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VITAMIN C ENRICHMENT APPLICATION TO REDUCE THE RISK OF CARCINOGENIC NITROSAMINE FORMATION IN SMOKED FISH

TÜTSÜLENMİŞ BALIKLARDA KANSEROJEN NİTROSAMİN OLUŞUM RİSKİNİ AZALTMAK İÇİN C VİTAMİNİ ZENGİNLEŞTİRME UYGULAMASI

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ABSTRACT

Vitamin C, with its antioxidant and prooxidant properties, can prevent carcinogenic nitrosamine by chelating residual nitrite. It decreases the formation of nitrosamine. The objective of the study was to determine the protective effect of vitamin C in fish in reducing the formation of biogenic amines and the formation of nitrosamines. In this context, smoked samples were treated with vitamin C and analyzed by preparing in portions with different experiments and stored at ±4°C for different storage conditions. In this regard, putrescine(PUT), cadaverine(CAD), tyramine(TYR), and histamine(HIS) changed during storage. On the other hand, analyzes were also made for nitrosodimethylamine (NDMA), nitrosopiperidine(NPIP), nitrosopyrrolidine(NPYR), nitrosodibutylamine(NDBA). The lowest biogenic amine values are detected in group N2. It was determined that groups had 2.41, 3.66, 0.13, and 1.17(µg/g) biogenic amine values after 30 days, respectively. The obtained results showed that vitamin C had lower histamine and putrescine values than other groups separately. In addition, NDMA, NPYR, and NPYR were detected in nitrite containing groups after 30 days. Finally, Vitamin C containing groups were found to be better in terms of biogenic amines and nitrosamines content. All these results show that vitamin C can be used as a preservative in foods to protect against carcinogens.

Keywords: Food, smoked fish, vitamin C, nitrosamine, carcinogen.

ÖZET

Antioksidan ve prooksidan olarak bilinen C vitamini, gıdalarda oluşabilecek kalıntı nitriti şelatlama özelliğine sahiptir. Bu özelliği ile kalıntı nitritten kaynaklanabilecek kanserojen nitrozamin radikallerinin oluşum riskini azaltma özelliğine sahiptir. Bu çalışmanın temel hedefi, füme balıklarda C vitamininin, biyojenik amin ve tüm bunlara bağlı olarak kanserojen nitrozaminlerin oluşumunu azaltmadaki koruyucu etkisini belirlemektir. Bu bağlamda, füme balık örnekleri C vitamini muamele edilmiş ve edilmemiş deney grupları halinde hazırlanarak incelenmiş ve farklı depolama sürelerinde ±4ºC'de depolanmıştır. Bu bağlamda, putresin (PUT), kadaverin (CAD), tiramin (TYR) ve histamin (HIS) değerleri depolama srasında değişmiştir. Öte yandan nitrosamin olarak, örneklerdeki nitrosodimetilamin (NDMA), nitrosopiperidin (NPIP), nitrosopirrolidin (NPYR), nitrosodibutilamin (NDBA) değerleri belirlenmiştir. Deneysel verilere göre en düşük biyojenik amin değerleri N2 grubunda bulunmuştur. Deney gruplarının 30 günlük depolamadan sonra sırasıyla 2.41, 3.66, 0.13 ve 1.17 (µg/g) biyojenik amin değerlerine sahip olduğu belirlenmiştir. Elde edilen sonuçlar, C vitamini eklenen grupların diğer gruplara göre daha düşük histamin ve putresin değerlerine sahip olduğunu göstermiştir. Ayrıca nitrit içeren gruplarda 30 günlük depolamadan sonra NDMA, NPYR, NPYR tespit edilmiştir. Son olarak askorbik asit içeren grupların biyojenik amin ve nitrozamin içeriği açısından daha iyi olduğu tespit edilmiştir. Tüm bu deneysel sonuçlar, C vitamininin gıdalarda kanserojen nitrozamin radikallerinin oluşumunu önlemek amacıyla koruyucu olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Gıda, füme balık, C vitamini, nitrozamin, kanserojen.

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INTRODUCTION

One of the many technologies used to increase sensory quality and safety in foods is smoking. The smoking process is a complex processing technology that uses many unit operations and some preservatives. In addition to unit operations such as heat treatment and drying, smoking also includes chemical preservative applications such as curing, salting, smoke, antioxidant, and antimicrobial treatment. It is known that some safety risks arise in smoked products, which are highly sought after by consumers as a traditional taste. The many risks that arise are usually because the product is smoked, nitrite used, semi-dried, or cold-processed product. Some of the many risks that may arise from direct consumption or storage are the risk of the formation of biogenic amines and nitrosamines. By reducing these and similar risks, it may be possible to further increase the demand for the product and consumer health. The main purpose of food smoke in contemporary societies is to improve the sensory qualities of the product, increase the shelf life, and increase the added value of the product (Kilic and Oztan, 2013, 2015).

In the processing and storage process, many unwanted changes and chemical metabolites are formed in the product due to quality losses. Biogenic amines, are formed as a result of the decarboxylation of amino acids and have the potential to form carcinogenic compounds as a result of their direct effects such as deterioration and poisoning, as well as their interaction with nitrite (Kilic and Oztan, 2013; Masoud et al., 2007). Biogenic amines and nitrosamines are risky, undesirable chemical metabolites for consumer health formed in the product. Biogen amines, which have extremely high risks in terms of food safety, are known as heterocyclics as toxic aliphatics and organic metabolites with biological activity (Vural and Oztan, 1996). Biogenic amines, one of the main indicators of quality loss, are compounds formed by decarboxylation of amino acids or transamination of aldehydes and ketones. Although hot smoking decreases amine production, this risk does not disappear in cold smoking (Kilic and Oztan, 2013; Flick et al., 2001). The use of vitamin C is one of the many protective effects used in foods (Wasson et al., 1991; Tömen et al., 1991, Kim et al., 2000, Hamre et al., 2003). Vitamin C, also known as ascorbic acid, can be used as a preservative, especially in products that use nitrite or to prevent nitrite use due to high nitrite accumulation (Kilic, Oztan, 213; Kilic, Oztan, 215).

Free biogenic amines in nitrite media react to form nitrosamines. This condition can occur in the gastrointestinal tract. Experimental studies have revealed that nitrosamines such as Ndma, Npip, Npyr, and Ndba are high-risk molecules that have a direct effect on cancer formation. Nitrous amines are toxic compounds with carcinogenic, mutogenic, and even teratogenic effects, that are formed by the combination of nitrite or nitrite salts with an amino group. Even if the products do not add nitrite, they can occur spontaneously in the body (Madhavi et al., 1996; Kilic, 2013, 2015).

While vitamin C acts as an antioxidant in high-density aqueous environments, it acts as a low-intensity pro-oxidant (Frankel, 1996; Yen et al., 2002). With this feature, it is necessary to increase the concentration of vitamin C to prevent oxidation in products. Vitamin C reacts with the free oxygen present in the product, protecting the molecular bonds in the surrounding bonds, and also exerts a synergistic effect against specific antioxidants such as tocopherols present in the product. On the other hand, it can chelate free nitrite left over from nitrite or nitrate naturally found in products or added for preservative purposes (Madhavi et al., 1996). Ascorbic acid, which has antioxidant, prooxidant, metal diluent, and reducing agent or oxygen collector properties, reduces the risk of nitrosamines in products where nitrite is used or in products where nitrite accumulation is high for various reasons. Although ascorbic acid is known to reduce autoxidation in fish, this effect may not be seen in cooked fish. The combination of these effects may dominate in most food applications. It acts as a high antioxidant concentration in aqueous environments. On the contrary, it functions as a prooxidant at low concentrations (Frankel, 1996). Deoxidizers such as ascorbic acid, ascorbyl palmitate, sulfites, and erythorbaceous react with free oxygen, keeping them bound to the environment. They show synergistic reactions to ascorbic acid and specific antioxidants such as ascorbyl palmitate and tocopherols. The use of ascorbate and erythorbate now reduces the amount of nitrite or residual nitrite (Madhavi et al., 1996; Kilic, 2013, 2015). Vitamin C shows not only its direct effect but also an indirect mechanism of action by showing a synergistic effect for vitamin E, which is known as a chelating agent. Vitamin C and E can decrease nitrosation together (Kilic and Öztan, 2013; Mirvish, 1995; Mirvish, et al., 1972; Mirvish et al., 1986; Ahn et al., 2004). Vitamin C not only inhibits nitrosation directly but also acts synergistically by showing a protective effect on vitamin E, which is also known as an anti-carcinogen and has a chelating feature for free nitrite. It has been proven by experiments that vitamins C and E show anti-carcinogenic properties by reducing nitrosation together (Kilic and Öztan, 2013; Mirvish et al., 1986).

The main purpose of this study is to determine whether ascorbic acid, which has various functional properties and protective effects, has protective advantages in terms of biogenic amine and nitrosamine formation. Experimental results obtained in the study revealed that ascorbic acid may have some protective functional properties in terms of biogenic amine and nitrosamine formation. In addition to all these protective properties, although the protective effects of vitamin C on the formation of biogenic amines and nitrosamines are estimated, a study in this scope has not yet been found in the literature. The study is important in terms of revealing the effects of ascorbic acid on the formation of biogenic amines and nitrosamines and completing the important deficiencies in the literature and food industry.

MATERIAL AND METHOD

The raw materials anchovy (*Engraulis encrasicolus*) was purchased by a private company (Subatan Alabalık Production Ltd. Şti., Kayseri). Before determining the chemical characteristics of the raw material, the condition factor was also determined for approximately 6 fish selected by random sampling. The same raw material was chosen to determine the chemical properties of the experimental product.

In the experimental studies, each analysis was repeated twice and 50 fish of approximately 250 g each were consumed in each trial. During the experimental studies, experimental sampling was done from four groups: 1. raw material, 2. processed product (day 0), 3. stored product at +4ºC (day 15), and 4. stored product at +4ºC (day 30). Figure 1 shows the procedures, experimental groups, additives, and parameters applied during the experiment.

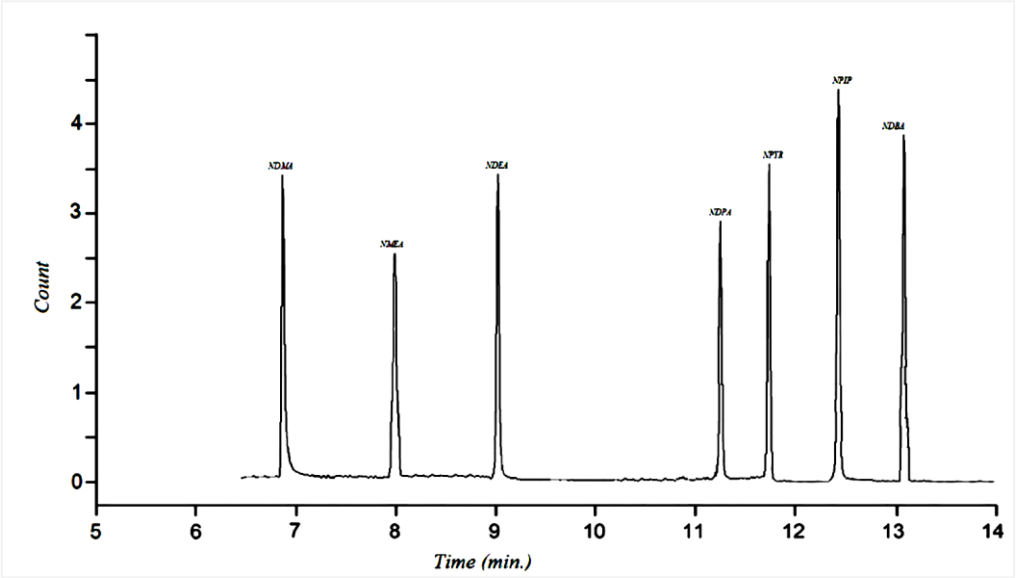

Figure 1. The Standard Chromatogram of Nitrosamines (1000 µg L^{-1} Solvent Standard).

The parameters of each process applied in the experimental groups are shown in Figure 1. 2% of salted fish were subjected to cold smoking after drying. The drying process was continued until a maximum of 5% drying was achieved. After the surface dried, the cold fumigation process was carried out.

Biogenic Amine and Nitrosamine Analysis

Ozogul et al., (2002) modified the method suggested by the UV/VIS detector and C18 Waters Sperisorp ODS-2 (125x 4.60 mm, 5 µm particle diameter) column equipped HPLC system. For this purpose, 5 g of sample was homogenized with 20 ml of trichloroacetic acid (6%), centrifuged at 12000 rpm for 10 minutes (4ºC), and subjected to filtration with a Whatman (no:1) strainer. The obtained sample was made up to 50 ml using distilled water of HPLC purity. The obtained extract was analyzed in HPLC system equipped with UV/VIS detector and C18 Waters Sperisorp ODS-2 (125x 4.60 mm, 5 µm particle diameter) column. Obtained chromatograms were compared with standard biogenic amine chromatograms and biogenic amine ratios were determined. As Carrier A; Acetonitrile was increased to 80% within 8 minutes by increasing the flow rate from 5%. HPLC distilled water was used as carrier B. The chromatograms obtained were compared with the biogenic amine standard and the biogenic amine ratios were

determined. Statistical evaluations were determined with the SPSS 11.5 for Windows package program and Duncan test was applied to the variables found to be important (Sümbüloğlu and Sümbüloğlu, 2002).

Nitrosamine analysis was determined by modifying the method applied by Andrade et al., (2005). In this context, after 2.5 g of homogeneous fish sample was cooked at 177ºC for 3 minutes, the extracts were placed in a blender with dry ice and placed in GC vials with 24 mL Teflon septa. The figure presents the standard chromatogram of nitrosamines (1000 µg L^{-1}solvent Standard). Figure 2 presents the standard chromatogram of nitrosamines (1000 µg L^{-1} solvent Standard).

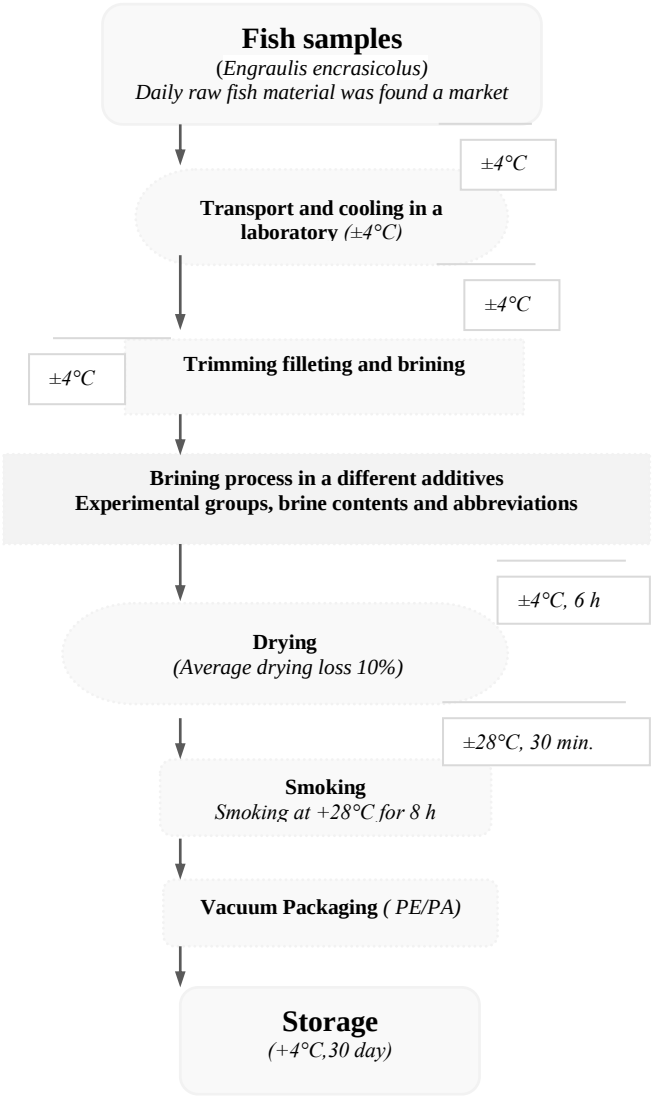

Figure 2. Experimental Groups And Production Flow Chart (*Modified from Kilic and Oztan, 2013, 2015*)

Chromatographic separations were determined by GC/MS according to a standard stock solution chromatogram with a column of Carbowax amine, 60 m x 0.53 ID, 1 µm film thickness.

RESULTS AND DISCUSSIONS

Biogenic amines, which have an important role in the biological functions of humans and animals, can show toxic effects when taken in large amounts with food. The most common poisoning caused by biogenic amines is histamine and tyramine poisoning. Tyramine mainly acts indirectly on the sympathetic nervous system by increasing blood pressure by peripheral vasoconstriction. Diamines such as putresin and cadaverine are considered mutagenic precursors due to their tendency to react with nitrites and the formation of potential carcinogen nitrosamines. These amines are converted by heat to pyrrolidine and piperidine, forming nitrozopyrrolidine and nitrozopiperidine. In the

study, nitrosamine amounts formed in experimental groups were determined. In the study, biogenic amine values such as *putrescine (Put)*, *histamine (His)*, *cadaverine (Cad)*, and *tyramine (Tyr)*, which are very important for fish quality, were determined. According to the results obtained, it was determined that there was a significant increase in biogenic amine values due to deterioration. The results obtained in the statistical analyzes will be determined and used in the preparation of the study for publication. If the values are to be examined, it is determined in the product. If the values are examined, it will be seen that there is a significant correlation between the quality parameters determined in the product and the biogenic amine values. Table 1 presents the variation in total biogenic amine values (µg/g) during shelf life.

Table 1. The Variation in Total Biogenic Amine Values during Shelf Life (µg/g)

	Biogenic amin (µg/g)											
	tyramine (Tyr)			*histamine (His)*			*putrescine (Put)*			*cadaverine (Cad)*		
Time (Day)	***0***	***15***	***30***	***0***	***15***	***30***	***0***	***15***	***30***	***0***	***15***	***30***
Experimental groups		*Ty*	*Ty*		*Hi*	*Hi*		*Pu*	*Pu*		*Ka*	*Ka*
Fresh (R)	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Brined (B)	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.76**	**0.00**	**1.87**	**1.32**	**0.00**	**1.04**	**0.99**
Control (C)	0.00	0.00	0.13^{a}	0.00	0.52^{ab}	1.56^{b}	0.00	3.53^{c}	3.72^{d}	0.00	1.05^{a}	3.22^{a}
LTHV4	0.00	0.00	0.86^{b}	0.00	0.54^{ab}	1.63^{bc}	0.00	3.21^{bc}	3.54^{bc}	0.00	1.33^{a}	3.28^{a}
LTHV10	0.00	0.00	0.85^{ab}	0.00	0.57^{b}	1.33^{b}	0.00	3.36^{bc}	3.66^{cd}	0.00	2.56^{b}	3.85^{c}
LTHV15	0.00	0.00	0.93^{b}	0.00	0.57^{ab}	1.47^{b}	0.00	3.54^{c}	3.74^{d}	0.00	2.44^{b}	3.46^{ab}
LTHV20	0.00	0.00	0.95^{bc}	0.00	0.51^{ab}	1.73^{c}	0.00	3.88^{d}	3.85^{d}	0.00	2.76^{d}	3.74^{bc}
N4	0.00	0.00	$\mathbf{0.19^{a}}$	0.00	0.30^{a}	$\mathbf{1.28^{ab}}$	0.00	2.35^{a}	$\mathbf{2.69^{c}}$	0.00	2.23^{ab}	$\mathbf{3.72^{b}}$
N2	0.00	0.00	$\mathbf{0.13^{a}}$	0.00	0.22^{a}	$\mathbf{1.17^{a}}$	0.00	2.46^{a}	$\mathbf{2.41^{ab}}$	0.00	2.31^{ab}	$\mathbf{3.66^{ab}}$
A	0.00	0.00	$\mathbf{0.51^{ab}}$	0.00	0.52^{ab}	$\mathbf{1.98^{c}}$	0.00	3.74^{cd}	$\mathbf{2.76^{c}}$	0.00	2.74^{cd}	$\mathbf{4.56^{ab}}$
N4A	0.00	0.00	$\mathbf{0.24^{a}}$	0.00	0.28^{a}	$\mathbf{1.16^{a}}$	0.00	2.71^{ab}	$\mathbf{2.44^{ab}}$	0.00	2.54^{bc}	$\mathbf{3.81^{cd}}$
N2A	0.00	0.00	$\mathbf{0.15^{a}}$	0.00	0.29^{a}	$\mathbf{1.39^{ab}}$	0.00	2.55^{a}	$\mathbf{2.\ 37^{a}}$	0.00	2.62^{c}	$\mathbf{3.89^{d}}$

*n= 2; a-d Mean values within a column with different letters are significantly different ($p < 0.05$).

Experimental data show that although the biogenic amine load could not be determined at the beginning of the storage or low values were determined, increases occurred after 15 days of storage, and even higher values were determined in 30 days. Especially Hi, Pu and Ka values were determined higher than Ty values in groups using preservatives and cold drying (N4, N2, A, N4A, N2A).

When these values were compared, it was determined that Pu and Ka values were lower when compared to control and raw materials. At the same rate, no differences were observed between drying and preservative added groups (LTHV4, LTHV10, LTHV15, LTHV20, N4, N2, A, N4A, N2A). Although the change in the quality values revealed significant differences between the groups, the same differences could not be determined in the biogenic amine values. In the presence of free biogenic amine formed in the fish's content, if residual nitrite is detected in its content, it means that nitrosation will occur under these conditions (Kilic and Öztan, 2013; Bruce Topkin, 2005). However, it was revealed that vitamin C would inhibit nitrosation. Vitamin C shows not only its direct effect but also an indirect mechanism of action by showing a synergistic effect for vitamin E, which is known as a chelating agent. Vitamin C and E can decrease nitrosation together (Kilic and Öztan, 2013; Mirvish, 1995; Mirvish et al., 1972; Mirvish, 1986; Ahn et al., 2004).

Nitrosamine Values

The retention times of *nitrosodimethylamine* (Ndma), *nitrosopiperidine* (Npip), *nitrosopyrrolidine* (Npyr), *nitrosodibutylamine* (Ndba) for NDMA was 6,9 mn, NPIP 12,50 mn, NPYR 11.7 mn, and NDBA 13,2 (30 µg mL^{-1}).

According to the obtained results, the samples treated with ascorbic acid have decreased especially histamine and putrescine values in comparison with other groups ($p<0.05$). In Addition, *NDMA, NPYR, and NPYR* were detected in all nitrite included experimental groups after 30 days storage. Consequently, the ascorbic acid including groups had better results for biogenic amines and nitrosamine content. It is suggested that ascorbic acid utilization be applied to preserve the fish quality.

The experimental determination of nitrite and ascorbic acid addition on nitrosamine formation is very important for the food industry and consumer health. Existing tests were carried out regarding the formation of nitrosamines in different groups in the products produced, and positive or negative results were obtained as shown in the table below. In the analyses made; for the Ndma, Npip, Npyr, and Ndba yes or no tests were performed. In particular, the results obtained in the study reveal the effect of additives as well as the effect of storage on nitrosamine formation. According to the results obtained from the study, nitrosamine formation of especially nitrite-containing groups was positive. Table 2 presents the detected nitrosamines in experimental groups depending on storage time (µg/kg).

Table 2. The Detected Nitrosamines in Experimental Groups Depended on Storage Time (µg/kg).

	Storage time (day)											
	0	*15*	*30*	*0*	*15*	*30*	*0*	*15*	*30*	*0*	*15*	*30*
Nitrosamine	***NDMA***			***NPIP***			***NPYR***			***NDBA***		
Raw (R)	-	-	-	-	-	-	-	-	-	-	-	-
Brined (B)	-	-	-	-	-	-	-	-	-	-	-	-
Control (C)	-	-	-	-	-	-	-	-	-	-	-	-
LTHV4	-	-	-	-	-	-	-	-	-	-	-	-
LTHV10	-	-	-	-	-	-	-	-	-	-	-	-
LTHV15	-	-	-	-	-	-	-	-	-	-	-	-
LTHV20	-	-	-	-	-	-	-	-	-	-	-	-
N4	-	-	+	-	+	+	-	-	+	-	-	-
N2	-	-	+	-	-	+	-	-	+	-	-	-
A	-	-	-	-	-	-	-	-	+	-	-	-
N4A	-	-	+	-	+	+	-	-	+	-	-	+
N2A	-	-	+	-	+	+	-	-	+	-	-	+

*n= 2; (-); Not dedected,

The values of the retention times for Ndma were 6,9 mn, Npip 12,50 mn, Npyr 11.7 mn, Ndba 13,2 (30 µg mL^{-1}). According to the results obtained, the samples treated with ascorbic acid have decreased especially histamine and putrescine values in comparison with other groups. In Addition, *Ndma, Npip, Npyr, and Ndba* were detected in all nitrite included experimental groups after 30 days of storage. Consequently, the ascorbic acid including groups had better results for biogenic amines and nitrosamine content. It is suggested that ascorbic acid utilization be applied to preserve the fish quality. Table 2 presents the detected nitrosamines in experimental groups depending on storage time (µg/kg). The results give good agreement with Kilic and Oztan (2013, 2016). The results obtained showed a good correlation between the residual nitrite ratios determined by Kılıç and Öztan (2013, 16) and the effect of ascorbic acid on the residual nitrite values. In their study, the researchers showed that the residual nitrite values in the experimental groups to which nitrite was added using ascorbic acid completely disappeared depending on time. Residual nitrite, which occurs spontaneously in the product or is formed due to nitrite added, is a possible expected result, in which nitrosation is eliminated and nitrosamine formation is prevented due to its elimination with ascorbic

acid. Experimental studies have shown that different nitrosamines can lead to the formation of different cancers. From this point of view, the data obtained show that nitrosamine compounds formed due to nitrite and free amine compounds that cause the risk of different cancer types such as different liver, lung, oesophagus, bladder, and pancreas (Li et al., 2013; Bogovski and Bogovski, 1981; Magee and Barnes, 1956).

CONCLUSIONS

Experimental studies reveal that although PUT, CAD, TYR, and HIS values are correlated with the quality loss due to preservative use as expected, this relationship is evident in raw material and control groups and the groups using drying and preservatives, but in the groups treated with preservative and drying. No significant or significant differences could be identified among them. On the other hand, it can be said that the histamine and putrescine values in the samples, especially in which ascorbic acid was applied, were lower than the other groups ($p<0.05$).

On the other hand, NDMA, NPYR, and NPYR were detected in all experimental groups containing nitrite after 30 days of storage in the NDMA, NPIP, NPYR, and NDBA analyzes. Briefly, enrichment of vitamin C, known as ascorbic acid, has better results for groups containing ascorbic acid, biogenic amines, and nitrosamine content and reduces the risk of nitrosamine formation. As a result, the following benefits are obtained with the addition of vitamin C in the Anchovy as a fish;

1. Long storage life,
2. Decrease in residual nitrite rate,
3. Decrease in biogenic amine ratio,
4. Decrease in nitrosamine formation,
5. Synergetic contribution to vitamin E,
6. Inhibiting nitrosamine formation,
7. Preservation of color and texture,
8. Nutrient enrichment.

It has been revealed that Vitamin C is recommended to be used in production technologies with its important advantages.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

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