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ISOTHIOCYANATES; SOURCES, PHYSIOLOGICAL FUNCTIONS AND FOOD APPLICATIONS

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Abstract

Foods are complex mixture of major and minor nutrients. They also contain some non-nutrient mixtures which exert beneficial effects to the human body known as phytochemicals. Phytochemicals are the chemical compounds that are synthesised by the plant cells to fight any kind of stress conditions. Isothiocyanates (ITCs) are the secondary metabolites produced by the plant from the enzymatic hydrolysis of glucosinolates by myrosinase during the plant tissue injury. They are present in Cruciferous vegetables, such as cabbage, broccoli and kale and are sulphur rich compounds. Isothiocyanates have been found to play an important role for the plant against microbial and pest infections. ITCs have been found to have beneficial activities like antibacterial, antifungal, bioherbicidal, and antioxidant, biopesticidal, anticarcinogenic and antimutagenic. Due to these activities they are having applications in food preservation like fruit juices and as an additive in Mayonnaise. Their recent application has been in the food packing's and in MAP. The present review gives the insight of these phytochemicals, their physiological functions and food applications

Keywords: Isothiocyanates, glucosionolate, sources, biocidal, food applications.

Introduction

Isothiocyanates are the type of phytochemicals that are produced from glucosinolates; a natural class of compounds containing sulphur and nitrogen. They are mostly present in Cruciferous vegetables containing varieties of glucosinolates, which yield different isothiocyanates when hydrolysed (Bischoff, 2016). Glucoraphanin and sinigrin are some of the glucoiolates that are present in broccoli. Glucoraphanin is the precursor of sulforaphane (SFN) and sinigrin is the precursor of allylisothiocyanate (AITC) (Higdon *et al.,* 2007). Gluconasturtiin, is present in watercress which is the precursor of phenethylisothiocyanate (PEITC), while garden cress is rich in glucotropaeolin, the precursor of benzyl isothiocyanate (BITC). Isothiocyanates (ITC) possess a typical pungent odour and a biting taste. It is formed from glucosinolate, by the action of the enzyme myrosinase, once the plant tissue is injured (Choesin and Boerner, 1991). They are mostly produced by the plant to protect against infections. Many studies show that certain phytochemicals have chemo preventive properties to counter cancer (Chawla *et al*., 2019; Dhull *et al*., 2019a, Dhull *et al*., 2019b; Agarwal *et al*., 2020). ITCs exhibit anticancer properties, with the restraining of phase I enzymes, initiating the action of phase II enzymes, prompting of cellular anti-oxidative systems along with the stimulation of apoptosis. Experimental and epidemiological studies have shown that ITCs have anti-inflammatory properties also (Das *et al*., 2013, Verkerk *et al.,* 2009). It is also shown to have a great anti-microbial property. Steam distillates of crushed and wetted seeds of mustered and horse radish are recognized as good antimicrobial substances (Arshad and Batool, 2017). It was shown to suspend the growth of microbes in a variety of packed foods like cheese, fish and meat at low concentrations in the vapour phase (Isshiki *et al.,*1992) and due to this characteristic property, it has been able to be used as food preservative in a number of foods stuffs.

Intake estimates

The consumption of ITCs is hard to estimate due to a great variability in the chemical composition in all the species of cruciferous plants. In some studies, the authors have mentioned that the total dietary intake of ITCs should be about 10–100 μmol/person and is about 0.14–1.42 μmol/kg body weight, taking the body weight of 70 kg. This has been measured on the basis of production of urinary metabolites from ITCs and dietary questionnaire (Seow *et al*., 1998; Fowke *et al*., 2002; Shapiro *et al*., 1998; Lin *et al*., 2017). In 2018, 5.9 g/capita/day of broccoli were consumed in the US (in pounds) (Statistica US 2018). The consumption has been declined from 7.5 g/capita/day in 2016 to 5.9 g/capita/day in 2018. The data on the development of the per capita consumption of kale, cauliflower, and broccoli in Germany from 2005/06 to 2015/16 was roughly 2.3 kilograms. In 2005/06, the normal per capita consumption of these vegetables was roughly 2.3 kilograms and by 2015/16, this figure had decreased to 2 kilograms. This can be attributed to the moods and the temperaments of the people for its consumption. The bitter and the pungent taste of these vegetables can also be one of the attribute. In Germany the intake of broccoli contributes mostly to the glucosinolate intake per day (19 percent of total glucosinolate intake) with indole, aliphatic, and aromatic glucosinolates contributing for 26, 16, and 3 percent , respectively of the daily intakes of the glucosinolate groups (Steinbrecher and Linseisen, 2009).

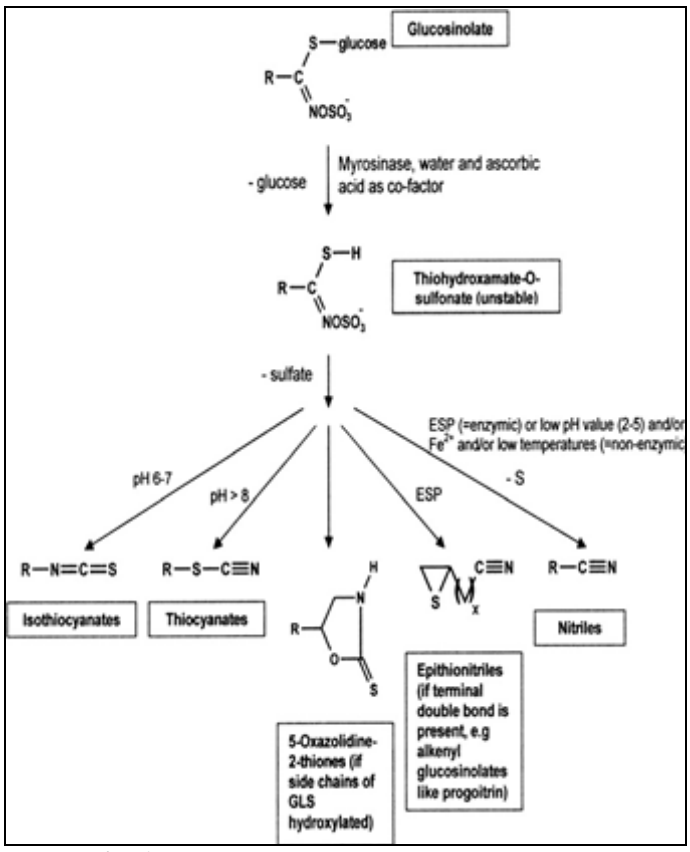

Fig. 1 : Hydrolysed products of glucosinolates

Glucosinolates enzymatic degradation

Any physical injury to the Cruciferous vegetables like chewing or cutting, results in the breakdown of glucosinolates through the action of myrosinase (β-thioglucosidase glucohydrolase, EC 3.2.3.1) which is stowed in the separate compartment of the plant cell and is released from ruptured plant cells (Fahey *et al.,* 2001). The glucose is produced as an intermediate and thiohydroxamate-O-sulfonate, is synthesised which undergoes spontaneous rearrangement into nitriles, isothiocyanates, elemental sulphur, epithionitriles, thiocyanates, indole compounds and oxazolidine- 2-thiones (Dinkova-Kostova and Kostov, 2012).

Glucosinolate precursors and their food sources

The concentration of isothiocyanates varies in foods formed from Glucosinolates mainly depending upon the food processing and the variety. The amount of the Glucosinolates in the cruciferous vegetables varies due to the differences in the agricultural practices and climatic conditions. These phytochemicals are mostly present in higher concentration as compared to others in the cruciferous plants. The main sources are the cruciferous vegetables like Broccoli, Brussel sprouts, Cabbage, horse radish, garden cress, Indian cress and Watercress. Broccoli, sprouts, Brussels, horse radish and Cabbage are the Glucosinolates precursors of Sinigrin and and glucoraphanin from which AllylIsothiocyanate (AITC) and Sulforaphane (SFN) are formed. Cabbage, garden cress and Indian cress contain Glucotropaeolin which is the glucosinolate precursor of Benzyl Isothiocyanate (BITC) while as Watercress containing Gluconasturtiin is the precursor of Phenethyl-Isothiocyanate (PEITC) (McNaughton and Marks, 2003).

Effects of processing methods

Glucosinolates are the bioactive compounds that are mostly water-soluble and are leached with the cooking water. Cruciferous vegetables when boiled for 9 to15 minutes cause a loss of about 18-59% of the total glucosinolate content. Most losses of glucosinolates in terms of isothiocyanates have been found more in the boiling water as compared to other processing methods like microwave, steaming and stir frying. Boiling greatly affects the compounds like myrosinase and β-thioglucosidase present in Glucosinolate (GL) containing plants. Cooking and boiling brassica vegetables, on the basis of consumer preference to final texture and for various lengths of time, the GL content gets substantially decreased (Oliviero *et al.,* 2018). This drop is caused by heat degradation. Storage of cruciferous vegetables in a domestic refrigerator for 7 days also has shown the reduction in its concentration. Therefore the enhanced bioavailability of isothiocyanates can be achieved by reducing the boiling of these vegetables (Song and Thornalley, 2007). These methods actually deactivate the myrosinaise activity and thus reducing its bioavailability.

The non-conventional methods of processing like Pulse electric Field treatment conditions of 4 kV cm^{-1} for 525 and 1000 μs were optimum to increase the glucosinolate levels in broccoli stalks (ranging from 110.6 to 203.0%) and flowers (ranging from 187.1 to 212.5%) respectively (Aguiló-Aguayo *et al*., 2015) and The results for high pressure (HP) treatment in one of the studies for the Seedlings of Brussels sprouts revealed that higher preservation of glucosinolate (85% of the untreated level) and inactivation of myrosinase was found after High pressure (HP) treatment at 600MPa, 60 °C, 10min. The maximum protection of myrosinase activity compared to untreated seedlings was after High pressure (HP) treatment of 100MPa, 30°C, 3min and 10min with low degree of cell permeabilization. It has been found that cruciferous vegetables (broccoli) when cooked with other vegetables like onion help in the retainment of Glucosinolates two folds higher than samples containing brocolli alone (Giambanelli *et al.,* 2015)

Physiological activities

Biocidal

The degradation products of Glucosinolates (GSL) not only possess pungent flavour but also are responsible for preventing the plant from various infections. They are responsible to show antibacterial and antifungal activities. As such the isothiocyanates are not present in plant cells but are present in the form of Glucosinolates which are kept detached from endogenous myrosinase, but in response to mechanical wounds or any injuries they are formed as hydrolysed products in situ. The biocidal effects of cruciferous vegetables/tissues on microorganisms have been mostly recognized due to degradation of volatile products of GSLs released from their plants (Vig *et al.,* 2009).They show biocidal activity against various pathogens such as bacteria, fungi and other insects and pests (Table 1) (Brown *et al.,* 1991; Vig *et al.,* 2009) as discussed below.

Fungicidal activities

Many bioactive compounds have been observed to have anti-fungal activity (Bashir *et al*., 2015; Dhiman *et al*., 2020). Isothiocyanates too has been observed to have a potential antifungal activity. In a study conducted by Angus (1994), He observed that the Brassica species possess the ability to regulate the growth of phytopathogenic fungi i.e. they have

antifungal activity. In the same way Drobnica *et al*. (1967) studied the effect of some eleven natural ITCs for their antifungal activity and they established that these compounds are effective against the growth of *Rhizopus oryzae, Aspergillus niger* and *Penicillium cyclopium*, as well as for 13 additional parasitic and saprophytic fungi. The fungal toxins like Fumonisins have been prevented by the application of allyl, benzyl and phenyl isothiocyanate in vitro in bread. It can be concluded from their study that these ITCs can play a big role in increasing the shelf life of the bread (Azaiez *et al.,* 2013). The ITCs like β-phenyl-ethylisothiocyanate and benzyl-isothiocyanate showed remarkable antifungal activity as compared to other analogues of ITCs. The toxicity of these phytochemicals against fungus has been mostly attributed to its high volatility and the changes in the Isothiocyanate-R-group. Thus these compounds can be used as a biological agent as bio fungicide which can not only prevent the damage caused by fungi but also can prevent the environmental pollution which is caused due to the spraying of fungicides. The basic mechanism of ITCs is linked to the inhibition of oxygen uptake by fungi and constraining the coupling of ITCs with phosphorylation reactions and electron transport chain and thus inhibiting synthesis of ATP and making the fungi cells nutrition deprived (Kojima and Oawa, 1971, Vig *et al.,* 2009)

Bactericidal activities

Isothiocyanates are potential inhibitors of bacterial growth. The activity may vary from one another mainly depending on their structure. Some ITCs are more volatile than others and show more activity. The effective activity is even depending on its dosage also. The maximum the dosage of ITCs more will be effect of the bacteria (Dias *et al.,* 2014). The range of activity and toxicity also vary with the type of microbe like ammonium thiocyanate prevents the bacterial growth in soil but increases the growth of fungi at higher concentrations (Smith *et al.,* 1945) similarly seed extracts derived from *B. napus* prevented the growth of *Aphanomyces euteiches* but increased the growth of propionibacterium (Rutkowski *et al.,*1972). *B. napus* seed meal extracts inhibited the growth of *Aphanomycese uteiches,* but slightly enhanced the growth of propionibacterium. The gram negative bacteria are usually less vulnerable than gram positive bacteria to ITCs (Mennicke *et al.,* 1988).The hydrolysed products of aryl GSL and alkyl are cytotoxic to *Salmonella typhimurium* (Tiedink *et al.,* 1991). Benzyl isothiocyanate are used to treat urinary tracts and respiratory infections. Dias *et al.* (2014) studied the antibacterial activity of Isothicyanates from Cruciferous Plants and they found that these compounds have a strong effect on the growth of *Staphylococcus aureus*. They also found a strong relation between the chemical structure of ITCs and their antimicrobial potency. Benzyl-isothiocyanates was found to possess maximum activity than others. The study concludes that the ITCs are remarkable biological agents and must be considered as an important tool to be used against Methicillin-Resistant *Staphylococcus aureus*. According to the reports the ITCs inhibit various cellular enzymes of the microbe by breaking the Sulphur bridges in the enzymes and also inhibiting the ATP synthesis (Kojima and Oawa, 1971, Zsolnai, (1966).

Insecticidal activities

Isothiocyanates are considered as bio fumigants in the control of pest and other invertebrates, as they are less toxic and fully degradable. Extracts from horse radish containing isothiocyanates have been found effective against the growth of maize weevil *Sitophilus zeamais*, *Rhizopertha dominica*, *Tribolium ferrugineum* and *Liposcelis entomophila* (Wu *et al.,* 2009). In the study, it was found that there was almost 100% adult mortality rate of all the pests studied after 72 hours of the ITCs exposure. Bhushan *et al.* (2016) worked on the biocidal effect of Isothiocyanato-1-propene against *Spodoptera litura* and revealed that this ITC affected the overall life cycle of the insect from larvel stage to the developmental stage. They concluded that allyl-isothiocyanate can be an effective tool for the crop management on large scale and can control the invertebrate growth at any stage of development. ITCs especially aromatic compounds have been mostly demonstrated for Insecticidal activity. Borek *et al*. (1995) revealed that cyclic ITCs are lethal to the black vine weevil eggs (*Otiorhynchus sulcatus*). Methyl-ITCs were reported to work effectively against white fringed weevil larvae (*Naupactusleucoloma*) (Matthiessen and Shackleton, 2000). Organic thiocyanates have been utilized as insecticide in grains to control the growth of weevils and to eradicate the flying insects like flies (Beekhuis, 1975). The work mechanism of the isothiocyanates has been attributed to cause respiratory hindrances and ATP production blockage, which ultimately leads to the death of the pest (Tsao *et al.,* 2002)

Antioxidant activity

Phytochemicals like polyphenolics, flavonoids and carotenoids act as direct antioxidants due to their direct affect in the neutralization of free radicals before harming the cells (Yaqoob *et al.,* 2020). However, Isothiocyanates are believed to work indirectly because they don't cause the neutralization of the free radicals to happen directly but modulate the action of cytochrome P450 enzymes and providing a long lasting antioxidant activity However in one of the studies (Haina *et al.,* 2010), it was found that Isothiocyanate extracts from Broccoli contained 1/3 times less antioxidant activity than vitamin C and the antioxidant activity was attributed to the presence of Sulforaphane. No antioxidant activity was found for benzyl-ITCs. Phase I enzymes present in the cytochrome P450 usually rise the reactivity of lipid soluble components and as a result of which, some reactive molecules are formed which may be more toxic than parent molecule. Phase II enzymes like aldehyde reductase, glutathione-S-transferase, S-methyl transferase and N-acetyltransferase increases their water solubility and stimulate their excretion from the body. Hence inhibition of phase I and induction of phase II enzymes protect the cells from reactive oxygen species and DNA damage (Fuentes *et al.,* 2015). Thus ITCs establish a significant defence mechanism for the body against the damaging effects of these free radicals. 4-methylsulfinylbutyl isothiocyanate have been found to work efficiently for the induction of phase II enzyme. SFN has been attributed indirect antioxidant activity which would arise from induction of quinine reductase, glutathione transferases, and hemeoxygenase (Fahey and Talalay, 1999).

Food applications

Isothiocyanates have found to have applications in the food industry. They have been mostly used as food

preservatives. Antimicrobial food packaging targets to decrease, prevent or delay in flourishing of micro-organisms present on food products (Sarma *et al*., 2018). AITC from mustard seeds is used both as an antimicrobial and as a flavouring compound. AITC is accountable for the pungent taste of wasabi, horseradish and mustard and sometimes it is added to some prepared meals from vegetable to improve the flavour. This evaporative pungent compound has been utilized as a natural food preservative in Japan since the late 20th century. AITC in vapour form has been found to be more effective than in their liquid form of AITC (Shin *et al.,* 2010), but due to its powerful odour, it restricts its utilization in food systems. The utilization of AITC as a flavouring substance has been analysed by the EFSA (European Food Safety Authority) Panel on Flavourings, food additives, Processing, materials in Contact with Food (AFC) and processing Aids and by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA). By this report conclusion was drawn that from AITC intake there were no safety concerns at the evaluated intake levels. Moreover, the use of 4- hydroxybenzyl ITC have been conducted and evaluated, which is present in white mustard essential oil (WMEO), originated from white mustard seeds (*Sinapis alba* L.), and is obtained by the hydrolysis of the glucosinolate 'sinalbin' by myrosinase. WMEO was found to decrease the number of Salmonella recuperate from chicken particulates in a dose dependent manner and inoculated frozen vegetables.

Delaquis *et al.* (1995) reported that with the use of allylisothiocyanates, good control can be achieved over the microorganisms on the surface of hen eggs and goose. Allylisothiocynates has also been used with decrease in heat treatments for preservation of vegetable and fruit juice. AIT in horse radish and Mayonnaise is used as a food additive for the enhancement in flavor**.** Methyisothiocyanates are used as an additive for wine in some countries, though it forms a toxic compound with alcohol (methythiourathane). Isshiki *et al.,* 1992 have reported that shelf life of different foods which were packaged in barrier bags of plastic was enhanced, when isothiocyanates were added in the packaging material. Isothiocyanite vapors have been utilized in modified atmospheric packaging nowadays. Some of the ITCs are also reported to be extremely pungent and some are found to be bitter which restricts their use in food. Recent works recommend that they can be utilized to control the growth of microorganisms in vapor phase at very minute concentrations. Some of the studies reported that Allyl isothiocyanate extracted from Horseradish (*Armoraciarusticana*) of Brassicaceae family was incorporated into tofu, the growth of microorganisms in control was 8 log CFU/g with comparison to tofu containing allyl isothiocyante at 200 ppm showing a significant inhibitory effect upto 4 log CFU/g.

Commercial allyl isothiocyanate was checked for its activity against *E.coli* O157:H7 in fresh ground beef packaged under nitrogen and stored at chilled and frozen storage temperatures. The mesophilic bacteria were not affected due to the addition of allyl-isothiocyanate, however E. coli O157:H7 was reduced to undetectable levels after 10 days or 18 days at 4°C. Samples inoculated with 6 log CFU/g revealed >3 log CFU/g reduction at storage 4°C temperature after 21 days while a 1 log CFU/g reduction was observed at 10 and -18°C after 8 and 35 days.

Another study on allyl and other isothiocyanates was conducted on mustard flour, for the evaluation of naturally present glucosinolates which could show an effect on *E.coli* O157:H7 inoculated in ground beef at different levels of 5, 10 and 20%. At these levels 3 log CFU/g *E.coli* O157:H7 were reduced to undetectable levels within 3-18 days and an inhibitory effect at 10% and 20% on the natural microbiota present in the meat was shown. This clearly demonstrates the potential use of isothiocyanates as plant antimicrobials improve the safety and quality of food products and extend the storage life (Sultanbawa, 2011)

Table 1: Functions of various isothiocynates

Bioactive compound	Functions	References
Allyl isothiocynate	Inhibition of fungal growth on bread and inactivation of *Salmonella* on cantaloupe	(Chen *et al*., 2012; Nielsen *et al*., 2000)
Benzyl isothiocynate	Control *Alternaria* rot in bell pepper	(Troncoso *et al*., 2005)
Ethyl isothiocynate	Antifungal vapour phase activity of Ethyl Isothiocynate against *Botrytis cinerea* and *Penicillium expansum* infection on apples	(Wu *et al*., 2011)
Phenylisothiocynate	Chemical reduction of fumonisins toxicity in food products, Determination of Taurine in health food	(Azaiez *et al*., 2013; Yan *et al*., 2012)
Methylisothiocynate	Control of Cylindrocladium black rot of peanut with soil fumigants Toxicity of Rapeseed Meal and Methyl isothiocyanate to Larvae of the Black Vine Weevil	(Phipps, 1990; Borek *et al*.,1997)

Conclusion

Cruciferous vegetables are the richest sources of Isothiocyanates. These compounds provide pungency to these vegetables due to the presence of sulphur groups. These bioactive compounds possess beneficial effects. They provide these benefits both to the plants as well as animals and provide defensive roles. Due to the side effects of the synthetic chemicals, the natural phytochemicals are in demand in the food industry. Isothiocyanates possess various biocidal functions like antibacterial, antifungal, bioherbicidal, antioxidant, biopesticidal, anticarcinogenic and antimutagenic. Due to the antimicrobial activities against food borne pathogens it makes them a suitable candidate to be used as food preservative. These natural compounds are environmental friendly and can be to be used as integrated pest management instead of the chemical pesticides. But their usage needs to be more explored both for food applications and agricultural usage. Due to the potential benefit in the

agriculture and medicine, the plant breeders are developing new varieties containing higher levels of Isothiocyanates. Studies need to be done on the protective effective of ITCs for other diseases and to understand the exact mechanisms responsible for these activities

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