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THE UNTAPPED POWER OF HIGH-VALUE CRUCIFEROUS VEGETABLES: FUNCTIONAL INSIGHTS

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ABSTRACT

Cruciferous vegetables, often hailed as superfoods, include crops like kale, collards, kohlrabi, and Brussels sprouts, known for their therapeutic properties. These vegetables are rich in secondary metabolites such as flavonoids, anthocyanins, carotenoids, polyphenols, vitamins, minerals, coumarins, antioxidant enzymes, and terpenes. Regular consumption of crucifers can help combat obesity, cancer, atherosclerosis, inflammation, and metabolic syndrome, while also lowering the risk of various diseases. Their phytonutrients are most bioavailable and active when consumed raw, though processing can reduce their efficacy, necessitating careful cooking methods. Scientific validation of these benefits requires further clinical research, a focus of recent studies.

To enhance production and ensure sustainable conservation, techniques like micropropagation, cryopreservation, tissue culture, somatic hybridization, DNA banking, mycorrhization, and genetic engineering are recommended. Additionally, biotic and abiotic stresses can boost secondary metabolite levels, which can be optimized to enhance functionality. Molecular breeding techniques, including marker-assisted selection, backcrossing, next generation sequencing, and gene editing, offer significant potential for improving crucifer yield and quality. This review explores the health benefits, processing impacts, conservation strategies, and advancements in the production and value addition of cruciferous vegetables over the past decade.

Keywords: Cruciferous vegetables,antioxidant enzymes, terpenes, and Molecular breeding techniques

1. INTRODUCTION

Cole crops, part of the *Brassicaceae* family and species *oleracea*, include vegetables like Kale, Collards, Kohlrabi, and Brussels sprouts, which are considered underutilized crops. Other underused *Brassica* crops, include red cabbage, Chinese cabbage, leaf mustard, leaf rape, swede, horseradish, arugula, and watercress. These cruciferous vegetables are packed with sulforane compounds, especially glucosinolates and S-methylcystine sulfoxide, which are key for their health benefits. They also contain nitrogen-based compounds, colorful flavonoids, anthocyanins, carotenoids, and are rich in polyphenols, vitamins, minerals, coumarins, antioxidant enzymes, and terpenes. Despite their nutritional value, these vegetables are not widely grown or traded for their health benefits, making them underutilized crops.

Underutilized vegetables, like certain cruciferous crops, are grown, traded, and eaten mostly in local areas, with recent entry into commercial markets. Despite their value, they are not widely used due to low awareness of their nutritional benefits, such as their rich phytochemicals, high antioxidant activity, and vibrant colors that promote health. These vegetables are low in calories but packed with vitamins, minerals, and protective nutrients, making them vital for improving nutrition through regular consumption. Increasing their use can help fight malnutrition, poverty, and boost economic growth, especially in developed countries where they are used to manage chronic diseases and nutrient deficiencies.

Malnutrition, including issues like stunting, wasting, and obesity, affects millions globally, with 52 million children wasted, 1.9 billion adults overweight, and 462 million underweight, according to the World Health Organization (2021). Underutilized cruciferous vegetables, rich in bioactive compounds, can help address these health issues.

Promoting these crops through breeding, biotechnology, and conservation can reduce harmful compounds and improve their varieties. However, there is still a lack of knowledge about their full nutritional potential and commercial use. Recent research has focused on their health benefits, how processing affects their compounds, and their interactions in the body. Combining modern processing with traditional knowledge can boost their commercial value.

This review includes the current status of underutilized vegetables, their bioactive compounds, ways to enhance their nutritional profile, the impact of processing, and scientific evidence supporting their health benefits, while summarizing progress over the last decade to guide future efforts in crop production, breeding, and food processing.

2. Nutritional and economic emergence of underutilized cruciferous vegetables

The rising global population, increasing incomes, and urbanization are driving up demand for staple crops, but relying on just a few crops makes our food supply fragile and risks both food security and nutrition. A lack of variety in the gene pools of underutilized crops leaves them vulnerable to diseases and environmental stresses. In the past, farmers in remote areas grew diverse crops alongside staples, many of which were nutritious but not widely known.

Recently, researchers have started exploring these underutilized cruciferous vegetables, like kale, Brussels sprouts, and kohlrabi, for their rich nutrients and health benefits, especially in developing countries. These crops grow well in marginal lands and provide essential nutrients often missing in staple foods, supporting healthy body functions.

The World Health Organization promotes eating traditional vegetables in places like Sub-Saharan Africa to fight malnutrition. For example, pakchoi contains glucosinolates (20 mg/100 g), which are health-promoting compounds, while kale offers high calcium (more bioavailable than milk), fiber, and minerals, helping prevent diseases like heart disease and cancer. Red cabbage has anthocyanins (up to 322 mg/100 g) with anti-diabetic and anti-cancer benefits, and watercress is rich in antioxidants like lutein and beta-carotene. Horseradish provides glucosinolates and is used as a preservative and condiment. These vegetables are packed with vitamins, minerals, and phytochemicals like kaempferol and quercetin, which help reduce chronic disease risks. Growing these crops can improve nutrition, create jobs, and boost incomes for rural communities. Unlike expensive off-season vegetables, these affordable, nutrient-rich options can ensure better health and food security. They are also resilient to stresses, making them ideal for diverse farming systems. Expanding their use, either mixed with staple crops or grown alone, can help underdeveloped countries tackle nutrient deficiencies, reduce healthcare burdens, and promote sustainable, diverse food production.

3. Bioactive compounds in underexploited cruciferous vegetables and their synthesis

Underutilized cruciferous vegetables are rich in bioactive compounds like glucosinolates, phenolics, flavonoids, tocopherols, and carotenoids, which have anti-cancer properties and aid in treating heart and brain-related diseases. While not considered essential nutrients, these compounds play a key role in promoting overall health. Their classification is challenging due to variations in their amounts and types across different crucifers, plant parts, and growth stages.

Understanding how these compounds are produced in various plant parts of underutilized vegetables is crucial for identifying their chemical makeup and determining environmental factors or genetic improvements that can boost their production. This section explores the pathways involved in creating these major secondary metabolites and discusses ways to enhance their synthesis through changes in growth conditions and genetic advancements.

3.1.Synthesis of glucosinolates

Cruciferous vegetables are rich in glucosinolates, important water-soluble and sulfur containing compounds made up of a β-D-glucopyranose unit linked to a β thioglucoside N-hydroxysulfate structure. These compounds are typically formed from amino acids, with specific types like methyl-, isopropyl-, and p-hydroxybenzyl glucosinolates originating from alanine, valine, and tyrosine, respectively.

Glucosinolates are produced through a distinct metabolic pathway guided by common enzymes that shape their core structure, with the entire process controlled by genetics. The side chain attached to the β-thioglucoside N-hydroxysulfate determines the type of glucosinolate, categorizing them into aliphatic, aromatic, or indole groups, resulting in over 130 unique glucosinolates.

Cruciferous vegetables contain all 130 known glucosinolates, with the highest levels found in wild and underutilized varieties. The unique flavors of lesser-known crucifers like swede, kale, Brussels sprouts, collards, and horseradish come from the breakdown products of these glucosinolates.

An enzyme called myrosinase (thioglucoside glycohydrolase EC 3:2:3:1), found in crucifers, triggers the breakdown of glucosinolates into various compounds. Normally, myrosinase is separated from glucosinolates, but when the plant is damaged or stressed, they interact, producing different metabolites. The type of breakdown products formed depends on factors like the glucosinolate's side chain structure, the presence of cofactors, hydrolysis conditions, and the soil or crop's pH level.

When glucosinolates (GLCs) are broken down through hydrolysis, an unstable intermediate called aglucon is initially formed, which then transforms into various compounds like isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidinethiones.

The specific product depends on the pH environment: in acidic conditions, aglucon tends to form nitriles, which can be toxic, instead of isothiocyanates; in neutral pH, it produces thiocyanates with an indolic structure; and in alkaline pH, sinigrin leads to allyl isothiocyanate. Indolic glucosinolates, such as glucobrassicin, yield indole-3-carbinol, while glucoraphanin produces sulforaphane. Isothiocyanates, formed through a process called lossen rearrangement, are the primary and most desired products of glucosinolate breakdown. However, isothiocyanates with β-hydroxyl groups are unstable and convert into oxazolidine-2- thione.

The formation of glucosinolates (GLCs) can differ depending on the substrate. For instance, in swede, the epithiospecifier protein lacks the necessary enzymatic activity, but with ferrous ions present, it can incorporate sulfur atoms into the glucosinolate side chain (Petroski and Tookey, 1982). In Brussels sprouts, the primary glucosinolates include glucoiberin, progoitrin, glucoraphanin, sinigrin, glucobrassicin, and 4-methoxyglucobrassicin (Heaney and Fenwick, 1980).

In watercress, gluconasturtiin and glucobrassicin are the dominant glucosinolates, with the anticarcinogenic effects of watercress and broccoli attributed to phenethyl isothiocyanate (PEITC) (Palaniswamy et al., 2003). Collards (Brassica oleracea var. sabellica) mainly contain sinigrin and progoitrin, with smaller amounts of glucoiberin and glucoraphanin (Carlson et al., 1987; Deng et al., 2015).

Kale is a rich source of aliphatic glucosinolates such as gluconapin, glucoraphanin, and progoitrin, while rutabaga primarily contains progoitrin, 4-hydroxyglucobrassicin, gluconapin, and glucobrassicin (Baenas et al., 2014). Kohlrabi (Brassica oleracea var. gongylodes) has lower glucosinolate levels, with major GLCs including glucoiberin, glucoraphanin, glucoalyssin, glucoiberverin, glucoerucin, glucobrassicin, gluconasturtiin, and neoglucobrassicin (Carlson et al., 1987; Deng et al., 2015).

Cruciferous vegetables have long been recognized for containing glucosinolates (GLCs), but their significance in daily nutrition has been thoroughly explored only in the past ten years. Research highlights GLCs' protective effects against bacterial, viral, and fungal infections, resembling antibiotic properties, with their anticarcinogenic benefits being the most extensively studied.

A 14-year study by Zhang et al. (2000) involving 88,410 women found that those consuming cruciferous vegetables more than five times weekly had a relative risk (RR) of 0.67 for non Hodgkin’s lymphoma compared to those eating fewer than two servings per week. Additionally, the antitumor effects of broccoli and collards have been widely investigated in human tumor cell lines (MCF-7 and HeLa) over the last decade.

Collard extracts exhibit greater antioxidant activity than broccoli extracts, with glucobrassicin (80.33 mmol 100 g^{-1} fw) as the primary glucosinolate in collards, while glucoraphanin (95.97 mmol 100 g^{-1} fw) predominates in broccoli (Zhang et al., 2000). Radošević et al. (2017) demonstrated that extracts from both broccoli and collards, at concentrations of 50 to 100 mg mL^{-1}, suppressed cell viability.

3.2. Synthesis of phenolic compounds

Phenolic compounds, vital phytochemicals, are widely found across the plant kingdom, with the highest levels in cruciferous vegetables, valued for their strong antioxidant properties. Their structure features aromatic rings paired with hydroxyl groups (Cartea et al., 2011; Li et al., 2018). These compounds are produced via the shikimic acid pathway, yielding phenylpropanoids, and the acetic acid pathway, generating simple phenols (Boudet, 2007; Kumar and Goel, 2019). The enzyme phenylalanine ammonia-lyase (PAL) plays a crucial role by converting phenylalanine into cinnamic acid, facilitating the synthesis of various phenolic acids (Mandal et al., 2010).

The phenolic content varies depending on the plant part analyzed and environmental factors, with leaves typically containing the highest amounts. Phenolic compounds are divided into two main categories: flavonoids and non-flavonoids. Flavonoids encompass flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, and isoflavones, while non-flavonoids include phenolic acids, hydroxycinnamates, and stilbenes, with flavonols, hydroxycinnamic acids, and anthocyanidins being prevalent in underutilized vegetables (Crozier et al., 2006). In Brassica vegetables, kaempferol and quercetin glycosides, along with hydroxycinnamic acids and sinapic acid derivatives, are particularly common (Vallejo et al., 2002).

3.3 Synthesis of flavonoids and non-flavonoids

Flavonoids, a class of polyphenols with a C6-C3-C6 structure, are primarily located in plant vacuoles and represent a significant portion of phenolic compounds (Cartea et al., 2011; Favela-González et al., 2020). Quercetin and kaempferol, two prominent flavonoids in cruciferous vegetables, are known for their antioxidant and anti
inflammatory properties, as well as their cardioprotective and antihypertensive effects.
Kale stands out among underutilized vegetables for its rich flavonoid content (Heimler et al., 2006; Cartea et al., 2008). In kale, the primary phenolic compounds include 3-O-sophoroside-7-O-glucoside and hydroxycinnamic acids (Llorach et al., 2003; Ferreres et al., 2005; Olsen et al., 2009). Glycosylation processes produce various flavonoid isomers with diverse health benefits. Kaempferol is a prevalent flavonoid in cruciferous vegetables, particularly abundant in pak choi (Brassica campestris L. ssp. chinensis var. communis) (Harbaum et al., 2007; Rochfort et al., 2006).

Pak choi also contains derivatives of kaempferol, quercetin, and isorhamnetin, with isorhamnetin being the dominant flavonoid in the Brassica rapa group (Romani et al., 2006). Quercetin and kaempferol are the main flavonoids in tronchuda cabbage and watercress (Sousa et al., 2007; Martinez-Sanchez et al., 2008). Isorhamnetin glycosides are absent in Brussels sprouts, cabbage, cauliflower, and Chinese broccoli, while cabbage contains minimal quercetin glycosides (0.07

mg/g dry weight of quercetin-diglucoside) (Li et al., 2018).

Rutin (quercetin-3-O-rutinoside) and nicotiflorin (kaempferol-3-O-rutinoside) are key flavonoids found exclusively in watercress, with trace amounts of rutin in rocket salad (Li et al., 2018). Anthocyanins, water-soluble pigments, are present in red cabbage and purple cauliflower, accompanied by significant amounts of cinnamic acids. Red cabbage contains 15 anthocyanin derivatives, with Cyanidin-3-O-(sinapoyl) (feruloyl)diglucoside-5-O- glucoside as the primary anthocyanin (Dyrby et al., 2001; Lo Scalzo et al., 2008; Jahangir et al., 2009; Mazza and Miniati, 1993). The diverse array of flavonoids in cruciferous vegetables makes them highly valuable for general nutrition and targeted health benefits.

3.4. Synthesis of hydroxycinnamic acids

Hydroxycinnamic acids, classified as non-flavonoid phenolic compounds with a C6- C3 structure, are widely present in plants. In cruciferous vegetables, common hydroxycinnamic acids include ferulic (4-hydroxy-3-methoxycinnamic), sinapic (3,5- dimethoxy-4-hydroxycinnamic), and p-coumaric (4-hydroxy-cinnamic) acids, which contribute to plant defense mechanisms (Cartea et al., 2011; Favela-González et al., 2020).

Derivatives of hydroxycinnamic acids, such as ferulic acid, caffeic acid, p coumaric acid, chlorogenic acid, sinapic acid, curcumin, and rosmarinic acid, are prevalent in crucifers. These compounds are synthesized through the mevalonate and shikimate pathways, with phenylalanine and tyrosine serving as precursors. The shikimate pathway, regulated by multiple enzymatic reactions, produces derivatives like caffeic, cinnamic, p-coumaric, sinapic, and ferulic acids, which also act as precursors for stilbenes, chalcones, flavonoids, lignans, and anthocyanins (El-Seedi et al., 2012; Zhao and Moghadasian, 2008).

In kale and broccoli, hydroxycinnamoyl gentiobiosides and hydroxycinnamoylquinic acids are particularly abundant (Vallejo et al., 2004). Hydroxycinnamic acids and their degradation products exhibit strong anti-collagenase, antioxidant, anti-inflammatory, antimicrobial, and anti-tyrosinase properties. Notably, their ability to shield skin from ultraviolet (UV) radiation supports their use as potential anti-aging, anti-inflammatory, preservative, and hyperpigmentation agents.

3.5. Synthesis of tocopherols and carotenoids

Tocopherols, natural secondary metabolites, are key forms of vitamin E, a fat-soluble phenolic compound. Together with tocotrienols, they constitute lipid-soluble vitamin E. Tocopherol biosynthesis involves two precursors: homogentisic acid (HGA), derived from the cytosolic shikimate pathway for the molecule’s head, and phytyldiphosphate (PDP), produced via the plastidic methylerythritol phosphate (MEP) pathway for the tail, with the process occurring in plant plastids.

Carotenoids, a diverse group of over 750 naturally occurring pigments, impart yellow, orange, and red hues to fruits and vegetables. They provide 40 to 50 dietary carotenoids, offering numerous health benefits. Carotenoids are synthesized from isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP), primarily through the plastidial MEP pathway in plants (Rodriguez-Concepcion, 2010). Cruciferous vegetables are rich sources of both tocopherols and carotenoids. Among underutilized vegetables, Brussels sprouts are notable for their high tocopherol and tocotrienol content (Piironen et al., 1986; Kurilich et al., 1999; Podsedek, 2007).

Carotenoids, serving as vitamin A precursors, include alpha-carotene, beta-carotene, and beta cryptoxanthin, which are abundant in crucifers and exhibit strong antioxidant activity due to their conjugated bonds (Favela-González et al., 2020). Kale, a key cruciferous vegetable, is particularly rich in carotenoids, with beta-carotene and lutein being predominant (Podsedek et al., 2006). Carotenoid levels vary among crucifers, with Brussels sprouts containing 6 mg/100 g fresh weight (FW), broccoli 2 mg/100 g FW, and red cabbage 0.5 mg/100 g FW, surpassing regular cabbage (Podsedek, 2007).

4. Functional benefits of underutilized cruciferous vegetables:

Shifting dietary patterns and modern lifestyles have led to numerous health issues, prompting health-conscious individuals to seek nutrient-rich foods that can lower disease risk through simple dietary adjustments. Vegetables play a crucial role in daily nutrition, with health organizations advocating for their regular inclusion in meals. Consistent consumption of diverse vegetables has been linked to reduced rates of lifestyle-related conditions such as obesity, atherosclerosis, and various cancers.

Research confirms that omitting fresh vegetables from the diet is directly associated with an increased risk of chronic illnesses, particularly cancers (Czapski, 2009). Both in vitro and in vivo studies demonstrate a strong connection between cruciferous vegetable intake and reduced cancer risk (Manchali et al., 2012). Additionally, cruciferous vegetables help lower the prevalence of cardiovascular diseases by regulating blood pressure and cholesterol levels (Liu, 2004). Recent studies highlight crucifers as rich sources of antioxidants, containing significant amounts of bioactive compounds like glucosinolates, carotenoids, and phenolics (Cartea et al., 2011). Antioxidant levels vary depending on the growth stage, with Chinese cabbage, for example, showing peak antioxidant activity during its juvenile phase. Among cruciferous vegetables, broccoli, kale, red cabbage, and Brussels sprouts exhibit higher antioxidant content than regular cabbage (Soengas et al., 2012).

These bioactive compounds serve as natural defenses in plants against bacteria, viruses, and fungi. Beyond chronic disease prevention, cruciferous vegetables mitigate factors contributing to the gradual development of such conditions. For instance, Tiku et al. (2008) found that Brassica campestris protects against gamma-radiation-induced chromosomal damage in mice. Overall, the regular inclusion of cruciferous vegetables, rich in bioactive compounds, significantly enhances health outcomes (Fig. 1). Their diverse health benefits and specific properties are detailed in Table 1 and summarized below:

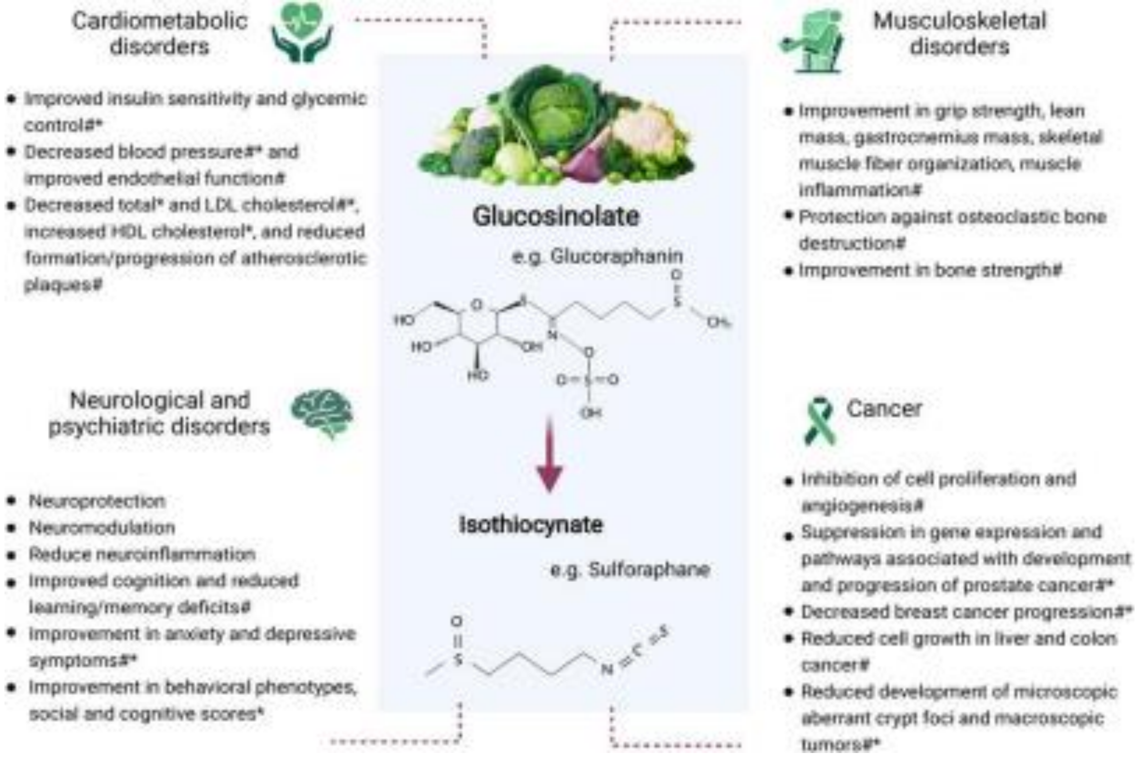

Fig 1. A Review of Bioactive Metabolites in Underutilized Vegetables and Their Implications for Human Health.

4.1.Anti-carcinogenic activity :

An unhealthy lifestyle heightens the risk of exposure to carcinogens, which may be present in the environment or consumed through regular diets. Cruciferous vegetables have long been valued for their potential to combat cancer. These vegetables are rich in bioactive compounds that aid in the body's detoxification processes. Among these, glucosinolates are particularly significant, as they influence various pathways to modify or break down carcinogenic substances.

The development and progression of different cancers largely depend on how carcinogens interact with bodily enzymes. Phase I and Phase II enzymes are critical in this process, with Phase I enzymes, including the cytochrome P450 family, modifying certain carcinogens, sometimes aiding in detoxifying procarcinogens (Esteve et al., 2020). Conversely, Phase II enzymes enhance the elimination of carcinogenic compounds, while Phase I enzymes can increase their reactivity. Effective carcinogen management requires deactivating Phase I enzymes and activating Phase II enzymes (Kumar and Andy, 2012).

Glucosinolates significantly boost Phase II enzyme activity. When hydrolyzed, glucosinolates produce compounds like isothiocyanates, which include benzyl isothiocyanate, phenethyl isothiocyanate, and sulforaphane, varying by crop. Histone deacetylase, an enzyme involved in histone acetylation and linked to cancer development, can be inhibited by compounds such as isothiocyanates and sulforaphane, which suppress its expression (Dashwood and Ho, 2007; Manchali et al., 2012). Additionally, cytochrome P450 enzymes (CYPs) contribute to the activation of carcinogens.

Benzyl isothiocyanate (BITC), derived from glucotropaeolin, is found in watercress and exhibits anticancer properties, particularly against blood cancer, by modulating extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) expression (Verkerk et al., 2009; Herz et al., 2016; Tang et al., 2015). BITC also plays a role in preventing cancers of the colon, brain, breast, and pancreas by regulating Nuclear factor-kB (NF-kB) and enhancing p53 transcription factor expression, which helps reduce osteolytic bone resorption (Pore et al., 2017). Under abnormal conditions, Nrf2, supported by genes like HO-1 (Heme oxygenase) and GCL (glutamate-cysteine oxygenase), suppresses NF-kB expression to inhibit breast cancer (Huang et al., 2013; Kim et al., 2010).

Phenethyl isothiocyanate (PEITC), produced from gluconasturtiin and abundant in watercress, targets cancer cells in the colon, prostate, ovary, breast, and blood by generating reactive oxygen species (ROS). The expression of Fas and Fas ligand, part of the death receptor and TNF families, respectively, triggers tumor cell death in blood cancer (Wang et al., 2014; Golstein, 2000). PEITC also reduces human epidermal growth factor receptor 2 (HER2) expression, curbing cancer cell proliferation in breast and ovarian cancers (Gupta and Srivastava, 2012), while promoting ferroptosis and upregulating 8-oxo deoxy guanine and pH2AX foci to prevent prostate cancer (Ikejiri et al., 2018; Kasukabe et al., 2016).

Sulforaphane suppresses CD44v6 expression, offering protection against brain tumors by inhibiting tumor initiation (Li et al., 2014; Jung et al., 2009). The activity of HDAC5 and LSD1 elevates breast cancer risk, but sulforaphane counteracts this by blocking HDAC5 transcription factor activity (Royston et al., 1980). In colon and prostate cancers, sulforaphane induces cell death by activating ERK1/2 and Akt kinases (Jakubíková et al., 2005; Zhou et al., 2018). Indole-3-carbinol (I3C), derived from indolyl methylglucosinolate, and its dimer, 3,3'-diindolylmethane (DIM), contribute to anticancer effects (Virtanen, 1965). I3C modulates nuclear transcription factors to promote an anticarcinogenic response, disrupting the G1 cell cycle of tumor cells and inactivating Rb protein and CDKs, which drive the transition from G1 to S phase in cancer cells (Sherr, 1996). Additionally, I3C delays multidrug resistance (MDR) by inhibiting the expression of the MDR-1 gene transcript P-glycoprotein (P gp).

4.2.Protection against cardiovascular diseases:

Cardiovascular diseases (CVD) remain the leading cause of mortality worldwide (Liu et al., 2013). Numerous studies indicate that cruciferous vegetables help lower the risk of CVD. Researchers have assessed methanolic and aqueous kale extracts for their potential in treating cardiovascular conditions, revealing that these extracts
inhibited lipid peroxidation in isolated very low-density lipoprotein and low-density lipoprotein from human samples (Kural et al., 2011).

4.3. Antiobesity activity :

Obesity is a condition marked by excessive body fat accumulation, resulting in weight gain and increasing the risk of diseases like type 2 diabetes and cardiovascular issues. It is defined by excessive lipid storage and reduced glucose tolerance (Chang et al., 2011). The anti-obesity effects of Brassica rapa were investigated in obese mice over an 8-week period, revealing that ethanolic extracts of Brassica rapa (50 mg/kg) reduced lipid buildup in adipocytes and inhibited beta(3)-AR-dependent lipolysis stimulation (An et al., 2010).

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Table 1: Bioactive Constituents of Underutilized Cruciferous Vegetables and Their Impacts on Human Health

Crop	Health Implications	Bioactive compound	Test modal	Findings	Refs.
Kale	Defense from oxidative stress	Glucoiberin, Progoitrin, Glucoraphanin, Sinigrin, Glucobrassicin, Neoglucobrassicin, Kaempferol-3-Osoph+, Kaempferol-3- O-(feruloyl)soph, Quercetin 3-O-(feruloyl)soph-7- Osoph+,	Rat fed with AIN-93 G standard diet containing 10% raw and 10% cooked lyophilized kale and with paraquat	Lowers down the lipid peroxide level	Sikora et al. (2013)
Brussels sprout	Defense from oxidative stress	Glucoiberin Progoitrin Glucoraphanin Sinigrin Gluconapin Glucobrassicin	Non-smoking volunteers after consumption of the sprouts @ 300 g.day_1	Restricted DNA-migration (97%) induced by the heterocyclic aromatic amine 2-amino-1-methyl-6-phenyl imidazo-[4,5-b]pyridine (PhIP)	Hoelzl et al. (2008)
Collards	Anti tumor potential	Glucoiberin, Progoitrin,	Two human anti-tumor	Exhibited inhibitory effect	Rado_sevi_c et al. (2017),

		Glucoraphanin, Sinigrin, Gluconapin, Glucobrassicin, Gluconasturtiin	cell lines MCF-7 and HeLa treated with 100 mg m L _1 collard extract	on tumor cells and protect from oxidative stress (21.62%) viability in MCFcells and 57.56% for HeLa cells)	Carlson et al. (1987), Deng et al. (2015)
Kholrabi	Anticarcinogenic	Glucoiberin, Glucoraphanin, Glucoiberverin, Glucoerucin, Glucobrassicin	HT-29 and Caco-2 colorectal cancer cell lines treated with 10_60 mg/mL kholrabi extracts	Induced cell cycle arrest in HT-29 cells at G and S phase and strong antiproliferative effect	Rizk and Zarzour (2013), Deng et al. (2015)
Red cabbage	Anticancerous	Progoitrin, Glucoraphanin, Sinigrin, Gluconapin, Glucobrassicin, Neoglucobrassicin, cyanidin-3-Odiglucoside 5-O-glucoside	Human cell line (HT-29, MCF-7, Caco 2 and HFF3) extract used at lower concentrations than 6.4 mg/ ml	Prevented the normal human cell damage	Tajalli et al. (2020)

Watercress	Nephrotoxicity inhibtion	Gluconasturtiin	Adult male wistar rats - watercress (250, 500 mg/ kg pretreatment	Alleviated increased serum creatinine and urea levels, MDA levels, kidney, and reduced creatinine clearance attenuated Vancomycin (VCM)-induced nephrotoxicity	Karami et al. (2018)
Rocket	Defense from oxidative stress	Glucoraphanin, Glucosativin, Dimeric glucosativin, Glucoerucin	Rat fed with 300 mg/kg. B.W. ethanolic extract	Improved the testicular functions and spermatogenesis in H_2O_2, stressed rats.	Nowfel and Al Okaily (2017)

4.4. Antidiabetic activity:

Type 2 diabetes mellitus is characterized by insulin resistance, primarily affecting individuals over 45, though it is increasingly prevalent among children and adolescents. It is closely linked to obesity, as inflammation in white adipose tissue, a hallmark of obesity, contributes to diabetes development. The antidiabetic effects of Brassica oleracea var. italica were evaluated in type 2 diabetic Sprague-Dawley (SD) rats over 28 days at doses of 200, 400, and 800 mg/kg body weight. The greatest reduction in blood glucose levels was observed at the highest dose of 800 mg/kg, demonstrating its potential as an antidiabetic agent (Shah et al., 2016).

4.5. Antioxidant and anti-inflammatory activity:

Phenolic compounds are the primary contributors to the antioxidant properties of cruciferous vegetables. Stress conditions often trigger the production of reactive oxygen species (ROS), which are oxygen radicals generated as byproducts of cellular reactions and are linked to ailments like atherosclerosis, aging, and neurodegenerative disorders. While low levels of ROS aid in regulating defense mechanisms, excessive amounts can harm cells. The antioxidant capacity of bioactive compounds in cruciferous vegetables depends on the presence and position of hydroxyl groups.

Oxidative stress and inflammation are closely related, as oxidative damage often provokes inflammatory responses. Antioxidant response elements (ARE) encode genes such as heme oxygenase 1 (HO-1), GST, UGT, and NAD(P)H quinone oxidoreductase 1 (NQO1), which play a role in detoxification (Keum et al., 2003; Xu et al., 2006). Compounds like allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), and phenethyl isothiocyanate (PEITC) suppress the expression of inflammation-related genes, including Ilb1, IL6, TNFa, nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX2) (Caglayan et al., 2019; Huang et al., 2013; Kim et al., 2010). The aryl hydrocarbon receptor (AhR) helps regulate inflammation by forming a heterodimer with the AhR nuclear translocator upon binding ligands from cruciferous vegetables, thereby modulating anti-inflammatory gene expression (Neavin et al., 2018).

Kequan and Liangli (2006) noted that kale exhibits the highest antioxidant potential among cruciferous vegetables, while Soengas et al. (2012) later highlighted the elevated antioxidant activity in underutilized vegetables like tronchuda cabbage, nabicol, and broccoli. Cabbage leaves have traditionally been used to reduce inflammation in breastfeeding women, with scientific evidence supporting this practice (Samec et al., 2011). Caglayan et al. (2019) found that AITC, a glucosinolate precursor, reduced infarct volume and brain swelling in a mouse model of traumatic brain injury by downregulating NF-kB, decreasing IL1b and IL6 production, and boosting antioxidative activity via the Nrf2 pathway.

These properties make underutilized cruciferous vegetables ideal candidates for breeding programs aimed at enhancing the quality of commercial vegetables and promoting their inclusion in daily diets. To fully establish their role as natural therapeutics, the specific bioactive compounds in cruciferous vegetables that manage metabolic syndromes require further study and clinical validation to improve their acceptance.

4.6. **Metabolic syndrome**:

Metabolic syndrome is a condition characterized by coexisting health issues such as hypertension, obesity, and diabetes, often influenced by dietary habits. Individuals with obesity, particularly those over 40, face a higher risk of developing diabetes, high blood pressure, and subsequent complications like stroke and heart disease. The transcription factor Nrf2 plays a significant role in mitigating type 2 diabetes and obesity. Glucosinolates in cruciferous vegetables help reduce oxidative stress and inflammation, thereby limiting the progression of metabolic syndrome by enhancing Nrf2 activity.

These glucosinolates inhibit adipocyte differentiation (Esteve, 2020), while Nrf2 upregulates genes such as Heme oxygenase 1 (HO-1), glutathione peroxidase, glutathione S-transferase A1, NAD(P)H quinone oxidoreductase, and glutamate-cysteine ligase, which support cell survival under oxidative stress (Salazar et al., 2006; Ishii et al., 2000). Conversely, glycogen synthase kinase 3 beta (GSK-3b) suppresses phase II gene expression, increasing oxidative stress.

Recent research has explored the role of underutilized cruciferous vegetables in managing metabolic syndrome. In a clinical study, Choi et al. (2014) found that sulforaphane from cruciferous vegetables prevented weight gain and reduced cholesterol levels in mice fed a high-fat diet (HFD), while also increasing adiponectin levels. Similarly, Chuang et al. (2019) reported that supplementation with 1 g/kg of benzyl isothiocyanate (BITC) or phenethyl isothiocyanate (PEITC) in HFD-fed mice over 18 weeks prevented weight gain by downregulating PPARg, LXRa, and SREBP1c transcription factors.

Limited human studies exist, but Bahadoran et al. (2012) conducted a trial with 81 placebo patients divided into three groups, finding that those receiving 10 g/day of broccoli sprout powder (BSP) experienced a significant reduction in serum insulin levels, aiding in the management of type 2 diabetes.

5. Conservation strategies:

Conservation efforts focus on safeguarding the genetic diversity of crops and plants to prevent genetic erosion, serving as a vital approach to protect wild relatives of modern cultivated varieties. Plant genetic resources face threats from factors such as the replacement of diverse local varieties, urban expansion, invasive species, climate change, globalization, natural disasters, introduction of new crops, overgrazing, and rising population pressures (Kaviani, 2011).

Limited data exists on the genetic resources of underutilized crops, which hinders their development and long-term sustainable conservation. Knowledge about the genetic potential of underutilized cruciferous vegetables, such as Brussels sprouts and kohlrabi, is particularly scarce, with only 702 accessions of Brassica oleracea var. gemmifera currently preserved in genebanks.

Biotechnological techniques play a crucial role in addressing these challenges by enabling the improvement and preservation of vulnerable germplasm through methods like micropropagation, cryopreservation, in vitro conservation, tissue culture, somatic hybridization, DNA banking, mycorrhization, and genetic engineering (Rao, 2004). Plant genetic resources are a key component of biodiversity, containing valuable genetic material essential for meeting current and future agricultural needs (Leisa, 2004).

Advances in agriculture, such as improved yields, disease resistance, and enhanced functionality, rely heavily on utilizing genetic material from superior related cultivars. The loss of these resources poses a significant threat to long-term food and nutritional security. Genetic resources for conservation can include reproductive or vegetative materials like seeds, stems, somatic tissues, pollen, or DNA (Ogbu et al., 2010).

Somatic hybridization in Brassica species has led to the development of numerous intergeneric somatic hybrids with promising future applications (Arumugam et al., 2000; Hu et al., 2000; Du et al., 2009). Such biotechnological tools offer innovative solutions for enhancing the functional properties of cruciferous vegetables, potentially improving their response to specific macro- or micro-molecules.

Plant conservation is categorized into in situ and ex situ approaches. In situ conservation preserves genetic resources in their natural environments, while ex situ conservation involves maintaining them under controlled, artificial conditions (Withers and Engelmann, 1998). Advanced biotechnological methods, such as in vitro culture and micropropagation, have significantly enhanced the preservation and management of plant genetic resources (Rao, 1994).

To date, 10,301 Brassica germplasm collections have been amassed from national and international sources, comprising 10,059 native and 242 foreign accessions, including 147 released varieties and 49 registered germplasms (Radhamani et al., 2013). Wild Brassica relatives are crucial for hybrid seed development, providing cytoplasmic male sterility and nuclear genes that confer disease resistance. In vitro propagation enables large-scale plant production by generating callus from explants like leaves, seeds, or tubers, followed by the formation of shoots, roots, and complete plantlets under regulated conditions (Rai, 2010).

This technique has facilitated the development of stress-resistant Brassica lines with improved yields and quality (Rai et al., 2011). For instance, in vitro culture has produced salt-tolerant *B. juncea* and *B. napus* varieties suitable for diverse soil types (Kirti et al., 1991; Rahman et al., 1995). It also supports the cost-effective production and maintenance of pathogen free germplasm for underutilized vegetables, with potential for further improvement. For vegetable species that lack seeds, are challenging to propagate vegetatively, or are endangered in their natural habitats, DNA banking at -20°C for short-term storage or - 80°C for long-term preservation provides an effective solution (Dulloo et al., 2006).

Cryopreservation, another key method, stores biological tissues at -196°C in liquid nitrogen, halting cellular activity to preserve germplasm for extended periods without altering its viability, vigor, or genetic composition, making it ideal for conserving woody plant species (Obgu et al., 2010; Panis and Lambardi, 2005). Genetic diversity in vegetables can be assessed using molecular marker technologies, such as Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Restriction Fragment Length Polymorphism (RFLP).

These cost-effective, sensitive, and reliable methods detect genetic polymorphisms and aid in identifying and conserving rare or endangered species (Karp et al., 1996). Alongside these modern techniques, involving local communities in traditional conservation practices within natural habitats can enhance preservation efforts. Depending on a crop's importance and sensitivity, one or more of these strategies can be employed for effective long-term conservation and utilization. These approaches are critical to ensuring that underutilized vegetables are preserved to meet the population's nutritional and functional needs.

6. **Methods for Functional Optimization**:

Over the past few decades, traditional breeding methods have been employed to enhance vegetable quality and yield. However, limitations such as genetic bottlenecks, loss of genetic diversity, and time-consuming processes have pushed researchers toward innovative breeding and biotechnology-based approaches to improve vegetable characteristics. Additionally, various biotic and abiotic stresses have been shown to hinder plant growth, significantly reducing both productivity and product quality.

A key challenge in modern cultivars is their limited resistance to pathogenic diseases and environmental stresses, which are critical concerns for production. To address these issues, advanced techniques such as marker-assisted selection, marker-assisted backcrossing, gene pyramiding, and genetic engineering have been widely adopted to enhance the quality traits of numerous vegetable crops.

Throughout the 20th century, research on vegetable improvement primarily targeted enhancing nutritional value and functional qualities to provide consumers with superior products. Various biotechnological methods have been employed to boost commercial benefits, including improvements in quality, nutrition, yield, and resistance to biotic and abiotic stresses. Huang et al. (2002) highlighted biotechnology's role in optimizing yield while maintaining high quality.

Through plant genetic engineering, specific traits can be introduced by transferring desirable genes, even from unrelated species, to achieve targeted functionalities. Numerous efforts using advanced scientific tools have aimed to enhance the properties of fresh vegetables and food products. For instance, Naeem et al. (2020) reduced the viscosity of mustard oil by transferring the EaDACT (diacylglycerol acetyl transferase) gene from Euonymus alatus to Indian mustard using a glycinin promoter via Agrobacterium-mediated transformation, enabling the synthesis of modified triacylglycerols. Similarly, metabolic engineering facilitated the transfer of the IFS gene (GMIFS2) from soybean to Brassica napus, increasing isoflavone genistein (with phytoestrogenic and antioxidant properties) in B. napus leaves to 0.72 mg/g (Li et al., 2011). Additionally, Vigeolas et al. (2007) overexpressed glycerol-3-phosphate (GLy3P) dehydrogenase in rapeseed under a seed-specific promoter, boosting GLy3P levels by three to four times and raising lipid content by 40%. Such advancements have been crucial for enhancing the availability and bioactivity of bioactive compounds in various crops, including cruciferous vegetables.

Plant breeders employ molecular marker-assisted breeding techniques to enhance vegetable characteristics by assessing germplasm and mapping genes, among other methods. This approach enables selection at the seedling stage, shortening the breeding cycle and allowing the development of improved vegetable varieties in less time. Numerous molecular markers associated with traits like low linolenic acid and high oleic acid have been identified in brassica vegetables, enabling quality improvements in cold pressed oils from crops such as rapeseed, used for salad dressings and other culinary
purposes (Jourdren et al., 1996).

Additionally, cutting-edge technologies like RNA interference, next-generation sequencing, and nanotechnology have significantly contributed to enhancing crop quality and yield. For instance, transgenic oilseed rape (Brassica napus) has been engineered to express the BnSCE3 gene, enhancing sinapine esterase activity, which breaks down sinapine into sinapate and choline, reducing sinapine levels by up to 5% compared to non-transgenic varieties (Clauß et al., 2011).

In the past, traditional breeding techniques, such as interspecific sexual hybridization and the use of natural or induced mutations, were the primary methods for enhancing vegetable quality traits (Chiang and Jacob, 1992). However, with the growing global population, there is now a pressing need to produce vegetables that are nutrient-rich, have extended shelf life, and are cultivated using minimal resources. Achieving these goals requires integrating conventional breeding with

biotechnological methods and marker
assisted selection techniques.

The development of stress-resistant crops through genomics, bioinformatics, and stress biology can significantly boost both yield and quality (Dickson and Wallace, 1986). To date, various molecular approaches, including gene pyramiding, linkage mapping, allele mining, recombinant DNA technology, genetic engineering, marker-assisted backcrossing, marker-assisted recurrent selection, genome
wide selection, and next-generation sequencing, have been employed to improve cruciferous vegetables (Fang et al., 2005). Genetic transformation has proven highly effective in developing vegetables with enhanced quality traits (Zou et al., 1997). For example, using a transgenic approach, the lysophosphatidate acetyltransferase gene from yeast was overexpressed in oilseed rape, resulting in a seed oil content increase of up to 10% under field conditions.

Molecular marker-assisted breeding involves leveraging molecular biology and linkage mapping to identify and enhance specific genes based on genotypic analysis, enabling targeted trait improvement (Jiang, 2013). This approach uses DNA markers to select genes associated with desired traits at the molecular level. Compared to conventional plant selection methods, DNA markers improve the accuracy and efficiency of identifying genes responsible for specific traits. Techniques such as marker-assisted selection, marker-assisted backcrossing, and marker-assisted recurrent selection are employed to modify a plant’s genetic composition for improved traits (Ribaut et al., 2010).

Markers can be morphological, biochemical, or molecular and are associated with specific plant characteristics (Rosyara, 2006). For instance, genome-wide analysis of the GRAS gene family, a group of transcriptional regulators in Chinese cabbage, categorized 48 genes into eight groups based on gene structure and phylogenetic analysis, providing insights into the roles of various genes in determining specific traits (Song et al., 2014).

Marker-assisted backcrossing is a technique used to enhance plant and crop traits by transferring specific genes or quantitative trait loci (QTLs) from a donor parent to a superior recurrent parent. This method relies on molecular markers linked to a target gene of interest within the plant. It has been extensively applied to improve traits such as drought resistance, biotic stress tolerance, and

quality in crops like soybean, barley, rice,
wheat, and tomato (Collard et al., 2005; Dwivedi et al., 2007).

This approach is particularly valuable for improving vegetable crop traits when phenotyping is challenging. Identifying QTLs that are critical for enhancing specific functional traits in crops is essential. To date, several QTLs have been discovered that play a key role in regulating oil content in cruciferous vegetables (Burns et al., 2003; Zhao et al., 2006), with some QTLs accounting for up to 10% of the variation in total oil content in improved vegetable varieties (Qiu et al., 2006; Delourme et al., 2006).

Marker-assisted gene pyramiding is a key approach for enhancing crop and plant traits by simultaneously targeting multiple genes. Plant genetic engineering involves inserting foreign DNA or genes into plant cells to develop varieties with specific desired characteristics (Newell, 2000). This technique is employed to improve various commercial attributes, such as resistance to biotic stresses (caused by viruses, fungi, bacteria, etc.) and abiotic stresses (resulting from non-biological factors like temperature, salinity, drought, or light) (Fagoaga et al., 2007).

Transgenic plants, created through genetic engineering and tissue culture, represent genotypes with these modified traits. Analyzing gene transcript profiles in response to heat in non-heading Chinese cabbage using qRT-PCR techniques can help enhance heat tolerance in plants (Wang et al., 2016). Additionally, Mei et al. (2020) described the development of Sclerotinia-resistant rapeseed lines by transferring and pyramiding three major QTLs from wild Brassica oleracea to Brassica napus using molecular marker-assisted selection, achieving 35% greater resistance than the wild type while maintaining high seed quality, yield, and oil content.

Analysis of gene expression through transcriptome studies has revealed insights into genes responsible for the purple coloration of leaves in Brassica juncea, enabling the development of vegetables rich in anthocyanins (Heng et al., 2020). Previous research has demonstrated the use of Agrobacterium-mediated genetic transformation to enhance quality traits in Brassica species, such as Brassica juncea (Barfield and Pua, 1991) and Brassica oleracea (De Block et al., 1989).

Additionally, elevated glucosinolate levels were achieved in transgenic hairy roots of watercress by overexpressing cabbage transcription factors (Cuong et al., 2019). Incorporating such nutrient-enriched vegetables into daily diets is increasingly vital, as it can help prevent or manage various chronic health conditions. Transgenic head cabbage resistant to diamondback moth larvae was developed using Agrobacterium tumefaciens-mediated transformation to introduce Bacillus thuringiensis (Bt) Cry genes (Jin et al., 2000). Similarly, transgenic Indian mustard expressing a tomato glucanase gene was developed to inhibit the growth of the fungus Alternaria brassicae, effectively addressing biotic stress (Mondal et al., 2007).

Genome editing involves precise, targeted alterations to specific genes, enabling the addition, removal, or modification of genetic material. This approach is extensively applied to enhance the functional qualities of various vegetable crops. Techniques such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas systems are commonly used. For instance, Shan et al. (2015) utilized
TALEN technology to knock out the OsBADH2 gene in rice, resulting in aromatic rice with improved flavor, as well as elevated mineral and vitamin levels compared to conventional varieties. Similarly, Shan et al. (2014) employed the CRISPR/Cas system to develop wheat resistant to powdery mildew. These methods allow precise gene modifications in vegetable crops to achieve desired traits. Sinapate esters, which can bind to proteins and reduce their bioavailability, were reduced by 60-70% in transgenic oilseed rape seeds by silencing the BnSGT1 gene using dsRNAi (H€usken et al., 2005).

Mao et al. (2014) enhanced the head shape of Chinese cabbage by regulating the BrpTCP gene with microRNA, halting cell division in leaf areas, which significantly increased its commercial value. The CRISPR-Cas9 system, known for its high specificity, ability to perform multiple edits, and user-friendliness, has become a powerful tool for crop improvement (Song et al., 2016).

Sashidhar et al. (2019, 2020) used CRISPR/Cas9 to knock out multiple paralogs of the inositol tetrakisphosphate kinase (ITPK) enzyme in B. napus seeds, reducing phytic acid, an anti-nutritional factor, in triple mutants. CRISPR/Cas9 has also been widely applied to study key traits in Brassica species. For example, Zheng et al. (2019) targeted the carotene desaturase gene in Chinese kale, achieving a mutation efficiency of 68.42% and producing an albino phenotype.

Additionally, Yusuf and Sarin (2007) overexpressed the g-TMT gene in Indian mustard cv. Varuna, boosting α-tocopherol content by sixfold.

7. Effect of processing on bioactive compounds:-

Vegetables are a vital part of the human diet, providing essential nutrients, promoting satisfaction, and helping to lower the risk of various health conditions. While fresh, unprocessed vegetables are often recommended, recent research indicates that the way vegetables are processed and cooked can significantly impact their nutritional value, either enhancing or diminishing the bioavailability and effectiveness of bioactive compounds.

Cooking methods, such as heat treatments and various processing techniques, are crucial for improving sensory appeal and digestibility but may negatively affect the quality of bioactive components (Junior, 2017). Vegetables are complex, containing a mix of macro- and micromolecules, and the processing methods used can lead to both beneficial and adverse interactions among these biochemical elements.

Thus, studying the transformations that occur in vegetables from preparation to consumption is essential for both scientific understanding and enabling consumers to make informed choices about vegetable preparation and cooking methods (Fabbri and Crosby, 2016). Common cooking techniques include steaming, roasting, boiling, frying, and using microwave or pressure cooking (Hailemariam and Wudineh, 2020).

Cruciferous vegetables are often eaten raw in salads or lightly cooked. When consumed raw, the enzyme myrosinase breaks down glucosinolates in the upper digestive tract into various metabolites that the body easily absorbs. However, cooking can alter the glucosinolate-myrosinase system by deactivating the plant’s myrosinase enzyme, causing glucosinolates to move to the colon, where gut microbiota break them down.

Factors like temperature, storage duration, packaging, and myrosinase inactivation processes can significantly affect the bioavailability and activity of glucosinolates and their derivatives (Rungapamestry et al., 2007; Barba et al., 2016; Ciska et al., 2015). Research by Ciska et al. (2015) first identified glucosinolate (GLS) breakdown products and explored the connections between these products (aliphatic, aralkyl, and indole) and their parent GLS in boiled Brussels sprouts. They found that thermal processing could reduce GLS levels by up to 67%.

In boiled Brussels sprouts, seven breakdown products were identified: indole-3-acetonitrile, indole-3-carbinol, ascorbigen, and 3,3'-diindolylmethane from glucobrassicin; 3-butenylnitrile from sinigrin; 4-methylsulfinylbutanenitrile from glucoiberin; and 2-phenylacetonitrile from gluconasturtiin. The levels of indole-3- acetonitrile and 3-butenylnitrile corresponded to approximately 54% and 9% of the GLS values, respectively. Thus, the high levels of unhydrolyzed GLS in Brussels sprouts make them a valuable source of bioactive compounds, which gut microbiota release in the digestive tract.

Suzuki et al. (2006) studied glucosinolate changes during the pickling process, where fresh and blanched watercress were soaked in a 3% NaCl solution for a week. They noted an increase in the proportion of indole glucosinolates relative to total glucosinolates, while the overall glucosinolate content dropped. Post-harvest handling also impacts crucifer quality. Song and Thornalley (2007) reported that storing Brassica vegetables at room temperature or in a refrigerator resulted in a modest glucosinolate loss (9–26%) over seven days. Kim et al. (2020) examined glucosinolate changes in Chinese cabbage stored at 24°C for three weeks, finding that GLS levels varied by storage temperature, with the smallest increase at 4°C (longest preservation) and the largest at room temperature.

Optimal storage conditions should be tailored to the intended storage purpose and duration. Preprocessing, such as shredding, can significantly reduce glucosinolate content. Boiling not only modifies or deactivates bioactive compounds in cruciferous vegetables but also leads to losses through leaching into cooking water, with up to 90% of glucosinolates detected in the water (Barba et al., 2016).

Baenas et al. (2019) investigated how domestic cooking methods affect glucosinolate and isothiocyanate (ITC) degradation in broccolini and kale. Stir-frying (120°C) and steaming (98°C ± 2°C) preserved about 50% of GLS and ITC compared to raw vegetables, while boiling retained only 20–40%. Cooking methods like stir-frying, steaming, and microwaving increased ITC yields in broccoli, cabbage, cauliflower, and kale by about four times, whereas boiling, stewing, and chipbaking reduced ITC by 58% (Wang et al., 2020).

The impact of processing on the bioavailability of bioactive compounds in lesser known cruciferous vegetables is summarized in Table 2.

Table 2: Influence of Processing on the Bioaccessibility and Bioactivity of Bioactive Compounds in Underutilized Cruciferous Vegetables

PIGMENTS	EFFECT OF PROCESSING
Polyphenols, Phenols	According to Armesto et al. (2018), steaming kale for 20 minutes preserved the highest amount of flavonoids (53.06 mg/100 g), compared to microwave and pressure cooking methods. Steaming resulted in the highest total phenol content in kale, reaching 19,157.7 mg GAE/kg dry weight, surpassing the levels observed after boiling, frying, and microwave heating (Akdaş & Bakkalbaşı, 2017), The retention of total phenolic content (TPC) in red cabbage increased by up to 6% due to the presence of metal ions after five weeks of storage in polyethylene bags at 37°C (Ratanapoompinyo et al., 2017). Steaming at 90°C for 5 to 15 minutes led to a significant rise in phenolic content in kale (an increase of 86.1%), while in red cabbage, it caused a notable reduction of 34.6% (Murador et al., 2016). Brussels sprouts kept in zipper bags retained a greater polyphenol content than those stored in plastic containers, showing about a 17% difference after three months of storage (Kapusta-Duch et al., 2014). After

	one year of storage, frozen kale products preserved 22–45% of polyphenols, whereas canned products retained around 24%, with values influenced by the extent of raw material comminution (Korus and Lisiewska, 2011).
Glucosinolates and isothiocyanates	Stir-frying and steaming were effective methods for retaining glucosinolates and isothiocyanates—preserving about 50% of the levels found in raw samples— whereas boiling significantly reduced their retention, maintaining only 20–40% of the original content in kale and broccolini (Baenas et al., 2019). Blanching Chinese cabbage at 95°C in a 10% lemon juice solution led to a significant rise in glucoassicin levels, reaching between 0.3 and 0.6 mg/g as the concentration of lemon juice increased (Managa et al., 2019) Microwaving and steaming watercress for 2 to 5 minutes caused only slight reductions in total glucosinolate content, indicating that these cooking methods help preserve higher levels of these compounds (Giallourou et al., 2016)
Pigments (Chlorophyll, Carotenoid,	Treatment with acidic electrolysed water containing 100 mg/L of available chlorine for 3 minutes significantly reduced the anthocyanin content and

Anthocyanin)	antioxidant activity in red cabbage, with pelargonidin levels decreasing by 18.5% (Chen et al., 2018). The rate of chlorophyll degradation in Brussels sprouts rose with increasing microwave power, ranging from 460 to 700W (Nakilcioglu-Taş and Otleş, 2018) Steaming resulted in the highest retention of chlorophyll content in kale (7227.6 mg/kg dry weight) compared to boiling, frying, and microwaving (Akdaş and Bakkalbaşı, 2017). Microwaving preserved carotenoid content in kale to the greatest extent (559 mg β-carotene equivalents/kg dry weight) compared to frying and boiling (Akdaş and Bakkalbaşı, 2017) Brussels sprouts that were microwaved had the highest levels of total carotenoids (0.35 mg/g) and chlorophylls (3.01 mg/g), with steamed and raw samples containing lower amounts (Hwang, 2017) Anthocyanin degradation during storage (in polyethylene bags that were heat sealed and kept in an incubator at 37°C for 5 weeks) was more pronounced in red cabbage samples containing Sn^{2+} (k = 0.03602 $week^{-1}$) compared to control samples (k = 0.00644 $week^{-1}$) (Ratanapoompinyo et al., 2017) Brussels sprouts frozen for 3 months and stored in two types of packaging— zipper bags made of low-density polyethylene and plastic boxes made of oriented polystyrene—showed similar β-carotene content

	(Kapusta-duch et al., 2014)
Antioxidative activity	The antioxidant activity of red cabbage, measured by 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging, was significantly decreased by 11.2% after treatment with acidic electrolyzed water containing 100 mg/L available chlorine for 3 minutes (Chen et al., 2018) Following 12 months of storage, frozen kale products maintained 43–56% of their original TEAC levels, whereas canned products retained only 23–25%, depending on the processing extent of the raw material (Korus and Lisiewska, 2011)

velop cooking techniques and offer guidance to consumers on preparing cruciferous vegetables in ways that maximize the retention of bioactive compounds, particularly in lesser-known varieties.

The impact of thermal processing on cruciferous vegetables varies by method, significantly affecting dietary fiber content. Boiling, for instance, increases cellulose and lignin levels in vegetables like red cabbage compared to steaming. Red cabbage leaves, richer in dietary fiber than other parts, can significantly enhance a healthy diet (Komolka et al., 2012).

Earlier studies by Leszczynska et al. (2009) explored changes in nitrate and nitrite levels in brassica vegetables (curly kale, broccoli, white and green cauliflower, and Brussels sprouts) under different processing conditions. Vegetables blanched and frozen for 48 hours showed either stable or increased nitrate levels with variable nitrite changes, while those frozen for four months exhibited reduced nitrate and elevated nitrite levels compared to blanched samples.

Boiling frozen vegetables (stored for 48 hours) further decreased nitrate content compared to raw frozen ones. Betoret and Rosell (2020) investigated interactions between Brassica napobrassica leaf powder and starch, assessing how processing conditions like pH and temperature affect phenolic compounds and antioxidant activity. Smaller powder particle sizes and maize starch enhanced interactions with phenolic compounds, protecting them from degradation and boosting antiradical activity.

Boiling and blanching white cauliflower caused notable losses in dry matter, proteins, minerals, phytochemicals, and DPPH radical scavenging, while steaming, stir frying, and microwaving better preserved nutrients and phytochemicals (Ahmed and Ali, 2013). Among cooked brassicas (broccoli, cauliflower, cabbage, and Chinese cabbage), steaming retained the highest antioxidant levels, followed by boiling and microwaving, with longer cooking times reducing phytonutrient content (Wachtel-Galor et al., 2008). In
turnip greens (Brassica rapa), boiling and high-pressure cooking led to comparable losses of total glucosinolates (64%) and phenolics (over 70%), with flavonoid losses of 64% and 67% for boiling and high-pressure cooking, respectively (Francisco et al., 2010).

Over the past decade, growing consumer interest in functional foods has heightened awareness of post-harvest physicochemical changes and processing-related nutrient losses. This has driven a shift toward fresh or minimally processed foods with higher bioactive compound levels. Non-thermal processing methods, such as high-pressure processing, pulsed electric fields, and ohmic heating, as well as hurdle technology and minimal processing, have been extensively studied to preserve vital bioactive compounds in processed products.

Recent studies provide valuable insights, enabling consumers to choose nutrient-rich foods and identify critical processing stages that affect quality. Selecting appropriate cooking or processing methods is crucial for preserving health promoting phytochemicals in cruciferous vegetables.

8. **Conclusion**:

Malnutrition remains a major global challenge, closely linked to diet related illnesses, especially among low- and middle-income households with limited access to fresh produce. Underutilized cruciferous vegetables are rich sources of phytochemicals and hold significant promise for improving food and nutritional security. These lesser-known brassica crops offer a variety of bioactive compounds, such as glucosinolates, flavonoids, anthocyanins, coumarins, and carotenoids, which promote health.

Many of these crops possess untapped genetic potential that could significantly boost vegetable production through sustainable methods. Research indicates that consuming these underutilized crucifers is associated with a reduced risk of various chronic diseases. However, value-added products and research into functional foods derived from these crops are largely unexplored. Efforts should focus on raising awareness and encouraging the consumption of these vegetables among the public.

Research should prioritize developing innovative processing techniques to enhance the bioactivity and availability of nutrients. Future strategies should aim not only to increase food production but also to enhance the nutritional quality of food through sustainable farming practices. Compared to major cruciferous crops, these underutilized varieties have received little attention in research and breeding programs.

Modern biotechnological methods to optimize the concentration and efficacy of bioactive compounds in these vegetables could be highly valuable. Long-term research and breeding initiatives using advanced biotechnological approaches are essential to ensure their market competitiveness. Coordinated efforts at local, regional, and global levels are needed to fully harness the potential of these underutilized crops.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

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