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# Effect of Antioxidant Compounds on Nitrites as Inhibitors of N-Nitrosamine Formation: A Short Review

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## ABSTRACT

N-nitrosamines are carcinogenic compounds that are very easy to find. N-nitrosamines are formed by the reaction between nitrogen oxides and secondary amines in food or the body. Inhibition of the formation of N-nitrosamines can be done by controlling the reaction on nitrites or nitrite-free radicals. One compound that can inhibit the formation of nitrite-free radicals is an antioxidant compound. This article aims to discuss the effect of antioxidant compounds on nitrites as inhibitors of N-nitrosamine formation. Writing articles is written using a deductive approach by collecting literature through Google Scholar and ScienceDirect with the keyword's antioxidant compounds and N-nitrosamines. A total of 57 articles were collected from the literature and only 17 relevant articles can be used. The search results of the article show that natural antioxidant compounds in the form of phenolic compounds, vitamin C, vitamin E, and vitamin A can capture nitrite-free radicals, accelerate the oxidation of nitrite into nitric oxide, and inhibit the activation of enzymes that form N-nitrosamines. Enzymatic antioxidant compounds in the form of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) function as DNA protectors from carcinogenic N-nitrosamine compounds by capturing oxygen radicals and hydroxyl radicals so that DNA strand termination by oxygen radicals can be inhibited.



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## 1. INTRODUCTION

N-nitrosamines are compounds that are classified as carcinogenic compounds by the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (US EPA). N-nitrosamines are very easy to find in water, air, food, beverages, cosmetics, tobacco, and packaging materials. N-nitrosamines are formed by the reaction between nitrogen oxides and secondary amines but are also produced during fermentation (Park *et al.,* 2015). N-nitrosamines are generally stable, especially in alkaline conditions. N-nitrosamines are not carcinogens that act directly but require metabolic activation through oxidative reactions to produce diazo alkylating compounds.

N-nitrosamines can be formed from the presence of excess nitrites. Nitrite or nitrate is a compound that is often used as a preservative in meat or processed products. In addition to maintaining the red color of the meat through the myoglobin fixation mechanism, the addition of these compounds also functions as an antioxidant (Ebulue, 2023). Excessive use of nitrites can produce residues that can react with secondary amines to produce N-nitrosamines (Kosim *et al.*, 2016).

In brewing through the fermentation process, microorganisms can reduce nitrates to nitrites, degrade proteins into amino acids, and create a slightly acidic environment. Decarboxylation of amino acids by microbial enzymes can produce precursors of cyclic N-nitrosamines. In addition, N-nitrosamines were also detected in water as a by-product of disinfection. The reaction between dimethylamine, a contaminant from wastewater, and monochlor amine as a substitute for chlorine, can form a derivative of the N-nitrosamine compound, namely N-nitroso dimethylamine (Park *et al.,* 2015).

The following is the reaction for the formation of N-nitrosamines:

$NO_2^- + H^+ \rightarrow HNO_2$

$HNO_2 + H^+ \rightarrow H_2NO_2^+$

$H_2NO_2^+ + NO_2^- \rightarrow N_2O_3 + H_2O$

$N2O_3^+$ (R) 2NH → (R) 2NN=O $+NO_2$

Antioxidants are compounds that can absorb or neutralize free radicals so that they can prevent degenerative diseases, such as carcinogenesis, cardiovascular and other diseases (Vijayarani *et al.,* 2023; Laroza *et al.,* 2021). Based on the source, antioxidant compounds are classified into three: antioxidants that have been produced in the body or known as antioxidant enzymes, synthetic antioxidants, and natural antioxidants obtained from plants. Antioxidant compounds can reduce and inhibit the formation of N-nitrosamine precursors in different ways and under other conditions. The purpose of writing this article is to discuss the effect of adding natural and enzymatic antioxidant compounds to nitrites as inhibitors of N-nitrosamine formation.

## 2. METHODS

Writing articles is written using a deductive approach, namely providing an overview of antioxidant compounds, then directed to more specific matters regarding the effect of these types of antioxidant compounds as inhibitors of the formation of N-nitrosamines by collecting literature through Google Scholar and ScienceDirect using the keywords antioxidant compounds and N. -nitrosamine. The total literature collected is 57 articles and only 17 relevant articles that can be used and discussed in this article.

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## 3. RESULTS AND DISCUSSION

### 3.1. Inhibition of N-Nitrosamines by Natural Antioxidant Compounds

Natural antioxidant compounds are obtained from plant parts such as wood, bark, roots, leaves, fruit, seeds, and even flower powder. Natural antioxidant compounds that are often found are phenolic compounds, vitamin A, vitamin C, and vitamin E. Antioxidant compounds can absorb free radical compounds that are damaging to the body. Free radicals are compounds that have reactive oxygen atoms or better known as Reactive Oxygen Species (ROS). One example of a free radical compound is nitric oxide (NO•). Nitric oxide is the result of the homolytic breakdown of covalent bonds in a molecule or lone pairs of electrons in an atom. Free radicals can cause inflammation or inflammation in the body. The function of antioxidant compounds is to prevent damage caused by free radicals to normal cells, proteins, and fats.

#### 3.1.1. Effect of phenolic compounds

Phenolic compounds are natural antioxidant compounds in the form of active compounds found in plants. Generally, natural antioxidant compounds are in the form of phenolic or polyphenolic compounds derived from the flavonoid group, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids (Simanjuntak, 2011).

Many studies have been carried out regarding the effect of phenolic compounds on the formation of N-nitrosamines. Zhou *et al*. (2020) investigated the effect of rosemary extract, grape seed extract (GSE), and green tea polyphenols (GTP) on the formation of N-nitrosamines and the quality of western-style smoked sausage during the curing process. The results obtained were that these three components could reduce nitrite residue and N-nitrosamine content in smoked sausages. Rosemary extract, GTP, and GSE are rich in phenolic compounds, especially in GSE. The increase in extract concentration is proportional to the increase in acids and phenols which will react with proteins and cause water retention. With a continuous increase in concentration, the content of residual nitrites decreased significantly. Likewise, the detected N-nitrosamine content decreased. One type of N-nitrosamine group, namely Nitrosodimethylamine (NDMA) can be inhibited due to an increase in extract concentration which will also increase the reaction between phenolic compounds in plant extracts, especially polyphenols, and flavonoids with amino acids in proteins, causing nitrite disintegration. The bond that occurs between phenol and amino acids causes the tendency of nitrite residues to bind to amino acids to decrease so that they cannot form N-nitrosamine compounds (Zhou *et al.,* 2020).

Suryanto et al. also examined the antioxidant activity of free phenolic and bound phenolic extracted from nutmeg shell flour using ultrasonic waves with ethanol (ET), distilled water (AQ), and untreated (TP). The use of ultrasonic waves causes the process of transferring bioactive compounds from the plant to the solvent to be faster. Phenolic compounds have the potential as antioxidants because of the presence of hydroxyl groups. The hydroxyl group functions as a hydrogen atom donor when it reacts with radical compounds through an electron transfer process so that the oxidation process can be inhibited. The higher the total phenolic content, the higher the antioxidant activity. Free phenolic antioxidant activity is quite high in counteracting free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azinobis-3-ethyl-benzothazoline-6-sulfonic acid (ABTS), while bound phenolic activity shows a low value. to counteract nitrite-free radicals (NO•). This is due to the acidic and alkaline conditions in the bound phenolic which can help the process of binding phenolic compounds with nitrite-free radicals so that the extract can reduce and donate electrons. With the binding

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of phenol to nitrite-free radicals, there will certainly be a decrease or inhibition of the formation of N-nitrosamine compounds (Suling *et al*., 2022).

### 3.1.2. Effect of vitamin C

Vitamin C or also known as ascorbic acid is needed by the human body and animals. Vitamin C is a strong reducing agent, which when oxidized becomes dehydroascorbic acid. Vitamin C easily reduces nitric acid (HNO2) to nitric oxide (NO) (Shader, 2017; Kamilah & Nandiyanto, 2023).

The content of vitamin C in plants such as fruit is widely used as an ingredient to inhibit the formation of N-nitrosamines. As was done by Haradito *et al.,* (2014) used lime fruit extracts (Ermawati & Utami, 2014) and red guava (Haradito *et al.,* 2021) as additives to reduce nitrite residue during the meat curing process (Ayu & Nandiyanto, 2023). Similar to the phenolic compounds, increasing the concentration of ascorbic acid from lime and red guava extracts gave significant results in reducing the amount of nitrite residue during the curing process. Vitamin C in fruit will accelerate the formation of nitrites into nitric oxide. Nitric acid will experience decomposition at an acidic pH and vitamin C will provide its electrons so that the nitrite is reduced to nitric oxide. In addition, the presence of vitamin C which is an antioxidant can inhibit the reaction between nitric oxide and oxygen which can reform into nitrites.

In addition to food, vitamin C affects the human body, such as acting as an antidote to free radicals on the skin as Pakaya (2014) said. Vitamin C as an antioxidant will provide electrons to neutralize reactive free radicals. Vitamin C plays an important role in connective tissue metabolism and other functions. Vitamin C can prevent the conversion of secondary nitrite and amines into N-nitrosamines, which are carcinogens. skin cancer problem (Pakaya, 2014; Rana, 2022).

Astija (2021) said that the content of vitamin C in food, especially fruit, can be affected by its level of ripeness. The more developed a fruit, the more its vitamin C content will be more and more. However, if the fruit is too ripe, the vitamin C content will decrease due to the oxidation of ascorbic acid to dehydroascorbic acid and will undergo further changes to diketogluconic acid. Oxidation of vitamin C Enzyme activity in plants causes vitamin C to be oxidized resulting in a decrease in vitamin C levels in ripe fruit. For this reason, the consumption of good fruit needs to be seen from the level of ripeness so that more and more vitamin C is consumed (Astija, 2021).

### 3.1.3. Effects of vitamin E

α-Tocopherol is the main component of vitamin E. α-Tocopherol is a highly lipophilic phenol, easily oxidized to α-tocoquinone, and functions in cells as a lipid antioxidant, scavenging free radicals, and lipid peroxide. α-Tocopherol also reduces NO2 to NO in organic solvents and lipids, and its emulsions in water reduce nitrite to NO. However, this reaction occurs when α-tocopherol is free, not its acetate (vitamin form) so inhibition of intragastric nitrosation requires free α-tocopherol. α-Tocopherol is a strong inhibitor of the formation of nitrosamides from N-butyl acetamide and NO2 or its dimers (N2O4), such as the food additive butylated hydroxytoluene (BHT) (Maulana *et al.,* 2021). The α-tocopherol phenol ring is fully substituted, making it a good nitrosation inhibitor (Shader, 2017).

The roles of vitamin E and vitamin C as antioxidants are never separated and are always related. The combination of these two vitamins makes the function of antioxidant compounds more effective, especially as an antidote to free radicals in cells. Chandra *et al.,* (2012) recommended the use of vitamin E as an antioxidant in oral lesions. Vitamin E can inhibit the reaction of specific N-nitrosamines in tobacco which are carcinogenic with specific activation

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and detoxification processes. The use of other antioxidants such as vitamin C, provitamin A, zinc, spirulina, and β-carotene is also believed to have a preventive role against oral cancer (Chandra *et al.,* 2012).

### 3.1.4. Effect of vitamin A

Vitamin A comes from provitamin A in the form of β-carotene and carotenoids, which become active compounds in the form of retinol ester derivatives, namely retinol and retinoic acid. A deficiency of vitamin A can increase susceptibility to the formation of carcinogenic compounds (Holmes, 1985). Vitamin A can reduce NNK genotoxicity by inhibiting the activation of enzymes for nitrosamines. NNK can induce DNA single-strand breaks in hepatocyte cells and metabolites formed through α-hydroxylation that mediate genetic toxicity. The activity of the P450 monooxygenase enzyme is inhibited due to vitamin A which can interfere with the oxidation process during NNK activation so that NNK metabolism decreases. For example, in rats that were given additional vitamin A, it significantly prevented the induction of SCEs and SSBs DNA in hepatocyte cells so that the NNK metabolic process was inhibited and decreased. NNK or 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone is one of the N-nitrosamine derivatives contained in tobacco. Smoking tobacco has been shown to increase hepatocellular carcinoma or liver cancer which occurs due to abnormal growth of hepatocyte cells (Alaoui-jamali *et al.,* 1991).

### 3.2. Inhibition of N-nitrosamines by enzymatic antioxidant compounds

Enzymatic antioxidant compounds or called endogenous antioxidants have been produced in the human body, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Enzymatic antioxidants function as free radical scavengers and stop the formation of free radicals.

Hepatocyte cells are very active in the metabolism of xenobiotics and drugs. Xenobiotic detoxification generates reactive oxygen and oxidative stress. Exposure to diethylnitrosamine (DEN) increases reactive oxygen metabolism which can cause DNA damage and cause cell death. Increased DEN can reduce the activity of SOD and other antioxidant enzymes. SOD is the body's first line of defense against superoxide radicals and is considered the most effective antioxidant. GPx is a second line of defense against hydroperoxides by catalyzing the reduction of organic hydroperoxides with glutathione catalysts. The decrease in enzyme activity by DEN may be due to the direct interaction of enzyme molecules and modifications to the transcription or translation stages in enzyme synthesis (Shaban *et al.,* 2014).

SOD is a metalloenzyme that catalyzes the reduction reaction of superoxide anion radicals (O2•) to hydrogen peroxide (H2O2) and oxygen (O2), while catalase is an enzyme composed of more than 500 amino acids that can catalyze the reduction reaction of hydrogen compounds peroxide (H2O2) to oxygen (O2) and water (H2O). SOD plays a major role in reducing the effects of nicotine use which is closely related to chronic inflammation. In addition to nicotine, tobacco contains the compounds Tobacco-specific nitrosamines (TSNA), nitrosonornicotine (NNN), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) which can produce superoxide anions due to induction by enzymes. xanthine oxidase. The xanthine oxidase enzyme can degrade purines by catalyzing hypoxanthine into xanthine and uric acid. Nitrosamines in tobacco have been shown to damage DNA, by observing an increase in the production of superoxide anions that is proportional to the increase in oxygen radicals (Effiong & Aya, 2022). The presence of SOD, catalase, and mannitol can protect target DNA (lungs) effectively from genotoxic oxygen radicals by canceling the DNA strand-breaking

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process by oxygen radicals through the capture of oxygen radicals and hydroxyl radicals (Weitberg & Corvese, 1993).

SOD can also inhibit the oxidation process of 1,1-diphenyl hydrazine to N-nitroso diphenylamine through an active oxygen capture process. Superoxide radicals greatly influence the oxidation process of hydrazine derivatives to nitrosamines in ocular tissue microsomes exposed to visible light in the presence of photosensitizers such as riboflavin, flavin adenine dinucleotide, flavin mononucleotide, lumiflavin, lumichrome, NAD+, NADH, NADP+, or NADPH (Nikaido *et al.,* 1992).

## 4. CONCLUSION

Antioxidant compounds are compounds that can absorb or neutralize free radicals. Natural antioxidant compounds consist of phenolic compounds, vitamin C, vitamin E, and vitamin A, while enzymatic antioxidant compounds consist of SOD, GPx, and catalase. Antioxidant compounds can function as inhibitors of the formation of carcinogenic N-nitrosamine compounds through various reactions. Natural antioxidant compounds can inhibit the formation of N-nitrosamines by capturing nitrite-free radicals, accelerating the oxidation of nitrites into nitric oxide, and inhibiting the activation of enzymes forming N-nitrosamines. Enzymatic antioxidant compounds function as DNA protectors from carcinogenic N-nitrosamines by capturing oxygen radicals and hydroxyl radicals so that the breaking of DNA strands by oxygen radicals can be inhibited.

## 6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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