PRODUCTION OF ANTIMICROBIAL PAPER WITH NATAMYCIN AND METHYLCELLULOSE AND EVALUATION OF ITS PRINTABILITY

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Abstract: *Food preservation has become one of the important research topics due to developing technology and decreasing resources. One of the methods used to increase the shelf life of food is to make antimicrobial packaging. A package should both promote the product well and protect it. For this reason, the production of printable and antimicrobial packaging paper was investigated within the scope of this study. In this study, methyl cellulose biopolymer containing natamycin in different amounts (0;2,5;5;7.5;10) to produce antimicrobial paper was successfully coated on office paper in a laboratory paper coating device and its antimicrobial properties were measured by disc diffusion method. The gloss and color values of the obtained papers were determined and offset test prints were made with IGT C1. Color and gloss properties of printed papers were measured. As a result, an effective antimicrobial paper was produced with natamycin. The antimicrobial properties of the produced papers increased due to the increased amount of natamycin. In addition, it has been found that the produced papers are suitable for offset printing.*

Keywords: *natamycin, biopolymer, paper coating, antimicrobial, printing*

INTRODUCTION

In recent years, we have been encountering more and more researches on increasing food quality and safety of food both in cultivation and during transportation and storage due to reasons such as increasing food need and population, awareness of people. Contaminations that occur after the production of food until it reaches the end consumer and the deterioration of food as a result of the growth of microorganisms lead to serious diseases in humans or economic losses resulting from the disposal of food without consumption [1].

The chemical changes in the structure of the food and the growing microorganisms during the transportation, storage and sale of the food cause the food to deteriorate and reduce the quality [2]. Unsuitable conditions and poor packaging can be counted among the main causes of these deteriorations in the storage and transportation of foods. These food spoilages increase the risk of diseases such as poisoning [3]. Contamination of foods by microorganisms poses great effect on the shelf life and sensory properties of the foods and also causes significant health and economic concerns.

Antimicrobial substances can be applied directly on the food in order to prevent the microorganisms that reproduce in the food under these unsuitable conditions. In these applications, dipping, spreading with spray, coating methods can be used. However, direct application of antimicrobial agents to food may cause some adverse effects in food. While researches for safer food processing continue, it is seen that there are different chemicals in the literature that prevent the growth of microorganisms. Among them, organic acids and their salts, ammonium compounds, amines and sulfites appear [4]. Because of people's health concerns, consumers do not want to use antimicrobial agents in direct contact with food. However, excessive use of antimicrobial agents causes problems by making microorganisms resistant [5]. Today, due to increasing health concerns, antimicrobial materials produced from natural substances such as milk and plants attract attention. Such materials are of edible origin. The use of naturally sourced antimicrobial packaging materials eliminates many environmental and health concerns [5] [6].

Biopolymers that can be used in smart packaging materials not only act as carriers against the antimicrobial agents used, but also add moisture and oxygen barrier properties to the packaging material [7] [8].

Cellulose is the most abundant biopolymer of natural origin. It is found in almost all plants. It is a polymer formed by the combination of many cellulose units with beta1,4 glycosic bonds. Cellulose in its unprocessed form is insoluble in water due to its polymeric arrangement [9]. However, some derivatives of cellulose become soluble by etherification through the hydroxyl groups on them [10]. Examples of water-soluble cellulose derivatives are methyl cellulose and hydroxypropyl cellulose. Thus, cellulosic films can be formed. These films from cellulose derivatives are generally transparent and have good barrier properties [11].

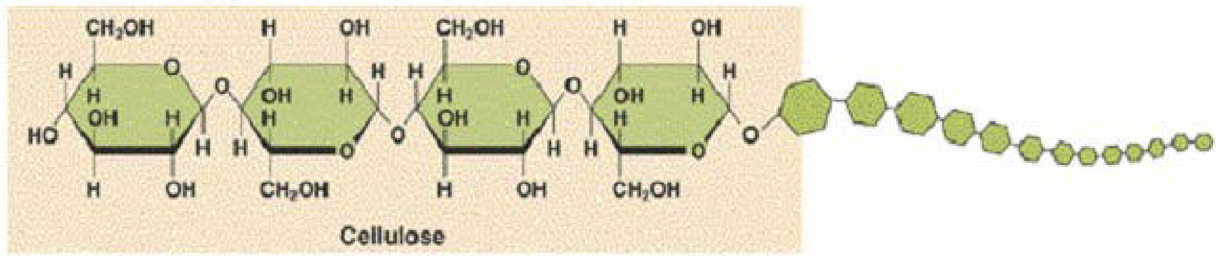

Figure 1: Structural of cellulose

Methyl cellulose is a water-soluble cellulose derivative. The synthesis of methyl cellulose is carried out by combining cellulose with methyl chloride at the appropriate pressure and temperature [10] [12]. The shape of methyl cellulose is given in figure 2. The films and coatings of methyl cellulose are transparent, flexible and have good barrier properties [11] [12].

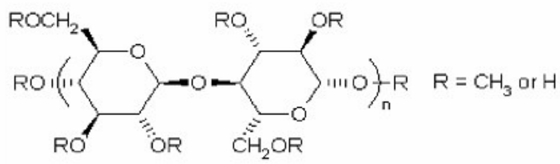

Figure 2: Chemical structure of Methyl Cellulose

The use of coatings formed with edible polymers and naturally sourced antimicrobial materials as active packaging material in the food industry attracts attention in terms of health and sustainability. Examples of naturally derived antimicrobial agents added to edible biopolymer are oils produced from plants (rosemary, mint, lavender), food additives nisin etc and some enzymes can be given [13].

Natamycin is a good antibiotic produced from Streptomyces natalensis and is used as a growth inhibitor in fungi and molds. (Stark 2003). The reason why natamycin is used in the food industry is that it has no negative effects on health and visibly reduces reproduction even in low amounts. Another reason for use is that it does not cause a change in taste and color in delicatessen products consumed in daily life. The fact that it does not inhibit the growth of good bacteria does not affect the ripening process in processed foods [14].

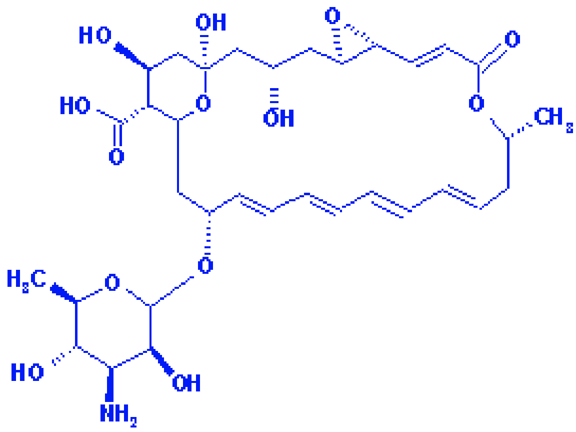

Figure 3: Chemical structure of natamycin

In this study, edible bio based polymer Methyl cellulose and Natamycin antimicrobial additive used to produced for active packaging applications. Natamycin was added to methyl cellulose at different rates as a solid additive and the resulting coated papers were treated with two different bacterial species and two fungi species. In addition, color and gloss of the obtained papers were evaluated. In addition, some printability properties that are absolutely necessary for packaging have been determined.

MATERIAL AND METHOD

In the first part of the study, coating formulations with biopolymer methyl cellulose binder and natamycin antimicrobial agent were prepared according to Table 1. The paper sample described as F0 is an untreated 80 g/m2 office paper. It was used as a control group.

Table 1: Paper coating formulations

Formulation Number	Methyl Cellulose (gram)	Water (gram)	Ethanol	Natamycin (gram)
F1	10	45	45	0
F2	10	45	45	2.5
F3	10	45	45	5
F4	10	45	45	7.5
F5	10	45	45	10

Before starting the coating process, the papers to be used were conditioned for 48 hours under laboratory conditions. In the preparation of coating formulations, a 10 % methyl cellulose / distilled water:ethanol mixture was first prepared. And while the mixture was stirred at 750 rpm with a magnetic stirrer, it was heated to 80 degrees Celsius and stirred at this temperature for about 10 minutes. Thus, the biopolymer fibers were separated from each other and F1 was obtained. This mixture was cooled to room temperature and the amount of antibacterial agent in Table 1 was added and mixed at 500 rpm and coating formulations were obtained. All the formulations obtained were coated with a laboratory type K303 model Multi-coater (RK Print Coat

Instruments Ltd, United Kingdom) using Mayer Rod 2 at a speed of 2 m/min at a rate of 0.1 g/m^2 on one side of 80 g/m^2 paper at room temperature. The average thickness of the coatings was set at 3 micrometers. The obtained coatings were dried at 25 degrees Celsius for 24 hours at 65% relative humidity. The chemical structure, color, gloss and surface morphology of the obtained coatings were determined by Perkin-Elmer ATR-FTIR, X-Rite eXact spectrophotometer and BYK Gardner glossmeter and Leica optical microscope, respectively.

In the second part of the study; Antibacterial test was performed for untreated paper (F0), surface sized paper (F1), surface coated papers (F2, F3, F4, F5). The antimicrobial activity of the surfaces was determined by the zone of inhibition method (disk diffusion method). Each bacterial culture was activated by inoculation in Tryptic Soy Broth (TSB) for 24 hours at 37 °C. The inoculum (0.1 mL) was spread on the surface of Mueller-Hinton (MH) agar petri dishes using the spread plate technique, then 6 mm diameter films cut from the prepared coated papers were placed on the petri dishes. Petri dishes were incubated at 37 °C for 24 hours. After incubation, petri dishes were checked for bacterial growth, inhibition zones around disc films were evaluated qualitatively and quantitatively. Quantitative evaluation was made according to the preventive zone diameter. The regions around the disc films were evaluated as an indicator of inhibition of bacterial growth. The paper coating, which produces a large zone of inhibition, is thought to exhibit a high antimicrobial activity.

In the third part of the study, background prints were made on uncoated, only surface sized and different coated papers using an equal amount of process magenta ink (DIN ISO 2846-1) with an IGT C1 offset printability test printing machine. The printing parameters were set as 300 N printing pressure, 0.2 m/s printing speed. The ink film thickness of all printed samples was measured as 8 µm. Color measurements of prints made on papers with different coatings were made using the CIE L*a*b* method according to ISO 12647-2:2013 standard using X-Rite eXact spectrophotometer. The measurement conditions of the spectrophotometer were determined with a D50 light source in the 400-700 nm range, with an observer angle of 2° and an open polarization filter in 0°/45° geometry. The difference between the colors of different prints is calculated according to formula 1 according to the CIE ΔE* 1976 ISO 13655 standard.

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

L* black (0) to white (100); a * denotes green (-) to red (+) and b * denotes blue (-) to yellow (+).

The gloss measurements of the coated papers were carried out in accordance with the ISO 8254-1: 2009 standard, with BYK Gardner GmbH micro gloss 75º geometry, and the gloss measurements of the prints were carried out with BYK Gardner GmbH micro Tri-gloss 60º geometry in accordance with the ISO 2813: 2014 standard.

RESULTS

Coating formulations were prepared using natamycin and methyl cellulose and coated on the paper surface under laboratory conditions. The chemical structures of the obtained coated papers were elucidated with ATR-FTIR. Figure 4 shows the ATR-FTIR spectra of F1, F2, F3, F4, and F5.

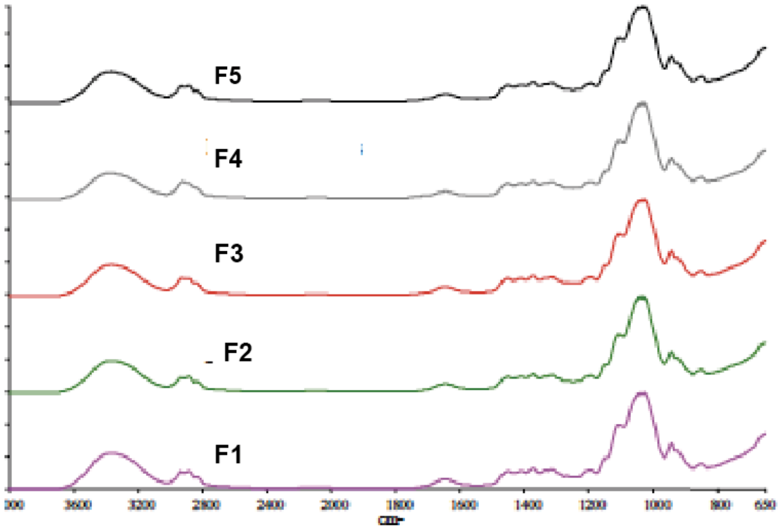

Figure 4: ATR-FTIR spectra of F1, F2, F3, F4, and F5

When the FTIR spectrum of methyl cellulose is examined, the hydroxyl vibration band at 3400 cm^{-1}, the carbon hydrogen vibration band at 2800-2900 cm^{-1}, the stress of carbon oxygen at 1650 cm^{-1} the vibrations of carbon hydrogen around 1450 cm^{-1}, the carbon oxygen carbon vibration at 1050 cm^{-1}clearly reveals. The results are consistent with the literature [15]. FTIR spectra of all natamycin added coatings gave the same result as pure methyl cellulose. This shows that the added natamycin does not react with methyl cellulose [16].

Table 2: Color and gloss values of coatings

Formulation number	L*	a*	b*	ΔE	Gloss
Base paper	95.46	2.91	-10.21	Standart	5.2
F1	91.98	3.6	-11.25	3.7	21.6
F2	91.70	3.7	-10.74	3.89	17.4
F3	90.94	3.6	-10.69	4.6	15.8
F4	90.98	3.7	-10.52	4.5	13.7
F5	90.43	3.5	-10.41	5.1	13.1

CIE L*a*b* color, gloss and color differences of the coatings are given in Table 2. When the colors of the samples are examined, it is seen that the color of the base paper slightly shifted to blue with the addition of surface sizing and natamycin. When the ΔE color differences were compared, it was determined that the surface sized was made compared to the uncoated paper and the coatings with natamycin added were within acceptable limits according to ISO12647-2. In addition, the gloss value of the coatings is given in Table 2. In the surface sizing process applied on the base paper, it was determined that the gloss increased 4 times. The gloss has also increased in natamycin added coatings, but this is less than the residual surface sizing process. Because natamycin did not react with methyl cellulose and created some roughness on the surface, which scattered the light and reduced the brightness a little. In the printing industry, gloss is required for attractiveness. In F1, the paper fibers are filled only with methyl cellulose and the surface is smoothed. Thus, diffuse reflection is reduced and full reflection is approached. This also increased the gloss. With the addition of natamycin to the coating formulation, the gloss decreased at first glance as the roughness would increase [17]. However, this decline has not been so drastic, due to the distinctive brilliance of natamycin.

Table 3: Antibacterial activity of coatings

Formulation number	Inhibition diameter S. aureus (mm)	Inhibition diameter E. coli (mm)	Inhibition diameter A. niger (mm)	Inhibition diameter P.roquefortii (mm
F0	0	0	0	0
F1	0	0	5	5
F2	0	0	7.5	18
F3	0	0	18	22
F4	0	0	23	29
F5	0	0	27	34

The antimicrobial activity of the prepared paper coatings was tested against both gram positive (S. aureus) and gram negative (E. coli) bacteria as well as A. niger and P. roguefortii fungi. The diameter of the inhibition zone of the samples is shown in Table 3. In the control samples, it was observed that all bacteria and fungi grew homogeneously in all parts of the petri dishes. It was determined that both S. aureus and E. coli did not produce any radius of inhibition, that is, natamycin did not have any effect against bacteria. Since there is no antimicrobial agent in F0, any inhibition diameter did not occur as expected. Some antifungal activity was seen in the F1 formulation. This showed that methyl cellulose was slightly inhibitory in fungi. It was found that natamycin had inhibitory effects against A. niger and P. roguefortii in all coatings containing natamycin. However, the best antifungal effect was seen in P.roguefortii. In addition, it was determined that active packaging was more effective against fungi with increasing natamycin amount.

The surface morphology of the coated paper containing the most natamycin belonging to F5 was examined by scanning electron microscopy. Figure 5 shows that the additives in the coatings are homogeneously distributed on the paper surface and the surface is regular and smooth.

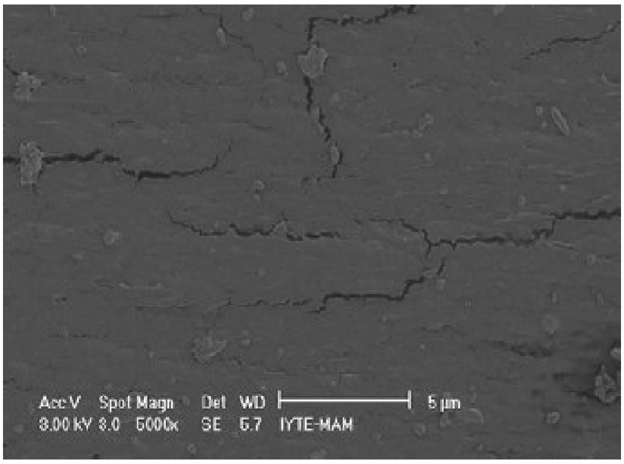

Figure 5: SEM image of F5

Offset test prints were made with IGT C1 on the surface of all coated papers. CIE L*a*b* color, gloss and color differences of the prints obtained are given in Table 4.

Table 4: Color and gloss values of prints

Formulation No	L*	b*	b*	ΔE	Gloss
Base Paper	47.68	74.20	-3.48	Standart	1.6
F1	44.26	74.93	-4.39	3.6	15.7
F2	44.13	74.89	-4.26	3.7	13.2
F3	44.18	74.91	-4.14	3.6	12.6
F4	43.99	74.80	-4.06	3.8	12.2
F5	43.56	74.79	-4.01	4.2	11.7

When Table 4 is examined, it is seen that natamycin tends the color slightly towards blue in printing, as in the colors of the coatings. The ΔE color difference in the coatings has decreased. This shows that the magenta tone of the ink tolerates the color difference. The reason for the decrease in the gloss of all prints is that the pigment in the ink scatters the light to some extent. Thus, the surface roughness and diffuse reflection increased and the brightness decreased.

CONCLUSIONS

In this study, natural antimicrobial agents added methyl cellulose binder paper coatings were produced that would not adversely affect human health even if migrated, and it was aimed to investigate the printability parameters.

Paper coating formulations were prepared using antibacterial agents such as natamycin and successfully coated on the paper surface. The chemical structures of the obtained coated papers were elucidated with ATR-FTIR.

When the color values of the coatings were examined, it was concluded that while natamycin did not cause a great change in color, it increased the gloss approximately three times. According to these results, it was found that the natamycin added coating was better than the uncoated papers in terms of printability. When the antimicrobial effect was examined, it was found that natamycin antimicrobial agent did not have any effect on gram positive and gram negative bacteria, but inhibited two different types of fungi and had a protective feature in delicatessen product packages such as cheese and salami.

Offset prints were made on coated papers containing natamycin, and it is determined that natamycin tends the color slightly towards blue in printing, as in the colors of the coatings. The ΔE color difference in the printings has decreased. This shows that the magenta tone of the ink tolerates the color difference. The gloss value of the coated papers in the printed samples was higher than the reference paper. However, it is inferior to unprinted papers. Because the pigments in the ink caused the light to scatter and reduced the gloss.

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