Cumhuriyet Science Journal
CSJ

e-ISSN: 2587-246X
ISSN: 2587-2680

Cumhuriyet Sci. J., Vol.38-4 (2017) 834-844

The Effects of Nisin and Natamycin on the Microbiological, Chemical and Sensorial Qualities of Meatballs

Emre HASTAOĞLU[1]*, Meryem GÖKSEL SARAÇ[1], Zehra SEBA KESKİN[1], Fazıl YOZGAT[2]

[1]Cumhuriyet University Yildizeli Vocational School, Sivas / TURKEY

[2]Cumhuriyet University Faculty of Economics and Aministrator Sciences, Sivas / TURKEY

Received: 01.08.2017; Accepted: 09.10.2017 *http://dx.doi.org/10.17776/csj.349817*

Abstract: Meatballs are known to be one of the foodstuffs most sensitive to microbiological deterioration due to their physical and chemical properties. Meat and meat products are known to be the leading sources of food-related diseases. Nisin and natamycin are natural additives which are known for their activities in the inhibition of microorganisms. In this study, an evaluation was made of the effects of nisin and natamycin on the properties of meatballs and the possibilities for use of these natural antimicrobials in meatballs were investigated. Chemical, microbiological and sensory analyses to determine quality were applied to meatball samples which included these natural antimicrobials at different ratios. Moisture, crispness, fat content, pH, total aerobic mesophilic bacteria count and sensory values of the meatball samples which included 0, 2.5 and 5 g nisin/kg and 0, 2.5 and 5 g natamycin/kg were analysed at 0^{th}, 5^{th}, 10^{th} and 15^{th} days, and changes in the product structure were recorded. The sensory properties of the meatballs were moisture, crispness, strange taste and odour, colour, flavour and overall taste. Multi-criteria decision-making techniques, SAW and TOPSIS tests were performed for the sensory analysis. The results showed that the acceptable consumption period of meatballs which included these antimicrobials was increased. According to the sensory analyses the most preferred meatball sample was that which included 5 g nisin.

***Keywords*:** Nisin, natamycin, meatball, natural antimicrobial, sensory analyse, multi-criteria decision-making

Köftenin Mikrobiyolojik, Kimyasal ve Duyusal Kalitesi Üzerine Nisin ve Natamisinin Etkileri

Özet: Köfte, fiziksel ve kimyasal özelliklerinden dolayı mikrobiyolojik bozulmaya en duyarlı gıdalardan biri olarak bilinir. Gıda kaynaklı hastalıklar arasında et ve et ürünleri en büyük orana sahiptir. Nisin ve natamisin mikroorganizmaların inhibisyonu için faaliyetleriyle bilinen doğal katkı maddeleridir. Bu çalışmada, nisin ve natamisinin köfte kalitesi üzerine etkileri ve bu doğal antimikrobiyallerin köftede kullanım olasılıkları araştırılmıştır. Bu doğal antimikrobiyal maddeleri değişik oranlarda içeren köfte örneklerin kalitelerini belirlemek için kimyasal, mikrobiyolojik ve duyusal analizleri yapıldı. 0, 2.5 ve 5 g nisin/kg köfte ve 0, 2.5 ve 5g natamisin/kg köfte içeren köfte örneklerinin depolamaya bağlı olarak 0, 5, 10 ve 15. günlerde nem, kül, yağ içeriği, pH, toplam aerobik mezofilik bakteri sayımı ve duyusal değerleri araştırıldı. Duyusal kriter olarak sululuk, gevreklik, farklı tat ve koku, renk, lezzet ve genel tat değerlendirildi. Çok kriterli karar verme tekniklerinden SAW ve TOPSIS testleri duyusal analizlere uygulandı. Sonuç olarak, bu antimikrobiyalleri içeren köftelerin kabul edilebilir tüketim süresinin arttırdığı bulunmuştur. Duyusal analizlere göre 5 g nisin içeren köfte örneği en sevilen örnek olmuştur.

***Anahtar Kelimeler*:** Nisin, natamisin, köfte, doğal antimikrobiyal, duyusal analiz, çok kriterli karar verme

* Corresponding author. *Email address:* *ehastaoglu@cumhuriyet.edu.tr*

1. INTRODUCTION

Meat has an important role in human nutrition because of the nutritional value, and particular flavour and smell. Due to the physical and chemical properties of meatballs, they are known to be a highly sensitive food product in respect of microbiological spoilage. It is well known that meat and meat products are the leading cause of foodborne diseases. The most common spoilage bacteria in meat are Gram negative aerobic psychotropic *Pseudomonas, Moraxella, Acinetobacter, Aeromonas* and facultative anaerobic *Alteromonasputra faciens,* Gram positive *Lactobacillus spp*. and *Brochotrix thermopsphacta.*

Microorganisms are the most important factors related to food safety [1]. Rates of foodborne illnesses and poisonings are increasing, associated with various factors such as changes in lifestyle, an increase in sensitive consumer groups, the development of exports and imports, new developments in the production of animal origin food, new applications in food processing, increased travel, global warming, trends for natural nutrition, and a high demand for additive-free food [2].

The main principle of food preservation is the inactivation, delayed growth or prevention of microorganisms which are pathogens and cause food spoilage. Of the many food spoilage prevention techniques which are in use, antimicrobial chemicals and heating processes are the oldest and the most common methods [3]. However, the negative attitudes or prejudices of consumers towards synthetic food additives and increasing demands for food products which have undergone a minimum processing but have a long shelf life has led to research into alternative methods of food preservation.

Although various antimicrobial additives are used for the elimination of pathogens in meat products, the use of these chemicals is restricted because of the negative effects on human health of adding antimicrobials such as nitride and nitrate over the permitted limit [4]. Consumer trends are for the use of natural antimicrobials rather than synthetic additives. Some of these natural antimicrobials are used in food preservation but some are still at the research stage. Lysozyme, ovotransferrin and avidin in eggs, lactoperoxidase and lactoferrin in milk, and transferrin in blood serum are examples of animal origin natural antimicrobials. Carvacrol, eugenol, thymol, cinnamicaldehyde and allicin are derived from herbs and spices, while essential oils and extracts are major herbal natural antimicrobials. Bacteriocins are antimicrobial polypeptides or proteins which are ribosomally produced by bacteria. The antimicrobial effect is related to the bacterium. These cationic molecules with 60 amino acid residue and resistance to heat [5] have a role in the inhibition of foodborne pathogens, the control of fermentation, shelf life extension and the provision of microbiological safety[6, 7]. Bacteriocin is used in the food industry as a direct addition to the food formulation, by immersion of the food in a bacteriocin solution, and by inoculation of the food with strains which produce bacteriocin [8]. Bacteriocins of lactic acid bacteria are classified in 4 groups; lantibiotics, heat stable bacteriocin, not heat stable, complex bacteriocins [6]. There are also natural antimicrobial bacteriocins such as nisin and pediocin which are derived from microorganisms [3, 9, 10].

Lactococcus lactis is a bacterium used as a fermentation agent in many dairy products and through controlled fermentation, the natural, polycyclic antimicrobial peptide, nisin can be obtained. This bacteriocin is extensively used in the protection of a wide range of food products against spoilage bacteria, and is permitted for use in food processes due to the absence of toxicity and the impact on pathogens and spoilage microorganisms. Due to these properties, nisin has become the subject of research [11]. However, restrictions of use are that it has no effect on Gram negative bacteria, fungi and yeasts, or on all Gram positive bacteria.

Food preservatives play important in meat preservation, role. According to the properties of the meat, nisin shows antimicrobial activity when

in contact with the meat matrix. The use of nisin in meat processing is mainly dependent on the presence of glutathione, which can inactivate nisin through the catalyst of glutathione S-transferase reaction [12]. In cooked meat, the glutathione inactivation is lower as free sulphydryl groups that act as the catalyst for the reaction between glutathione and proteins, are lost during the heating process [13]. The inactivation of nisin also occurs though proteolytic enzymes, which are generally found in fresh meat [12]. Furthermore, the antimicrobial efficacy of nisin can be reduced by interaction with the meat fats, in addition to glutathione and the presence of proteolytic enzymes.

Natamycin is a natural, macrolide polyene antifungal product, which is obtained through the fermentation of *Streptomyces natalensis*. As a food additive, it has a role in the control of yeast and mould growth, mainly on the surface of cheese, meat and sausages. Natamycin (INN) is a naturally occurring antifungal agent, also known as pimaricin, which is commonly found in soil. Natamycin is used in the food industry as a natural preservative, as a patented antibiotic against carcass decontamination and as an alternative to chemicals such as trisodium phosphate and chloride. The antifungal effect occurs through combining with sterol in the cell walls of yeasts and moulds. As bacteria have no cell wall sterol, natamycin is not effective against bacteria [14, 15]. In the food industry, natamycin has been used for many years to inhibit fungal outgrowth in dairy products and other foods. The potential advantages for the use of natamycin could include the replacement of traditional chemical preservatives, as a neutral flavour impact, and that the efficacy is less dependent on pH, as is common with chemical preservatives. Natamycin can be applied to foods by various methods. These can be sprayed or immersed the aqueous solution into the product or powder form can be added into meat mixture. More specific uses of natamycin are the common use in products such as cottage cheese, sour cream, yogurt, shredded cheeses, cheese slices, and packaged salad mixes. One of the reasons for food manufacturers to use natamycin is to replace the artificial preservative sorbic acid. Natamycin has been approved for different applications at different levels throughout the world, and is approved in over 150 countries worldwide. Natamycin does not have acute toxicity. The EFSA has concluded that the use of natamycin as a food additive has no relevant risk for the development of resistant fungi.

The aim of this study was to investigate the effects of nisin and natamycin on the microbiological, chemical and sensory properties of meatballs and to present these antimicrobial functions to the meat industry.

2. MATERIALS AND METHODS

2.1. Materials

Meatball samples were prepared with the following ingredients: meat (Yıldız Et, Sivas, Turkey), nisin and natamycin (Maysa Gıda, Istanbul, Turkey), salt (Yılmazlar Tuz, Sivas, Turkey). The meatball samples were prepared in the manner of traditional butcher meatballs. The meat samples were minced and included 20g/kg granule salt and temperature was measured as 0-4^{0}C, were. Nisin (NS) and natamycin (NT) at different concentrations were added to the mixture. The control samples NS-0 and NT-0 did not include any antimicrobials, samples NS-2.5, NS-5.0, NT-2.5 and NT-5.0 included nisin 2.5 g/kg, nisin 5.0 g/kg, natamycin 2.5 g/kg and natamycin 5.0 g/kg respectively. The meatball mixture was prepared by manual kneading then stored for 24h at 4^{0}C for marination. The mixture was homogenized again through a meat grinder and shaped by hand. The meatball samples were then stored at 4^{0}C in sealed polythene bags for 15 days.

2.2. Methods

2.2.1. Proximate Analyses

Moisture and crispness [16], fat content [17] and pH values [18] of the 5 different meatball samples were determined using analytical methods. Moisture (g water/100 g sample) was determined by drying a 3g sample at 105 °C in a drying oven (Nüve, MF120, Turkey) for constant weight. Crispness analysis was applied at 550 °C for 12 h (g ash/100 g sample) in a furnace (Nüve, MF120,

Turkey). The fat content analysis was applied as the basic component using ether extraction methods. The pH values were determined by blending 10 g sample with 50 mL deionized water for 2 min. The pH of the resultant suspension was measured with a pH meter (Hach, ABD).

2.2.2. Microbiological Analyses

For total aerobic mesophilic bacteria counting, a 25g sample and 225 mL sterile 0.1% peptone water were homogenized for 2 min. Then, 0.5 mL of the mixture was inoculated onto plate count agar (PCA) and incubated for 48-72 h, at 32-35^{0}C. Data were expressed as "$\log_{10}$ cfu/g".

2.2.3. Sensory Analyses

As sensory quality criteria, moisture, crispness, strange taste and odour, colour, flavour and overall taste were evaluated by 20 panellists (15 females, 5 males, with an average age of 22.7±3.4 years) who were students in the Food Technology Department in Yıldızeli Vocational School, Cumhuriyet University, Sivas, Turkey. The panellists were currently in good health and volunteered for the testing. The sensory analysis was applied in a room at a controlled temperature. Before the analysis, the panellists were educated about the general properties related to hot meatballs. The panellists tasted a sample of each meatball labelled with a three-digit random number. Evaluation was made of consistency, flavour, colour, moisture, strange taste – odour and overall like using a 9-point hedonic scale (1=disliked extremely; 5=neither liked nor disliked; and 9=liked extremely).

2.2.4. Application of SAW and TOPSIS Test

The judgment diagram of the meatball selection is shown in Figure 1.

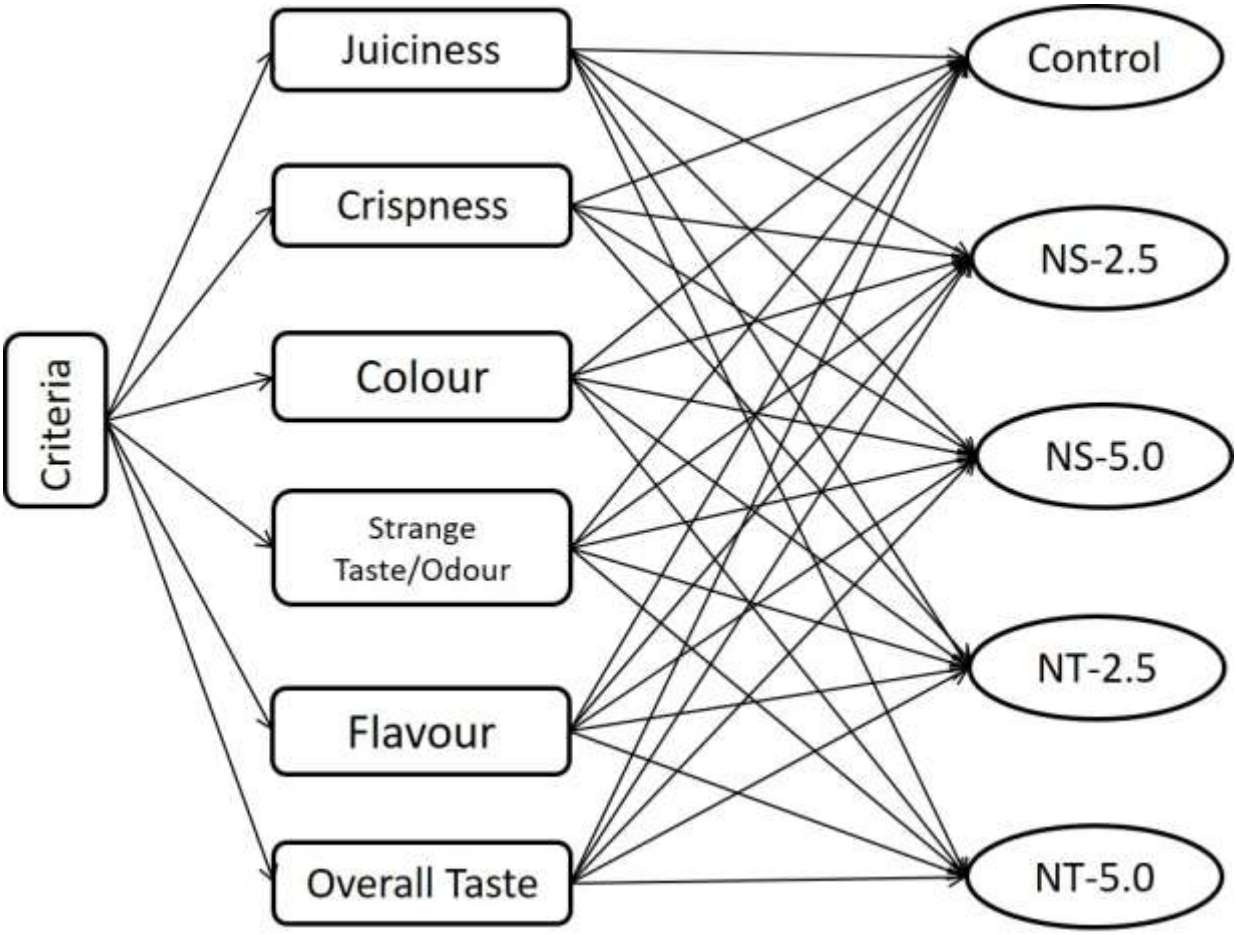


Figure 1. Decision diagram of the meatball selection.

The SAW (simple additive weighting) test method used to compare the criteria separately.

1. Create of pairwise comparison matrix (*m* x *n*) based on Saaty's 1-9 scale.
2. Selection of the important criteria for each comparison and scoring to illustrate how much more important it is.
3. Create of a decision matrix with m alternative and n criteria.
4. Composing of the weighted normalized matrix with the equation:

$$Ai = \sum wixij \quad (1)$$

where x_{ij} is the score of the *i*th alternative with respect to the *j*th criteria, and w_j is the weight of the criteria [19]

5. Determining the total of the weighted normalized vectors to determine the ranking of the alternatives.

TOPSIS (technique for order preference by similarity to ideal solution) is a method developed by [20] to be used as an alternative to ELECTRE. The selected alternative should have the shortest distance from the negative ideal solution in geometric media. The order of alternatives preferred is provided by comparing the Euclidean distances [21]. To simplify the process of locating the ideal and negative ideal solutions, the basic assumption of the method is that each attribute can only increase or decrease in a single direction

1. Normalization of the decision matrix: Converting the various dimensional attributes to a non-dimensional version. The normalized decision matrix (r_{ij}) can be calculated as:

$$r_{ij} = \frac{a_{ij}}{\sqrt{\sum_{k=1}^{m} a_{kj}^2}} \quad (2)$$

2. The decision maker decides on a set of weights $W=(w_1, w_2, w_3, \ldots, w_N)$, (where $\sum_{i=1}^{n} w_i = 1$) to be applied to the decision matrix to generate the weighted normalized matrix Y_{ij} as follows:

$$Y_{ij} = \begin{bmatrix} w_1x_{11} & w_2x_{12} & \ldots & w_nx_{1n} \\ w_1x_{21} & w_2x_{22} & \ldots & w_nx_{2n} \\ . & & & . \\ . & & & . \\ . & & & . \\ w_1x_{m1} & w_2x_{m2} & \ldots & w_nx_{mn} \end{bmatrix}$$

3. Performance data for n alternatives over k criteria are calculated. Raw measurements (rij) are standardized (n_i) by the following formula:
4. For each criterion, a set of importance weights (wk) are formed. Although the basis for the weights is generally ad hoc reflecting the relative importance of each criterion, it can be set as anything. When step 1 standardization is successful, there will be no problems in scaling.
5. The determination of positive (A^+) and negative (A^-) ideal solutions

$$A^* = \{v_1^*, v_2^*, \ldots, v_n^*\} \quad \text{(Maximum values)}$$

$$A^- = \{v_1^-, v_2^-, \ldots, v_n^-\} \quad \text{(Minimum values)}$$

v is the weighted normalised values.

6. The calculation of the distance from positive and negative ideal solutions of the alternatives.

$$D_i^* = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^*)^2} \quad (3)$$

$$D_i^- = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^-)^2} \quad (4)$$

v_{ij},v_j* and v_j^- are the positive and negative ideal solutions as weighted normalized values. D_i* and D_i^- are the respective distances from the positive and negative ideal solutions.

7. Finding out a ratio of R for each alternative is equal, which is fixed dividing the distance to the nadir by the total of the distance to the nadir and the distance to the ideal

$$R_i^* = \frac{D_i^-}{D_i^- + D_i^*} \quad (5)$$

8. Ranking of the alternatives using the R values. The best alternative is the sample with the highest R value.

2.2.5. Statistical Analysis

All the analyses except sensory analyses were carried out in triplicate and data were emphasized as mean ± DF. Tukey was used to determine the effect of antimicrobial type and the concentration by SPSS 22.

3. RESULTS

3.1. Chemical Properties

The dry matter content of the samples increased depending on the storage period for all samples as shown in Table 1. At the end of the 15th day, the sample including 5g/kg of nisin was the highest dry matter content. The moisture content changes of the meatball samples were not statistically significant according to antimicrobial addition.

Table 1. Dry Matter Contents of Meatball Samples.

	% Dry Matter			
Samples	0th day	5th day	10th day	15th day
Control	36.04±0.8 Aa	41.72±0.5 Aa	46.05±0.6 Ba	46.95±0.8 Ba
NS-2.5	41.97±0.7 Ab	41.84±0.6 Aa	44.63±0.4 Aa	48.43±0.7 Ba
NS-5	37.89±0.5 Aa	43.08±0.3 Ab	46.05±0.7 Aa	56.74±0.6 Bb
NT-2.5	44.85±0.7 Ab	41.60±0.5 Aa	46.37±0.5 Aa	46.83±0.4 Ba
NT-5	36.11±0.6 Aa	44.54±0.4 Ab	46.40±0.4 Aa	48.99±0.7 Ba

On the table, the same letters in lines and columns express no difference statistically, different letters express a difference statistically. Capital letters were coded for lines; small letters were coded for columns.

As seen in Table 2, the sample with the highest ash content was NS-5. Changes in ash content were not seen until the 5th day, and after the 10th day, the ash content of the samples increased depending on the storage time.

The pH values and fat contents of meatball samples were not statistically significant (Table 3). The pH values of the control sample more decreased, depending on the storage period and the NT-5 sample had the lowest pH value. The fat content of the meatball samples did not change with the effect of nisin and natamycin in comparison with the control group (Table 4).

Table 2. Ash Contents of Meatball Samples.

% Ash Content				
Samples	0th day	5th day	10th day	15th day
Control	2.03±0.5 Aa	2.37±0.5 Aa	2.56±0.6 Ba	2.60±0.9 Ca
NS-2.5	2.08±0.3 Ab	2.56±0.6 Ab	2.70±0.8 Bb	2.97±0.5 Cb
NS-5	2.23±0.5 Ac	2.68±0.5 Ac	3.09±0.4 Bc	3.16±0.7 Cc
NT-2.5	2.06±0.4 Ad	2.40±0.3 Ad	2.65±0.2 Bd	2.70±0.7 Cd
NT-5	2.41±0.2 Ae	2.53±0.1 Ae	2.61±0.4 Be	2.68±0.6 Ce

On the table, the same letters in lines and columns express no difference statistically, different letters express a difference statistically. Capital letters were coded for lines; small letters were coded for columns.

Table 3. pH Values of Meatball Samples.

pH Values				
Samples	0th day	5th day	10th day	15th day
Control	5.75±0.4 Aa	5.65±0.6 Aa	5.44±0.4 Aa	5.22±0.5 Aa
NS-2.5	5.74±0.3 Aa	5.78±0.5 Aa	5.84±0.3 Aa	5.93±0.5 Aa
NS-5	5.42±0,4 Aa	5.47±0.3 Aa	5.50±0.3 Aa	5.52±0.7 Aa
NT-2.5	5.53±0.2 Aa	5.53±0.8 Aa	5.52±0.5 Aa	5.51±0.6 Aa
NT-5	5.37±0.1 Aa	5.33±0.7 Aa	5.27±0.7 Aa	5.21±0.5 Aa

On the table, the same letters in lines and columns express no difference statistically, different letters express a difference statistically. Capital letters were coded for lines; small letters were coded for columns.

Table 4. Fat Contents of Meatball Samples.

% Fat Content				
Samples	0th day	5th day	10th day	15th day
Control	19.78±0.6 Aa	19.98±0.5 Aa	20.13±0.6 Aa	20.13±0.7 Aa
NS-2.5	19.49±0.7 Aa	19.66±0.6 Aa	19.98±0.8 Aa	19.98±0.4 Aa
NS-5	20.03±0.4 Aa	19.98±0.6 Aa	20.11±0.4 Aa	20.09±0.2 Aa
NT-2.5	19.85±0.6 Aa	19.83±0.4 Aa	19.89±0.7 Aa	19.88±0.5 Aa
NT-5	19.96±0.6 Aa	19.97±0.5 Aa	19.96±0.3 Aa	19.98±0.4 Aa

On the table, the same letters in lines and columns express no difference statistically, different letters express a difference statistically. Capital letters were coded for lines; small letters were coded for columns.

3.2. Sensory Properties

From the evaluation of the sensory scores, there was no statistically significant difference between the nisin added samples and the control group. Differences were observed between the natamycin added meatball samples and the nisin added samples and the control group. The meatballs with the highest scores were the nisin added meatball samples, as shown in the table.

SAW method used for specifying the alternatives were directly used and normalized of the sensory scores. The normalized and weighted normalized values of the analyses results are shown in Table 5.

Using the SAW technique, the NS-5 sample had the highest value indicating that NS-5 was the best sample.

Table 5. Sensory Property Results of Meatball Samples.

	0^{th} day	5^{th} day	10^{th} day	15^{th} day
		Juiciness		
Control	6.87±0.3 Aa	5.35±0.5 Aa	5.29±0.5 Aa	5.00±0.4 Aa
NS-2.5	6.88±0.4 Aa	6.78±0.7 Ab	6.56±0.5 Ab	6.46±0.3 Ab
NS-5	7.50±0.5 Aa	7.01±0.6 Ab	6.79±0.7 Ab	6.66±0.7 Ab
NT-2.5	7.77±0.6 Ba	7.68±0.6 Bb	7.00±0.6 Bb	6.89±0.6 Bb
NT-5	6.41±0.4 Ca	6.35±0.6 Cb	5.67±0.6 Ca	5.45±0.6 Ca
		Crispness		
Control	6.00±0.6 Aa	6.00±0.6 Ab	5.98±0.4 Aa	5.87±0.6 Aa
NS-2.5	6.04±0.5 Aa	6.00±0.7 Ab	5.97±0.7 Aa	5.80±0.4 Aa
NS-5	6.13±0.3 Aa	6.00±0.5 Ab	5.98±0.6 Aa	5.89±0.4 Aa
NT-2.5	6.12±0.2 Ba	6.10±0.6 Bb	6.09±0.7 Bb	6.00±0.7 Bb
NT-5	5.50±0.8 Cb	5.45±0.4 Ca	5.00±0.6 Ca	4.86±0.5 Cc
		Colour		
KT	6.17±0.6 Aa	6.00±0.6 Ab	5.97±0.6 Aa	5.87±0.6 Aa
NS-2.5	6.96±0.5 Aa	6.87±0.6 Ab	6.78±0.5 Ab	6.50±0.5 Ab
NS-5	7.08±0.5 Aa	7.00±0.4 Ab	6.98±0.8 Ab	6.50±0.4 Ab
NT-2.5	6.65±0.7 Ba	6.30±0.3 Bb	6.21±0.4 Bb	6.00±0.8 Bb
NT-5	5.95±0.9 Cb	5.76±0.6 Ca	5.53±0.4 Ca	5.21±0.8 Ca
		Strange taste and Odour		
Control	6.52±0.5 Aa	6.32±0.7 Ab	6.00±0.6 Ab	5.98±0.7 Aa
NS-2.5	6.68±0.4 Aa	6.56±0.8 Ab	6.45±0.8 Ab	6.56±0.5 Ab
NS-5	7.42±0.3 Aa	7.40±0.9 Ab	7.34±0.5 Ab	7.00±0.6 Ad
NT-2.5	7.12±0.2 Ba	7.00±0.6 Bb	6.78±0.7 Bb	6.50±0.6 Ba
NT-5	5.18±0.7 Cb	5.09±0.7 Ca	4.99±0.3 Cc	4.87±0.6 Cc
		Flavour		
Control	6.65±0.5 Aa	6.34±0.4 Ab	6.12±0.2 Ab	6.00±0.5 Ab
NS-2.5	7.08±0.6 Aa	7.00±0.6 Ab	6.87±0.4 Ab	6.56±0.7 Ab
NS-5	7.50±0.4 Aa	7.45±0.7 Ab	7.00±0.5 Ab	6.98±0.7 Ab
NT-2.5	6.65±0.5 Ba	6.45±0.8 Bb	6.30±0.4 Bb	6.00±0.9 Bb
NT-5	4.55±0.5 Cc	4.34±0.4 Cc	4.00±0.6 Cc	3.99±0.6 Cc
		Overall taste		
Control	7.05±0.4 Aa	7.00±0.6 Ab	6.98±0.6 Ab	6.94±0.2 Ab
NS-2.5	7.52±0.2 Aa	7.45±0.3 Ab	7.30±0.7 Ab	7.17±0.2 Ad
NS-5	7.46±0.7 Aa	7.36±0.5 Ab	7.12±0.6 Ab	7.00±0.4 Ad
NT-2.5	6.85±0.6 Ba	6.54±0.6 Bb	6.34±0.7 Bb	6.00±0.2 Bb
NT-5	5.00±0.6 Cc	4.98±0.7 Cc	4.87±0.7 Cc	4.65±0.1 Cc

On the table, the same letters in lines and columns express no difference statistically, different letters express a difference statistically. Capital letters were coded for lines; small letters were coded for columns.

Table 6. Pair wise comparison matrix of alternatives based on the criteria and overall score of the alternatives obtained from SAW.

Normalized Comparison Matrix					
Sample	Colour	Juiciness	Strange taste and odour	Flavour	Overall taste
Control	0.45	0.53	0.45	0.41	0.39
NS-2.5	0.44	0.39	0.41	0.41	0.45
NS-5	0.51	0.49	0.55	0.62	0.59
NT-2.5	0.41	0.41	0.41	0.38	0.38
NT-5	0.41	0.40	0.39	0.36	0.39
Weighted Normalized Matrix					
Sample	Colour	Juiciness	Strange taste and odour	Flavour	Overall taste
Control	0.076	0.030	0.050	0.115	0.153
NS-2.5	0.074	0.021	0.046	0.115	0.177
NS-5	0.085	0.027	0.062	0.173	0.228
NT-2.5	0.068	0.023	0.046	0.105	0.148
NT-5	0.068	0.022	0.043	0.099	0.151

Table 7. Distance from positive (D^+), negative (D^-) and ratio values of each alternative for technique for order preference by similarity to ideal solution (TOPSIS) techniques.

Sample	D^+	D^-	R
Control	0.0961	0.0214	0.1821
NS-2.5	0.0801	0.0337	0.2961
NS-5	0.0024	0.1121	0.9787
NT-2.5	0.1076	0.0071	0.0618
NT-5	0.1097	0.0037	0.0323

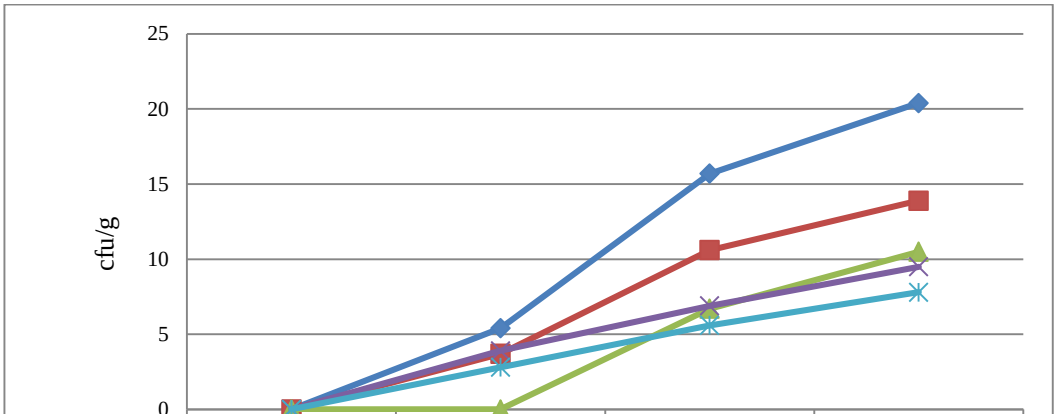
The calculations of Eqs. 3, 4and 5 were made using the negative (D^-), positive and (D^+) ideal solution values of the alternatives as shown in Table 7. With the aim of arranging the alternatives, Eq. 5 was used to obtain the R value. When compared to the other methods, the best sample was seen to be NS-5 (sample included 5g nisin). The results obtained using TOPSIS and SAW are presented in Table 8.

Table 8. Scores of the meatball samples on the sensory alternatives with different multi-criteria decision techniques.

Samples	SAW	TOPSİS
Control	3	3
NS-2.5	2	2
NS-5	**1**	**1**
NT-2.5	4	4
NT-5	5	5

3.3. Microbiological Properties

The Total Aerobic Mesophilic Bacteria Count (TAMB) value must be under 5 $\log_{10}$ cfu/g, according to microbiological criteria of the Turkish Food Codex. Using this reference, the microbiological qualities of the meatball samples which included natural antimicrobials were determined during the storage period and the time, when the TAMB value of the samples reached 5 $\log_{10}$ cfu/g. During the storage period, the TAMB of the meatball samples increased but this rise was less in samples including nisin and natamycin. The amount of antimicrobial increase and the decrease in number of microorganisms are shown in Figure 2.



	0th day	5th day	10th day	15th day
Control	0	5,40	15,7	20,4
NS-2.5	0	3,7	10,6	13,9
NS-5	0	0	6,7	10,5
NT-2.5	0	3,9	6,9	9,5
NT-5	0	2,8	5,6	7,8

Figure 2. Total Aerobic Mesophilic Bacteria.

4. CONCLUSION

In this study, nisin and natamycin were added to meatball formulations and the effects were evaluated by physical, chemical, sensory and microbiological analyses. As a result of the analyses on 0^{th}, 5^{th}, 10^{th} and 15^{th} days, the samples were compared with control groups and statistical differences between the control groups and the samples were detected in respect of moisture, ash, fat content and pH values.

The microbiological results showed less total aerobic mesophilic bacteria in samples including nisin and natamycin in comparison with the control groups and there was an inverse ratio of the amount of antimicrobial to the number of microorganisms. The samples including nisin and natamycin had similar sensory scores to the control groups but the most accepted one was the meatball sample with 5g nisin added.

Many different enzymes and antimicrobials produced by microorganisms are used for the elimination of foodborne pathogens which threaten consumers and public health. The utilisation of antimicrobials such as nitrite, nitrate and sorbate over permitted limits to meat products has a negative effect on human health and this inhibits the use of these additives. Therefore, it has become more common to use enzymes and microbial products which have been determined to be safe by a series of studies and have been accepted by the United States Food and Drug Administration (FAO). In addition to antimicrobial activities, to be natural, colourless, and odourless are important for the properties of meat products. These additives have a protein and peptide structure and ae therefore affected by the gastric secretions and proteolytic pancreatic enzymes rendering them digestible by humans.

The results of this study showed that the use of these antimicrobials is acceptable in meatballs and these antimicrobials can also prolong the shelf life of meatballs. As seen on results of analyses, no physical or chemical differences were identified between the samples, the sensory property values of the 5 g nisin added group were the best according to the panellists.

ACKNOWLEDGMENTS

The study was founded by Cumhuriyet University Scientific Research Projects (CUBAP-YMYO-002).

REFERENCES

[1] Eilers J.R., New foods provide new food safety challenges. Food Process. 1990; 51: 104-108.

[2] Smith-Palmer A., Stewart J., Fyfe L., The potential application of plant essential oils as natural food preservatives in soft cheese. Food Microbiol. 2001; 18: 463-470.

[3] Gould G.W., Industry perspectives on the se of natural antimicrobials and inhibitors for food applications. J. Food Prot. 1996; 59: 82-86.

[4] Hampikyan H., Çolak H., Nisin ve gıdalardaki antimikrobiyal etkisi. TSK Koruyucu Hekimlik Bülteni 2007; 6: 142-147.

[5] Bruno M.E., Montville T.J., Common mechanistic action of bacteriocins from lactic Acid bacteria. Appl. Environ. Microbiol. 1993; 59, 3003.

[6] Andersson R., Biopreservation of foods by means of starter cultures. International Seminar Progress in Food Microbiology 1989.

[7] Foegeding P.M., Thomas A.B., Pilkington D. H., Klaenhammer T. R., Enhanced control of Listeria monocytogenes by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 1992; 58: 884.

[8] Okereke A., Montville T., Bacteriocin inhibition of Clostridium botulinum Spores by Lactic Acid Bacteria. J. Food Prot.1991; 54: 349-353.

[9] Davidson P.M., Harrison M.A., Microbial adaptation to stresses by food preservatives. Microbial Stress Adaptation and Food Safety, 3rd edition, CRC Press, 2003.

[10] Lemay M.J., Choquette J., Delaquis P.J., Gariépy C., Rodrigue N., Saucier L., Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. Int. J. Food Microbiol. 2002; 78: 217-226.

[11] Akkoç N., Şanlıbaba P., Akçelik M., Bakteriyosinler: alternatif gıda koruyucuları. Erciyes Üniversitesi Fen Bilim. Enstitüsü Derg. 2009; 25: 59-70.

[12] Rose N.L., Sporns P., Stiles M.E., McMullen L.M., Inactivation of nisin by glutathione in fresh meat. J. Food Sci. 1999; 64: 759-762.

[13] Stergiou V.A., Thomas L.V., Adams M.R., Interactions of nisin with glutathione in a model protein system and meat. J. Food Prot. 2006; 69: 951.

[14] Yılmaz L., Kurdal E., Peynir muhafazasında kullanılan doğal bir antimikrobiyal: natamisin. Gıda /The Journal of Food 2005; 30: 6.

[15] Ünlütürk A., Turantaş F., Gıda Mikrobiyolojisi. İzmir: Meta Basım, 2003.

[16] Gökalp H.Y., Kaya M., Tülek Y., Zorba Ö., Et ve Ürünlerinde Kalite Kontrolü ve Laboratuar Uygulama Kılavuzu, 2nd edition. Erzurum: Atatürk Ünv. Ziraat Fak. Ofset Tesisi, 1995.

[17] Soyer A., Et İşleme Teknolojisi Dersi Uygulama Notları. Ankara, 2008.

[18] Sallam K.I., Ishioroshi M., Samejima K., Antioxidant and antimicrobial effects of garlic in chicken sausage. LWT - Food Sci. Technol. 2004; 37: 849-855.

[19] Afshari A., Mojahed M., Yusuff R., Simple additive weighting approach to personnel selection problem. Int. J. Innov. Manag. Technol. 2010; 1: 511-515.

[20] Hwang C.L., Yoon K., Methods for multiple attribute decision making. Lecture Notes in Economics and Mathematical Systems 1981; 58: 191.

[21] Pohekar S.D., Ramachandran M., Application of multi-criteria decision making to sustainable energy planning-A review. Renew. Sustain. Energy Rev. 2004; 8: 365-381.