	Parentage	Duration Days	Yield (kg/ha)		Special features
				Rainfed	Irrigated	
TMV 1	1939	Mass selection from Palani (local)	85	300	600	Erect, fairly bushy with moderate branching, 4 loculed reddish brown to black seeds. Oil 50%
TMV 2	1942	Nagpur white x Sattur	80	300	—	Open, moderate branching 6–8 loculed, cylindrical big sized capsules dark brown to black seeds. Suitable for cold weather 52% oil.
TMV 3	1943	South Arcot variety x Malabar Variety	80	350	700	Bushy with profuse branching 4 loculed, dark brown to black seed 51% oil.
KRR1	1967	Pureline selection From Karur Paramathy	120	450	—	Bushy with profuse branching, 4 loculed, brown seeds, oil 52%.
KRR 2	1970	Karur local x Bombay white	110	—	—	Bushy with profuse branching4 loculed, oil 52%, dull white seeds.
TMV 4	1977	Pureline selection Sattur (local)	85	—	700	Bushy with profuse branching, 4 loculed, brown seeds, 51% oil.
TMV 5	1978	Pureline selection from Srivaikuntam local	80	400	750	Erect with moderate branching, 4 loculed, brown seeds, 51% oil.
TMV 6	1980	Selection from Andhra local	85	—	750	Erect with moderate branching, 4 loculed, brown seeds, 54% oil.
CO 1	1983	(TMV 3 x Si 1878) x Si 1878	85–90	600	900	Bushy plant, 4 loculed, black warty seeds, 51% oil content Notification No: 596(E)/13.08.1984
Paiyur 1	1990	Si2511 x Si 2314	90	—	644	Resistant to powdery mildew, 4 loculed, bushy suited for irrigated condition, black seed, oil 50%.
SVPR 1	1992	Selection from "Western ghat white"	80	—	800	White seeded, 4 loculed, high yielding variety suitable for irrigated tracts of Tamil Nadu, oil content 50%
VRI 1	1995	Pureline selection from Tirukkattupalli local	75	—	700	Short duration crop, 4 loculed, suited specially for rice fallows, oil content 51%
VRISV2	2005	US9003 x TMV6	80–85	706	726	Moderately resistant to shoot webber, 4 loculed, high oil content (51.9%)
TMV (Sv) 7	2009	Si 250 x ES 22	85–90	750	820	High yield, 4 loculed, tolerant to root rot disease, Lustrous brown testa, oil content 50%
VRI 3	2017	SVPR 1 x TKG 87	75–80	995	1055	Moderately resistant to phyllody and root rot diseases White seed 50.1 per cent oil content.

Source: https://sites.google.com/a/tnau.ac.in/cpbg/oilseeds/sesame-varieties

Table 4.
Details of sesame varieties released from TNAU, Coimbatore, Tamil Nadu, India.

to exploit the hybrid vigour needs identification of diverse germplasm both by morphological and genetic markers. A diversity analysis was performed on the germplasm of sesame to evaluate yield and associated traits. Fourteen qualitative and six biometric traits were evaluated phenotypically on 270 sesame lines from TNAU using the NBPGR descriptors.

The morphologic characters stem and leaf hairiness, branching, plant growth habit and type exhibited variations. Whereas the six quantitative characters had significant diversity similar to the finding of Abate et al. [20]. The flower colours were white with pink shade suggesting its origin and several of them were shattering type which is prevalent in Indian germplasm [21]. Parental selection was performed based on their mean performance for traits such as days to 50% flowering, days to maturity and plant height. For characters, primary branches, capsules and seed yield per plant grand mean + SE was good. Early maturing phenotypes are the choice in recent years to evade pest and diseases and to accommodate more crops per year. Short duration genotypes, RSS-379-2, SI-3296, GUN-3-NL-1, G-10, NIC-8317, NIC-8283, IC-131651 and SI-2143 with 93–95 days for maturity were selected since they matured early and performed well than the control TMV7. Similarly dwarf genotypes which resist to lodging were preferred. The following genotypes recorded <85 cm height SI-2144, SI-440, JLSC-96, SI-1712, SI-345, SI-395 and SI-2143. Highest number of branches of 10 was observed in ORM-7, SI-395, KMS-4343, SI-533, NIC-1610 and SI-2143. High number of primary branches, high capsule number and yield were recorded together in genotypes, SI-395, NIC-1610 and SI-2143. Yield obtained was 23.09 g, 21.5 g and 21 g in SI-395, NIC-1610 and SI-2143 respectively. SVPR variety recorded a slightly lower yield of 18. 8 g. A positive correlation of primary branches, number of capsules and yield was observed similar to the results reported by Ozcinar et al. [22]. Genotypes SI-395 and SI-2143 were high yielding and dwarf genotypes with a negative direct effect between height and yield as indicated by Agarwal et al. [23, 24]. Variability measures in terms of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) help us to evaluate the contribution of genetic and environmental factors. The phenotypic character, days to 50% flowering showed a moderate GCV and PCV as reported by Iqbal et al. [1], Parmeshwarappa et al. [25] and Sumathi and Muralidharan [26]. However, the same trait recorded highest heritability and genetic advance and similar observations were also made by Kiruthika et al. [27]. In contrast, the days to maturity recorded low GCV and PCV with high heritability and low genetic advance as that of Hika et al. [28] and Sourey et al. [29] respectively. Plant height exhibited moderate GCV and PCV with high heritability and GA in this study as observed by earlier studies by Parmeshwarappa et al. [25].

Genetic divergence by Mahalanobis D2 analysis [27] in these 270-sesame clustered them into 16 groups and 10 of them are single monogenotypic suggesting the diverse nature of germplasm. Genotypes of different geographical origin also have clustered together similar observation recorded by Tripathi et al. [30]. Analysis of α-linolenic acid, sesamin and sesamol content from Tamil Nadu sesame germplasm collection was reported by Chellamuthu et al. [31] enabling the choice of varieties for medicinal purposes.

5. Sesame Indian Scenario

Varietal Development: Eighty-three varieties have been developed for different agroecological situations. Seed yield of these varieties range from 800 to 1000 kg/ha, days to maturity 80–95 and oil content 48–52%. State wise recommended varieties are shown in **Table 5**.

State	Varieties
Gujarat	Gujarat Til-1, Gujarat Til-2, Gujarat Til-10, Gujarat Til-3
Madhya Pradesh	TKG-21, TKG-22, TKG-55, JTS-8, PKDS-11, PKDS-8, PKDS-12, TKG-306, TKG-308
Chattisgarh	TKG-21, TKG-22, Uma, RT-54, TKG-55, JTS-8
Rajasthan	RT-46, RT-54, RT-103, RT-125, RT-127, RT-346, RT- 351,
Maharashtra	Phule Til-1, Tapi, Padma, AKT-64, AKT-101, PKV-NT-11,JLT-408
Uttar Pradesh	T-12, T-13, T-78, Sekhar, Pragati, Tarun
Tamil Nadu	TMV-3, TMV-4, TMV-5, TMV-6, CO-1, TSS-6, Paiyur-1, VRI-1,
West Bengal	Savitri, Rama
Orissa	Uma, Usha, Nirmala, Prachi, Amrit
Andhra Pradesh	Madhavi, Rajeshwari, Varaha, Gautama, Swetha, Chandana, Hima, Sarada
Kerala	Kayamkulam-1, Thilak, Thilathara, Thilarani
Karnataka	DS-1, DSS-9
Punjab	Punjab Til-1, TC-25, TC-289
Bihar	B-67, Krishna
Haryana	Haryana Til-1
Himachal Pradesh	Brijeshwari

Table 5.
List of sesame varieties available in India.

6. Molecular markers of sesame

Molecular marker technologies have been exploited for sesame genotyping and breeding. The first class of molecular markers including random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) were planned and employed for genetic diversity reports [32]. The second class of markers include simple sequence repeats (SSR) types such as Inter-Simple Sequence Repeats (ISSR), Expressed Sequence Tags-SSR(EST-SSR), cDNA-SSR, Genome sequence –SSR, Chloroplast-SSR [33–36]. There are more than 7000 validated and 1, 00,000 non- validated SSR markers were listed and acquired for sesame research. These markers were used for genetic and association mapping, molecular breeding and genetic diversity studies of sesame. Recently, next generation sequencing (NGS) technology, the third class of molecular markers were initiated. SNPs are very valuable genetic markers than other conventional markers because they are most plentiful and steady form of genetic variation in genome. Restriction site-associated DNA sequencing (RADseq), Specific length amplified fragment sequencing (SLAF-seq), RNA-seq, Whole genome sequencing (WGS), Genotyping by sequencing (GBS), insertions/deletions (Indels) has also been stated in sesame [37–40].

6.1 Genome Resources

The nuclear genome of sesame containing of 54.5 Gb of high quality data from the cultivar "Zhongzhi No.13" through Illumina sequencing [41]. The draft genome including of 27,148 genes dispersed on 16 linkage groups (LG) with 274 Mb of size. This genome consist of contig N50 of 52.2Kb and a scaffold with 2.1 Mb has been recently improved to reach 13 pseudo chromosomes, 94.3% of the

estimated genome size and 97.2% of predicted gene models [42]. Another genome sequencing project was started parallel under Sesame Genome Working Group (SGWG). They assembled the variety "Yuwhi 11" a genome size of 293.7 Mb out of 354 Mb estimated in sesame and predicted the function of 23,713 genes. Recently, two new genome sequences from landraces "Baizhima" and "Mishuozhima" have been announced [43]. In addition, a team from National Bureau of Plant Genetic resources, India ensued in the genome sequencing of India variety "Swetha". Nearly 1000 sesame accessions were re-sequenced to provide genome-wide information [44–46]. Gene cloning and molecular breeding, genome wide association studies (GWAS), genome variation and evolution studies are possible nowadays [47, 48]. Novel breeding methods like genomic selection (GS) could be performed for crop improvement in sesame [49].

6.2 Transcriptome Assembly

The first transcriptome summarizing began with 3328 ESTs obtained from cDNA library of 5–25 days old immature sesame seeds. These reports bring out the metabolic pathways implicated in lignan biosynthesis including sesamin and sesamolin [50]. On the other hand, sesame productivity is severely influenced by different biotic and abiotic stresses; studies have been pointed to find out some potential genes to convey stress tolerance in root tissues to waterlogging stress in sesame [51]. Another significant abiotic stress spoiling sesame productivity is drought stress, for that gene expression changes were examined in two sesame genotypes (tolerant and sensitive) through Illumina Hiseq 4000 sequencing platform [52]. RNA-seq study was applied for resistant and susceptible sesame cultivars inoculated with *Fusarium oxysporum* to shed light on molecular mechanism of sesame resistance to Fusarium wilt. It is one of the major diseases in sesame accounting to a yield loss of 15–30% [40].

7. Conventional Breeding methods

Conventional breeding approaches mainly involve the existence of wild relatives, elite cultivars, and landraces to enable the assortment of superior lines for quality enhancement (**Figure 3**). Genetic diversity studies can be carried out by several methods such as biochemical, morphological and molecular markers. Genetic differences examined using morphological markers is also an essential tool among sesame genotypes. A few investigations dependent on morphological markers have shown the presence of high genetic diversity in sesame populations [21, 49]. The high level of genetic diversity prevalent among the 58 Indian collections is probably indicative of the nativity of this crop species [50]. As part of broadening the genetic base of sesame (*Sesamum indicum* L.) in India through germplasm enhancement, National Bureau of Plant Genetic Resources has made initiatives [51]. A selection was made of 24 of the most diverse and unadapted parental lines, including one accession of the wild species *S. mulayanum*, and these were intercrossed in various combinations to maximize genetic diversity and to develop locally adapted pools of genetic resources. Genetic analyses on sesame crosses have shown the presence of additive, dominance and epistatic gene interactions for yield and its components. Molecular marker techniques such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), inter simple sequence repeats (ISSR) are extensively used for genetic diversity studies. AFLP markers showed a low level of genetic diversity (0.14–0.21) among 36 sesame germplasm collections [53]. Characterization of Indian sesame

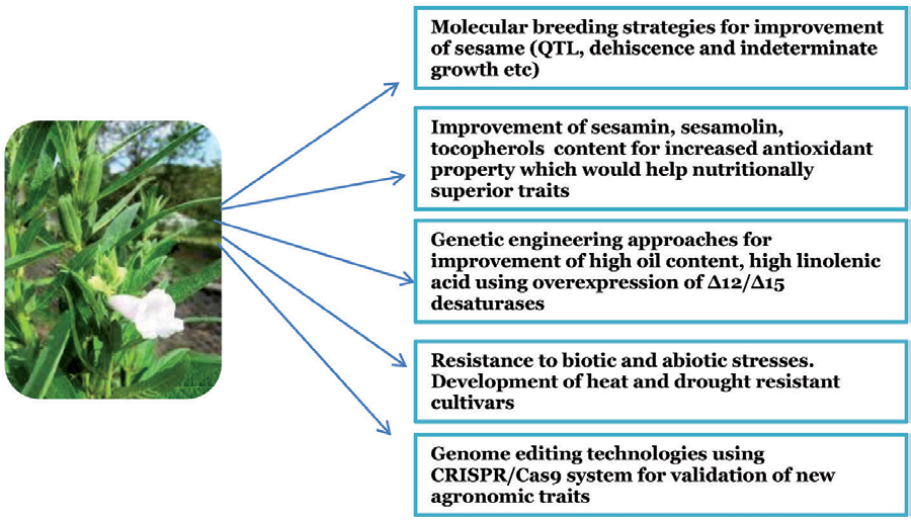

Figure 3.
Future perspectives for improvement of sesame.

varieties was performed using SSR and ISSR markers. Results indicated that the varieties were clustered independently of their geographical locations [52]. Single nucleotide polymorphism (SNP) plays an imperative role in genotyping and these are the most abundant molecular markers which are widely dispersed throughout the genomes with a variable distribution among species [54]. Expressed sequence tags (EST) from *Sesamum indicum* and *Arabidopsis thaliana* resulted in similar and different gene expression profiles during seed development and 41,248 ESTs for developing seeds of sesame cDNA library has been generated [55].

7.1 Genetic Engineering approaches for sesame improvement

For oil quality improvement fatty acid composition and enzymes involved in metabolism are very important, so for our gain like to increase or decrease the quantity of particular fatty acid through genetic engineering we can reduce or increase the expression of endogenous enzymes by various means. It proved that genes for membrane-bound fatty acid modifying enzymes not only from plants but also from bacterial, animal, yeast have been shown to function in transgenic plants. The enzymes such as fatty acid synthase, thioesterases, elongases, desaturases, stearoyl-ACP desaturase, Δ12-desaturase, Δ15- Desaturase, acyltransferases and Hydroxylases are important in fatty acid manipulation. Suppression of the oleate Δ12-desaturase gene (which normally converts 18:1 to 18:2) in soybean, sunflower, cotton and canola has resulted in the production of oils with a high oleic acid content, which have greater oxidative stability and improved performance in high temperature cooking application [56]. Yadav et al. [57] reported for the first-time successful recovery of fertile transgenic plants of sesame with transformation frequency of 1.01%. From cotyledon explants inoculated with *A. tumefaciens* carrying a binary vector pCAMBIA 2301 that contains a neomycin phosphotransferase gene (nptII) and a β-glucuronidase (GUS) gene (uidA) interrupted with an intron. The most efficient gene transformation protocol using de-embryonated cotyledon of sesame (cultivar VRI-1) was reported by Chowdhury et al. [58]. Shoot regeneration from cotyledons was reported recently in Indian sesame genotypes [59]. Enhancement of omega 3 fatty acid content of sesame using *Fusarium moniliforme* Δ12/Δ15 bifunctional

desaturase gene through genetic engineering approach (Unpublished data).Yeast is an excellent model for lipid biosynthesis related studies. Functional characterization of DGAT and PDAT genes of sesame using yeast H1246 oil synthesis deficient mutant and their oil accumulation were analysed [60]. Co-expression analysis of DGAT1 and PDAT1 genes with omega 3 desaturase genes were characterized in the yeast expression system for oil quality enhancement (Unpublished data). These are some of the strategies for improvement of sesame through genetic engineering.

7.2 CRISPR/Cas9 Applications in Plants

The accumulation of complete genome sequencing data has enabled the targeted genome editing strategy using CRISPR/Cas9 system for oil improvement in many oil seed crops. In allotetraploid *Brassica napus*, the efficiency of the CRISPR/Cas9 mutation was examined for 12 paralogous genes, BnaA9.RGA, BnaC9.RGA, BnaA6. RGA, BnaC7.RGA, BnaA2.DA2.1, BnaA2.DA2.2, BnaC6.DA2, BnaC5.DA1, BnaA6. DA1, BnaA9.FUL, BnaC2.FUL, and BnaC7.FUL. They determined the specificity and heritability of the CRISPR/Cas9 mutants. The result showed that the targeted mutation in the T0 generation was stably inherited into the progeny and the mutation frequency ranged from 27.6% to 96.6% and no off-target mutation was identified. FAD2–2 is a desaturase gene which uses the substrate oleic acid and converts it to linoleic acid. In soybean this gene was mutated using CRISPR-Cas9 system in order to improve the seed oil. The result showed that the mutation efficiency was 21% and the content of oleic acid was increased to 65.58% from 17.34%and the level of linoleic acid was reduced to 16.08% from 59.54% [61]. *Camelina sativa* is considered as one of the most important sources of cooking and industrial oil. The total oil content of Camelina is found to be 32–40%. Increase in oleic acid and decrease in poly unsaturated fatty acids such as linolenic acid and linoleic acid contents can provide better suited oil for many industrial purposes and mainly biofuels. A research group in United States attempted the CRISPR/Cas9 gene-editing system to increase the oleic acid content and decrease both linolenic acid and linoleic acid content by knocking out the FAD2 gene in Camelina. The allohexaploid Camelina genome contains a total of six FAD2 genes. They designed sgRNA constructs to knockout both allelic copies of FAD2 genes in Camelina. The result showed that seeds had over 50% vs. 16% oleic acid and less than 15% polyunsaturated fatty acids in T4 generation. This work provided the proof of concept that the FAD2 genes in oil seed plants can be successfully edited using the CRISPR/Cas9 system to yield plants capable of producing commercially valuable oils [62]. In bread wheat *Triticum aestivum*, two genes, inositol oxygenase (inox) and phytoene desaturase (pds), were targeted using CRISPR/Cas9 gene-editing system. Two sgRNA constructs were used to target each gene. The efficient production of insertions and deletions were observed in wheat cell suspension cultures with each of two sgRNA constructs. When the two sgRNA genes were placed together in a single expression cassette, the gene fragment between the two target sites was deleted. This study demonstrated that creation of gene knockout and gene fragment deletions in hexaploid wheat were also possible using CRISPR/Cas9 [63]. In rice, FAD2–1 gene was mutated using CRISPR/Cas9 to produce high oleic acid and low linoleic acid in bran oil. The results showed that the content of oleic acid was increased twice the wild type which was80% vs. 32% [64]. There are many constraints for molecular and biotechnological approaches in developing elite varieties in sesame. Besides, seldom available transgenic plants and approaches are not well received by public. These are some of the strategies will help the researchers to generate superior sesame traits through CRISPR/Cas9 based targeted editing and mutation breeding.

8. Prospects of Sesame improvement

1. Development of large number of varieties suitable for our agro climatic conditions.

2. Improvement of value added products in sesame will enhance the economic value in the world market.

3. Development of sesame plants with high lignans, tocopherol, and omega 3 fatty acid content will help to reduce the risk of cancer, diabetes, cardiovascular problems.

4. Conventional breeding methods, advancement in next generation sequencing will help to develop tools for genetic improvement of sesame.

5. Enhancement of oil quality through CRISPR/Cas9 to generate superior varieties in sesame.

9. Conclusion

Nowadays vegetable oil demand was increasing globally and oil consumption was expected to be doubled in 2030. There is lot of room to improve the sesame varieties for yield, oil content and quality. Besides oil, other lignans such as sesamin and sesamolin contents in Indian varieties add unique flavor and value to the sesame oil. Sesame is used as a promising target oilseed for biofuel applications, pharmaceutical etc.

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Conflict of interest

The authors declare no conflict of interest.

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Nuts, including peanuts, have always been an important part of the human diet. They are nutrient-dense food products containing health-friendly lipids, beneficial phytonutrients, and other essential vitamins and minerals. Basic, clinical, and epidemiological research is now being directed towards understanding the mechanisms by which nuts influence human health and developing dietary guidelines for their optimum consumption. Research is also being directed towards the issues of fungal contamination of nuts, associated risks to human health, and methods of minimizing such risks. This book addresses these topics in chapters written by international experts in the field.

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