Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi
Iğdır University Journal of the Institute of Science and Technology

Gıda Katkı Maddesi Olan Sodyum Sülfitin *Tubifex tubifex*'te Katalaz ve Glutatyon–S-Transferaz Enzim Aktivitelerine Etkileri

Filiz ALANYALI[1]

ÖZET: Sodyum sülfit gıda ürünlerinin taze görünümünü korumak için gıda endüstrisi tarafından kullanılır. Bu çalışmada, sodyum sulfit sublethal dozunun suda yaşayan oligochaeta grubundan *Tubifex tubifex* üzerinde etkileri araştırılmış, Katalaz ve Glutatyon–S-Transferaz enzim aktivite değişiklikleri değerlendirilmiştir. Antioksidan enzim aktivite değişiklikleri, *Tubifex tubifex'te* farklı sodyum sülfit konsantrasyonlarında (2.5mM, 5mM ve 10mM) 2, 4 ve 6 saat maruz bırakılmasının bir sonucu olarak araştırılmıştır. Katalaz ve Glutatyon–S-Transferaz aktivitesinin, veriler kontrol gruplarıyla karşılaştırıldığında zamana bağlı arttığı gözlenmiştir.

Anahtar kelimeler: Katalaz, glutatyon–S-transferaz, sodium sülfit, *Tubifex tubifex*

Effects of Sodium Sulfite as a Food Additive on Catalase and Glutathione-S-Transferase Enzyme Activities in *Tubifex tubifex*

ABSTRACT: Sodium sulfite is used to keep the food product with fresh appaerence by food industry. In this study, the effects of sodium sulfite sublethal dose on aquatic oligochaeta *Tubifex tubifex* were investigated and enzyme activity changes of Catalase and Glutathione-S-Transferase were evaluated. The antioxidant enzyme activity changes were searched as a result of 2, 4 and 6 hour exposure within various concentrations (2.5mM, 5mM and 10mM) of sodium sulfite in *Tubifex tubifex*. Catalase and Glutathione-S-Transferase activity increased depending on the time when data were compared with the control groups.

Keywords: Catalase, glutathione-S-transferase, sodium sulfite, *Tubifex tubifex*

Cilt/*Volume*: 7, Sayı/*Issue*: 2, Sayfa/*pp*: 83-88, 2017
ISSN: 2146-0574, e-ISSN: 2536-4618 DOI: 10.21597/jist.2017.133

[1] Anadolu Üniversitesi, Fen Fakültesi, Biyoloji, Eskişehir, Türkiye
Sorumlu yazar/Corresponding Author: Filiz ALANYALI, fsusuz@anadolu.edu.tr

Geliş tarihi / *Received:* 01.12.2016
Kabul tarihi / *Accepted:* 06.02.2017

INTRODUCTION

Food additives are not included in the natural structure of the food according to WHO/FAO and CAC description. They are added into food during various operations such as production, processing, packaging, transportation (Altuğ T, 2001). The use of food additives is not a new phenomenon. People use food additives for centuries; for example, it is known that salt was used to store meat products in the year B.C.3000. Salt, sugar and vinegar were the first food additive materials used to store a long time (Bradford, 1976; Altuğ, 2001).

Because of the number of people entering the working life and the reduction in raw materials supplying the food industry; the production and consumption of fast food has become obligatory (Claiborne, 1985). Therefore, food additives used in rapidly evolving food technology are very important for public catering. The last decades, particularly in the developed countries, the number of chemicals involved in food has increased rapidly. There are around six thousand additives including aroma / flavor additives. These substance consumptions increase results for some diseases (Çalışır ve Çalışkan, 2003).

The usage of food additives in the food industry was originated from the different production techniques brought by the evolving technology and gain diversification of consumer tastes accordingly. These all works aim the most economical way to diet as well as the healthiest way for consumers; it should also have the technology to prevent risks arising from food additives used. (Claiborne, 1985; Çalışır ve Çalışkan, 2003)

Paracelsus described toxicity ‘ All substances are poisons; there is no non- toxic substances. The dose toxicity and usefulness will determine the status of a substance as defined poison’ in the 16th century. This dose of harmlessness is determined with current toxicological studies for food additives (Atman ÜC, 2004). Food additives are used as long as they are effective, and can be analyzed with measurable results and finally have to be safe for animals (Claiborne, 1985). Food additives; increasing doses of the substances should be tested whether they accumulate in the body. If they accumulate, the resulting damage is reversible or not reversible should be searched. Toxicological assessments have to be applied and all ingredients have to be kept under continuous control for additives. Usage conditions can be update according to the results of the new scientific findings (Altuğ, 2001).

E “numbers are used as a practical method of encoding of food additives in the European Union (Bradford, 1976). This code is set by “the Scientific Committee on Food” which is a subcommittee of the European Union. There are food additives over eight thousand, only 350-400 of these have “E” number (Claiborne, 1985).

Sodium Sulfite has crystal or powder, white appearance and reducing property with the chemical formula Na_2SO_3. Sodium sulfite shows bleaching, de-sulfurizing, and dechlorinating activities. It is used to keep the food product with fresh appearance by food industry (Efsa, 2010). Sulfites, E 221, are widely used as preservatives and antioxidants in food and drugs, often without specification. For this purpose, this is used for control of microorganisms, inhibition of the activity of microorganisms and elimination of their harm, suppression of various enzymatic reactions, and non-enzymatic browning including enzymatic browning, and also as the reducing agent in pulp bleaching, pH control and stabilizers (Boğa ve Binokay, 2010). Sulphites are used as food additive but their usage is, nowadays, an issue of concern, since certain sensitive individuals showed adverse reactions to sulphite residues in foods (Taylor and at all, 1986). Sulfite can lead to severe hypersensitivity reactions, asthma being obviously the most frequent symptom, but also urticarial, angioedema, or other anaphylactoid symptoms may occur. Furthermore, allergic leukocytoclastic vasculitis and exacerbation of an atopic eczema have been observed (Günes, 2014).

Catalases and Glutathione S-transferases are antioxidant system enzymes. These enzymes are very important in protecting the organ and body against toxic agents (Fazio and Warner, 1990).

Catalases (CAT; E.C.1.11.1.6) are protective enzymes responsible for the degradation of H_2O_2 before it can damage cellular components. They are present in all aerobic organisms and many anaerobic organisms. The catalytic reaction takes place in two steps resulting in the resting state enzyme, water and oxygen (Przybilla

and Ring, 1987). The glutathione-S-transferases (GSTs; E.C.2.5.1.18.9) are an enzyme group, function in the detoxification mechanism. All examined living organisms including plants, animals, and bacteria have these proteins. Most GST is soluble enzymes, and there are also verified microsomal GST, and mitochondrial GST (Eaton and Bammler, 1999). Many different kind of chemicals and carcinogens are detoxified by GST (Switala and Loewen, 2002).

In this study, the effects of sodium sulfite on Catalase and Glutathione-S-transferase enzyme activities of *Tubifex tubifex* (Muller, 1774) (Oligochaeta: Tubificidae), an important species in aquatic food chains, were investigated. This experimental animal is appropriate since it is easy to find, to expose the food additive and assay the enzyme activity. Tubificid worm, *T. tubifex*, Tubifex tubifex is a fresh water form. It appears to ocur in marginal habitats, it is not as common as it is taught, it is generally widespread in colder climates. It is often found in mixed species environments, but in the absence of some leeches it reaches high numbers. It lives in these conditions; temperature 6.8-24.6 ° C, pH 7.24-9.45, salinity ‰ 0.022-1.949, dissolved oxygen is 2.3-11.9 mg l^{-1} and alkalinity is 0.0-35.0 meq l^{-1} in mud, sand, detritus, stone, gravel. It is a cosmopolitan, sexual breeding species. Tubificid worms are considered as a sensitive test organism for aquatic and sediment toxicity tests. It is suggested that tubificid worms are important members of benthic fauna in the aquatic environment, and any harmful effects on these organisms are likely to be reflected in the whole ecosystem; therefore their use as a bioassay test organism is a logical one (Armstrong, 1997; Eaton and Bammler, 1999).

MATERIAL AND METHODS

Tubifex tubifex were collected from Porsuk River, Eskisehir. The worms were maintained in an aquarium with no sediment under spring water. The water was changed weekly and the worms were fed with tetraMin flakes (Tetra Werke, Melle, Germany) once a week. The lighting was 12 h dim light 12 h darkness and the temperature was 21 ± 1 C. The water was continuously aerated and the worms were cultured for 1 week before experiments for adaptation laboratory conditions.

Glutathione-S-transferase (GST) and Catalase (CAT) Assay

The control groups were not exposed to sodium sulfite.

The experimental groups, sodium sulfite exposure groups were put under 2.5mM, 5mM, 10mM concentrations for 2, 4, 6 hour period.

To evaluate protein and enzyme activity, for all control and experimental groups, 1 g of aquatic worms were homogenized in potassium phosphate buffer (25mM, pH: 7.4), centrifuged (12000g, +4C°, 10 min) and the supernatant was used for Bradford assay, GST and CAT enzyme assays. Total protein content of the extract was determined by dye binding method (Bradford, 1976) using bovine serum albumin as a standard.

The activity of GST was assayed as the increase of absorbance at 340 nm due to the conjugation of glutathione (GSH) to 1-chloro-2.4-dinitrobenzene (CDNB) according to Regoli et al. (1997). Sigma Kit was used for the experiments. 96-well microplate reader system was used to determine the enzyme activity of Glutathione S-Tranferase. Firstly, 100 mM CDNB was prepared in 96% of ethanol and it was used as substrate in the experiment. Potassium phosphate buffer (1X PBS) (pH 6.5) and 100 mM reduced glutathione (GSH) were used. Cocktail was prepared in order to put together with samples in the 96-well plate. Cocktail was kept in ice, 980 µl PBS, 10 µl CDNB and 10 µl GSH were pipetted into the plate and shaken. For the detection of enzyme activity, 0.001 mg ml^{-1} of protein from the sample is required to be put into the each reaction well. For this purpose, the protein amount from each sample is calculated according to 0.001 mg ml^{-1} and the amount of the cocktail, which will need to include a total of 200 µl mixtures pipetted in the 96-well plate. After pipetting to the wells, at A_{340} nm, for 30 minutes and with 1 minute intervals, the absorbance changes were recorded.

CAT activity of protein was determined by the decrease of absorbance at 240 nm due to H_2O_2 as described by Claiborne (1985). 0.001 mg ml^{-1} amount of protein was added into quartz cuvette. Appropriate amounts of buffer and H_2O_2 are added in 1ml of quartz cuvette and measured at 240 nm wave length for 10

minutes with 10 second intervals and the absorbance change in activity is determined. Statistical analysis was performed using the SPSS statistical program.

In all cases, three independent experiments were conducted According to the linear variations between the absorbance values, the formulas were calculated;

GST specific activity;

$$\mu mol\ ml^{-1}\ min^{-1} = \frac{(\Delta A_{340})\ min^{-1} \times V(ml) \times dil}{\varepsilon_{mM} \times V_{enz}\ (ml)}$$

where;

dil = the dilution factor of the original sample

ε_{mM} ($mM^{-1}\ cm^{-1}$) = the extinction coefficient for CDNB conjugate at 340 nm.

V = the reaction volüme

$V_{enz=}$ the volume of the enzyme sample tested

CAT specific activity;

$$Units\ ml^{-1}\ enzyme = \frac{(3.45)\ df}{time(0.1)}$$

where;

3.45 = decomposition of 3.45 µmoles of hydrogen peroxide in a 3.0 ml reaction mixture producing a decrease in the A240 from 0.45 to 0.40

df = dilution factor

time = minutes required for the A240 to decrease from 0.45 to 0.40

0.1 = milliliter of enzyme solution added to the cuvette

RESULTS AND DISCUSSION

In this study, the effects of sodium sulfite on Catalase and Glutatyon-S-transferase enzyme activities of a freshwater *Tubifex tubifex* (Muller, 1774) (Oligochaeta: Tubificidae), an important species in aquatic food chains, were investigated. This experimental animal is appropriate since it is easy to find, to expose the food additive and assay the enzyme activity and also Tubificid worms are considered as sensitive test organism for aquatic and sediment toxicity tests (Armstrong, 1997; Eaton and Bammler, 1999).

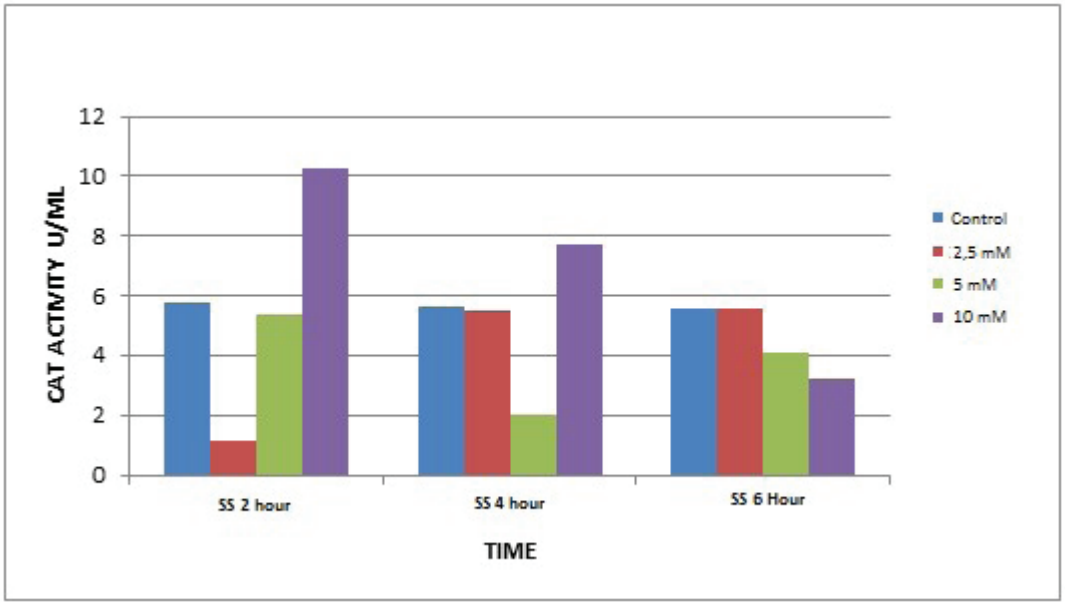

Figure1. Effects of various concentrations of sodium sulfite on catalase (CAT) activity of *Tubifex tubifex* for 2, 4 and 6 hours exposures. Control contains no added sodium sulfite

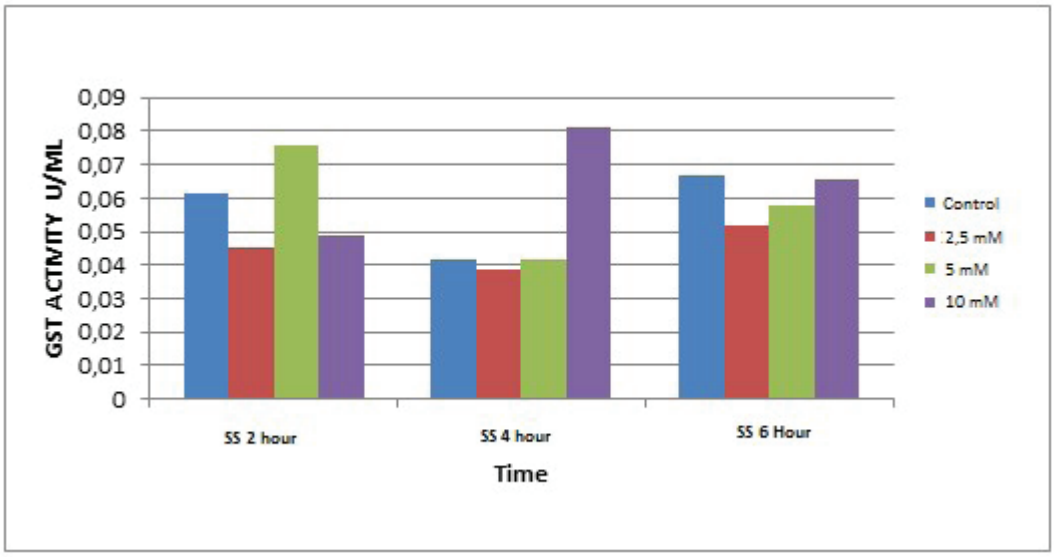

Figure2. Effects of various concentrations of sodium sulfite on glutathione-S-tranferase (GST) activity of *Tubifex tubifex* for 2, 4 and 6 hours exposures. Control contains no added sodium sulfite

As a result of performing exposure and activity measurement of catalase (CAT); there was an increase in enzyme activity in 10mM sodium sulfite concentrations (Figure 1). The enzyme activity increased with increasing duration of exposure and concentration. The enzyme activity decreased in 10mM sodium sulfite concentration with increasing time period and concentration, as the result of higher concentration. Consequently there was H_2O_2 destruction activity loss, exceeding decreased in the potential of living of the organism's defenses and is considered to be the starting point to the emergence of chronic damage. These results showed sodium sulphite concentrations above 10mM may cause damage to *Tubifex tubifex*, aquatic organisms.

The highest GST activity was in 4 hour exposure of 10mM sodium sulfite (Figure 2). The decrease 6 hour exposures can be interpreted as the start to lose the

activity of the organism depending on the concentration and exposure time increases.

Kayraldız and et al. investigated the effect of sodium sulfite by Ames test. In the presence or absence of S9 mixture, they found sulfites were not mutagenic for TA98 and TA100 strains (Kayraldız, 2006).

CONCLUSION

The usage of food additives should be determined according to toxicological evaluations, continual control of all additives in use and re-evaluation of usage conditions in accordance with new scientific findings if necessary, restriction of the use of an additive in the recommendation list or food standards for certain foods, certain conditions and purposes. It should be used according to the amount that can be taken in the day.

Despite the use of food additives for many years, the expansion of a diet for population growth and fast food consumption has become a topic receiving more attention today because of the increased consumption of fast food. Although there are still many regulations related to patterns of use and dose of several ingredients, investigations continues. The main purpose of these investigations is to find out effects of these substances on human health. However, these studies could not be carried out on human, thus investigations are usually carried out on animal group. According to all data, depending on the consumption amount and the exposure time sodium sulfite may cause a risk to living organisms.

The effects of sodium sulphite on GST and CAT enzymes has been shown for the first time in *Tubifex tubifex*, aquatic organisms, in order to help further studies that can be done with higher organisation organisms.

REFERENCES

Altuğ T, 2001. Gıda Katkı Maddeleri. Meta Basım Matbaacılık, İzmir, Turkey. 286p.

Atman ÜC, 2004. Gıda Katkı Maddeleri ve Gıda Kontrolü. Sürekli Tıp Eğitim Dergisi (Sted) 13 (3): 86-88.

Armstrong RN, 1997. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem. Res. Toxicol, 10: 2-18.

Boğa A, Binokay S, 2010. Gıda Katkı Maddeleri ve Sağlığımıza Etkileri. Arşiv 19: 14.

Bradford MM, 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry. 72: 248-254.

Claiborne A, 1985: Catalase activity. In: Handbook of Methods for Oxygene Radical Research. 283-284.

Chapman PM, Farrell MA, Brinkhurst RO, 1982: Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aquat. Toxicol, 2: 47.

Çalışır EZ, Çalışkan D, 2003. Gıda Katkı Maddeleri ve İnsan Sağlığı Üzerine Etkileri. Ankara Ecz. Fak. Dergisi 32 (3):193-206.

Eaton DL, Bammler TK, 1999. Concise Review of the Glutathione S-Transferases and their Significance to Toxicology. Toxicological Sciences, 49: 156-164.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2010. Scientific Opinion on the use of potassium sulphate and sodium sulphate as sources of respectively potassium and sodium added for nutritional purposes to food supplements. EFSA Journal 8(12): 1940

Fazio T, Warner CR, 1990. A review of sulphites in foods: analytical methodology and reported findings. Food Addit Contam. 7(4): 433-54.

Günes FE, 2014. Sülfitler ve Gıda Katkı Maddesi Olarak Kullanılması. Akademik Gıda 12(2): 114-119.

Kayraldız A, Kaya FF, Canımoğlu S, Rencüzeoğulları E, 2006 Mutagenicity of five food additives in Ames/Salmonella/ microsome test. Annals of Microbiology. 56 (2): 129-133.

Przybilla B, Ring J, 1987. Sulfite hypersensitivity. Hautarzt 38(8): 445-8.

Rathore RS, Khangarot BS, 2003: Effects of Water Hardness and Metal Concentration on a Freshwater *Tubifex Tubifex* Muller. Water, Air, and Soil Pollution. 142 (1): 341-356.

Regoli F, Principato G,Bertolli E, 1997.Biochemical characterization of the antioxidant system in the scallop *Adamissium colbeki*, a sentinel organism for monitoring the Antarctic environment. Polar Biology.17, 251-258.

Switala J, Loewen PC, 2002. Diversity of properties among catalases. Archives of Biochemistry and Biophysics. 401(2): 145–154.

Taylor SL, Higley NA, Bush RK, 1986. Sulfites in foods: uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity, and hypersensitivity. Adv Food Res 30: 1-76.