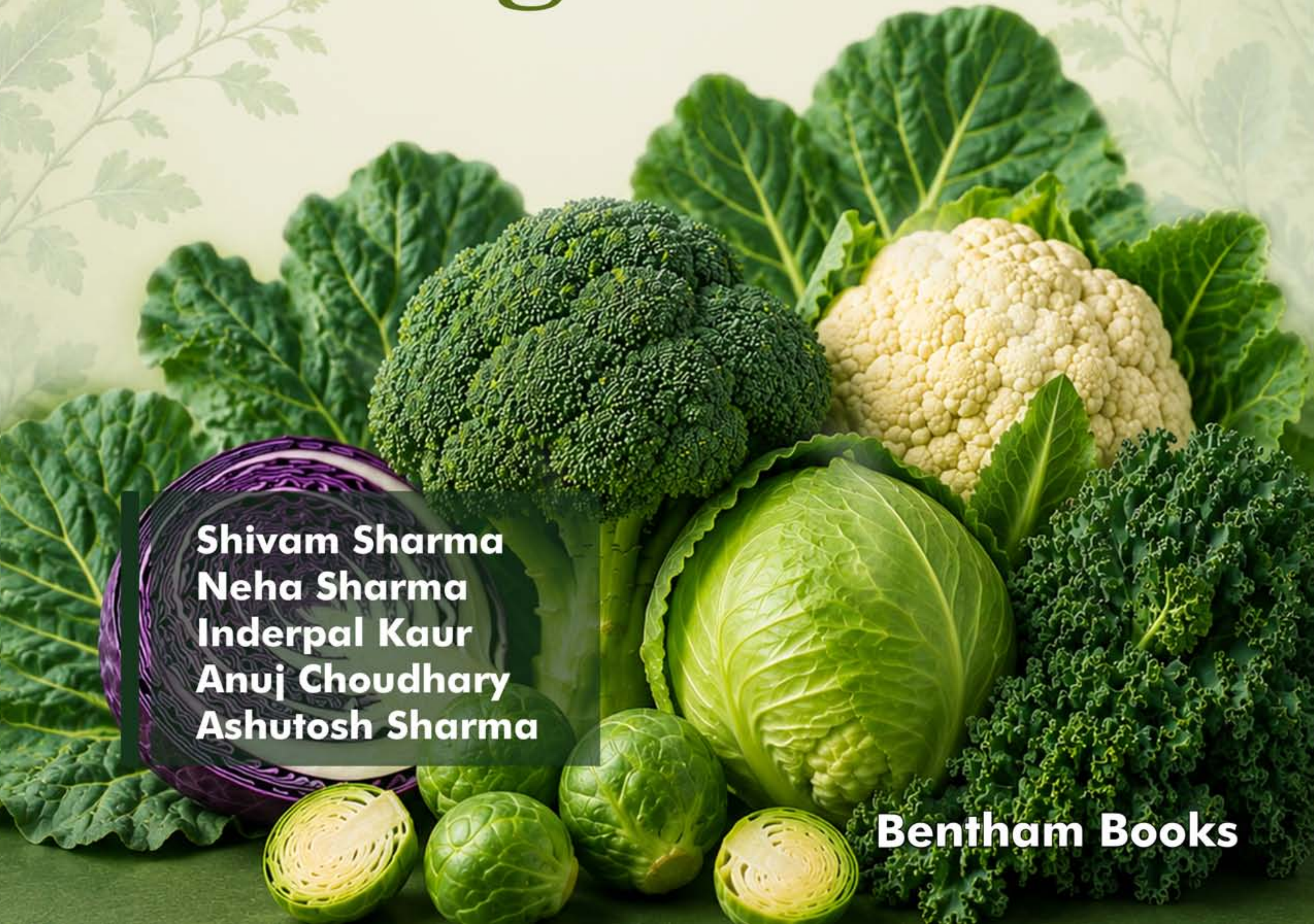
Nutritional Biochemistry and Health Benefits of Brassica Vegetables



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FOREWORD

It is with great pleasure that I introduce the book 'Nutritional Biochemistry and Health Benefits of Brassica Vegetables' written by Sharma *et al*., which is a timely and comprehensive resource published by Bentham Science Publishers. I have witnessed the growing global interest in plant-based human nutrition and in the role of Brassica vegetables in nutritional security. Brassica species, including familiar staples like broccoli, cabbage, cauliflower, and kale, have long been recognized for their distinct flavors and culinary versatility. However, their true value extends far beyond the plate. These remarkable plants are powerhouses of essential vitamins, minerals, and, critically, a unique array of bioactive compounds such as glucosinolates, phenolics, and carotenoids. Scientific inquiry has increasingly linked these compounds to a wide spectrum of health benefits, including potent antioxidant, anti-inflammatory, anticancer, and cardioprotective effects, as well as their contribution to supporting the immune system.

This book meticulously unveils the intricate nutritional biochemistry of Brassica vegetables, providing in-depth analyses of their constituent compounds and elucidating the mechanisms through which they confer health advantages. It serves as an invaluable resource, synthesizing cutting-edge research to provide a holistic understanding of how these vegetables support human health.

I congratulate the authors for their valuable efforts in compiling such a comprehensive and insightful work. This book will undoubtedly become an indispensable academic resource for researchers, nutritionists, public health professionals, and indeed, anyone interested in leveraging the health benefits of Brassica vegetables. It represents a significant contribution to the growing knowledge on functional foods and their vital role in preventive healthcare. Once again, I congratulate all the authors for compiling comprehensive information on nearly every aspect of the nutritional biochemistry of Brassica vegetables.

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PREFACE

Research on nutrition and its effects on human health has evolved considerably in recent decades, with growing emphasis on bioactive compounds. Among the various vegetable families that enhance human health, Brassica vegetables are particularly notable for their distinctive blend of essential nutrients and bioactive phytochemicals with health-promoting properties. This publication, titled 'Nutritional Biochemistry and Health Benefits of Brassica Vegetables', seeks to offer an in-depth analysis of these extraordinary vegetables from a biochemical standpoint. It integrates the most recent scientific findings regarding their molecular structure, assesses their contributions to human health, and examines their contributions to human health. Each chapter focuses on the essential nutrients, antioxidants, vitamins, minerals, and bioactive compounds present in Brassica species, including glucosinolates, flavonoids, and carotenoids, and the physiological effects these compounds may have on human health.

The objective of this book is to connect laboratory findings with practical applications. It provides insights into incorporating these vegetables into daily diets to promote well-being. By examining their biochemical characteristics, this work also highlights the health advantages of Brassica vegetables within the framework of contemporary nutritional science. We explore how specific compounds in Brassicas interact with metabolism, immune function, and the gut microbiome. It also explores their potential roles in chronic disease prevention. It is my aspiration that this book will be a valuable reference for students, researchers, healthcare practitioners, and anyone interested in the significant influence of diet on health. As the body of evidence continues to grow, it becomes increasingly evident that Brassica vegetables not only form a crucial part of a nutritious diet but also represent a promising direction for future research aimed at preventing and managing a variety of chronic diseases.

As readers embark on this journey through the fascinating world of Brassica vegetables, I invite you to explore both their science and their practical applications. May this book inspire readers to incorporate more of these nutrient-dense vegetables into their diets and to consider their potential for fostering better health outcomes for all. This preface sets the stage for a deep dive into the biochemical properties of Brassica vegetables and highlights their significance in health and disease prevention. It aims to engage both scientific and general audiences by making the content accessible while grounding it in rigorous research.

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CHAPTER 1

Introduction to Vegetable Brassicas

Abstract: Vegetables play a vital role in nutritional security and overall human health. Their role in the human diet was yet again realized after the COVID-19 pandemic period. Their daily consumption aids in overcoming chronic diseases like cancer, heart disease, and stroke. Besides their documented health benefits, inadequate vegetable consumption displays a life-threatening global health challenge. Among winter vegetables, Brassicaceae is the most important plant family, containing highly valued vegetables such as broccoli, Brussels sprouts, cabbage, cauliflower, kale, and kohlrabi. These vegetables are a rich source of vitamins, dietary fibers, and secondary metabolites. These Brassica vegetables have significant nutritional and biochemical diversity. Recent studies have emphasized the pharmacological effects (anticancer, antidiabetic, and antiaging effects) of Brassica vegetables. These properties are primarily regulated by glucosinolates and phenolic acids. This highlights the direct potential of brassica vegetables as functional foods and as nutraceuticals for targeting global nutritional security.

Keywords: Brassica, Biodiversity, Evolutionary history, Pharmaceutical properties, Triangle of U.

INTRODUCTION

The major role of vegetables in the maintenance of human health is highlighted by alarming global statistics displaying insufficient dietary intake. At the start of this decade, the Food and Agriculture Organization (FAO) declared the year 2021 as the ‘International Year of Fruits and Vegetables’ to raise awareness of their health and nutritional benefits among people in the post COVID-19 pandemic world. But the reality remains stark, *i.e.,* 660 million people may still face hunger by 2030 owing to the long-lasting effects of the COVID-19 pandemic on global food security [1]. Additionally, the World Health Organization (WHO) recommends the consumption of at least 400 g of fruits and vegetables daily, with an average serving size of 80 g, *i.e.,* approximately five times a day [2]. Insufficient intake of fruits and vegetables is estimated to cause approximately 14% of deaths from gastrointestinal cancer worldwide, approximately 11% of deaths from ischemic

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heart disease, 9% of deaths from cardiac strokes [3]. In addition, it would result in a 30-40% rise in cancer cases by 2030 [4]. Increasing agricultural productivity has simultaneously imposed serious negative impacts on human health due to the inappropriate and indiscriminate use of agrochemicals (plant protectants and chemical fertilizers), decline in the groundwater table, and soil degradation [5, 6]. Considering these global health challenges, the production and consumption of brassica vegetables is a need of the hour. Over the past 20 years, consumer ideotype for vegetable availability has shifted towards quality production [7]. As a result, brassica vegetables have gained significant momentum and attention [8]. Among these, Brassicaceae is the most popular family of winter vegetables, which includes cole vegetables such as cabbage, broccoli, cauliflower, knol khol, kale and several others [9]. Due to their enormous potential to ensure global nutritional security and the wide array of phytochemicals they contain, cole crops are referred to as the 'Crown Jewel of Nutrition'. Furthermore, they are rich in vitamins and dietary fiber, low in fat and calories, and rich in secondary metabolites that can boost the human immune system [10]. Different species of the Brassicaceae family are commercially cultivated.

The most important genus of the family, namely *Brassica*, has a considerable degree of variability, which is reflected in the wide range of cultivated forms. This diversity also influences the categorization of these crops for commercial purposes. The FAO estimates for the global commercial production of *B. oleracea* for human consumption are aggregated into two groups: 'cabbages and other Brassicas' and 'cauliflowers and broccoli' [11]. The promising biodiversity among commercial brassica vegetables confirms their easy availability in the market. In 2020, the total yield estimates for these two items combined amounted to over 96 million tonnes (70.86 and 25.53, respectively) from approximately 3.76 million hectares (2.41 and 1.35, respectively). The relationship among 6 cultivated Brassica forms was studied between 1922 and 1937 by Karpechenko, Morinaga, and his student Nagaharu U *i.e.*, so called Triangle of U (Fig. **1**) [12]. Understanding the evolutionary history of these diverse vegetables is mandatory not only for commercial classification and production but also for unlocking their true genetic potential. Bridging on this heritage, the genus *Brassica* proposes an interesting case study in plant evolutionary history and regional domestication. Therefore, the rich evolutionary history of *Brassica* vegetables has fascinated breeders for centuries, chiefly because of the huge diversity among commercial forms (Table **1**).

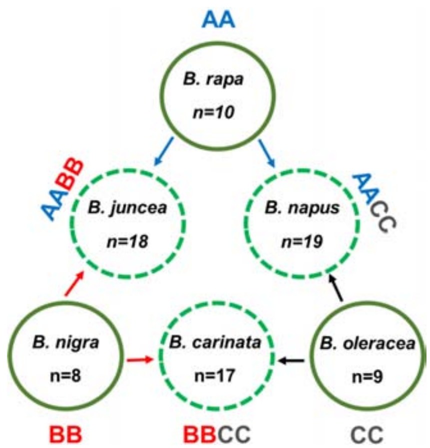

Fig. (1). A schematic representation describing the relationship among six cultivated Brassica forms, commonly called the 'Triangle of U.'

GENUS BRASSICA

The evolutionary history of *Brassica* has fascinated researchers for centuries owing to the huge diversity among cultivated types (Table **1**). The primary challenges in identifying progenitor species include the number of wild species that share a single cytodeme and are interfertile with *B. oleracea* (2n=18; similar genomic organization, referred to as the 'C' genome). The 'C' genome represents a distinct inherited set of chromosomes characteristic of *B. oleracea* and its close relatives. Several wild coastal Brassica taxa possess the C genome cytodeme and exhibit interfertility with cultivated *B. oleracea*. These taxa include forms historically classified as *B. oleracea* subsp. *oleracea* (wild cabbage), *B. incana*, *B. villosa*, *B. insularis*, and *B. cretica*, which complicates the definitive identification of a single progenitor [13]. Both classical and molecular studies have suggested *B. oleracea* var. *oleracea*, *B. incana*, *B. rupestris*, or *B. cretica* as primary ancestors.

However, recent genome-scale analyses indicate a preference for Eastern Mediterranean Aegean endemics, supported by substantial evidence of gene flow between crops and wild and crop–feral kale populations [14]. In addition, Chen *et al*., [15, 16] suggested tri-genomic bridges for Brassica improvement, while other researchers have occasionally proposed different wild progenitors [17]. Most recently, Mabry *et al*., [18] tracked the evolutionary history of wild, domesticated, and feral *Brassica oleracea* using newly generated RNA-sequenced data for a diversity panel of 224 accessions. It represents 14 different *B. oleracea* crop types and 9 potential wild progenitor species, and reported *B. cretica* as the closest living relative of cultivated *B. oleracea*, supporting an origin of cultivation in the Eastern Mediterranean region. Their research also identified several feral lineages interspersed among crop clades, suggesting the frequent reversion of cultivated

CHAPTER 2

Brassicas as the 'Crown Jewel' of Nutrition

Abstract: Vegetables are an integral part of the human diet and provide all essential nutrients. Among them, Brassicas are rich sources of essential vitamins, minerals, and antioxidants that contribute to disease prevention and overall well-being. Kale exceeds other leafy greens in calcium, magnesium, and vitamin A content, while broccoli is a significant source of selenium. Vegetable brassicas contain high levels of hydrophilic and fat-soluble antioxidants, and exhibit multiple pharmacological properties. Pertaining to their richness in Selenium, they exhibit inhibition of SARS-CoV-2 activity. Furthermore, Brassica extracts showed potential cosmetic and therapeutic applications, including anti-aging, skin repair, and obesity control. Overall, if vegetables are the crown of human nutrition, Brassicas can be considered the 'the jewel' in the middle of that crown.

Keywords: Antioxidant, Nutritional security, Phytochemicals, Selenomethionine, Skin health.

INTRODUCTION

Vegetables are an integral component of the human diet and are valuable in ensuring the nutritional security of mankind by supplying essential minerals and vitamins. These vitamins and minerals constitute basic but essential nutritional requirements for supporting disease-free, healthy lifestyles in human beings [1]. Among different vegetable families, those belonging to the family Brassicaceae, generally referred to as '*Brassica* vegetables', are at the top, as they are rich in pharmaceutically active ingredients as well. Water, which is a major component of these vegetables, ranges from 89% to 92%, while containing negligible fat (Tables **1** and **2**).

NUTRIENT COMPOSITION OF SPECIFIC BRASSICA VEGETABLES

Kale (borecole or farmer's cabbage), followed by broccoli, is rich in almost all components of a balanced diet, *i.e.,* vitamins, protein, and major macro- and micronutrients [4]. It stands out among Brassicas for its significantly higher calcium and magnesium content, with approximately six times more calcium and

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twice as much magnesium as other varieties [5]. Among green leafy and temperate vegetables, kale accumulates high levels of minerals, particularly potassium (K) [6, 7]. It is also a rich source of vitamins A and B-complex. Broccoli is notable for accumulating most of its selenium from the soil [8]. Moreover, broccoli has approximately 130 times more vitamin A per 100 g fresh weight than cauliflower and 22 times more than cabbage. Brussels sprouts and broccoli are the highest-rated sources of folate-rich diets, contributing approximately 110-135 and 70-90 μg/100 g fresh weight, respectively [9]. Brussels sprouts, kale, and broccoli and cauliflower contain the highest amounts of vitamin C, reflecting their radical-scavenging and antioxidant activities [10]. In addition, both these vegetables, along with red cabbage, exhibited the highest Total Antioxidant Capacity (TAC), while common cabbage showed the lowest Total Antioxidant Capacity (TAC) among the group [11]. Kale, broccoli, Brussels sprouts, cauliflower, red cabbage, and common cabbage are all nutrient-rich members of the Brassica family. However, they exhibit distinct differences in mineral, vitamin, and antioxidant profiles [12].

Among the various commonly consumed varieties of *Brassica oleracea*, kale stands out for its high concentrations of vitamin C, provitamin A carotenoids, and a range of B vitamins, in addition to its overall rich vitamin and mineral profile. In this regard, broccoli and brussels sprouts also exhibit remarkable nutritional value. Both kale and broccoli are excellent sources of vitamin K and folate, with kale providing high potassium and > 100 mg of calcium/100 g of fresh weight [13]. Compared with other brassica vegetables, kale consistently shows higher levels of Ca and Mg. Similarly, broccoli offers a significant quantity of calcium, potassium, and iron, vitamins A, C, and K, making it a multifaceted vegetable [14].

Table 1. Proximate nutrient compositions of Brassica vegetables.

Crop	Macronutrients (g/100g FW) *						Micronutrients (mg/100g DW)**						
	Energy (Kcal)	Water (g)	Protein (g)	Fat (g)	CHO (g)	Fiber (g)	Ca (mg)	P (mg)	Fe (mg)	Mg (mg)	Na (mg)	Se (μg)	K (mg)
Arugula	25	91.7	2.58	0.66	3.65	1.6	160	52	1.4	47	27	0.3	369
Broccoli	34	89.3	2.8	0.3	6.6	2.6	47	78	1.1	21.0	36	1.6	303
Brussel sprout	45	85.2	4.9	0.4	8.3	2.5	36	80	1.5	23.0	25	1.6	389
Cabbage	25	92.1	1.2	0.09	5.8	2.2	40	29	0.4	12.0	18	0.3	170
Cauliflower	25	92.0	1.9	0.2	4.97	2.0	22	56	1.1	15.0	30	0.6	299

(Table 2) cont.....

Crop	Macronutrients (g/100g FW) *						Micronutrients (mg/100g DW)**						
	Energy (Kcal)	Water (g)	Protein (g)	Fat (g)	CHO (g)	Fiber (g)	Ca (mg)	P (mg)	Fe (mg)	Mg (mg)	Na (mg)	Se (µ g)	K (mg)
Chinese cabbage	14	95.3	1.2	0.2	2.1	1.0	105	40	0.6	19.0	65	0.5	252
Collard	32	89.62	3.02	0.61	5.42	4	232	25	0.47	27	17	1.3	213
Horseradish	48	85.08	1.18	0.69	11.2	3.3	56	31	0.42	27	420	2.8	246
Knol Khol	3	90.3	1.5	0.2	6.6	4.0	41	51	0.5	27.0	20	0.7	350
Kale	35	89.6	6.0	1.4	4.4	4.1	254	93	2.9	32.7	53	0.9	348
Radish	16	95.3	0.68	0.1	3.4	1.6	25	20	0.34	10	39	0.6	223
Turnip	28	91.9	0.9	0.1	6.2	3.6	30	27	0.3	11	67	0.7	191

*Expressed on a fresh weight (FW) basis; **Expressed on a dry weight (DW) basis

Table 2. Proximate bioactive compounds and vitamins levels in different Brassica vegetables.

Crop	Bioactive compounds [a]				Vitamin (mg) [b]						
	GLs (mg)	PC[c] (mg)	β-carotene (β-car) (µg)	L+Z (µg)	A (IU)	B1 (mg)	B2 (mg)	B3 (mg)	C (mg)	E (mg)	K (µg)
Arugula	7.96[d]	0.10	1420	3560	2373	0.04	0.08	0.3	15	0.43	109
Broccoli	47	45.33	93	745	2500	0.10	0.23	0.9	89.2	0.15	102
Brussel sprout	36	73.78	450	1590	550	0.10	0.16	0.9	102	0.88	177
Cabbage	40	79.33	42	30	130	0.05	0.05	0.3	36.6	0.15	76
Cauliflower	22	7.17	0	1	60	0.11	0.10	0.7	48.2	0.08	15.5
Chinese Cabbage	105	0.06	2680	40	150	0.05	0.04	0.6	45.0	0.09	45.5
Collard	11.4-36.4*	0.08	2991	2323	6670	0	0	0	35.3	2.26	437.1
Horseradish	8.9-12.5*	2.76	1	10	2	0.01	0.02	0.39	24.9	0.01	1.3
KnolKhol	41	4.13	22	0	20	0.06	0.04	0.3	62.0	0.48	0.1
Kale	254	34.45	2870	6260	10000	0.16	0.26	2.1	93.4	0.66	390
Radish	676	0.66	4	10	0	0	0	0	14.8	0	1.3
Turnip	698	0.13	0	0	11587	0.07	0.6	0.38	21	0.03	0.1

Where: [d] mg/g; *µmol/g dry weight Source: USDA [2]; [c] Phenol-Explorer 3.6 [3]
[a] Expressed on a dry weight (DW) basis; [b] Expressed on a fresh weight (FW) basis

On a 100 g fresh weight basis, Brussels sprouts and broccoli offer approximately 110–135 µg and 70–90 µg of folate, respectively. This makes them ideal brassica vegetables for preparing folate-rich or folate-supplemented diets [15]. Since all brassica vegetables provide amounts of vitamin B (thiamine, niacin, and pyridoxine), their concentrates may be extracted to prepare health supplements for

CHAPTER 3

Brassica Microgreens and Sprouts as Superfoods

Abstract: Sprouts and microgreens have gained popularity and momentum in recent years owing to their rapid growth cycles and high nutritional value. Glucosinolate content usually decreases after reaching harvest maturity due to biochemical conversions. However, sprouts or microgreens offer significant health benefits to consumers. Sprouts are naturally harvested within 2-7 days and comprise shoots and rootlets without true leaves. In contrast, microgreens are harvested later, at 7-21 days after the development of true leaves. Both of them are well-suited and preferred for indoor cultivation. They are a rich source of secondary metabolites such as glucosinolates, phenols, and carotenoids. As a result, synergistically, these compounds display pharmaceutical effects. Additionally, microgreens or sprouts mostly exceed their mature counterparts in terms of nutrient density. During the COVID-19 pandemic, the global population gained awareness regarding the nutritional richness of these tiny plants. However, bottlenecks still exist in this area. These include limited bioavailability, linked anti-nutritional effects (goitrogenicity), and insufficient research. Such an area showcases significant research gaps and needs to be addressed before reaching a conclusive remark. Additionally, future research must increase the current understanding of processing methods to support overall health and food security.

Keywords: Bioavailability, COVID-19, Glucosinolates, Microgreens, Pharmaceutical properties, Sprouts.

INTRODUCTION

The rising global challenges of climate change and the prevalence of chronic diseases have already alarmed the growing population across the globe. In such a scenario, indoor cultivation of brassica sprouts and microgreens has gained momentum and emerged as novel food sources [1, 2]. Brassica sprouts and microgreens are renowned for their rapid growth and better nutritional content. The nutrient density of the growing substrate delivers indispensable building blocks and stress signals, which, in turn, influence the production of health-promoting phytochemicals. Their suitability for indoor cultivation not only ensures a year-round supply but also parallelly match the growing demand for sustainable genetic resources. Therefore, the escalating global interest is largely due to their remarkable array of bioactive constituents. However, interesting

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indications from *in vitro* and animal models support these claims. However, a critical assessment of human clinical data is a need of the hour. This is mandatory for fully validating their efficacy and for framing optimal consumption strategies for diverse global populations.

Brassica sprouts and microgreens display 4-40 times higher concentrations of vitamins and minerals and rapid 2–21-day growth cycles [3]. However, in real world scenario, factors such as cultivar, growing environment, and harvest stage influence their nutritional status. For example, light quality and intensity efficiently regulate photosynthetic pathways and secondary metabolite biosynthesis. Additionally, temperature influences myrosinase enzymatic activity and growth rates, thereby acting as a key factor for concentrate accumulation. This leads to conflicts in the tested nutritional profiles and poses challenges for their standardization and therapeutic applications [4]. Even though multiple *in vitro* and *in vivo* studies have emphasized the potential health benefits. However, a significant research gap in direct human clinical data, often leads to conflicts or overestimations of real-world efficacy. Although indoor hydroponic or vertical systems provide better advantages in reducing water use by almost 90-95% less and eliminating pesticides [5]. But a critical question always remains unsolved: how exactly controlled environments regulate the growth pattern and accumulate within the cells and tissues of microgreens or sprouts, compared with field-grown counterparts. In line with this, studies of the optimal parameters for achieving specific health benefits are also important. This book chapter resynthesizes the current understanding of Brassica sprouts and microgreens as nutraceuticals.

BRASSICA SPROUTS AND MICROGREENS

Currently, brassica sprouts, microgreens, and baby greens are becoming popular owing to their early availability and nutritional value [1, 2]. A drastic decrease in total glucosinolate content is observed after germination, primarily due to enzymatic hydrolysis by myrosinase, leading to the decomposition and transformation of glucosinolates into bioactive compounds [6]. Sprouts are distinct from microgreens, although both products are consumed at an immature stage of development. Sprouts constitute shoots and rootlets obtained from germinated seeds that grow for 2-7 days and are harvested when the cotyledons are still underdeveloped, and the true leaves have not yet emerged [7, 8]. Apart from sprouts, microgreens are distinguished by the full expansion of the cotyledon leaves with the appearance of the first true leaves. This generally occurs nearly 7-21 days after sowing [9]. The duration of days for baby greens extends further, *i.e.,* bridging microgreens and juvenile plants. However, all stages offer superior nutritional profiles, ranking brassica varieties as “superfoods” for functional nutrition [10, 11]. For instance, research by Xiao *et al.* [12] demonstrated that broccoli microgreens can contain 2-5 times higher glucoraphanin than kale microgreens. Similarly, red cabbage microgreens show higher anthocyanin content (1.5-3 times) than green cabbage. This difference could be due to genetic variations in the pigment biosynthesis pathway [13].

However, the exact quantity of these secondary metabolites can vary significantly between species. For example, broccoli microgreens contain high glucoraphanin content, *i.e.,* 100-300 µmol/g DW. Unlike broccoli, red radish microgreens contain lower levels of glucoraphanin but higher levels of sinigrin, *i.e.,* 50-150 µmol/g DW [14, 15]. The glucoraphanin-to-sulforaphane conversion rate varies from 60% to 90%, depending on processing methods and myrosinase activity [16]. The glucoraphanin content in broccoli sprouts could peak around the first 8 days of germination, *i.e.*, reaching 200 µmol/g DW, and decrease by 30-50% at harvest maturity [17]. To exemplify this variability, Table **1** provides a relative overview of the major bioactive constituents and their concentrations across different Brassica species. For example, broccoli sprouts can contain glucoraphanin levels up to 10-fold higher than those in mature broccoli (200 µmol/g DW vs. 20 µmol/g DW). On the other hand, red cabbage microgreens steadily show higher anthocyanin content, *i.e.,* 50-150 mg/100 g FW [18]. Broccoli microgreens can contain up to 825 mg GAE/100 g FW. Unlike broccoli, radish microgreens might exhibit lower phenolic content (300-500 mg GAE/100 g FW) and higher anthocyanin levels (30-70 mg/100 g FW) [12, 19]. Moreover, the nutrient solutions can be improved to act as chemical elicitors, resulting in the production of secondary metabolites [20]. Ntsoane *et al.* [21] reported that LED light recipes (especially blue light) boost phenolic compounds (25-60%) and glucosinolates (up to 30%) in Brassica microgreens.

Table 1. Brassica microgreens or sprouts with bioactive constituents and pharmaceutical properties.

Crop	Bioactive Compounds	Conc. at Full Maturity	Conc. at Microgreen/ Sprouts	Pharmaceutical Properties	Reference
Kale	Total glucosinolates	359-980 (nmol g^{-1} FW)	10.4 ± 0.7-14.8 ± 0.4 (mg/100 g FW)	Anticancer	[30, 12]
Red cabbage	Total carotenoids (mg/100 g FW)	0.11±0.009	10.4 ± 0.3 (microgreens)	Antioxidant	[13]
	β-carotene (mg/ 100 g FW)	11.5 ± 1.2	0.044 mg/100 g FW in microgreens	Antioxidant	[31, 18]
	lutein/zeaxanthin (mg/100 g FW)	8.6 ± 1.0	0.3 mg/100 g FW in microgreens	Anti-aging	[18]

CHAPTER 4

Brassica By-products as Functional Mines

Abstract: Brassica vegetables and their by-products, encompassing leaves, stalks, and stems, are rich and largely unexplored resources with high nutritional and functional potential. Although the florets of vegetables such as broccoli and cauliflower are traditionally utilized, valuable bioactive compounds are retained in the underutilized, generally discarded parts. Several research findings have demonstrated that Brassica by-products are loaded with antioxidants, vitamins, minerals, phenolic compounds, flavonoids, glucosinolates, and carotenoids. Broccoli leaves, in particular, exhibit potent antioxidant and anticancer properties, whereas broccoli stems exhibit elevated levels of sulforaphane. Other underutilized by-products of *Brassica* spp., such as cauliflower leaves and cabbage waste, also possess valuable bioactives, namely, cynaropicrin and inulin. These by-products exhibit potential health-promoting properties, including anti-inflammatory, antimicrobial, and antiviral activities, as well as protection against skin photoaging and the influenza A virus. Industrial applications have demonstrated that incorporating broccoli leaf powder into food products, such as pasta, enhances their nutritional value without compromising sensory quality. Valorizing these by-products supports sustainability goals and promotes a circular economy through innovative waste repurposing methods. This chapter highlights the nutritional richness and diverse health-promoting properties of *Brassica* spp. by-products, advocating their incorporation and integration into a wide array of functional foods and nutraceutical products.

Keywords: Bioactive compounds, Circular economy, Glucosinolates, Health-promoting properties, Nutritional composition, Phytochemicals.

INTRODUCTION

Traditional culinary practices involving *Brassica* spp. predominantly focus on the edible florets or other common edible parts of *Brassica* vegetables, inadvertently discarding valuable portions, such as stalks and leaves, as waste. However, as global imperatives shift towards circular economy models and heightened sustainability, the substantial nutritional and bioactive value of these *Brassica* by-products warrants attentionand can no longer be neglected [1]. This chapter explores the potential of these often-discarded components, challenging conventional perceptions of what is 'edible' and 'valuable' within the *Brassica* family. Contrary to the widespread belief, these by-products are far from mere

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refuse as they represent rich reservoirs of essential nutrients and potent bioactive compounds. While florets are well established for their concentrations of amino acids and specific glucosinolates, such as glucoraphanin [2], studies suggest that leaves and stalks often outperform them in terms of some critical phytonutrients. For example, *Brassica* leaves are often richer in carotenoids, chlorophyll, vitamins E, ascorbic acid (AA), Vitamin K, and total phenolic content (TPC), and exhibit higher antioxidant activity [2 - 4]. In addition to their general nutritional value, specific by-products demonstrate unique benefits. For example, inflorescence extracts, particularly from flower buds, exhibit strong inhibitory effects against food-borne microbes, attributing to their high glucosinolate content [5], whereas broccoli stems contain significantly higher levels of sulforaphane than florets [6]. This growing body of evidence highlights the imperative to re-evaluate and repurpose *Brassica* by-products, and transform them from 'agricultural waste' into 'valuable resources' for human nutrition, functional food development, and industrial applications. Throughout this chapter, '*Brassica* by-products' refers to the non-primary harvested parts of *Brassica* plants (*e.g.*, leaves, stalks, and stems) that possess significant nutritional and bioactive value. These materials are often discarded as 'agricultural waste,' a term we use to denote their unutilized status. The term 'residues' is used more broadly to refer to any biological material left after primary harvest or processing. While the presence of these bioactive com- pounds in *Brassica* by-products is well established, their ultimate health benefits are significantly influenced by their bioavailability, that is, how efficiently they are absorbed (*e.g.*, *via* specific transporters or passive diffusion), distributed, me- tabolized, and excreted by the body. Understanding and enhancing the bioavai- lability of these compounds is crucial for maximizing their therapeutic potential.

TYPES OF *BRASSICA* BY-PRODUCTS

Plant parts of *Brassica* spp. are often overlooked and discarded as 'waste,' but are important sources of nutrients and bioactives (Table **1**). Broccoli is considered 'a multi-faceted vegetable' for health [7], and its various parts, which are not usually consumed, are also rich sources of these valuable constituents. However, currently, only florets are consumed, while stalks and leaves are generally not utilized. Leaves and stalks contain not only nutrients but also bioactive substances [3]. In addition, Liu *et al.* [2] reported that broccoli florets have higher concentrations of amino acids, glucoraphanin, and neoglucobrassicin than other tissues. In contrast, carotenoids, chlorophyll, vitamin E and K, and total phenolic content with elevated antioxidant activity are higher in the leaves. Gudino *et al.* [5] reported findings similar to those of Liu *et al.* [2]. They also found that inflorescence extracts, particularly at the flower bud stage, had higher glucosinolate content and showed strong inhibitory effects against *Bacillus* *cereus*, *Staphylococcus aureus*, and *Listeria innocua*. This inhibition is attributed to the high content of fatty acid derivatives.

Table 1. Bioavailability and uses of various bio-actives from *Brassica* by-products.

S. No.	Crop	By-product	Bioactive Compounds	Bioavailability	Uses	Reference
1.	Kale and Cabbage	Tops (F+L)	TGC	47.75 µmol g^{-1} DW	Anticancer, anti-inflammatory, and anti-neurodegenerative	[8]
			TPC	1902.20 mg GAE/100 g DW		
2.	Red or purple Cabbage	Young shoots	TCC	123.3 mg/100 g DW	Antioxidant and anticancer	[9] `
			TPP	3201.5 mg/100 g DW		
			TA	336.7 mg/100 g DW		
			AA	186.7 µmol Trolox/g DW		
			C	795.9 mg/100 g DW		
3.	Green Cabbage	Flower buds	TPC	4.14 mM g^{-1} DW	Antioxidant and anticancer	[10]
		Leaves	TCC	0.28–12.51 µM g^{-1} DW		
4.	Broccoli	Stems	SL	76.8 µg g^{-1}	Anticancer	[6]
			TGC	7.45 µmol g^{-1} DW	Anticancer and antimicrobial	[2]
			TCC	15.6 µg g^{-1} DW	Anticancer and cardiovascular	
			TPC	939 mg GAE/ 100 g extract	Antioxidant, hepatoprotective and anti-inflammatory	[5]
			AA	882 mg Trolox/100 g dry extract	-	
		Inflorescence	TPC	1074 mg GAE/ 100 g extract	Antioxidant	
			AA	985 mg Trolox/100 g dry extract	-	

CHAPTER 5

Glucosinolates and their Biological Significance

Abstract: Glucosinolates are nitrogen- and sulfur-containing secondary metabolites that represent a class of diverse molecules (more than 130 reported to date) that are mainly found in Brassica vegetables and have numerous potential health benefits. When the plant cell is disrupted, an enzyme called 'myrosinase' quickly hydrolyzes them into relatively more bioactive forms such as isothiocyanates, indoles, nitriles, and thiocyanates, which possess anticancer, anti-inflammatory, antidiabetic, and antimicrobial properties. Among these hydrolysis products, sulforaphane, an electrophilic compound, specifically activates Nrf2 signaling by covalently modifying the cysteine residues on Keap1, leading to Nrf2 stabilization and subsequent upregulation of antioxidant and detoxifying genes, thereby protecting against oxidative stress and cancer progression. Their chemopreventive role has been highlighted by various researchers in treating cancer and fighting multidrug-resistant tuberculosis. Moreover, glucosinolate degradation products regulate phase I and II detoxifying enzymes, providing protection against various carcinogens. The present chapter explores and highlights the biochemical pathways, health benefits, and therapeutic potential of glucosinolates, emphasizing their significance as bioactive constituents in vegetable Brassicas for disease prevention in humans.

Keywords: Anticancer activity, Bioactive, Glucosinolate degradation products, Isothiocyanates, Myrosinase, Nrf2, Sulforaphane.

INTRODUCTION

Brassica spp. are rich in bioactive constituents, which, in one way or another, promote human health. Among these, 'glucosinolates' are a major but diverse group of more than 130 secondary metabolites that contain both nitrogen (N) and sulfur (S) and are found almost exclusively in Brassica vegetables [1]. Various types of glucosinolates exist, some of which are responsible for the characteristic pungent taste of these foods. When the plant cell is disrupted, an enzyme called 'myrosinase' quickly hydrolyzes the thioglucosidic bond of glucosinolates, releasing a glucose molecule and an unstable aglucone molecule. This aglucone then spontaneously rearranges, primarily into isothiocyanates, nitriles, and thiocyanates, depending on the side chain and reaction conditions (*e.g.*, pH), which represent relatively more bioactive forms [2]. The strong anticancer activity

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of Brassica vegetables is mainly attributed to the presence of glucosinolates. In addition to their anticancer properties, specific glucosinolate derivatives, such as sulforaphane, have many other bioactive properties, including antidiabetic, anti-inflammatory, antioxidant, and antimicrobial properties. This chapter describes the multifaceted roles and mechanisms of glucosinolates and their hydrolysis products in promoting human health.

GLUCOSINOLATES AND THEIR BIOLOGICAL ACTIVITIES

Glucosinolates are a group of over 130 diverse N- and S-containing secondary metabolites found almost exclusively in vegetable Brassicas [1]. Of these, only five are found in food for human consumption: glucobrassicin, glucoraphasatin, glucoiberin, glucoraphanin, and sinigrin [3]. The sharp taste of mustard seed was the first observation of glucosinolates in the 17th century [4]. These are categorized into acryl aliphatic, aliphatic, and indole types, depending on their amino acid precursors (aromatic, methionine, and tryptophan derived), of which methionine is of health significance and is most common among *Brassica* spp. [5]. In the *Brassica* genus, aliphatic compounds are primarily found, whereas indole compounds are present in the vegetative parts of *Brassica* species but are absent in the seeds [6]. The plant enzyme myrosinase hydrolyzes glucosinolates and yields isothiocyanate (contains S), indoles (contains no S), nitrile, and thiocyanate when they reach the stomach [2]. These hydrolytic products modulate the activities of phases I and II enzymes. Phase I enzymes generally increase the reactivity of lipophilic compounds, whereas phase II enzymes enhance water solubility and facilitate the removal of metabolites from the body [7]. To protect cells from DNA damage caused by carcinogens and reactive oxygen species (ROS), the inhibition of phase I enzymes and the induction of phase II enzymes are required.

Cancer and multidrug-resistant TB (MDR-TB) are among the deadliest diseases worldwide. However, cancer is a primary cause of death worldwide, with the global cancer burden rising to 18.1 million new cases and 9.6 million cancer deaths, as explained by Walia *et al.* [8]; therefore, Brassica vegetables with anticancer activity can be a breakthrough (Table **1**).

Table 1. Biological activity of some glucosinolates present in Brassica vegetables with supporting references. *Indicates that the study is based on *in vitro* experiments.

S. No.	Crop	Principal Glucosinolate	Biological Activity	References
1	Broccoli, Brussels Sprouts	Sulforaphane	Anti-COVID-19*, Anti-inflammatory, Anti-depression*, Anticancer*, Antidiabetic	[9 - 14]
		Sulforaphane, glucoraphanin	Anticancer; Antidiabetic, Anti-obesity	[15 - 20]
		Isothiocyanates	Antimicrobial, Anticancer, Detoxification, Antidiabetic	[21 - 28]
		Glucobrassicin, indole---carbinol	Chemopreventive and Antibacterial (*Citrobacter rodentium*)	[29 - 31]
		Glucocapparin, Gluconasturtiin, Glucobrassicin and Sinigrin	Against Crimean-Congo hemorrhagic fever virus, Anti-proliferative and Anticancer	[32, 33]
2	Kale, Cauliflower, Broccoli and Cabbage	Sulforaphane	Anti-cardiovascular, Neurodegenerative, Chemopreventive and Antidiabetic	[34 - 38]
3	Radish, Turnip, Watercress, Broccoli	Gluconasturtiin, phenethyl isothiocyanate, gluconapin	Antidiabetic, Anti-obesity	[39 - 41]
4	Turnip	Gluconasturtiin, glucobrassicanapin, gluconapin	Anticancer, Antimicrobial	[42 - 44]
5	Red and White Cabbage (shoots)	Sinigrin, progoitrin, glucoiberin, glucobrassicin	Antioxidant, Anticancer, Anti-proliferative	[45 - 47]
6	Chinese Cabbage, Knol Khol, Rocket, Argula	Glucoerucin, erucin	Anti-obesity* and Anti-hypertension	[48, 49]
			Anti-cardiovascular	[50]
			Apoptosis in colorectal cancer cells.	[51]
7	Rocket	Glucoraphanin, glucoerucin and Glucosativin	Prevention of free radicals	[52]
8	*Sinapis alba* (White mustard) and *Sinapis nigra* (Black mustard)	Sinigrin, allyl isothiocyanate	Antimicrobial, Anti-inflammatory*,	[3, 53 - 55]
9	Watercress	Gluconasturtiin	Nephrotoxicity and oxidative stress inhibition	[56, 57]

In vitro Studies

To date, several *in vitro* studies involving human cell lines have suggested the health benefits of Brassica vegetables. Sulforaphane (an isothiocyanate) was

CHAPTER 6

Exploring the Multifaceted Role of Glucosinolates: Metabolism, Bioactivity, and Therapeutic Potential

Abstract: Glucosinolates (GSLs) are sulfur-containing compounds found in plants of the Brassicaceae family, such as broccoli and Arabidopsis. Although GSLs are biologically inactive in their intact form, they undergo hydrolysis by myrosinase enzymes to produce bioactive compounds, such as isothiocyanates (ITCs), thiocyanates, and nitriles. These hydrolytic products contribute to the distinctive taste and aroma of Brassicaceae plants and exhibit biocidal properties against pathogens, pests, and fungi. GSL content in plants is influenced by various abiotic and biotic factors, including sulfur and nitrogen levels, salinity, herbivore stress, and pre-harvest treatments. Upon ingestion, GSLs are hydrolyzed by myrosinase from plant sources or the gut microbiota, and the resulting bioactive compounds are absorbed and further metabolized, giving rise to diverse biological effects. ITCs, particularly sulforaphane, exhibit anti-inflammatory, neuroprotective, anti-obesity, and anticancer properties through mechanisms such as the inhibition of NF-κB activation, activation of phase II detoxification enzymes, and modulation of the gut microbiota. However, the bioavailability of GSLs and their derivatives is affected by factors such as the human microbiome and interactions between the microbiota, enterocytes, and hepatocytes. Several pre-consumption factors, such as cooking practices, storage conditions, and the complexity of the plant tissue matrix, also influence the bioavailability of GSL. To maximize the health benefits from Brassica consumption, further research is needed to optimize GSL preservation and bioavailability.

Keywords: Bioactive compounds, Bioavailability, Brassicales, Glucosinolates (GSLs), Gut microbiota, Myrosinase.

INTRODUCTION

Plants employ both physical traits, such as latex and trichomes, and chemical defenses through secondary metabolites to protect themselves from herbivores [1]. Among these defenses, glucosinolates (GSLs) are key bioactive compounds found in Brassicales plants, such as oilseed rape, broccoli, and Arabidopsis, and play a crucial role in pest resistance. GSLs are anionic compounds composed of a glucopyranosyl unit, sulfate, thiohydroximates, and variable amino acid-derived side chains. GSLs are categorized as aromatic, aliphatic, or indolic based on their

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amino acid sources. GSLs are biologically inactive in their intact form and serve as sulfur storage sites. However, their degradation products, such as isothiocyanates (ITCs), thiocyanates, nitriles, and epithionitriles, are biologically active and exhibit a wide range of effects [2]. These hydrolytic products contribute to the pungent aroma and bitter taste of Brassicaceae plants [3] and possess biocidal properties against pathogens, pests, and fungi. They also have the potential to enhance human and animal health, particularly in terms of disease prevention and treatment. Nonetheless, excessive GSL hydrolytic products in animal feed can lead to adverse health effects, including thyroid dysfunction, goitrogenicity, liver toxicity, and kidney toxicity [4].

Glucosinolates are sulfur-containing compounds that are abundant in Brassica vegetables. Recent studies have identified several genes involved in glucosinolate (GSL) biosynthesis in *Arabidopsis thaliana* [5]. In Brassica crops, glucosinolates are primarily synthesized from amino acids such as phenylalanine, methionine, tryptophan, and tyrosine. These amino acids are vital for the formation of aliphatic, indole, and aromatic GSLs derived from methionine, phenylalanine, and tryptophan, respectively [6]. Isothiocyanates (ITCs) have been found to disrupt cell cycle progression and induce apoptosis in cancer cells by activating caspases and modulating Bcl-2 family proteins. Additionally, glucosinolates and ITCs are identified for their epigenetic effects, which influence gene expression through mechanisms such as DNA methylation and histone modification. For example, ITCs can inhibit histone deacetylases (HDACs), leading to histone hyperacetylation and upregulation of genes associated with cell cycle arrest and apoptosis.

When glucosinolates are broken down in the digestive system, isothiocyanates swiftly enter the bloodstream. They then bind to plasma proteins, facilitating their entry into cells through the plasma membrane (Fig. **1**). Once inside, isothiocyanates bind to glutathione in a reaction catalyzed by glutathione S-transferase (GST). Outside the cell, enzymes such as γ-glutamyl transferase and dipeptidases remain active in the degradation of the compound. After these enzymatic processes, the resulting metabolite is transported to the liver, where it enters the mercapturic acid pathway.

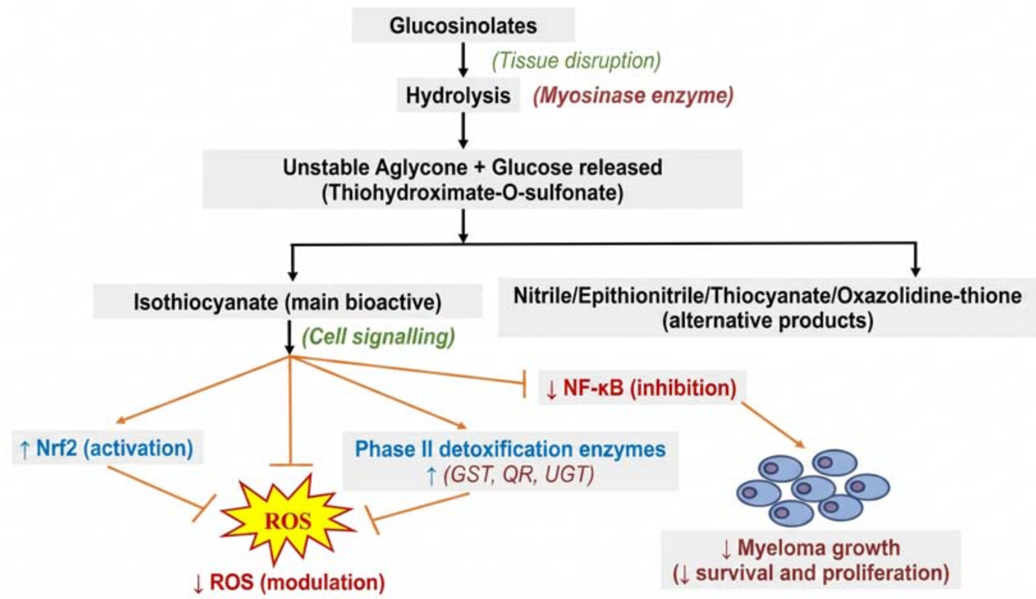

Fig. (1). Pathway showing the production of isothiocyanates and alternative products from glucosinolates and their effects on the production of reactive oxygen species (ROS) and myeloma cells.

In this pathway, N-acetyltransferases modify the metabolite, producing either an N-α-acetyl derivative or mercapturic acid, which is then excreted through the kidneys into the urine, thereby completing the elimination process [7, 8]. Additionally, recent investigations indicate that glucosinolates may offer neuroprotective benefits, as demonstrated by a study [9] that investigated the effects of sulforaphane on oxidative stress and neuroinflammation in a mouse model of Parkinson's disease. Their results revealed that sulforaphane administration reduced oxidative stress and neuroinflammation, further suggesting thepotential role of glucosinolates in the prevention and treatment of neurodegenerative diseases. In addition to their direct health benefits, glucosinolates may also influence the bioavailability and metabolism of other nutrients.

IMPACT OF ABIOTIC AND BIOTIC FACTORS ON GLS LEVELS

The glucosinolate (GLS) content in plants is governed by species and variety and is profoundly modulated by the conditions in which they are grown. The main factors that lead to variations in GLS content are abiotic and biotic factors [10].

CHAPTER 7

Phenolic Compounds: From Dietary Sources to Therapeutic Applications in Disease Prevention

Abstract: Phenolic compounds encompass a diverse array of over 8,000 substances, each containing at least one aromatic ring with one or more hydroxyl groups. These secondary metabolites are produced by plants *via* the shikimic acid pathway. Phenolic compounds are divided into flavonoids and non-flavonoids, with flavonoids comprising approximately two-thirds of the total phenolic content. Phenolic acids, which are the primary focus of this review, comprise the remaining one-third. Although their absorption in the small intestine is limited to 5-10%, phenolic compounds offer numerous health benefits, including anti-inflammatory, anticancer, and antidiabetic properties. However, excessive intake may lead to toxicological effects owing to their pro-oxidant nature, highlighting the importance of establishing safe consumption levels through clinical studies. Phenolic acids are further classified into hydroxybenzoic and hydroxycinnamic acid derivatives, each providing a range of health benefits, including antidepressant, antihypertensive, and neuroprotective effects. Tannins, another class of phenolic compounds, are divided into hydrolyzable and condensed types and possess antioxidant properties with various health benefits, including cardioprotective, antitumor, anti-inflammatory, and antimicrobial effects. Recent studies have also underscored the effectiveness of natural phenolic compounds in anti-COVID-19 therapies. In conclusion, phenolic compounds found in foods have the potential to enhance health and prevent diseases, with applications in food preservation, bioactive packaging, natural dyes, and various industries such as pharmaceuticals and materials engineering.

Keywords: Bioavailability, Flavonoids, Non-flavonoids, Phenolic acids, Therapeutic potential Gut microbiota.

INTRODUCTION

Phenolic acids are a prominent group of bioactive compounds found abundantly in various plant sources, such as fruits, spices, and vegetables. These compounds enhance the sensory qualities of foods by contributing to their color, flavor, and astringency. Phenolic acids offer health benefits, including anti-inflammatory, anticancer, and antidiabetic effects [1]. They are used as functional additives in food to prolong shelf life by inhibiting microbial growth and lipid oxidation [2]. Although the phenolic acid content in fruits and beverages has been extensively

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researched [3], vegetables, despite being rich in these compounds, have received comparatively less attention because of their lower concentrations. Vegetables are a vital part of the diet, providing vitamins, fibers, and minerals, and serving as a significant source of phenolic acids [4]. The phenolic acid content varies according to maturity, environmental stress, and variety. Research has shown that phenolic acid levels are higher in flowers, roots, leaves, and stems than in fruits and vegetables. Additionally, phenolic acid content is influenced by the morphological characteristics of vegetables, as demonstrated by the higher concentration of phenolic acids in purple carrots than in orange carrots [5] and pigmented potato cultivars [6].

While the phenolic acid content in fruits and beverages, especially in berries, grapes, and coffee, has been extensively studied [7], vegetables, despite being rich in these compounds, have received comparatively less attention. This is because phenolic acids often occur at lower concentrations and within more complex matrices. Vegetables are a vital part of the diet, providing vitamins, fibers, and minerals, and serving as a significant source of phenolic acids and other phenylpropanoids that contribute to overall antioxidant intake [8]. The phenolic acid content in vegetables varies with maturity, environmental stress (light, temperature, drought, and salinity), agronomic practices, and variety or cultivar, reflecting both genetic and environmental regulation [4, 9, 10]. Research has shown that phenolic acid levels are often higher in flowers, roots, leaves, and stems than in fruit and vegetables, consistent with their roles in defense, UV protection, and structural functions in plants [11]. In addition, phenolic acid content is strongly influenced by the morphological and pigmentation characteristics of vegetables. For instance, [12] and [13] reported several-fold higher total phenolic content and phenolic acids in purple and black carrots than in orange carrots, associated with higher anthocyanin and phenylpropanoid pathway activity. Similar trends have been observed in pigmented potato cultivars, where colored-flesh varieties exhibit up to an order of magnitude higher phenolic acid levels and antioxidant capacity than white or yellow-fleshed potatoes, with chlorogenic acid and its related derivatives predominating [14, 15]. These findings highlight the potential of exploiting varietal diversity and targeted breeding to develop vegetable cultivars with enhanced phenolic acid content and improved functional and nutritional properties [16].

Phenolic compounds encompass a vast array of over 8,000 distinct substances, each characterized by at least one aromatic ring with one or more attached hydroxyl groups [17]. Phenolics are synthesized in plants as secondary metabolites *via* the shikimic acid pathway, in which phenylalanine ammonia-lyase (PAL) facilitates their production from the aromatic amino acid phenylalanine [18]. Phenolic compounds are classified based on the number and arrangement of their carbon atoms into flavonoids (such as flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, and isoflavones) and non-flavonoids (including phenolic acids, hydroxycinnamates, and stilbenes) [19, 20]. Flavonoids perform numerous functions in plants, including photoprotection, UV screening, modulation of auxin transport, defense against pathogens, and contribution to flower and fruit coloration, which attracts pollinators and seed dispersers [21]. In humans, dietary flavonoids and phenolic acids exhibit strong antioxidant properties through radical scavenging, metal chelation, and antioxidant enzyme modulation. They can protect low-density lipoproteins (LDL) from oxidative modification, thereby reducing cardiovascular and diabetic risks [22]. In addition to their antioxidant activity, these compounds influence cell signaling pathways, inflammation, and endothelial function, supporting their potential in the prevention or adjunct management of chronic diseases [23 - 25].

ANTIOXIDANT POTENTIAL OF PHENOLS POSING HEALTH BENEFITS

Currently, a diet rich in glucosinolates and phenols is essential for combating SARS-CoV-2 [24]. Recent studies have highlighted the efficacy of natural phenolic compounds with anti-COVID-19 properties [25-27]. Additionally, quercetin has been acknowledged for its anti-inflammatory effects, as it modulates the inflammatory response during infections [28]. *In vitro* studies in mice have validated the antidiabetic properties of these compounds [29-30]. The use of phenolic compounds to inhibit angiotensin-converting enzymes has been applied in managing hypertension, whereas the inhibition of carbohydrate-hydrolyzing enzymes shows promise for treating type 2 diabetes mellitus, and cholinesterase inhibition has been employed in the treatment of Alzheimer's disease [31]. Abu et al. [32] explored the health benefits of green and purple cabbages, focusing on their antioxidant and cytotoxic properties. Kaempferol, a compound found in Brassica vegetables, has been shown to significantly lower blood glucose and HbA1c levels while enhancing insulin sensitivity [33]. Further *in vitro* studies have suggested that kaempferol boosts glucose uptake and glycogen synthesis, likely due to the upregulation of GLUT4, an insulin-responsive glucose transporter (Fig. **1**) [34]. Quercetin, a flavonol commonly found in Brassica vegetables such as broccoli, is known for its ability to inhibit LDL oxidation by binding to metal ions and neutralizing free radicals. Its potential to activate enzymes that detoxify various carcinogens, along with its antioxidant properties, may help prevent chronic inflammation, atherosclerosis, and cancer. Several epidemiological studies have suggested that a diet rich in broccoli lowers the risk of developing prostate cancer. Evidence indicates that consuming one or more servings of broccoli per week can significantly reduce both the incidence and progression of prostate cancer, from less severe to more aggressive forms of the

CHAPTER 8

Carotenoid Biosynthesis and its Function for Human Health and Longevity

Abstract: Carotenoids, a diverse group of pigments found in various organisms, play a crucial role in human health by reducing the risks of cardiovascular diseases, cancer, and eye disorders. Brassica vegetables, particularly kale, are rich in carotenoids, and their levels are influenced by genotype, farming practices, and environmental conditions. The human body can convert certain carotenoids (α- and β-carotene) into vitamin A. In plants, phytoene synthase and carotenoid isomerase are the key enzymes involved in carotenoid biosynthesis, mainly *via* the methylerythritol phosphate (MEP) pathway. In a living system, physical aging is characterized by a decline in function ability, an increase in disease vulnerability, and a disruption in cellular processes. Carotenoids can neutralize harmful reactive oxygen species, reduce oxidative stress, and increase cell survival, potentially delaying aging. They may also influence gene expression *via* various signaling pathways. Carotenoids have been used as nutraceuticals and antioxidants in traditional medicine across different cultures. Research supports their protective significance in various health conditions, and modern science is exploring ways to enhance their efficacy through targeted therapies, nanoformulation, and encapsulation. Further research is needed to optimize the administration and delivery methods of carotenoids for human and animal health.

Keywords: Antioxidants, Brassica, Carotenoids, Methylerythritol phosphate pathway, Phytoene synthase.

INTRODUCTION

Carotenoids, composed of 40 carbon atoms (C40), are found in bacteria, plants, algae, and fungi. Whereas apocarotenoids are also a class of carotenoids with fewer than 40 carbon atoms [1]. Broadly, carotenoids have been categorized as xanthophylls and carotenes. In carotenes, α-carotene, β-carotene, γ-carotene, and lycopene are the primary carotenoids [2]. Among these, α- and β-carotene are the most abundant carotenoids. In plant cells, chloroplasts and chromoplasts are the primary sites of carotenoids. Carotenoids play an essential role in human health by reducing the risks of cardiovascular diseases, cancer, and eye problems [3]. Brassica vegetables are especially rich in carotenoids, abundant in β-carotene and lutein [4]. Among *Brassica oleracea* cultivars, kale has the highest carotenoid

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content (>10 mg per 100 g), followed by Brussels sprouts (6.1 mg per 100 g) and broccoli (1.6 mg per 100 g) [5]. The carotenoid content of Brassica crops is affected by genotype, environmental factors, and agronomic techniques [6]. The human body can convert α-carotene, β-carotene, and β-cryptoxanthin into retinol (vitamin A) [7]. Carotenes, which are fat-soluble/non-polar compounds, are situated on the periphery of the cell membranes, whereas xanthophylls are polar molecules. Notably, xanthophylls like lutein and zeaxanthin cannot be converted into vitamin A. Among *Brassica species*, kale is recognized as the most abundant source of β-carotene and lutein [8]. Sun *et al.* [9] revealed that the carotenoid content of *Brassica oleracea* var. *gemmifera* ranged from 0.88 mg/kg in the swollen flesh stem to 335.02 mg/kg in the lateral bud leaves, and was positively correlated with antioxidant activity. A diet rich in carotenoids has been linked to a reduced risk of cancer (Table **1**). Drozdowska *et al.* [10] found a significantly higher concentration of total carotenoids in young shoots of red cabbage than in white cabbage [11]. In addition, medical professionals recommend that COVID-19 patients consume a diet high in β-carotene to strengthen their immune systems [12]. Theories of oxidative stress, skin photoaging, inflammation, and metabolism are crucial for exploring the causes and mechanisms of skin aging. A clinical study by Meinke *et al.* [13] involved volunteers who were given natural kale extracts rich in carotenoids. The findings of this study indicate a notable increase in dermal carotenoid levels and a better collagen I/elastin aging index at 5 and 10 months after the start of supplementation.

Table 1. Epidemiological research studies on carotenoids for pharmacological effects.

S. No.	Activity/Disease	Principal Carotenoid	Mechanism of Action	References
1	Anticancer	Astaxanthin	Triggered apoptosis of cancerous cells, especially astroglioma multiforme (GBM) cells	[14, 15]
		Lycopene	High intake inversely correlated with gastric and prostate cancer disease	[16, 17]
		β-carotene	Reduced risk of breast cancer	[18, 19]
2	Neurodegenerative	Astaxanthin	Inhibits endoplasmic reticulum stress and prevents Parkinson’s disease	[20]
		Lycopene	Inhibits oxidative stress and neuronal apoptosis to prevent Alzheimer's disease	[21]
		Crocin	Hinder NF-kB and NLRP3 signaling pathways to relieve anxiety and depression	[22]
3	Osteoporosis	β-carotene and cryptoxanthin	Inhibit bone resorption and improve bone mineral density	[23, 24]
4	Cardiovascular	Lycopene	Regulates blood pressure	[25, 26]

(Table 1) cont.....

S. No.	Activity/Disease	Principal Carotenoid	Mechanism of Action	References
5	Antiinflammatory	Lutein	Decreased C-reactive protein (CRP) level revealed anti-inflammatory activity.	[27]
6	Eye and skin	Astaxanthin, phytoene, and phytofluene (Colourless)	UV protection and improved skin texture	[28 - 30]
		Lutein and zeaxanthin	Maintenance of eye health	[31]
7	Antiviral (SARC-CoV-2)	Siphonaxanthin	Viral infection decreased with increased siphonaxanthin intake	[32]
8	Antiobesity	Lycopene	Increased lipolysis and decreased insulin resistance	[33, 34]
		Lutein	Positively correlated with body and visceral fat loss	[35, 36]
		Astaxanthin	Decreased malondialdehyde content reflected its lipid peroxidation and antioxidant potential.	[37]
		Fucoxanthin	Suppressed *Erysipelotrichaceae* and *Lachnospiraceae* activity, while boosted growth of *Bifidobacterium* and *Lactobacillus*	[38]

BIOSYNTHETIC GENES

Studies have shown that carotenoids are essential for plant growth. In plants, with the help of the Methylerythritol phosphate (MEP) pathway, isopentenyl diphosphate and dimethylallyl diphosphate (DMAPP) are produced that serve as substrates for the synthesis of geranylgeranyl diphosphate (GGPP), a carotenoid precursor [39]. The condensation of two GGPP molecules, facilitated by phytoene synthase, leads to the formation of 15-cis-phytoene. Desaturases convert phytoene into prolycopene, which is further transformed into lycopene by carotenoid isomerase (CRTISO) (Fig. **1**). Lycopene undergoes cyclization to form α-carotene and β-carotene, and lutein is synthesized from α-carotene *via* the action of Cytochrome P450 enzymes [40]. Cao *et al.* [41] identified 62 carotenoid biosynthesis genes (CBGs) in cabbage, of which 46 were mapped to 9 chromosomes. PSY genes in cabbage are localized to the chloroplasts and exhibit tissue-specific expression. The 'Or' gene mutation in cauliflower affects PSY, thereby influencing carotenoid accumulation [42].

CHAPTER 9

Tuberculosis in the Modern Era: Diagnosis, Drug Resistance, and Brassica's Promising Applications

Abstract: Tuberculosis (TB) remains a significant global health challenge, with over 80% of cases and deaths occurring in low- and middle-income countries. The emergence of drug-resistant strains, such as multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), is a major issue that requires more efforts to control this disease. Rapid and precise diagnosis is essential for effective treatment and prevention of disease transmission. Modern molecular diagnostic tools, such as Xpert MTB/RIF and next-generation sequencing (NGS), provide high-speed, high-sensitivity TB diagnosis, whereas culture-based drug susceptibility testing (DST) remains the gold standard. However, challenges such as cost, interpretation expertise, and maintenance hinder their wide use. Potential research on Brassica plant-based TB vaccines and the role of nutrition in improving treatment has shown promising results. Studies have shown that consuming vegetables rich diet, such as Chinese cabbage, radish, and cauliflower, may significantly reduce the risk of TB treatment failure. Furthermore, certain isothiocyanate compounds found in Brassica plants exhibit growth-inhibiting properties against Mycobacterium tuberculosis, including MDR strains. To develop new therapeutic strategies and meet the United Nations Sustainable Development Goal of eliminating the TB epidemic by 2030, robust research, collaboration, and improved access to care are essential.

Keywords: Brassica, Diagnosis, MDR-TB, Molecular diagnostic tools, Tuberculosis, XDR-TB.

INTRODUCTION

Tuberculosis (TB) mainly spreads through airborne particles, as individuals infected with the disease release bacilli from their lungs into the environment [1]. These bacilli, when inhaled by susceptible individuals, including those in close contact and those with weakened immune systems, become the source of infection (Fig. **1**) [2]. Once inhaled, the bacteria multiply in the lungs, triggering an immune response that leads to granuloma formation [3]. Approximately 10% of infected patients develop active, symptomatic TB, whereas 90% develop latent, non-transmissible TB [4]. Approximately 5–10% of immunocompetent individuals with latent TB infection (LTBI) will develop active TB during their

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lifetime, with the highest risk within the first 2–5 years after infection; this risk can increase by more than 20-fold in the presence of HIV or other causes of severe immunosuppression [5]. Latent TB is characterized by viable but dormant bacilli within granulomas that can reactivate when host immunity declines, as in the case of HIV, malnutrition, advanced age, silicosis, or the use of TNF-α inhibitors [6]. The Tuberculin Skin Test (TST) and interferon-gamma release assays (IGRAs) remain widely used tools for detecting LTBI, although BCG vaccination and non-tuberculous mycobacteria can cause false-positive results in TST. IGRAs offer higher specificity but at a higher cost, especially for drug-resistant tuberculosis [7]. Latent TB can potentially reactivate, especially under conditions that weaken the immune system, such as HIV infection and malnutrition [8]. Reactivation occurs when bacteria within granulomas become active due to immunosuppression [9]. Tuberculin Skin Test (TST) is a widely recognized method for detecting TB exposure by assessing the immune response through delayed hypersensitivity to a purified protein derivative from heat-inactivated bacilli. The TST remains the standard method for diagnosing latent TB infection [8]. Various laboratory techniques are available for TB diagnosis, including phenotypic methods such as acid-fast smear microscopy, microscopically observed procedures, and agar proportion testing [10]. To effectively combat tuberculosis (TB), a regimen of antibiotics, including isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin, is typically recommended for daily use for four to six months. Completing the entire treatment course is crucial for preventing drug resistance [11]. Eradication of the TB epidemic is one of the health targets of the United Nations Sustainable Development Goals (SDGs) by the year 2030 [12].

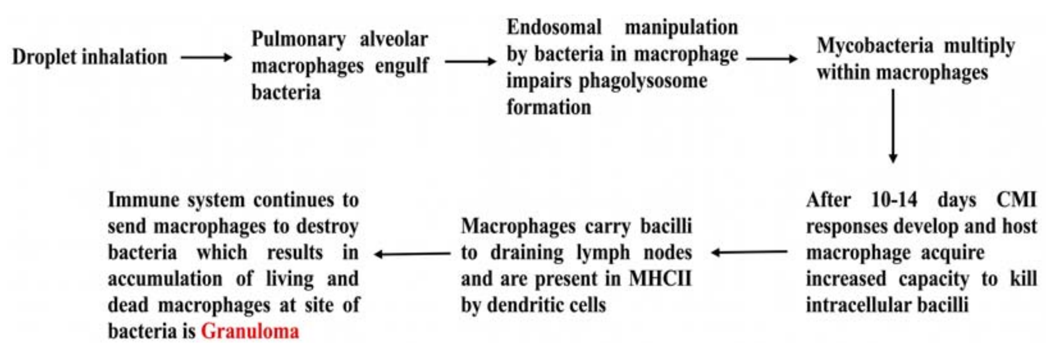

Fig. (1). TB infection and granuloma formation process.

Phenotypic diagnostic methods include sputum smear microscopy using Ziehl–Neelsen or fluorochrome staining, which is rapid and inexpensive but insensitive, especially in HIV co-infection and pediatric diseases. Culture on solid or liquid media and drug susceptibility testing (DST) are considered reference standards; however, they are slow and resource-intensive. Rapid molecular tests, such as Xpert MTB/RIF/Ultra and Truenat MTB, have transformed TB diagnosis by detecting *M. tuberculosis* DNA and rifampicin resistance within hours, improving case detection and enabling earlier initiation of appropriate therapy [13, 14]. The standard first-line treatment for drug-susceptible TB typically consists of a 2-month intensive phase with isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by a 4-month continuation phase with isoniazid and rifampicin, with a recommended total duration of six months. Shorter 4-month regimens based on rifapentine and moxifloxacin have shown non-inferiority in selected patients; however, the WHO still recommends the 6-month regimen as the global standard while the scale-up of shorter regimens is ongoing. Strict adherence is essential to prevent relapse and the emergence of drug-resistant TB, which is more difficult and costly to treat [35]. TB remains a major global health challenge; in 2023, an estimated 10.3 million people fell ill with TB, and 1.3 million died, with more than 80% of cases and deaths in low- and middle-income countries in regions such as Africa and Southeast Asia. The United Nations Sustainable Development Goals (SDGs) and WHO's End TB Strategy aim to reduce TB incidence and deaths by 80% and 90%, respectively, by 2030, compared to 2015 levels; however, current progress is insufficient, requiring intensified efforts in diagnosis, treatment, prevention, and social determinants of health [5].

TDR-TB, a severe form of the disease, was identified first in Italy in 2006 and subsequently detected in India at Hinduja Hospital in Mumbai in the year 2012 [15]. Multidrug-resistant tuberculosis (MDR-TB) poses a significant challenge due to its resistance towards primary treatment medicines, such as isoniazid and rifampicin, thereby necessitating the use of second-line medications, which are comparatively more expensive and linked to more severe adverse effects than first-line medications. Drug resistance can be linked to the improper use of tuberculosis medications, including incorrect prescriptions, substandard drug quality, and early treatment cessation. MDR-TB remains a critical public health problem threatening health security, with only approximately 40% of patients being able to receive treatment by the year 2022. In 2015, the World Health Organization (WHO) reported a total of 484,000 cases of drug-resistant tuberculosis (TB), with multidrug-resistant TB (MDR-TB) showing resistance to first-line antibiotics, such as isoniazid (INH) and rifampicin (RIF), which are often found associated with poor treatment adherence [16]. Extensively drug-resistant TB (XDR-TB), which is resistant to fluoroquinolones as well as second-line injectable drugs, has been detected in 127 countries, with a notable prevalence in Russia and South Africa, where it is worsened by HIV coinfection [12, 15]. MDR-TB treatment requires 20–30 months of second-line pharmacotherapy, whereas XDR-TB is treated with a combination of second-line

CHAPTER 10

The Role of Plant-based Diets and *Brassica* spp. in Diabetes Mellitus Control

Abstract: Diabetes mellitus is a chronic condition affecting millions of people globally. It is characterized by insufficient insulin production or utilization, leading to hyperglycemia. Plant diets rich in *Brassica* spp. have shown promising results in the management of diabetes. This chapter explores the mechanisms by which vegetable-based diets affect diabetic control, with a focus on the role of Brassica vegetables. The diagnosis of diabetes involves hemoglobin A1c, fasting plasma glucose, and an oral glucose tolerance test. Plant-based diets are higher in fiber and lower in calories, helping regulate body weight and improve insulin sensitivity by reducing glucose absorption and promoting a healthy gut microbiome. Replacing meat with plant-based proteins in the human diet can be achieved by increasing the consumption of fruits, vegetables, and whole grains. It additionally provides essential nutrients to enhance glycemic control. Various *in-vitro* studies have highlighted the potential of Brassica vegetables to manage post-prandial hyperglycemia by inhibiting the activities of α-amylase and α-glucosidase. Bioactive compounds such as glucosinolates, phenolic compounds, and sulforaphane have demonstrated anti-diabetic effects through various mechanisms. These include activating Nrf2, increasing GLUT4 expression, and inhibiting dipeptidyl peptidase IV. The preclinical evidence supports the biological potential of *Brassica* spp. in modulating glucose regulation and improving insulin sensitivity. However, further clinical trials are required to validate these findings and establish Brassica vegetables as a choice of foods for diabetes management.

Keywords: *Brassica* species , Fasting plasma glucose, Hyperglycemia, Hemoglobin A1C, Plant-based diets, Insulin sensitivity.

INTRODUCTION

Diabetes mellitus is a chronic condition resulting from either inadequate insulin production or the inability of the human body to effectively use insulin, affecting approximately 537 million adults aging between 20 and 79 years, globally. This number is expected to increase to 783 million by the end of the year 2045 [1]. The poorly managed diabetes mellitus at early stages often results in hyperglycemia, which is marked by elevated blood glucose levels. Further, the global rise in obesity, frequently associated with sedentary lifestyles, high-calorie diets, obesity,

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and increased life expectancy, is also worsening the situation. Diabetes is primarily categorized into three types, *i.e.*, type 1, type 2, and gestational. Out of these, type 2 is linked with long-term health risks. It is a chronic condition commonly associated with lifestyle factors and is the most prevalent form of diabetes throughout the world. Excessive carbohydrate intake can lead to insulin resistance, which can subsequently cause hyperglycemia [2]. Synthetic drugs are often used for the prolonged management of degenerative diseases, including diabetes, generally considered 'silent-killers'. The traditional drug discovery regimes typically follow a 'one-disease, one-target, one-drug' model. However, complex diseases like diabetes can be largely benefited from the 'multi-component therapeutics' that employ various nutraceuticals targeting multiple pathways, a concept gaining traction in the scientific discourse. Plant-based medicines generally provide a safer alternative with relatively fewer side effects [3].

Chronic hyperglycemia, generally resulting from inadequate insulin secretion, disrupts the energy metabolism in humans. Clinically, it manifested as increased appetite, polydipsia, dysuria, weight loss, and visual disturbances. However, some individuals in the early stages of type 2 diabetes may remain asymptomatic [4]. Further, the uncontrolled diabetes may result in coma or even death, due to ketoacidosis or non-ketotic hyperosmolar syndrome [5]. Diabetes mellitus is also linked with several other severe complications, including blindness, renal failure, myocardial infarction, stroke, and lower limb amputation. Preventing these adverse outcomes requires an understanding of crucial health factors leading to diabetes [6].

DIAGNOSIS

Diabetes mellitus (DM) is mainly diagnosed using hemoglobin A1c (HbA1C), fasting plasma glucose (FPG), and oral glucose tolerance test (OGTT). The DM diagnosis is confirmed when the FPG is ≥126 mg per dL, 2h of OGTT is ≥200 mg per dL, or HbA1c is ≥ 6.5% (48 mmol per mol) [**7,** 8]. HbA1c-based diagnosis is preferred for its stronger correlation with microvascular complications and its greater stability compared to the FPG or OGTT [9]. When HbA1c is the only diagnostic test used, it is to be repeated within two weeks in asymptomatic patients. Further, ethnicity also influences the diagnostic cut-off values, leading to variations across countries or demographics [10]. Although some studies suggested HbA1c as an identifier in fewer patients than FPG or OGTT, whereas, others reported the opposite [11].

MECHANISTICS OF VEGETABLE-BASED DIET ON DIABETES CONTROL

Vegetarian diets, which emphasize more on the high-fiber foods, including whole grains, vegetables, and legumes, have been shown to improve insulin sensitivity and manage blood glucose levels. Additionally, plant-based diets generally have a lower glycemic index (including non-starchy vegetables and certain fruits), which may help to stabilize the blood sugar levels. On the other hand, low-processed foods, which are rich in healthy fats (like nuts, seeds, and avocados), can also help in diabetes management by reducing inflammation and improving metabolism. The specific therapeutic outcomes depend on the macronutrient composition (mainly carbohydrates, fats, and proteins), the presence/absence of specific micronutrients (such as magnesium), the concentration of dietary antioxidants, and the overall bulk characteristics (presence of dietary fibers) of the plant material. Studies have shown that low-fat vegan diets may offer more significant benefits than more moderate vegetarian diets that include dairy and eggs, suggesting that plant-based diets could be more effective in improving insulin resistance and associated oxidative-stress markers over the conventional diets in individuals with type 2 diabetes.

Regulation of Body Weight

The plant-based foods have a higher fiber content (mainly the soluble-fiber from vegetables, legumes, fruits, and whole grains) and low caloric density. This fiber is crucial for the regulation of blood glucose levels by retarding glucose absorption. The gradual release of glucose helps in checking the sharp spikes in the blood sugar levels and contributes to maining a stable energy level. Moreover, plant-based diets often replace high-calorie foods (mainly rich in fats and carbohydrates) with micronutrient-rich, low-calorie alternatives, which in turn may help with weight management. Even a modest reduction in body weight may significantly improve insulin sensitivity and glycemic control [11]. The benefits of a plant-based diet extend beyond weight management and offer additional therapeutic properties. They can be helpful in reducing inflammation, as they are rich in antioxidants and can reduce oxidative stress. The oxidative stress is a key factor contributing towards the development of insulin resistance. Additionally, plant-based diets also promote a healthy gut microbiome. Collectively, all the above factors suggest that shifting to plant-based diets offers a comprehensive strategy for diabetes prevention and management, involving multiple physiological pathways to improve overall energy metabolism and glycemic control [12].

CHAPTER 11

Brassica Vegetables as Natural Anti-aging Agents: Mechanism and Clinical Applications for Enhancing Skin Health

Abstract: Brassica vegetables (broccoli and kale) have attracted significant interest as natural anti-aging agents due to the abundance of bioactive compounds and antioxidant properties. These vegetables reduce oxidative stress, support cell regeneration, and activate genes associated with longevity. Antioxidants found in Brassica vegetables include vitamins C and E, phenolic compounds, and glucosinolates, which protect skin cells from oxidative damage. It primarily contributes to aging. Sulforaphane (a glucosinolate derivative) activates pathways like Nrf2, boosting antioxidant defence system and reducing inflammation. Extracts from related studies have demonstrated significant antioxidant properties and extended lifespan in model organisms, suggesting potential anti-aging effects. Consumption of Brassica vegetables or their extracts can increase skin collagen levels, reduce wrinkles, and protect against UV-triggered damage, as evidenced by clinical trials. The effectiveness as anti-aging agents is influenced by gut microbiota, individual metabolism, and processing approaches. To maximize health benefits, the consumption of young, fresh, and minimally processed Brassica products is recommended. More research is needed to fully understand the mechanisms and potential implications of Brassica vegetables in anti-aging contexts. It is paving the way for innovative dietary and skincare methods to promote healthy skin.

Keywords: Anti-aging, Antioxidants, Bioactive compounds, Collagen, Skin aging, Sulforaphane.

INTRODUCTION

The skin is the primary organ of the body that remains in direct contact with the external environment. It acts as a smart barrier and a visible predictor of aging [1]. The extent of skin aging mirrors an individual's health status and is recognized as an important indicator of individual life expectancy [2]. Skin aging poses an overall health and economic challenge. This issue affects over one billion people worldwide. Rising life expectancies and increasing ultraviolet (UV) exposure driven by climate change have intensified this issue. Therefore, driving innovation in natural remedies is an actual need of the hour. Currently, the anti-aging market

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for natural products is valued approximately $8 billion (in 2025) and is estimated to reach $12 billion by 2030. This underscores the global demand for plant-based beauty products. The skin is affected by multiple internal and external factors (oxidative stress, skin photoaging, inflammation, metabolism, and pollution) that lead to aging [3]. UV radiation harms the skin through DNA damage, inflammation, reactive oxygen species (ROS) production, and compromises the skin's antioxidant defenses [4]. As a result, most approaches and products are aimed at enhancing antioxidant defenses.

Brassica vegetables have gained attention as potent natural anti-aging agents because of their rich bioactive components and antioxidants. These vegetables help mitigate oxidative stress, support cell renewal, and trigger the activation of genes connected to longevity. Brassica vegetables are rich in antioxidants, including vitamins C and E, along with phenolic compounds. It protects cells from oxidative damage, a major contributing factor to aging [5]. The health benefits of these vegetables are primarily attributed to glucosinolates and their derivatives, particularly isothiocyanates. These compounds activate cell-related pathways, such as Nrf2, enhancing antioxidant defenses, reducing inflammation, and slowing cellular aging [6]. The health importance of broccoli sprouts stems from the hydrolysis of glucosinolates by myrosinase, which is converted into isothiocyanates. Sulforaphane potently activated Nrf2/Keap1 dissociation. This resulted in the transcription of HO-1, NQO1, and GCLC. Thereby, it amplifies glutathione synthesis. It quells NF-κB-driven inflammation and delays cellular senescence [7, 8]. Recent studies suggest that bitter taste receptors located away from the oral cavity, especially in the gut, may affect the health importance of Brassica vegetables by targeting hormone regulation and individual responses. Extracts from Tsai Tai vegetable have shown significant antioxidant properties and extended lifespan in *Caenorhabditis elegans*, indicating probable anti-aging effects [8]. Regular consumption is directly linked with a lower risk of chronic diseases such as cancer, cardiovascular disease, diabetes, and neuro-degenerative disorders [9]. Although animal, model organism, and cell line studies are promising, human clinical trials have yielded inconsistent results. This is possibly because of alterations in the gut microbiota and human personal metabolism of Brassica compounds.

ROLE OF SKIN ANTIOXIDANTS

Antioxidants play a vital role in defending against damage caused by ROS and related oxidative stress responses. These are significant contributors to skin aging, inflammation, and diseases, including cancer (Fig. **1**). These antioxidants nullify harmful free radicals, reduce inflammation, and aid cellular repair. Thereby, they maintain skin health and integrity. The skin is often exposed to ROS stress due to UV exposure, pollution, and internal metabolic processes. Both enzymatic and nonenzymatic antioxidant systems in the skin detoxify ROS, protect DNA, proteins, and lipids from damage, and lessen the risk of skin cancer as well as aging [10]. Enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, convert ROS into less reactive species. Nonenzymatic antioxidants such as vitamins C/E, glutathione, ubiquinone, carotenoids, and polyphenols directly remove the radicals and also chelate the metals. They inhibit lipid peroxidation chain reactions [12]. This dual impact protects DNA from dangerous mutations, proteins from carbonylation, and lipids from peroxidation products. It triggers photoaging by upregulating MMP-1 and promoting collagen degradation [11]. Antioxidants (melanin and carotenoids) protect against oxidative damage caused by UV radiation, a major factor in photoinduced aging and skin cancer. They help in repairing damaged biomolecules and maintaining cell homeostasis, thereby preventing the accumulation of oxidative damage that leads to degenerative skin conditions [12]. Antioxidants help lessen wrinkles, pigmentation, and other related signs of aging by combating cell-related changes induced by ROS [10]. They also lessen the risk of skin-related disorders by protecting against DNA and lipid peroxidation and by modulating inflammatory pathways. Topical and oral antioxidants are commonly used in skincare to enhance photoprotection, improve hydration, and support the skin barrier.

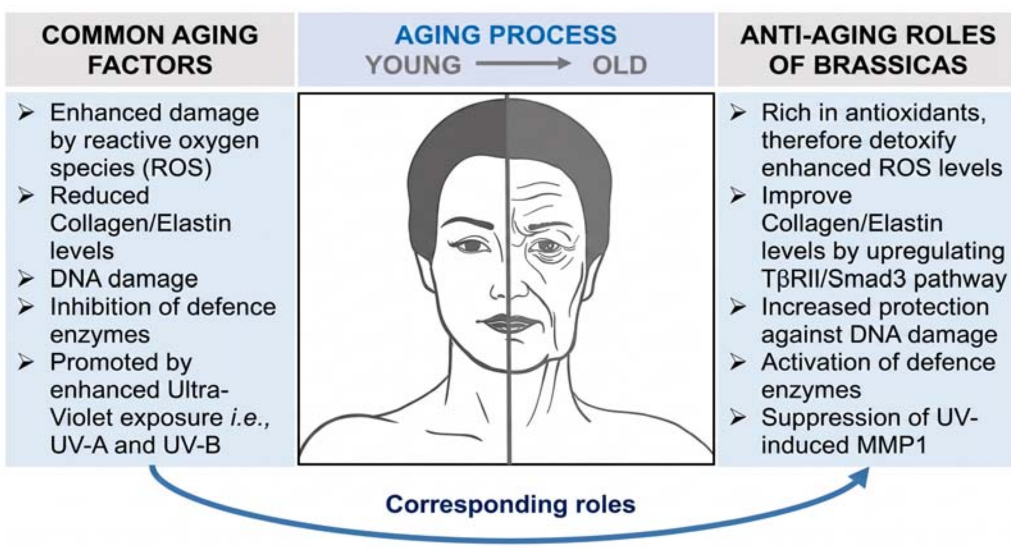

Fig. (1). Diagrammatic illustration showing the anti-aging properties of Brassica targeting ROS detoxification, collagen or elastin preservation, DNA damage mitigation, and repression of UV-related steps.

CHAPTER 12

Insights Into Multifaceted Relationship Between Brassica Intake and Thyroid Function: The Dual Nature of Brassica Consumption

Abstract: Brassica vegetables (broccoli, kale, and Brussels sprouts) have been suggested for their key nutritional benefits by offering a diverse range of vitamins, minerals, and phytochemicals. However, there are shortcomings regarding their goitrogenic effects, which could affect thyroid metabolism and overall health. This article aims to thoroughly dissect the existing literature on the nutritional profile and goitrogenic properties. This highlights the complex relationship between these factors and human health. The goitrogenic effects of Brassica vegetables are primarily linked to glucosinolates and their derived products. This interferes with iodine absorption and thyroid hormone synthesis. Research suggests that excessive consumption in raw form, in the context of iodine deficiency, may result in hypothyroidism as well as thyroid gland enlargement. Nevertheless, the risks associated with consumption can be mitigated by ensuring adequate iodine intake and using appropriate cooking methods. It is also important to consider individual dietary needs and health conditions when incorporating vegetables into a balanced diet. Future research should aim to elucidate the mechanisms underlying the goitrogenic effects and explore the long-term health implications of their consumption. By understanding the intricate network of nutritional benefits and potential risks of Brassica vegetables, evidence-based dietary guidelines can be developed to enhance their health benefits while minimizing their negative effects on thyroid function.

Keywords: Brassica vegetables, Goitrogenic effects, Iodine intake, Nutritional benefits, Thyroid function.

INTRODUCTION

The global shift from human to plant-based diets is governed by the rising incidence of chronic diseases. Brassica vegetables have been shown to have vital components for health-oriented dietary regimens [1]. However, concerns about raw Brassicaceae consumption, particularly their goitrogenic impact, which impedes iodine uptake and thyroid function, must be well addressed along with their nutritional potential to avoid compromising health-related benefits. Iodine deficiency impacts ~ 190 M people globally (2021), and this number is expected

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to rise despite interventions, with 62 M women of reproductive age at high risk. In South Asia and sub-Saharan Africa, it is reportedly associated with developmental disabilities and thyroid disorders. Brassica goitrogens fuel this by impeding iodine uptake in at-risk populations. Vegetables, including broccoli, kale, sprouts from Brussels, and cauliflower, are highly valued for their potential nutritional value. They are known sources of Vitamin C, with potent antioxidant potential; Vitamin K, which is crucial for bone health; and minerals, including potassium and iron, which are important for cardiovascular health and anemia prevention, respectively [2]. Moreover, phytochemicals, mainly glucosinolates in these vegetables, have been considered for their cancer-protective properties and have been included in discussions regarding diet and disease combat [3]. Despite their nutritional potential, secondary metabolites (glucosinolates) do not always benefit health but can interfere with iodine uptake and harm thyroid function when Brassica vegetables, especially raw forms, are consumed in excessive amounts.

This chapter reviews the existing information on the nutritional profiling of Brassica vegetables and their goitrogenic impacts. It highlights concerns regarding raw human intake and how these crucial points interact with the human working physiology. Our objective was to illuminate the mechanism by which Brassica vegetable consumption affects thyroid function and display strategies to balance their nutritional significance with potent health risks. By observing glucosinolate chemistry, cooking processes, and individual requirements, we provide evidence-based dietary recommendations. Here, we discuss the importance of individual dietary needs and the effect of cooking and processing methods on mitigating the potential risks associated with raw Brassicaceae consumption. In conclusion, this chapter offers recommendations for future research and dietary guidelines to ensure the benefits and precautions of Brassica vegetable consumption.

GOITROGENIC EFFECT

In a previous study [4], the impact of glucosinolates on hypothyroidism and low fertility in rats was reported to be negative. For example, an author [5] conducted a study based on a 200,907 type-2 diabetic-free population, including 88,293 men and the remaining women, in which higher glucosinolate consumption was positively linked with a 19% increased risk of developing type-2 diabetes. Moreover, glucosinolate toxicity is primarily related to the formation of thiocyanates, oxazolidinones, and nitriles [6]. These compounds interfere with iodine uptake (thiocyanates) and the synthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4), leading to hypothyroidism and enlargement of the thyroid gland. In contrast, progoitrin in broccoli, Brussels sprouts, and kale shows antithyroidal and antiproliferative activities [7]. Another study [8] revealed that decreased T3 and T4 levels and follicular atrophy adversely affected the thyroid glands and gonads of rabbits fed with cruciferous vegetables. The same trend was reported in another study [9], in which a lack of Se diet was correlated with low fertility, hypothyroidism, and cancer.

Some authors [10] studied the intake of cruciferous vegetables and its association with symptoms of thyroid dysfunction in 100 healthy college students selected from the University of Mysore campus, of whom 21 were obese with a body mass index (BMI) > 23.1. Based on the average consumption of cruciferous vegetables per week, 61% of the population consumed a high amount of cabbage, *i.e.,* 110-150 g/week, in the form of the most popular locally available fast food "Manchurian", followed by cauliflower (61%) and radish (43%). Hair loss (53%) was the most common symptom, followed by poor short-term memory, a deeper voice, and difficulty losing weight. Contrary to the above-mentioned studies, a study [11] evaluated and reported the goitrogenic content of the investigated vegetables within the range of acceptable daily intake and considered the involvement and interaction of other chemicals in causing endemic goiter in Southwest Nigeria. The consumption of rutabaga sprouts can worsen the adverse clinical symptoms associated with hypothyroidism, especially when combined with iodine deficiency and sulfadimethoxine [12]. Glucosinolates found in Brassica plants serve as a defense mechanism against pests and herbivores; however, their effects on human health and agriculture depend on their chemical composition and concentration [13]. Brassica vegetables are significant in lowering the total cholesterol content in adults. However, notable impacts on triglyceride content, low-density lipoprotein cholesterol content, high-density lipoprotein cholesterol, fasting blood sugar, or glycated hemoglobin contents have been missing [14]. Vegetarians face a high risk of thyroid issues due to their higher consumption of tofu, plant-based meats, and soy-based drinks compared to conventional diets [15]. A diet supplemented with Brassica vegetables is directly linked to a reduction in sulfate-reducing bacteria. It may have beneficial outcomes for gastrointestinal health [16].

Brassica has high glucosinolate and isothiocyanate concentrations, which contribute to its goitrogenic effect. However, when these vegetables are consumed with normal iodine intake, they do not interfere with human thyroid function [17] (Table **1**). Brassica vegetables offer both beneficial and potentially adverse health effects, although crops such as turnip tops, commercial broccoli, and kale pose minimal negative effects to thyroid functioning [18].

CHAPTER 13

Optimizing Cooking Methods for Maximizing Health Benefits of Brassica Vegetables

Abstract: Vegetables belonging to the family Brassicaceae are generally cooked before their consumption in a variety of ways that include blanching, boiling, frying, microwaving, roasting, or steaming. Each of these cooking methods could have a significant bearing on the sensory appeal, nutritional value, and health benefits. Although heat during cooking generally inactivates/kills harmful microbes, improves digestibility, and enhances the bioavailability of specific nutrients/ bioactive constituents, it may sometimes lead to the loss of certain vital nutrients and formation of toxic compounds. It has been suggested that stir-frying and steaming are the two most effective methods for retaining bioactive constituents, such as glucosinolates and isothiocyanates, whereas boiling may considerably reduce their concentration, especially in broccoli and kale. On the other hand, cooking methods like microwaving and/or frying may lead to significant losses of pigments (chlorophyll and carotenoids) and flavonoids in products like broccoli-based bars. Furthermore, steaming has been suggested as the most effective method for preserving bioactive compounds such as glucosinolates and phenolic compounds in Brussels sprouts. A brief treatment with heat at about 60 °C may increase the formation of isothiocyanates, thereby improving the health benefits linked to the consumption of vegetable Brassicas. However, an extended period of heating at temperatures increasing up to 99 °C may cause thermal breakdown of glucosinolates, resulting in enhanced levels of nitriles. Therefore, the best cooking method for maximizing health benefits depends on factors such as the type and cut size of the specific brassica vegetable, the chosen cooking method, cooking duration, and cooking temperature.

Keywords: Bioactive compounds, Boiling, Brassica vegetables, Cooking methods, Steaming.

INTRODUCTION

Although vegetables belonging to the family Brassicaceae can be eaten raw, they are typically cooked before consumption using a variety of methods, *viz*., blanching, boiling, frying, microwaving, roasting, and/or steaming. These cooking processes can affect the nutritional value, health benefits, and appeal of vegetables both positively and negatively. Blanching at about 80-100 °C in water or steam, and occasionally through microwave, infrared, or radio waves for brief periods, is

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generally followed by rapid cooling. The process leads to the inactivation of certain food enzymes, preserving their sensory attributes, and preventing the loss of vitamins and some other key metabolites during the processing and on-shelf storage. Blanching also helps remove air, prevent oxidation, and kill or deactivate microbes that may lead to food spoilage. Blanching at higher temperatures may not only stabilize against enzymatic degradation for extended periods but also lead to the loss of some vital nutrients and bioactives in addition to altering cellular structure. Heat treatments generally inactivate harmful microbes, improve digestibility, and enhance the bioavailability of specific nutrients and bioactives, sometimes increasing their concentrations as well. However, it may also result in nutrient loss and the formation of toxic compounds, which negatively affect certain sensory quality parameters and consumer acceptance [1, 2]. Baenas *et al.*, [3] studied the effects of various cooking methods on the concentrations of glucosinolates and isothiocyanates in broccoli and kale, as these compounds are typically unstable in cooking environments. Among the various cooking methods studied, stir-frying and steaming were found to be highly effective in retaining these bioactives (50% of the raw samples), whereas boiling markedly decreased their retention (only 20-40%). The above experiment suggested that selecting an appropriate cooking method is crucial for preserving the health-promoting benefits of brassica vegetables like broccoli and kale during cooking to achieve the positive effects of heat as mentioned above.

COOKING METHODS AND BIOAVAILABILITY OF BIOACTIVES

Brassica spp. is generally considered an important source of nutraceuticals, as it is a rich source of glucosinolates, phenolic compounds, carotenoids, vitamins, and minerals. However, various cooking methods may directly or indirectly affect the concentration of these nutrients during processing (Fig. **1**). The interplay of several factors, *viz.*, cooking duration, cooking temperature, and heat transfer mechanisms, plays a vital role in determining the fate of nutritional and bioactive constituents, thereof. Both boiling and steaming procedures utilize different methods of heat transfer (facilitate heat transfer from the surface to the interior), *i.e.*, conduction and convection, respectively.

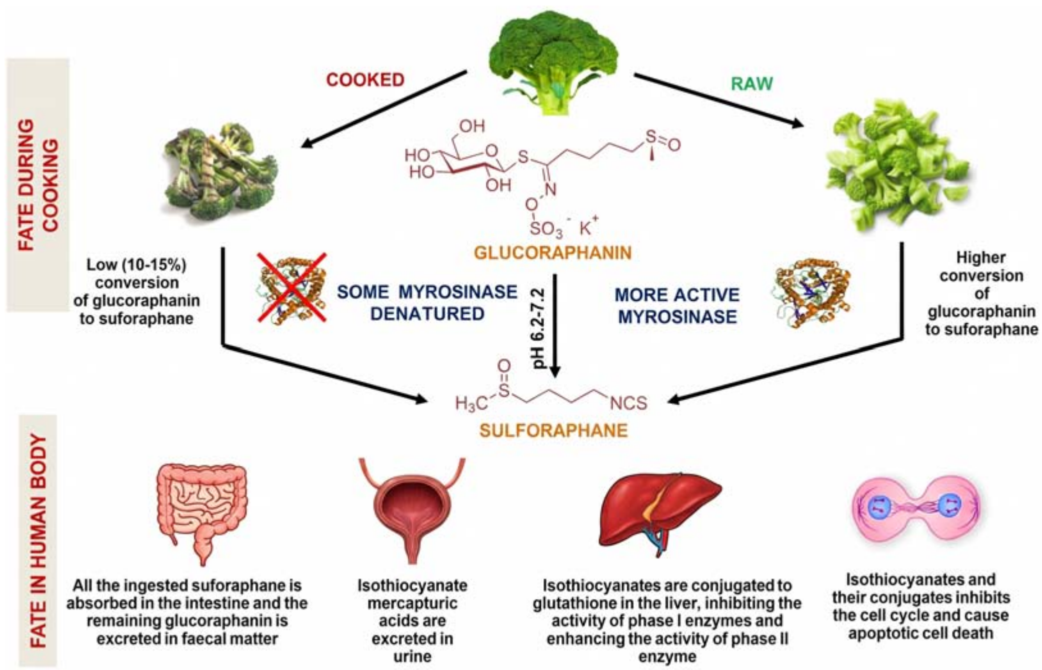

Fig. (1). A flow chart explaining the fate of glucoraphanin (a glucosinolate) and its degradation product, sulforaphane (an isothiocyanate) during cooking, as well as in the human body.

The effect of different thermal processing methods on the ascorbic acid (vitamin C) content of vegetables is quite important. Elevated temperatures and extended cooking times typically result in greater nutrient depletion [2, 4 - 6]. A number of studies examining the effects of various blanching and cooking techniques on the vitamin C content of vegetables suggested that cooking methods significantly affect its concentration in the final product. Kapusta-Duch *et al.* [7] observed that blanching reduces vitamin C content in Brussels sprouts by about 7%. Further, a comparative analysis of different cooking methods indicated that both stir-frying and boiling result in a significant decline in vitamin C content. Whereas, the milder techniques *viz*., microwave cooking and steaming, are quite effective for high retention of vitamin C content, with steaming causing minimal loss, *i.e.*, ~0.2%.

Several other studies involving different plant species also support the finding that cooking methods can significantly affect vitamin C retention in vegetables. Bureau *et al.*, [8] observed that both stir-frying and boiling led to lower vitamin C retention post-cooking; whereas, both microwave cooking and steaming were associated with much lower vitamin C losses. Similarly, Armesto *et al.*, [9] reported that boiling and vacuum cooking resulted in a considerable reduction in

CHAPTER 14

Bioavailability and Metabolism of Bioactive Compounds in Brassica Vegetables

Abstract: Brassica vegetables are rich in glucosinolates, anthocyanins, and carotenoids, which confer potential health benefits. However, the bioavailability and metabolism of these compounds are influenced by various factors, including cooking methods, food matrices, and individual gut microbiota. Glucosinolates are broken down by myrosinase, yielding beneficial hydrolysis products, such as isothiocyanates. Cooking deactivates myrosinase, thereby reducing the conversion of glucosinolates to their breakdown products. The bioavailability of isothiocyanates in fresh Brassica vegetables is approximately three times greater than that in cooked vegetables. Gut bacteria can also break down glucosinolates; however, their efficiency is lower than that of plant-derived myrosinases. Anthocyanins from Brassica vegetables, such as red cabbage, have low bioavailability owing to their instability in the digestive tract. However, acylated anthocyanins are more stable and are absorbed more easily. The absorption of anthocyanins is influenced by the food matrix, with raw red cabbage showing higher recovery rates than red cabbage extract. The bioavailability of carotenoids from Brassica vegetables is generally low because of the resistance of carotene-protein complexes, plant cell walls, and fibers to digestion. Chopping and cooking can increase carotenoid bioavailability by releasing carotenoids into the food matrix. Steaming and microwaving are the most suitable cooking techniques for preserving glucosinolates, isothiocyanates, and carotenoids in Brassica vegetables. Understanding the factors that influence the bioavailability and metabolism of these compounds is crucial for optimizing their health benefits.

Keywords: Bioavailability, Brassica vegetables, Cooking methods, Food matrix, Glucosinolate, Myrosinase.

INTRODUCTION

Glucosinolates, present in Brassica vegetables, are unique compounds that can be broken down by myrosinase to yield various beneficial products. When consumed, a small amount of intact glucosinolates is absorbed through the gastrointestinal lining, whereas most of the glucosinolates are metabolized in the gut. Eating raw Brassica vegetables allows the plant myrosinase to break down glucosinolates in the upper gastrointestinal tract, producing metabolites such as isothiocyanates, nitriles, oxazolidine-2-thiones, and indole-3-carbinols. However,

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cooking deactivates myrosinase; therefore, glucosinolates are degraded by the gut microbiota in the colon [1]. The bioavailability of glucosinolates and their breakdown products is influenced by various factors, including the type and level of glucosinolates present, the concentration and stability of myrosinase, and the solubility and physicochemical characteristics of each glucosinolate or its derivative. In addition, factors such as the degree of cell breakdown during chewing and the processes in the stomach and small intestine play a role in determining the availability of these compounds. Cooking Brassica vegetables not only deactivates myrosinase but also reduces the conversion of glucosinolates into beneficial breakdown products. Domestic processing has a dual effect: it can improve palatability and safety but frequently reduces both total glucosinolate content and myrosinase activity, thus limiting in situ isothiocyanate formation [2, 3]. Steaming and brief microwave cooking generally preserve more intact glucosinolates than boiling or stir-frying because leaching into cooking water and prolonged high temperatures are minimized [4]. Steaming cabbage and broccoli can retain 80–97% of their initial glucosinolates, whereas boiling can cause losses of 50–70%. Storage variables, including duration, temperature, and modified-atmosphere packaging, also influence glucosinolate stability, with chilled, short-term storage under atmospheres such as ~5% CO_2 and ~3% O_2 often maintaining higher glucosinolate levels than ambient or prolonged storage [5, 6].

Factors such as storage duration, temperature, and packaging atmosphere can also affect the bioavailability of glucosinolates and their breakdown products [7]. To find a solution [8], engineered broccoli crops in which glucosinolate hydrolysis occurs even at elevated cooking temperatures. Furthermore, cooking with a low water level, steaming, and microwave irradiation for brief periods are widely regarded as optimal methods for retaining the quality of glucosinolates. These transgenic lines supported significant isothiocyanate production after heating at temperatures approximately 20°C higher than those required to abolish hydrolysis in wild-type broccoli, demonstrating proof-of-concept for genetically tailoring crops to deliver bioactive hydrolysis products despite the necessary culinary processing. These strategies can be combined with breeding to develop favorable glucosinolate profiles, thereby enhancing chemopreventive potential without compromising sensory quality [8]. From a dietary perspective, combining small amounts of raw *Brassica* spp. (to supply active plant myrosinase) with lightly cooked vegetables or adding exogenous myrosinase-rich ingredients, such as mustard powder, to cooked dishes has been shown to enhance isothiocyanate formation and systemic exposure [9]. Short steaming or microwaving with minimal water, avoiding prolonged boiling and discarding of cooking water, and consuming fresh rather than long-stored produce is widely recommended to preserve glucosinolates and favor beneficial hydrolysis products. Ultimately, the realized health impact of Brassica-derived glucosinolates reflects an interaction between food preparation, crop traits (including emerging engineered varieties), the gut microbiome, and host metabolism, underscoring the value of a diversified, regularly consumed Brassica-rich diet rather than reliance on a single "superfood" [10].

BIOAVAILABILITY AND METABOLISM OF BIOACTIVE COMPOUNDS

A crossover study in healthy adults showed that sulforaphane (SFN) and erucin (ERN) metabolites appeared earlier and at substantially higher levels in plasma and urine after ingestion of fresh broccoli sprouts than after an equivalent dose of broccoli supplements lacking active myrosinase, confirming markedly lower bioavailability and delayed kinetics from supplements [11]. These findings underscore that myrosinase-free supplements, often perceived as equivalent to sprouts, deliver considerably less bioactive isothiocyanates, which is critical for both consumers and trial design [12, 13]. Human feeding studies indicate that SFN bioavailability from fresh or myrosinase-active broccoli preparations is approximately 3–4 times higher than that from cooked broccoli, in which myrosinase has been inactivated, with fresh broccoli soups yielding up to tenfold higher SFN equivalents than soups made from commercially blanched frozen florets [14]. Steaming generally preserves more glucosinolates and ITCs than boiling, and very mild heat (or brief steaming of sprouts) may even enhance conversion efficiency without inactivating myrosinase [15]. Although the gut microbiota exhibits myrosinase-like activity and can convert residual glucosinolates to ITCs, microbial conversion is slower, more variable among individuals, and usually less efficient than plant myrosinase, leading to lower and more delayed systemic exposure [1, 16].

Clarke *et al.* [11] studied the bioavailability and excretion of sulforaphane and erucin from broccoli in a crossover study with 12 participants who consumed 40 g of fresh broccoli sprouts and 6 pills of a broccoli supplement, separated by a 1-month washout period. Four additional participants served as the negative controls. Blood and urine samples collected over 48 h showed significantly lower bioavailability and delayed plasma concentration peaks and urinary excretion of sulforaphane and erucin from supplements compared with those from sprouts. Broccoli supplements without myrosinase activity do not produce the same levels of bioactive isothiocyanates as fresh sprouts, which is crucial for supplement users to consider. A study [6] demonstrated that the bioavailability of isothiocyanates from fresh broccoli is approximately three times greater than that from steamed broccoli, where myrosinase is inactivated. Although the gut microbiota can also produce isothiocyanates, its efficiency is lower than that of plant myrosinases [17]. Food products containing active myrosinase, such as

CHAPTER 15

Biotechnological Interventions and Effects of Abiotic Stress on Phytochemical Profiles of *Brassica* Spp.

Abstract: Biotechnological interventions and abiotic stress can significantly influence the phytochemical profile of *Brassica species*. Biofortification with selenium has been shown to enhance glucosinolates, flavonoids, anthocyanins, and phenolic compounds in broccoli and other *Brassica species*. Breeding efforts have enabled the development of low erucic-acid, mustard varieties and bio-fortified cauliflower rich in beta-carotene, anthocyanins, and chlorophyll. Wild relatives of Brassica have been exploited for their high glucosinolate and carotenoid content. Gene editing techniques, such as CRISPR-Cas9, have been used to modify traits including phytic acid, oleic acid, and glucosinolate content in rapeseed and Chinese kale. Overexpression of transcription factors, such as MYB28 and MYB29, has been shown to augment glucosinolate levels in broccoli. Abiotic stresses, such as UV light, methyl jasmonate (MeJA), elevated CO_2, and salinity, can boost the production of functional compounds in *Brassica* spp., However, cooking methods such as boiling and microwaving can reduce beneficial phytochemicals; whereas, lighter cooking methods such as steaming can preserve or even increase their levels. *In silico* studies have identified promising phytocompounds in broccoli for the treatment of hyperthyroidism. Molecular markers and new breeding techniques such as RNAi and CRISPR-Cas9 offer rapid and precise ways to improve various traits of Brassica crops, including nutritional quality and stress tolerance. Overall, a combination of biotechnological interventions, abiotic stress management, and appropriate postharvest processing can maximize health-promoting phytochemicals in Brassica vegetables.

Keywords: Abiotic stress, Anthocyanins, Biofortification, Breeding, Gene editing, Glucosinolates, Phenolic compounds.

INTRODUCTION

Brassica species, including broccoli, cauliflower, and mustard, play a significant role in global agriculture and the human diet due to their nutritional benefits. The growing concerns over food security and nutritional quality under changing climate conditions, understanding how biotechnological strategies and environmental stressors can modify health-promoting phytochemicals in Brassica

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vegetables is essential. Several studies have documented the synergetic effect between micronutrient bio-fortification and bioactive-compound enhancement [1 - 3]. Brassicaceae can accumulate selenium (Se) *via* the sulfur metabolic pathway [4]. A wide range of reviews and research articles on Se [5, 6] have reported an increased content of flavonoids and anthocyanins [7], phenolic compounds [8], and glucosinolates [9], which were observed to be biofortified at appropriate concentrations [10].

This chapter provides an in-depth review of recent advances in biotechnological interventions for enhancing the nutritional quality of *Brassica* spp., examines the impact of abiotic stresses on their phytochemical profiles, and discusses the implications of these modifications on human health. The highlights of this chapter include (a) biotechnological interventions and abiotic stress that significantly influence the phytochemical profiles of *Brassica* spp.; (b) biofortification with selenium enhances glucosinolates, flavonoids, anthocyanins, and phenolic compounds in *Brassica* spp.; (c) breeding efforts have developed low-erucic acid mustard and biofortified cauliflower-rich nutrients; (d) gene editing approaches, such as CRISPR-Cas 9, have been used to modify traits in Brassica spp; (e) overexpression of transcription factors such as MYB28 and MYB29 augment glucosinolate levels in broccoli; (f) abiotic stresses such as UV light, methyl jasmonate (MeJA), and salinity can enhance bioactive compounds in *Brassica* spp.; and (g) cooking methods affect phytochemical levels, with light cooking preserving beneficial compounds better than heavy cooking.

BREEDING AND BIOTECHNOLOGICAL INTERVENTIONS

The current trend and pressing need among health-conscious communities worldwide is the use of mustard oil with a minimal erucic acid content. In this context, pureline var. Pusa mustard 30 (low erucic acid, < 2%) and Pusa Double Zero Mustard 31, India's first canola quality mustard with erucic acid (↓) and oil content of 41% (↑), are landmarks [11, 12]. The primary gene involved in the production of erucic acid is FAE1. Sequencing of FAE1 in cultivars with both low and high levels of erucic acid showed base pair deletions, including 24 AT-rich base regions within a 1,300-bp segment located upstream of the FAE1 start codon promoter [13]. Bulgakov *et al.* [14] reported the ability of *rolB* to activate secondary metabolism in the *Arabidopsis calli*. In addition, targeting bio-fortified varieties is needed to advance the bio-fortification breeding [15]. For instance, beta carotene-rich yellow gene '*Or*' is introgressed for developing Cheddar, Orange Bouquet and Pusa Beta Kesari *i.e.,* 1st bio-fortified cauliflower variety [16, 17]; anthocyanin-rich purple gene '*Pr*' for Sinician violet, Graffiti, Violet Queen and Purple Cape in cauliflower [18]; chlorophyll-rich green gene '*Gr*' for

ACX800 broccoflower [19]; anthocyanin rich red gene '*MYB*' for Kinner red in red cabbage [20] and anthocyanin rich purple gene '*BrMYB2*' for 11S91 in Chinese cabbage [21] are milestones of biofortification breeding. Jin *et al.* [22] observed phenotypic expression and synthesis of anthocyanin pigmentation by abscisic acid (ABA) and ethylene (C_2H_4). The candidate genes *BoNCED2.1*, *BoNCED2.2* for ABA biosynthesis, and *BoACS11*, and BoACO*4* for ethylene biosynthesis can be utilized for purple pigmentation in the head and leaves of cabbage. Li *et al.* [23] reported that the *Arabidopsis thaliana* gene *PAP1* was efficient in enhancing antioxidant and phenolic content in *Brassica napus* leaves. Similarly, Li *et al.* [24] found that ectopic overexpression of bol-miR171b increased chlorophyll levels, leading to male sterility in broccoli. In addition, White *et al.* [25] investigated the limits of biofortification with Zn from 2014-17 and reported that *Brassica* leaf vegetables contribute to 5% of dietary Zn uptake, and bio-fortification of *Brassica* spp., with >0.100 mg Zn g^{-1} dry weight (DW) was the most suitable for enhancing the dietary intake of Zn.

Among wild relatives, *Brassica villosa*, methionine-derived glucosinolate, and high glucoraphanin [26, 27], *Brassica juncea* var. *integrifolia* with high levels of gluconasturtin and progoitrin [28]; *Brassica oleracea* var. *alboglabra*, with high carotenoid content [29]; DC 306, DC 383 (in leaves), DC 326, DC 383 (in curd) for high sinigrin content [30]; and DC 522 for high ascorbic acid content [31] linked to secondary (2°) and tertiary (3°) gene pools. For the utilization of these gene pools, embryo degeneration is a limiting factor that can be avoided through polyploid breeding using colchicine, and certain tissue culture-based methods can also be employed to overcome this. Indeed, another landmark is isothiocyanate-enriched broccoli 'Benforte', developed through three QTL introgressions from B. villosa *via* marker-assisted selection (MAS) and now commercialized in the UK [32]. A study [33] successfully isolated recombinant inbred lines (RILs) with an elevated level of 4-(methylsulfinyl) butyl and a low content of anti-nutritional factors, namely, (R)-2-hydroxybut-3-enyl glucosinolate, derived from a segregating *Brassica oleracea* population through three different crosses: broccoli with cauliflower, collard with broccoli, and collard with cauliflower. Moreover, another [34] confirmed similar results for the above-mentioned nutritional and anti-nutritional factors by utilizing RNA interference (RNAi) targeting the *GSL-ALK* gene in *Brassica napus.*

Several studies conducted on *A. thaliana* (a model plant of Brassicaceae) have shown enhanced glucosinolate content upon the overexpression of key target genes *i.e.*, *CYP79D2* for enhanced isopropyl, methyl propyl, and resistance towards *Erwinia carotovora* [35] and *AOP2* for aliphatic alkenyl conjugates [36], *AtPAP1* for flavonoids and phenolic acids in *B. napus* [37], *CYP79F1* for aliphatic glucosinolates [38], and *MYB29* for aliphatic glucosinolates in *B.*

Shivam Sharma

Dr. Shivam Sharma is an acclaimed vegetable breeder and Assistant Professor of Horticulture (Vegetable Science) at DAV University, Jalandhar. Specializing in vegetable breeding, his pioneering research led to the development of India's first public sector broccoli hybrid. He holds a Ph.D. from CSK HPKV Palampur and has maintained an elite academic record, securing All India Rank 16 in the competitive ICAR-SRF examination. A recipient of the prestigious Young Vegetable Breeder Award, Dr. Sharma has published over 20 research papers, 2 short communications, 2 books, and 10 book chapters and 9 review articles in high-impact national and international journals, holds a registered Indian design patent for advanced soil monitoring devices, and currently guides multiple postgraduate research scholars. Additionally, he serves as a life member of prominent agricultural societies.

Neha Sharma

Dr. Neha Sharma completed her doctoral degree in Vegetable Science from CSK HPKV, Palampur. During her academic journey, she was honoured with Best M.Sc. Thesis, Best Poster Presentation and Best Oral Presentation awards. Her research interests focus on vegetable crop improvement through breeding and genetics, enhancement of yield and quality traits, disease resistance, sustainable nutrient management and innovative production technologies. She has published 16 research articles in reputed national/international journals, along with 2 book chapters and attended two international conferences. Driven by a strong commitment to advancing and disseminating scientific knowledge, she hopes that this book will serve as a valuable resource for students, researchers, academicians and professionals in the field of Vegetable Science.

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Dr. Ashutosh Sharma is currently working as an Associate Professor at the Faculty of Agricultural Sciences, DAV University, Jalandhar, India, specializing in the field of plant biotechnology, plant-microbe interactions and plant stress biology. With over 14 years of experience, he has authored 150+ publications (Research papers, review articles and book chapters), edited seven books, and authored two. He is a recipient of the Punjab Academy of Sciences Young Scientist Award for developing a sensitive detection system for Alternaria alternata. He has secured major research grants from DST-SERB and CSIR. He has contributed nucleotide sequences to GenBank and microbial strains to the Microbial Type Culture Collection (MTCC). He has four design patents and has supervised 15 master's and three PhD students. He has qualified GATE (percentile score 99.18), CSIR-UGC NET-JRF, and ICAR-NET examinations.