THESES OF THE PH.D. DISSERTATION

DEVELOPMENT OF CELLULOSE-BASED SHEETS CONTAINING ACTIVE COMPONENT FOR PACKAGING TECHNOLOGY APPLICATIONS

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ABSTRACT

In the course of the research, potassium humate was made from Dudar brown coal, and cellulose fiber-based test sheets were prepared by the addition of humate and copper sulfate. The purpose of composite fabrication was to produce sheets of higher copper content utilizing the cationic and releasing properties of humate and to analyze their applicability as active packaging.

The characteristics of the prepared test sheets were determined by various test methods, such as FT-IR spectroscopy, color composition test, short span compression measurement, tensile and bending test, examination of porosity, scanning electron microscopy, surface composition determination. The antioxidant capacity of the test sheets was investigated by DPPH method. In addition, the antimicrobial activity of the test sheets was also inspected with various bacterial and fungal isolates. The difference in time of blackberry deterioration was observed for packed blackberry grains. The amount of copper dissolved in both distilled water and pH 5 solution as well as the VIS spectra of the solutions were also examined.

Based on the experienced results, it was found that the humate improved the mechanical properties of the test sheets. The results of FT-IR, SEM and EDS measurements showed that humate increased the copper content of the test sheets. The copper sulfate added to the fiber suspension was present in the test sheets as various copper particles. Based on the results of the microbiological measurement, the test sheets prepared by adding humate and copper sulfate indicated good antimicrobial

activity. Similar results were observed in the deterioration of blackberries packed in the same airspace with test sheets. The test sheet prepared with the addition of humate only was not effective in microbiological inhibition, yet it slowed the deterioration and mold growth of blackberry.

INTRODUCTION

Shelf life of packaged foods is mainly influenced by the presence of microorganisms, fungi and bacteria. The most serious disease is caused by Listeria genera among the bacteria that cause deterioration which can reproduce even under chilled conditions, and modified atmosphere packaging (MAP) does not inhibit its reproduction either. So, even frozen vegetables can be a source of human listeriosis. In 2018, several frozen vegetables were recalled by the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC) to curb an epidemic caused by an aggressive bacterial variant of Listeria monocytogenes (EFSA 2018).

1.3 billion tons of food waste is produced every year (Food and Agricolture 2015, Babbit et al. 2017) which has economic consequences and harmful to the environment. The production of food waste leads to wasting valuable resources and has a negative impact on the environment. Generating and disposing waste entails the release of 170 million tons of CO_2, and 8% of greenhouse gases are generated during disposal (Islam et al. 2016, Salim et al. 2017).

It is an important task to prevent food from becoming waste and to prevent developing food borne diseases, in which developing active packaging that increases shelf life has a major role to play. The modern packaging materials should not only protect the product, the food, from physical damage, but they should also provide active microbiological protection for shelf life of the food (Dashipour et al. 2014, Sahraee et al. 2017).

THE AIM OF THE RESEARCH

The aim of the doctoral research was to produce an economical, cellulose-based, antimicrobial active packaging containing copper, which can be easily integrated into the papermaking process. The sheets were prepared by the addition of humate and copper sulfate pentahydrate. The purpose of the research was to study the extent which the copper content of the sample sheets could be increased by the addition of humate to the sheets during sheet forming. The prepared sample sheets were tested for antimicrobial activity both in vivo and in vitro. A precondition for conducting the in vitro experiment is to develop a novel microbiological measurement method that can also be used for slow-acting substances.

MATERIALS AND METHODS

During the research, potassium humate was prepared from Dudari brown coal. First, the brown coal was ground to powder and then 50 ml of 5M KOH solution was added which was stirred for 3 hours with a magnetic stirrer. It was left at room temperature for one night. To obtain the humate, the material was centrifuged the next day at 2400 rpm for 10 minutes.

During the preparation of the fiber suspension, copper sulfate pentahydrate and potassium humate were added to the linter cellulose fiber suspension. Then, from the suspensions thus prepared, sample sheets were formed using a sheet former.

To prepare the fiber suspension, $CuSO_4$ was added to 250 ml of cellulose (containing 7 g of dry matter) fiber suspension (stirred in a magnetic stirrer) so that the pH of the suspension was 4.5 (~ 5 g). After leaving it at room temperature for one day, various amounts of potassium-humate solution were added and stirred for 80 minutes at 200 rpm with a magnetic stirrer. The fiber suspension made with or without the addition of copper sulfate (control) was also stirred for 80 minutes at 200 rpm. The fiber suspension M 2 contained 5 g of copper sulfate pentahydrate. The composition of the sample sheets produced is shown in Table 1.

Table 1: Composition of suspensions for preparation of different test sheets

Test sheet	Content (dry weight ratio)
S 1	Control
S 2	Copper-cellulose
S 3	1:0,1 Copper-cellulose : potassium-humate
S 4	1:0,25 Copper-cellulose : potassium-humate
S 5	1:1 Copper-cellulose : potassium-humate
S 6	1:5 Copper-cellulose : potassium-humate
S 7	1:10 Copper-cellulose : potassium-humate
S 8	1:1 Copper-cellulose : potassium-humate

Various tests were performed to determine the properties of the sheets produced, including mechanical properties, air permeability, surface morphology and composition, antioxidant capacity, antimicrobial activity in in vivo and in vitro experiments, and the amount and composition of particulate solutes. Furthermore, the amount and composition of the particles dissolved from the test sheets were examined.

SUMMARY OF RESEARCH RESULTS

Summarizing the results, it can be stated that the composite test sheets produced have antibacterial, antifungal and antioxidant properties and are, therefore, able to extend the shelf life of vegetables and fruits packaged in the produced paper.

Surface composition, scanning electron microscope images, and FT-IR spectra indicate that a bond was formed between humate and copper. Based on the surface composition analysis, humate increases the copper content of the sample sheets – by ~ 80-220 percent compared to the sample sheet made with copper sulfate only. SEM images of the surface of the test sheets contain particles of various shapes and sizes, suggesting that there are several types of copper-based compounds present in the sample sheets. Those two new absorbances, in the wavenumber ranges of 1681-1659 cm^{-1} and 1525-1522 cm^{-1}, observed in the FT-IR spectrum of the test sheets made with the addition of humate and copper sulfate, indicate bond formation between the humate and the copper particles.

The dissolution test showed significantly higher copper ion dissolution from test sheets M 3 and M 4 in distilled water and pH 5 media than from the other samples prepared with the addition of copper sulfate (M 2, M 5-7). It is presumable that copper is also present in these sample plates in the form of $CuSO_4$, as shown by the VIS absorption spectra In the case of sample sheets with a higher content of humate, the more the humate content increases, the more the copper dissolution decreases in both media. The reason is that higher amounts of humate have already formed the structure of the micelle, which

contains copper ions that have not dissolved in distilled water and pH 5 media.

Based on the results of the mechanical stress test of the samples, the addition of copper sulfate pentahydrate to the cellulose suspension deteriorates the mechanical properties of the sheets. This is due to acidic hydrolysis, which removes fine, thin fibrils from the fiber surface. The humate added to the test sheet increases the interactions between the fibers, thereby increasing the tensile and compressive strength of the test sheets.

The test sheets have an antioxidant capacity which depends on the humate content of the sample sheet. Sample sheets containing humate in the weight ratios of 1: 5 (M 6) and 1:10 (M 7) had the highest antioxidant capacity. The sample sheet M 8 also has measurable antioxidant capacity, which was made by adding humate only. During the study the antioxidant capacity of the sample sheets were compared at the 1st, 4th and 24th hours. The results showed that the values increased by 24 hours, indicating the slow mechanism of action of the sample sheets.

Due to the slow mechanism of action of the sample sheets, it was necessary to develop a new type of microbiological method in order to test their antimicrobial activity. According to the new type of disk diffusion method, the control (M 1) and the test sheet (M 8) made only with the addition of humate did not inhibit the growth of bacterial and fungal isolates, and had no antimicrobial activity. In contrast, sample sheets (M 3-7) made with the addition of copper sulfate and humate effectively inhibited the growth of bacteria and fungi on the agar plates.

The isolates tested were most effective in inhibiting the growth of molds, which soon appeared on the surface of vegetables and fruits, which started to deteriorate, it is therefore important to protect against them, thereby increasing food safety. The sample sheets M 3-7 were also effective in inhibiting the growth of Listeria genera, which can cause dangerous human listeriosis in the human body. The results of the in vivo study of blackberry showed similar results to the new disk diffusion method. The sample sheets M 2-8 efficiently slowed down the deterioration of blackberry compared to the control sample; the sample sheets M 3-5 were most effective.

According to the results of the antimicrobial test, the test sheet (M 2) made only with the addition of copper sulfate showed a lower level of antimicrobial activity with measurably higher copper (II) ion dissolution, than those sample sheets showing lower (M 5-7) copper dissolution (in pH 5 medium). In distilled aqueous media, the dissolution of sample sheets M 2 and M 6 were approximately the same, while sample sheets M 5 and M 7 showed less, but their antimicrobial properties (M 5-7) were better compared to the sample sheet M 2. The results suggest that other copper compounds that are not present as copper (II) ions may also play a role in antimicrobial activities.

THESES OF THE DISSERTATION

Based on the results of the detailed experimental studies, I make the following scientific findings:

1. It was found that the mechanical properties of cellulose-based sheets can be improved with adequate humate content. Compared to the sheet (M 2) made with the addition of copper sulfate only, sheets containing humate (M 3-6) in a weight ratio of 1: 0.1, 1: 0.25, 1: 1 or 1: 5 increased their elongation at break by ~ 6-40 percent, tear index by ~ 7-21 percent, SCT index by ~ 4-44 percent, and bending index by ~ 18-54 percent. Compared to the control sheet (M 1), the sheet containing humate (M 3-6) in a weight ratio of 1: 1 increased its elongation at break by ~ 6 percent, tear index by ~ 32 percent, SCT index by ~ 13 percent, and bending index by ~ 52 percent. [3,4]

2. Based on the SEM-EDS results it can be stated that humate can be used to increase the copper content of cellulose-based sample sheets. Copper and humate compounds are present in the form of particles of different sizes and shapes. The new bands appearing in the FT-IR spectrum of the composite sample sheets indicate the presence of new bonds between the humate and copper components. [1, 2]

3. It was found that composite sheets have antioxidant capacity. Increasing the humate content of the sheets increases the stable DPPH radical binding. Comparing the capacities measured at the 1st, 4th and 24th hours, it can be concluded that the antioxidant capacity of the composite sheets is significantly higher at the 24th hour, compared to the control sheet, which proves the time-dependent mechanism of action of the sheets.

4. I have proved that the developed modified disk diffusion method can be successfully applied to study the antimicrobial activity of slow-acting substances. The modified test method can be easily integrated into the measurement procedures currently used in microbiology. [1]

5. In contrast to bacterial and fungal isolates, humate-containing sheets with the addition of copper sulfate pentahydrate exhibit better antimicrobial activity than that prepared by adding $CuSO_4 * 5H_2O$ only. These results were also supported by the in vivo study of blackberries, where composite sample sheets were the most effective in slowing down the degradation process. [1]

6. It was confirmed by VIS spectra of solutions soaked in distilled water and pH 5, as well as by the determination of the amount of dissolved copper (II) ion that the amount of dissolved Cu (II) ion

depends on the pH of the medium. The absorbance characteristic of copper sulfate could only be observed in the VIS spectra of solutions of low humate content sheets. New absorbances indicating the humate copper complex can be seen in the spectrum of each composite sheet. [1]

PUBLICATIONS

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