

Synthesis and Application of Iso Thio cyanates

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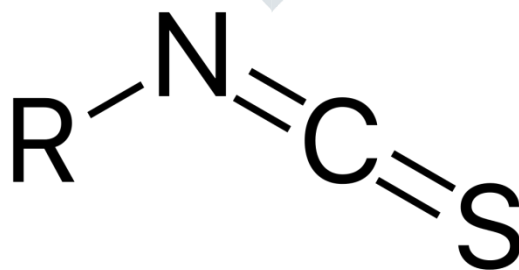
ABSTRACT

The uses of antimicrobial agents which are derived from plants have been investigated in depth. Several plant species belonging to the families Caricaceae, Capparaceae, Brassicaceae produce Isothiocyanates. They act as a defense system against pathogen attack. They are produced when glucosinolates are hydrolyzed by the enzyme of myrosinase. This article presents synthesis of Isothiocyanates and the applications of Isothiocyanates in the field of food science and medical research. The purpose of this article is the study of the potential use of Isothiocyanates. Isothiocyanates play important role on human health.

KEY WORDS

Myrosinase, glucosinolates, dithiocarbamate, allyl isothiocyanate, Cruciferous

INTRODUCTION



General Structure of Isothiocyanate

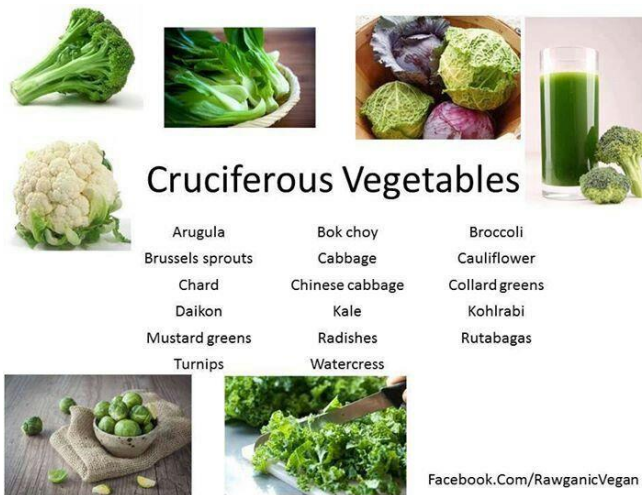
Isothiocyanates is the group-(NCS) which are linked by double bond. It is prepared when the oxygen molecule is added in the Isocyanate group along with the sulphur. Many plants

produce natural isothiocyanates which are obtained by conversion of the myrosinase enzymes (which act as catalyst) of intermediate end products of metabolism called metabolites, which are known as glucosinolates. Cruciferous vegetables for example broccoli, cabbage, cauliflower they all are the rich sources of glucosinolates which reduce the risk of cancer and heart diseases. Isothiocyanates are obtained from nature and are widely used in medical research and food science.

There are two types of Isothiocyanates; one is natural and other is artificial. The natural Isothiocyanates like allyl Isothiocyanates is called as mustard oil. Mustard oil is the transparent oil and is responsible for the pungent taste of mustard, radish. It is used in foods as flavoring agents. An artificial or synthetic Isothiocyanate like phenyl Isothiocyanate can be used for sequencing of amino acids in Edman degradation. Synthetic allyl isothiocyanate is used in agriculture as insecticides.

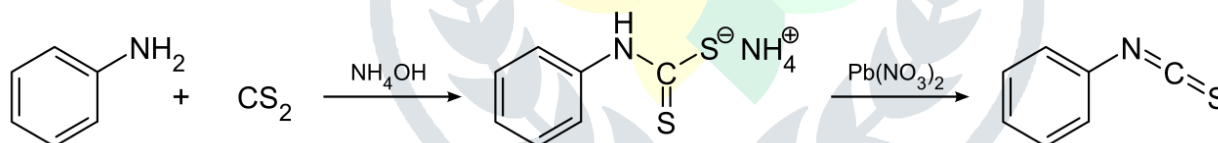
Every glucosinolates when hydrolyzed give different forms of isothiocyanate. For example, glucoraphanin is present in broccoli.

When the isothiocyanates are absorbed in the body then there is the formation of water soluble derivative which is combined with other compound mainly glutathione which consist three peptides which are glutamate, cysteine and glycine present in the liver. It is further metabolized in the mercapturic acid pathway and then it is excreted in the form of urine. The enzymic activity processed in the metabolic activity and the activity of the elimination of various cancer causing agents can be changed by the help of isothiocyanates. Isothiocyanates also exhibits in the form antioxidants and anti-inflammatory activities in the animal cells.

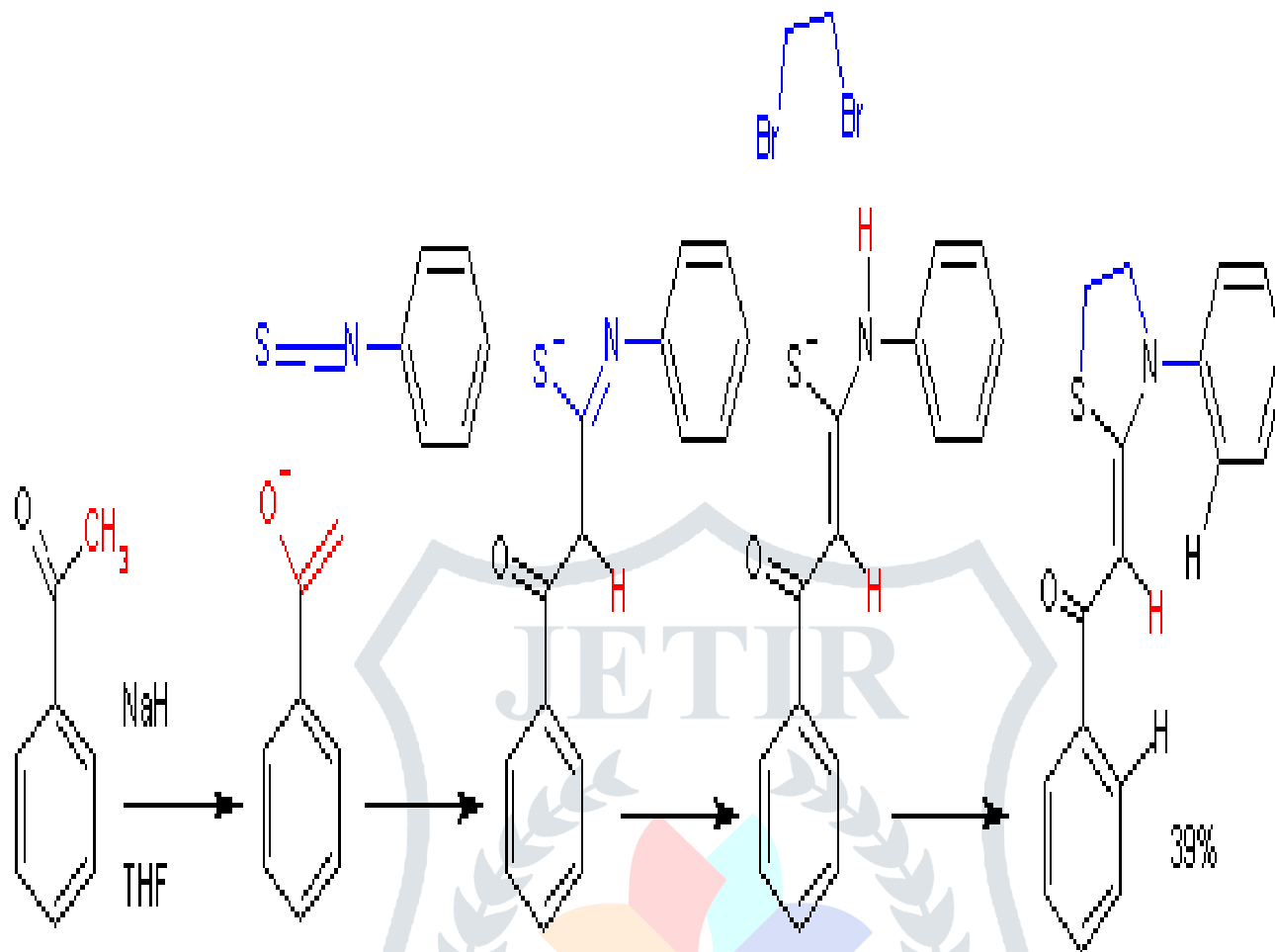


SYNTHESIS AND REACTIONS OF ISOTHIOCYANATE

The procedure for preparation of isothiocyanate involves the interaction between the aniline and carbon disulphide in the presence of hydrated ammonia which leads to formation of precipitate of the ammonium dithiocarbamate salt.¹ Then it is followed by treatment with lead nitrate to produce the isothiocyanate.



Isothiocyanates can also be prepared by thermally-induced fragmentation reactions 1,4,2-oxathiazoles. This procedure is also applicable to a polymeric synthesis of isothiocyanates.



They are electrophilic in nature and can undergo hydrolysis.

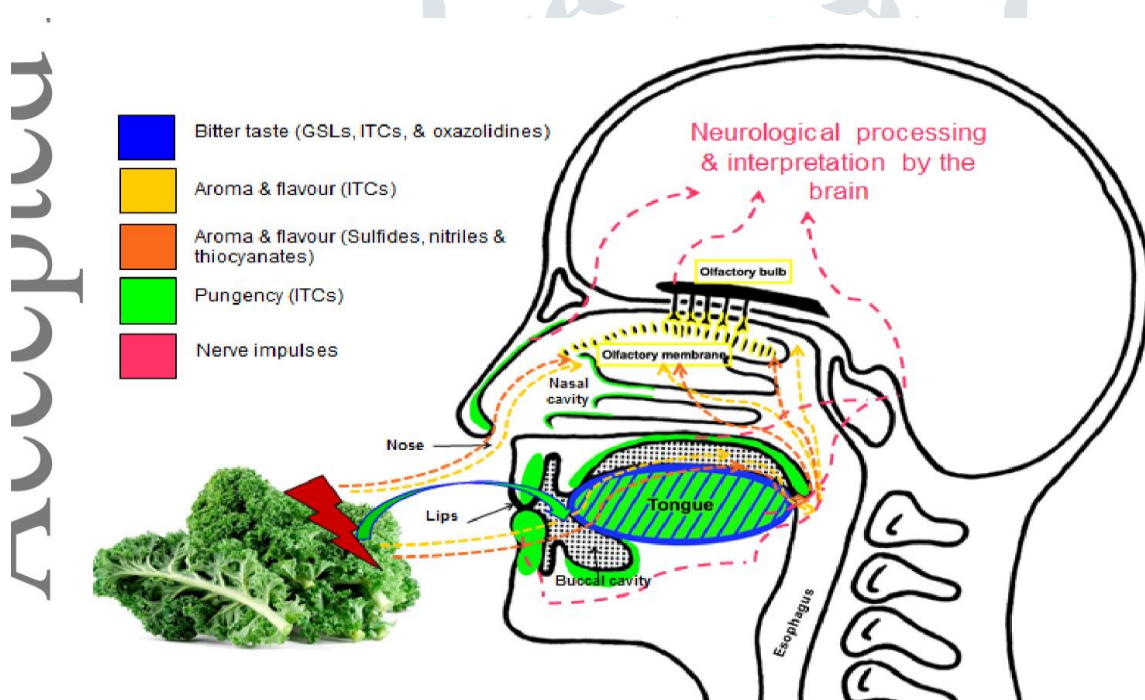
APPLICATIONS OF ISOTHIOCYANATES

1. RESEARCH IN FLAVOUR

Isothiocyanates are found in nature and is mainly used for medical research and the food science. There are some vegetables like broccoli, cabbage, cauliflower, muster, radish, papaya seeds, kale, horseradish, have characteristic flavors due to presence of isothiocyanates. They generate isothiocyanates in different proportions which is responsible for different flavours. They all belong to members of Brassicales and are produced by help of glucosinolates and myrosinase enzymes and this act upon glucosinolates which further produce isothiocyanates.

- The precursor to allyl isothiocyanate is Sinigrin.
- The precursor to benzyl isothiocyanate is Glucotropaeolin.
- The precursor to phenethyl isothiocyanate is Gluconasturtiin.²
- The precursor to sulforaphane is Glucoraphanin.

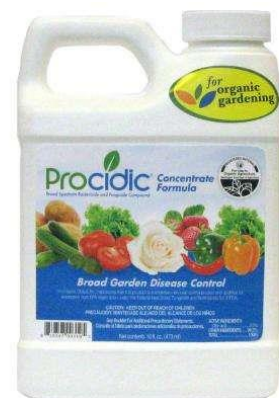
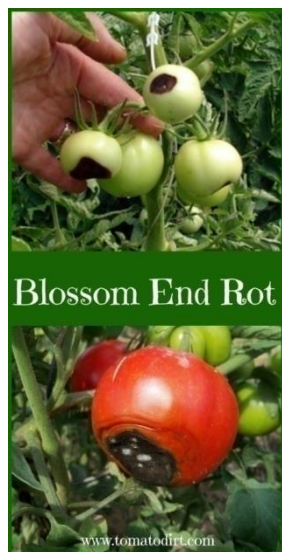
The compounds in which glucosinolates is present gives bitter taste characteristics. When glucosinolate compounds are hydrolyzed by the myrosinase enzymes present in plants, they give many bioactive compounds and a pungent aroma. The key odorant in cooked cauliflower was found to be allyl isothiocyanate.



TASTE AND FLAVOR PERCEPTIONS OF GLUCOSINOLATES, ISOTHIOCYANATES AND RELATED COMPOUNDS

2. ALTERNARIA ALTERNATA (BLACK ROT, BLACK SPOT)

Isothiocyanates are produced by plants acts as a system of defense against pathogen attack. The pathogen attack arises because of the hydrolysis of glucosinolates. Isothiocyanates have biological effects like antifungal activity. There have been studies about the postharvest of the isothiocyanates on control of fungal diseases in fruits and vegetables and the control of diseases caused by the fungus by the help of isothiocyanates. To control the black rot in tomato, benzyl isothiocyanate was used and there were further studies made of the effect of this compound on postharvest physiology and quality. Captan, which is a commercial fungicide, was used as a control.³



A similar study was made in bell peppers. A mixture of isothiocyanates in the presence of base to the relative amounts of isothiocyanates was detected in cabbage leaves were used to control alternate infection of bell pepper fruits.



3. CONTROL OF SOIL BORNE DISEASE

The soil borne diseases in agriculture systems can be reduced by methyl isothiocyanates. There was investigation made for the use of glucosinolates which is the precursor to isothiocyanate as one of the alternative processes. The glucosinolates and their breakdown products played a vital role in selecting the natural rhizosphere community. There is diffusion of the glucosinolates and their products of hydrolysis from the roots into the rhizosphere which were degraded by extracellular myrosinase. The modification in the content of glucosinolate of the plant is another way for the use of pesticides.



ITC spray application

Incorporation in soil

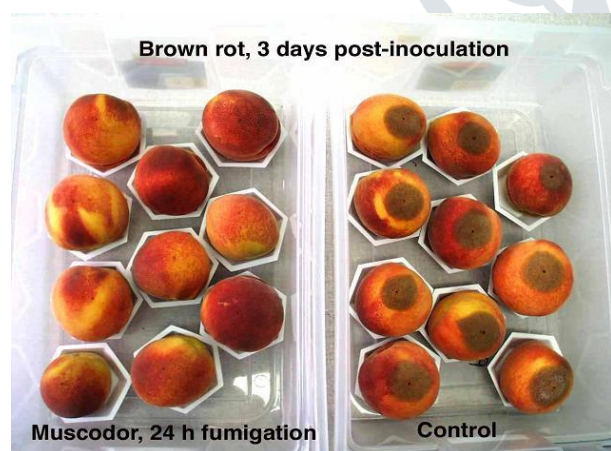


Bed formation and plastic laying

Transplanting after 3 weeks

4. USE OF NATURE OBTAINED ANTIMICROBIALS TO INCREASE THE SAFETY AND QUALITY OF FRESH FRUITS AND VEGETABLES

Isothiocyanates prevents the food from spoilage from several fungi, pathogenic and food spoilage bacteria like Bacillus, Salmonella etc. gram-negative bacteria reacts with these isothiocyanates more easily than gram-positive bacteria.⁴ The antimicrobial action isothiocyanates have mechanism which can be related with the interaction of the compounds with the enzymes which contain sulfhydryl which plays a vital role in cell and damage cellular structures. Isothiocyanates have volatile nature and this property can be used in prevention of vegetables and fruits. Isothiocyanates were applied as vapours in storage operations or in modified atmosphere packaging. The artificially inoculated peaches developed fungal infection called brown rot which could be easily treated and reduced by vapors of natural allyl and butyl isothiocyanates. To control the fungal infection in apples, allyl isothiocyanate and ethyl isothiocyanate were applied singly or in combination as vapors.



5. INHIBITION OF ENZYMIC OR REGULATORY ACTIVITIES

The functions of thioredoxin reductase and acetate kinase in body of living organism were affected by allyl isothiocyanates which inhibits specific enzymic activity.⁵ Sulforaphane inhibits urease activity in a gastric pathogen called *Helicobacter Pylori* and it produces ammonia which further neutralizes gastric acidity. A member of sulfur containing compound has been found as a new class of QS inhibitors. For example, ajoene from garlic extract with disulfide and sulfinyl groups. Isothiocyanates have been discovered which shows Quorum Sensing (QS) activity which is the ability to respond to cell population density by regulation of genes.



6. SUSCEPTIBILITY OF FLUOROCHROMES TO HYDROLYSIS

Fluorochrome is a chemical compound which is fluorescent in nature. Fluorochrome on excitation can re-emit light.⁶ Fluorochromes contain aromatic groups or cyclic molecules with pi-bonds. Fluorochromes are bonded to macromolecules covalently which serves as a marker for affine or bioactive reagents like antibodies, peptides, nucleic acids. The isothiocyanate of Fluorochromes are likely to be hydrolyzed, even by the moisture present in the air. They must be protected from light and must be stored in sealed containers. If it is stored in cold conditions, before the bottle is opened, they must be kept at temperature so that there is no moisture. It is necessary that they must not be kept wet. During the preparation of Fluorochromes, it must have necessary amount of hydrolyzed material to attain the required conjugation ratio. There is a competing hydrolysis reaction during the conjugation of Fluorochromes to antibodies. At very high concentration of protein, conjugation to protein is highly favored, and high efficiency of coupling can be attained.

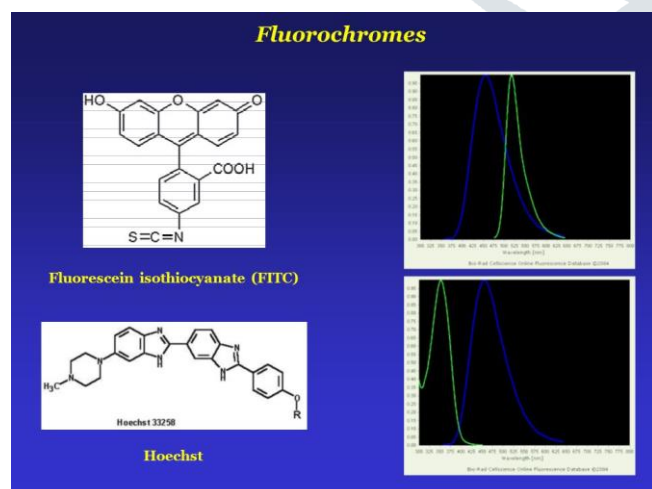
At very lower concentration of protein, the hydrolysis becomes very significant. It is easy to conjugate antibodies at protein concentration. It is important to add more Fluorochrome and the precaution must be taken that all untreated Fluorochrome is removed.

fluorochrome
e.g. FITC

detection
antibody

antigen

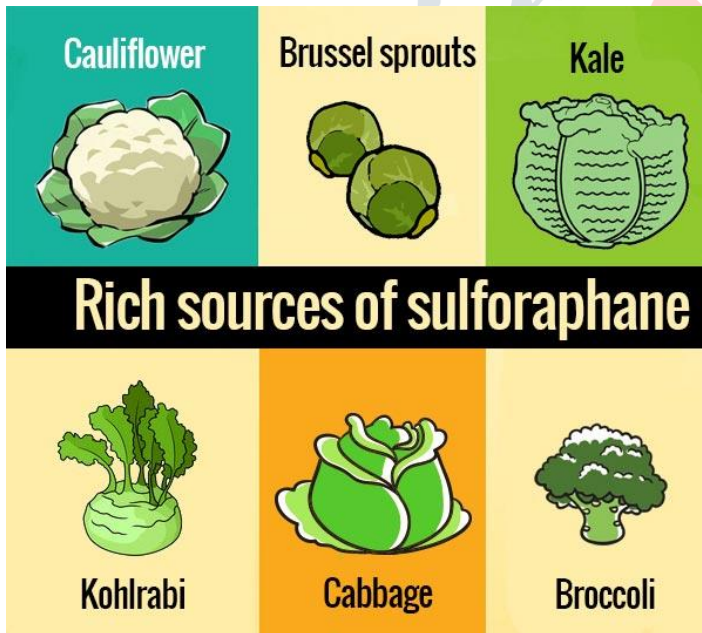
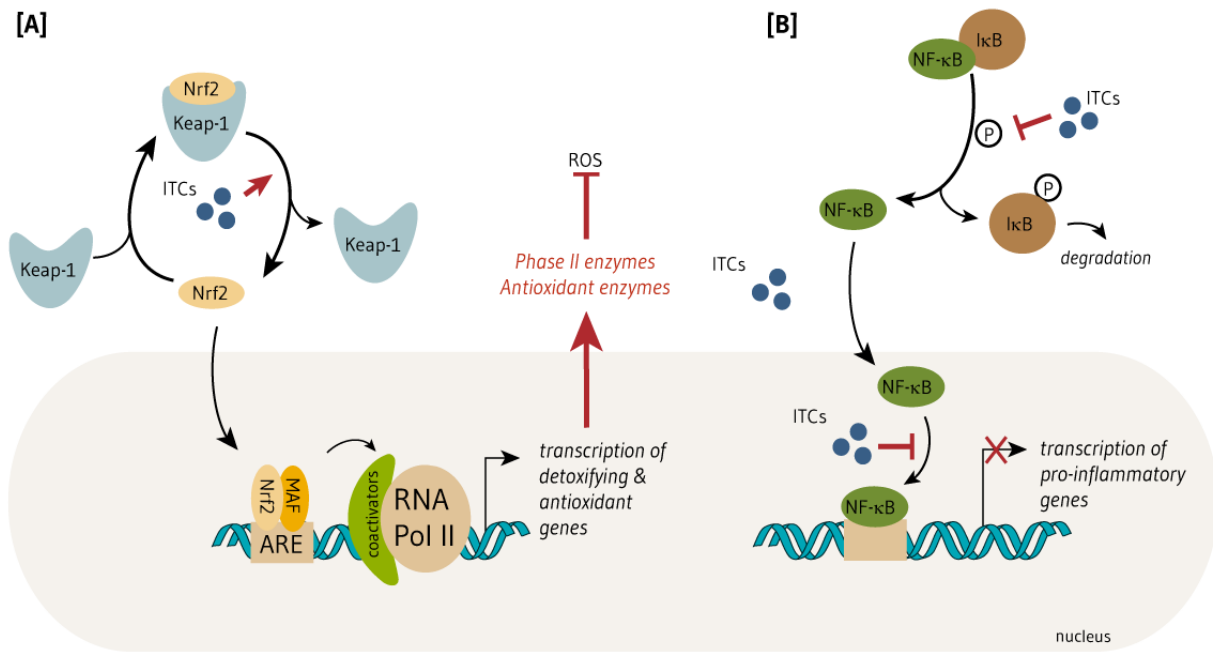
They
air-tight
then
room



7. PREVENTION FROM CANCER

Cruciferous vegetables contain isothiocyanates which on consumption has an essential role in prevention of cancer and cardiovascular benefits. The difference in the sequence of coding of gene for Glutathione S-transferases contains a family of eukaryotic and prokaryotic phase II metabolic isozymes may affect the activity of GSTs. These variations have been found in humans. Individuals having two copies of GSTM1-null or GSTT1-null alleles (alternative forms of a gene that arise by mutation) are unable to produce GST enzymes. If the GST activity in the individuals is reduced then it could slow the rate of excretion of isothiocyanates. This could increase the tissue exposure towards isothiocyanates when cruciferous vegetables are consumed. GSTs detoxify harmful substances from body like carcinogens. If the GST activity is reduced there are chances of cancer. The isothiocyanates help in the activity of GSTs which act as protective mechanism against oxidative stress and damage that is caused by cancer and cardiovascular disease. The oxidative stress can be reduced by the help of Sulforaphane and it is connected to activation of the nuclear factor E2-related factor 2 (Nrf2) dependent pathways.⁷ Naturally occurring isothiocyanates plays a crucial role in the prevention from liver, lung, stomach, small intestine cancer.

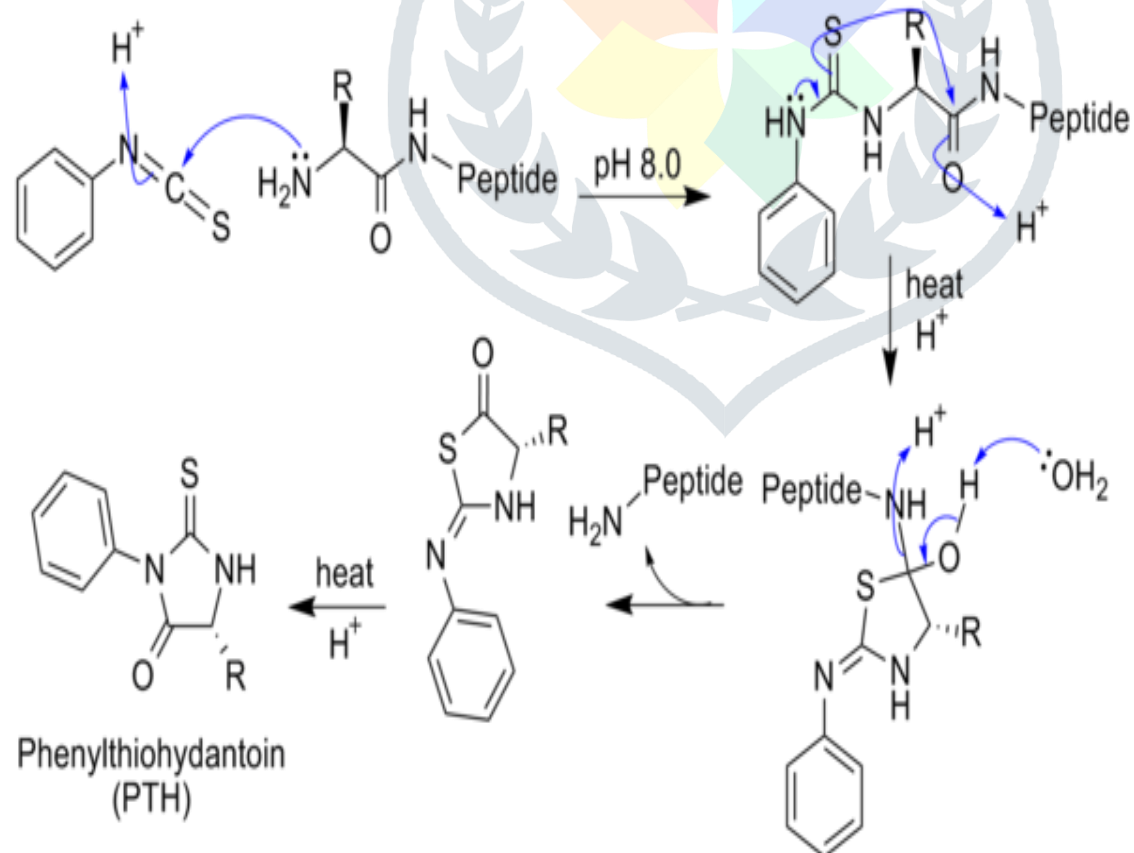
Figure 3. Isothiocyanates Target Nrf2 and NF-κB Pathways



8. USE OF PHENYL ISOTHIOCYANATE IN EDMAN DEGRADATION

The method in which there is sequencing of peptides with amino acids is known as Edman Degradation. There is labeling of left over amino-terminal and there is cleavage from the peptide without disturbing the peptide bonds between other left over amino acid.

N-terminal amino group which is not charged is reacted with phenyl isothiocyanate, keeping the conditions mildly alkaline so that it forms a derivative of cyclical phenylthiocarbamoyl. There is cleavage of this derivative of the terminal amino as a thiazolinone derivative under the acidic conditions. Then in an organic solvent, the thiazolinone amino acid is selectively treated. Then it is reacted with acid to form the stable phenylthiohydantoin (PTH)-amino acid derivative. Chromatography or electrophoresis is the method for its identification. On repeating this procedure again, we can detect the next amino acid.



Role of Aminoacids

CONCLUSION

The past studies have shown that isothiocyanates are chemo preventive. According to the evidence from the studies conducted in cell culture, animal models have shown important role of isothiocyanates in diet which protect from several cancers. The isothiocyanates have the ability to prevent the formation of tumors and its target to multiple pathways which include oxidative stress, apoptosis is a kind of program associated with lifeless cells generally in multiple cell organisms, cell cycle machinery, and MAPK pathway. This depends on many factors like isothiocyanates structures, the species of the animals, special tissues and the particular cancer causing substances used. It also has a quality of anti cancerous activity. The future studies should target upon

- Novel clinical trials
- Molecular target screening
- In vivo animal experiments
- In-depth mechanistic studies
- Novel compound designing based on isothiocyanate.

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