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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Designing New Multifunctional Food Pads Using Red Cabbage (*Brassica oleracea*) Extract

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Abstract: Packaged fresh food is one of the factors that negatively affect the shelf life; It is at the forefront that the liquid percolated by food accumulates inside of the package, accelerating chemical and microbial spoilage and finalized with reducing food quality. In order to prevent the accumulation of liquid in the package, the absorbent pads disposed therein are partially presented as a solution of the problem. The aim of this research is to enrich absorbent pads with naturally derived antioxidants to make them more functional and extend the shelf life of food. It is also possible to easily inform the consumer about the quality of food with the expected color change in the absorbent pad. This is the first study by making use of the extract of red cabbage plant and preparing absorbent pads by combining with cellulose for examination of color change on the pad through microbial growth by time. In this study, 25 gr of trout sample were used to observe color change on the fresh prepared food pads. Each pad was prepared using 5 ml of red cabbage extract (1:1 ratio) and dried. *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25893, and *Staphylococcus aureus* 25922 were inoculated to each fish sample to make the microbial growth faster. After 12 hours, color change on the pad was observed with a naked eye that purple-red cabbage color was changed to greenish-blue. However, pH measurements also showed a similar result with the color change and pH value (6.3) observed in the fresh fish increased to 9.0 on day 3.

Keywords: Absorbent pad, food safety, pH indicator, sensor, shelf life, red cabbage.

Kırmızı lahana (*Brassica oleracea*) özütü kullanılarak yeni çok fonksiyonel gıda pedi tasarımı

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Öz: Gıdadan süzülen sıvının ambalajın içinde birikmesi, kimyasal ve mikrobiyal bozulmayı hızlandırması ve gıda kalitesini düşürmesi ile sonuçlanması paketlenmiş taze gıdaların raf ömrünü olumsuz yönde etkileyen faktörlerden biridir. Ambalajın içinde sıvı birikmesini önlemek için, gıda paketinin içine yerleştirilmiş emici pedler kısmen problemin bir çözümü olarak sunulmaktadır. Bu araştırmanın amacı, emici pedleri doğal olarak elde edilen antioksidanlarla daha işlevsel hale getirmek ve gıdanın raf ömrünü uzatmaktır. Emici ped de beklenen renk değişikliği ile tüketiciyi gıda kalitesi hakkında kolayca bilgilendirmek de mümkündür. Kırmızı lahana bitkisinin ekstraktının kullanılması ve mikrobiyal büyüme sonucunda ped üzerindeki renk değişiminin incelenmesi için selüloz ile birleştirilerek emici pedlerin hazırlanmasıyla yapılan ilk çalışmadır. Bu çalışmada, hazırlanan taze gıda pedlerinde renk değişimini gözlemlemek için 10 gr alabalık örneği kullanılmıştır. Her ped için 5 ml kırmızı lahana özütü (1:1 oran) kullanılarak hazırlandı ve pedler kurutuldu. Mikrobiyal büyümeyi daha hızlı hale getirmek için her balık örneğine *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25893 ve *Staphylococcus aureus* 25922 inoküle edildi. 24 saat sonra çıplak gözle ped üzerinde renk değişimi gözlendi ve mor kırmızı lahana rengi yeşilimsi maviye dönüştü. Bununla birlikte, pH ölçümleri de renk değişimi ile paralel sonuç gösterdi ve taze balıklarda gözlenen pH değeri (6,3) 3. günde 9.0'a yükseldi.

Anahtar kelimeler: Gıida güvenliği, gıda pedi, kırmızı lahana, pH indikatörü, raf ömrü, sensör.

INTRODUCTION

Worldwide, food poisoning as a consequence of bacterial deterioration threatens human health and can cause death. Therefore, the aim of producing the healthiest and suitable food packaging materials is to provide the most natural, high quality, and low-cost materials that will not endanger human health and harm the environment. Many researchers are focused on increasing the quality and safety of food for years based on the processing steps and also packaging materials such as coating films, pH-indicator sensors and food additives (Benbettaieb et al., 2019; Cai et al., 2015; Fernadez-Saiz et al., 2013; Fernandes 2016; Hao et al. 2017; Sveinsdottir et al., 2020; Kaewprachu et al., 2015; Kim et al., 2017; Li et al., 2012; Li et al., 2019; Lu et al., 2010; Luchase et al., 2017; Mohan et al., 2012; Netam et al., 2018; Özen & Soyer, 2018; Pezeshk et al., 2015; Prietto et al., 2017). Some researchers are generally used plant extract as an additive to inhibit the lipid and protein oxidation, positively affected on the sensory quality and physicochemical stability, and also inhibition on microbial communities during frozen storage or refrigerator conditions (Rafiq et al., 2018; Hao et al., 2017; Sveinsdottir et al., 2020; Li et al., 2012; Li et al., 2019; Lu et al., 2010; Özen and Soyer,, 2018; Prietto et al., 2017; Sanchez-Alonso et al., 2007; Sanchez-Alonso et al., 2008; Pazos et al., 2005). Some of those researchers also focus on developing smart packaging materials such as bioactive edible films and sensors based on pH or temperature (Benbettaieb et al., 2019; Fernadez-Saiz et al., 2013; Kaewprachu et al., 2015; Kim et al., 2017; Luchase et al., 2017; Song et al., 2011; Prietto et al., 2017; Pourjavahera et al., 2017). The accumulation of liquid into the package could be be reduced the shelf life of packaged fresh foods, especially fish meat. To ensure food safety and extend shelf life, the food product must not come into contact with liquid. As a solution to this problem; the cellulose-based absorbent pads in the packaging have been used as a protective barrier for absorbing these unwanted liquids and have succeeded in minimizing bacterial growth. Moreover, cellulose is the most abundant on earth, a harmless, natural biopolymer of homopolysaccharides. Cellulose also could make intermolecular and intramolecular hydrogen bonds. Cellulose does not show allergic reactions of chitosan, which is another natural biopolymer and is used very actively. In terms of the consumer; clean, attractive and liquid-containing packaged products will be preferred as a manufacturer for long-term consumer mass is very important to have. However, it should be paid attention to produce natural, recyclable and economically suitable materials that do not threaten the health of the consumer and harm to the environment in the materials used in food packaging. In this context, the fact that the absorbent pads are in direct contact with food must be guaranteed to the customer that safe materials are being used and that there are no health hazards to humans and the environment. In this proposed research, it is aimed to design the functional food pad using natural antioxidants obtained from red cabbage extract and apply them to the cellulose absorbent pad. Thus, the absorbent pads will not only absorb unwanted liquids in the food package but are likely to develop on the food by red cabbage antioxidants; it could be prevented or minimized bacterial activity and to extend shelf life of especially perishable food. It is also contemplated that anthocyanins obtained from red cabbage extracts were applied to the absorbent pads to add two additional functions. Thus, the first function is the idea that absorbent pads with antioxidants would help prevent microbial growth in the ready to eat fish sample. The second and essential function is; anthocyanins (components that give the plant red and purple color) to assume the role of pH-indicator of the pads by dyeing and changing the color of these absorbent pads and to give the consumer the color of the pad from purple (pH = 7, neutral) to green-yellow (pH = 11-14, basic). The idea is to give information about the freshness of the food product quickly with a naked eye. All these include protecting the food product against the development of bacteria, extending its shelf life and giving information to the consumer about the freshness of food in a comfortable, natural, and economical way.

MATERIAL AND METHOD

Materials: Cellulose pads were purchased from M&N Hygiene factory in Kocaeli, Turkey. Red cabbages were purchased from the local organic market. *Escherichia coli* ATCC 25893, *Staphylococcus aureus* 25922 and *Salmonella typhi* ATCC 14028 were provided from the Faculty of Pharmacy at Erciyes University.

Extraction of Red Cabbage: Red cabbages (*Brassica oleracea*) were purchased from organic market and copped to fine pieces approximately (1–2 cm). 100 grams of copped red cabbage was weighed in 100 mL distilled water and boiled at 100 °C for 30 minutes. The aqueous extract was filtered by Whatman paper 1 and stored at -20 °C for further analysis and experiments.

Preparation of Food Pad and Sterilization: 5 ml of obtained aqueous red cabbage extract was absorbed into each cellulose pad. All prepared pads that including red cabbage extract were dried in an oven at 45 -50 °C. Dried pads were sterilized and packed one by one for further analysis.

***pH Measurement*:** During pH measurements of sample groups; 3 ml deionized water was added on 3 g sample and the sample was torn into pieces with a blender

for 1 min. And then, pH of the sample was measured at room temperature with a pH meter (Hanna, HI83141. Germany).

Microbial Analysis: Firstly, all the controls of fish samples were taken before being contaminated with the pathogenic microorganisms and then preactivated. In the control samples, *S. aureus*, *E. coli*, and *S. typhi* were investigated according to AOAC (method number: 966.23) methods. For the detection of *S. aureus*, a 25g portion of sample was enriched in Tryptic Soy Broth (DIFCO Laboratories, Detroit, MI) containing 10% NaCl and plated on Mannitol Salt Agar (DIFCO Laboratories). Yellow opaque colonies were selected, and identification of *S. aureus* was confirmed using a biochemical method. For the detection of *Salmonella typhi*., a 25-g portion of the sample was enriched in selenite F broth (Oxoid) and streaked on Eosin Methylene Blue Agar (Oxoid). Typical colonies were purified, and biochemical analysis was carried out using conventional methods according to the AOAC Official Method (AOAC,2002). To identification of *E. coli*, 1 ml suspension was replaced onto the center of film base. *E. coli* colonies appear as blue colonies associated with gas bubbles. A pure culture of three standard strains (*Escherichia coli* ATCC 25893, *Staphylococcus aureus* 25922 and *Salmonella typhi* ATCC 14028) were used to contaminate ready to eat fish. The samples placed on sterile pads (including red cabbage extract and free pads). Then they were inoculated individually with selected pathogenic bacteria, at a density of 0.5 McFarland, and incubated at 37°C. Surviving bacterial populations were evaluated using a non-selective medium (Tryptic Soy Agar (TSA)) for each bacterium. The ready to eat fish samples contaminated with pathogenic microorganisms was checked again after 24 hours. It was seen that the growth of microorganisms exceeded 0.5 McFarland density in terms of colony-forming units per mg/ml. The microorganism's replications were followed at 2 ,4, 6, 12 and 18 hours.

Disk diffusion methods: For determining the antibacterial activity of red cabbage extract, the agar disk diffusion method was applied, as previously reported studies (Baldemir et al., 2017). Briefly, red cabbage extract at 0.5 µg/mL were used to prevent *Escherichia coli* ATCC 25893, *Staphylococcus aureus* 25922 and *Salmonella typhi* ATCC 14028 growth. Bacterial cultures at a concentration of 1.0×10^8 CFU/mL were inoculated with the same concentrations of free red cabbage extract. After incubation of the culture plates at 37 ° C for 24 h, the inhibition region of bacterial growth was measured in millimeters. Independent tests were conducted as triplicates for each bacterial strain.

RESULTS AND DISCUSSION

Some studies conducted suggest that the antioxidants obtained from plant used are not only effective in adding a flavor or taste, but they are also used for the pH indicator effect in food conservation (Kim et al., 2017; Luchase et al., 2017; Prietto et al., 2017; Pourjavahera et al., 2017). In this study, as shown in Figure 1, red cabbage extract was applied to the cellulose food pad as pH sensor. 5 ml red cabbage extract was absorbed each free cellulose pad in figure 2-A, food pads were dried at 45-50 °C until dry as shown in figure 2-B, and then properly dried food pads were sterilized as seen in figure 2-C.

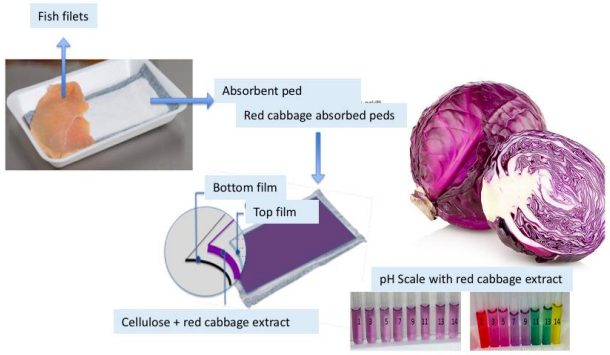

Figure 1. Graphical illustration of red cabbage absorbed food pad preparation.

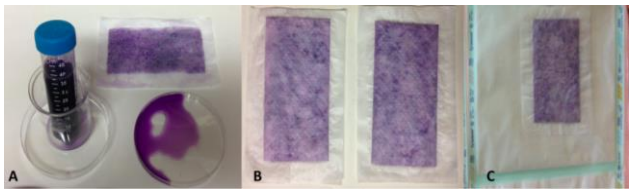

Figure 2. 5 ml red cabbage extract was absorbed each free cellulose pad (A), Dried food pads (B), Sterilized food pad (C).

It was determined that the microorganisms mentioned in the controls did not grow. The fish samples were not contaminated with these pathogenic microorganisms (*S. aureus*, *E. coli*, and *S. typhi*) according to AOAC methods before that were contaminated in laboratory. The microorganism's replications were followed at 2 ,4, 6, 12 and 18 hours. It was shown that microorganisms growth continued ready-to eat fish up to 18 hours. The color change on the pads was obliviously detected with the naked eye 12 hours later.

The color of the pads was turned from purple to green-blue by time at room temperature (Figure 3).

At the same time, both free red cabbage extract and control group (free disc) were tested for comparison of how to effect against microbial activity in Figure 4. There is an apparent difference was measured between control and red cabbage extract absorbed bacterial disc for *S. typhi* (A), *E. coli* (B), and *S. aureus* (C) in Figure 4 and 5. The

inhibition zone of red cabbage extract was respectively measured as 9.00 ± 0.03, 8.60 ± 0.04, and 12.00 ± 0.01 mm for *S. typhi* ATCC 14028, *E. coli* ATCC 25893 and *S. aureus* ATCC 25922.

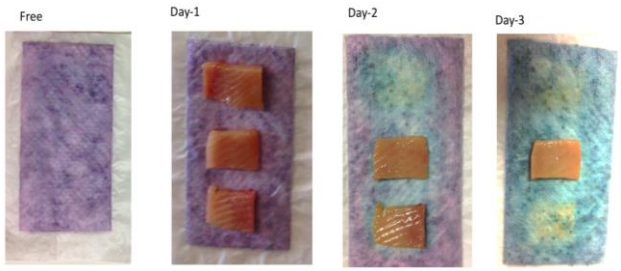

Figure 3. Fillets changed the color of the pad with growing bacteria and changing pH from day-1 to day-3.

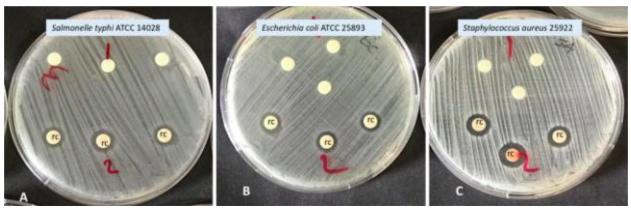

Figure 4. Photos of microbial growth by Disc test of control (without extract) and red cabbage extract (rc) for each bacteria type.

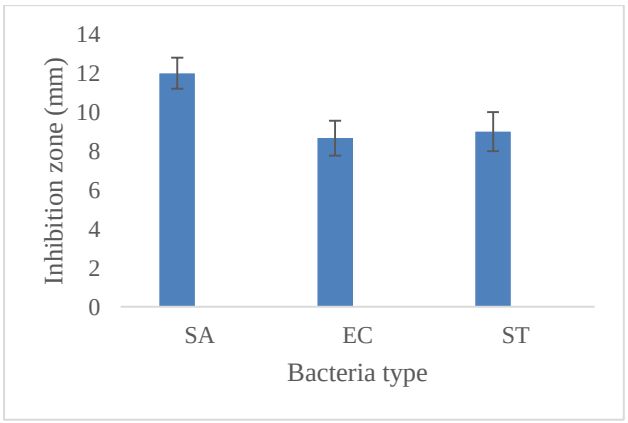

Figure 5. Disc results; inhibition zone (mm) of free red cabbage extract against to SA: *S aureus* ATCC 25922, EC: *E. coli* ATCC 25893, ST: *S. typhi* ATCC 14028.

The transition to an alkaline environment, such as a transition to an acid environment, is stressful for bacteria as shown by how *Escherichia coli* responds to alkaline by SOS and heat shock-like responses (Maurer et al., 2005; Schuldiner et al., 1986; Taglicht et al., 1987). In response to alkali it is important to distinguish the difference between survival and growth. Survival does not include a clear increase in live cells in the high pH test and is usually accompanied by some vitality losses. Survival is monitored by live-cell numbers as colony forming units in a neutral environment. It should be noted that well buffered cultures show a marked increase in pH in many conventional growth media, eg LB, because survival is often observed in the stationary phase (Farrell & Finkel, 2003). In this study, it is aimed to develop a system that can be easily detected bacterial growth in ready to eat fish by changing the pads color as a result of changing the pH for the bacteria to maintain their lives.

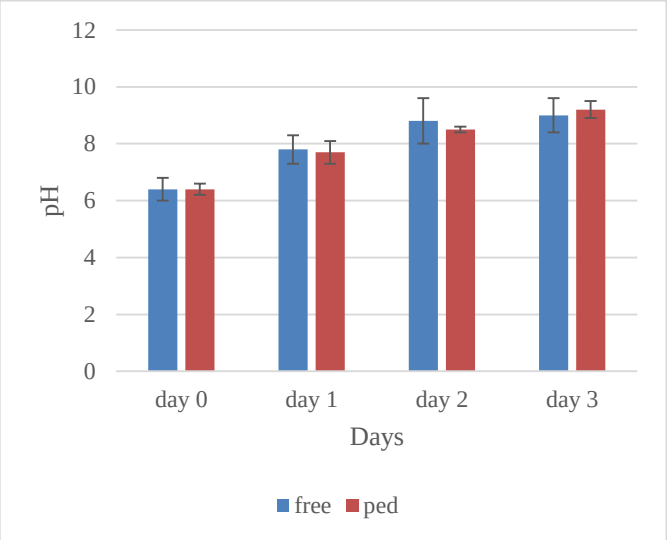

Figure 6. pH contents of free fish samples and fish fillets on the food pad absorbed red cabbage extract from day-1 to day-3 (day-0 = initial value of pH).

Some researchers focus on determining the pH value in the freshness of the fish mussel. According to Ludorf and Meyer (1973), the acceptable upper limit for the pH of fish is 6.8–7.0. The pH values of freshly caught fish are between 6-6.5. According to Ludorf and Meyer (1973) reported that fish could be consumed until the pH value in their mussel increases to 6.8. The pH values of spoiled fish muscle are above 7 as a base. However, some fish have an alkaline reaction even when they are kept fresh. Therefore, it is not correct to decide based on only pH value in the fish muscle. As it can be seen in Figure 6, pH values for free sample in control, initial (day-0), day-1, day-2, day-3 were, respectively, measured as 6.40 ± 0.02; 7.80 ± 0.04; 8.80 ± 0.00, and 9.00 ± 0.02. For the samples on the pads that red cabbage extract absorbed, pH values in initial, day-1, day-2, day-3 were respectively measured as 6.40 ± 0.02, 7.70 ± 0.05, 8.50 ± 0.03, and 9.20 ± 0.01. For all product groups that were stored at room temperature, no statistically significant difference was observed on the free samples and samples on the prepared food pads during storage day ($p > 0.05$). But there was a significant difference between initial and day-1 on pH values.

CONCLUSION

The packaging materials which are to be used as human food should not contain any risk which threatens public health. The effects of antioxidants (natural or synthetic) and spices which are used as additives in the

food production on the storage of products were examined for increasing the shelf life of food. This is the first time, red cabbage extract absorbed pads were used as a pH indicator and natural sensor for presenting information about the freshness of the food. It is considered that the new multi-functional food pad results on the experimental groups of red cabbage extract absorbed were obtained clearly color change with increasing pH value. The inhibition microbial growth on red cabbage extracted free bacterial disc indicated that the promising result was much more effective than the free sample. At the end of the storage (day-3), color of the pad was changed from purple to greenish-blue.

Moreover, in addition to obtained data, it is foreseen that further studies should be conducted on using different storage conditions (such as refrigerator storage, frozen storage etc.) and packaging methods (modified atmosphere packaging, vacuum packaging etc.) for the prepared experimental groups. Furthermore, various studies on the amount of plant extracts and spices used as coating films or additives in the experiments are needed to be conducted. As a result of the study guided, the importance of the alternative smart packaging materials, which is obtained from red cabbage extract and cellulose which has a high potential of production in Turkey in the food industry, pointed out. Moreover, it is considered that it will make a positive contribution to increasing the annual consumption rates of fresh food especially seafood products. It is predicted that a new multi-functional food pad and its possibility of becoming widespread may make a contrubition in the processed seafood sector.

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