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A Colorimetric Sensor Approach for Accurate Meat and Fish Freshness Monitoring

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Anahtar Kelimeler

Bromotimol mavisi (BTB)
Sensör
Et
Balık
Gıda tazeliği

Graphical/Tabular Abstract (Grafik Özet)

This study developed low-cost paper sensors using bromothymol blue to monitor meat and fish freshness. Color changes triggered by spoilage-induced pH increases were analyzed using RGB and ΔE methods. The sensor offers a fast, practical, and intuitive alternative for food safety. / Çalışmada, et ve balık tazeliğini izlemek için bromotimol mavisi kullanan düşük maliyetli kağıt sensörler geliştirmiştir. Bozulma kaynaklı pH artışıyla tetiklenen renk değişimleri RGB ve ΔE yöntemleriyle analiz edilmiştir. Sensör, gıda güvenliğini korumada hızlı, pratik ve sezgisel bir alternatif sunmaktadır.

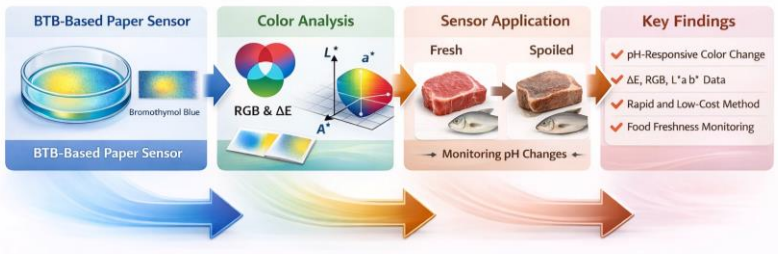

Figure A: *Graphical abstract of a BTB-based colorimetric sensor for rapid meat and fish freshness monitoring/* ***Şekil A:*** Et ve balık tazeliğinin hızlı izlenmesi için BTB tabanlı kolorimetrik sensörün grafik özeti

Highlights (Önemli Noktalar)

- *A BTB-based paper sensor enables rapid freshness monitoring of meat and fish. / BTB tabanlı kâğıt sensör ile et ve balık tazeliği hızlı izlenmiştir.*
- *Colour changes were quantitatively evaluated using ΔE, RGB, and L*, a*, b*. / Renk değişimleri ΔE, RGB ve L*, a*, b* ile nicel olarak değerlendirilmiştir.*
- *BTB concentration affects sensitivity; the system is low-cost and practical. / BTB konsantrasyonu duyarlılığı etkiler; sistem düşük maliyetli ve pratiktir.*

Aim (Amaç): *To monitor spoilage-related pH changes in meat and fish products, a BTB-based paper colorimetric sensor was developed and its performance was quantitatively evaluated using ΔE, RGB, and L*, a*, b* color spaces. / Et ve balık ürünlerinde bozulmaya bağlı pH değişimlerini izlemek amacıyla, BTB içeren kâğıt tabanlı bir kolorimetrik sensör geliştirilmiş ve performansı ΔE, RGB ve L*, a*, b* renk uzaylarında nicel olarak değerlendirilmiştir.*

Originality (Özgünlük): *This study directly reports L*, a*, and b* color coordinates in BTB-based paper sensors, compares performance on meat and fish, and systematically evaluates the effect of BTB concentration on sensor sensitivity. / BTB tabanlı kâğıt sensörlerde L*, a*, b* renk koordinatlarını doğrudan raporlayan, et ve balık üzerinde karşılaştırmalı testler yapan ve BTB konsantrasyonunun duyarlılığa etkisini sistematik olarak inceleyen nadir çalışmalardandır.*

Results (Bulgular): *Sensors with higher BTB content showed strong ΔE responses and clear color changes to spoilage-induced pH increases. / Yüksek BTB içeren sensörler, bozulmaya bağlı pH artışlarını güçlü ΔE tepkileri ve belirgin renk değişimleriyle göstermiştir.*

Conclusion (Sonuç): *The BTB-based paper sensor enables rapid, low-cost, and reliable monitoring of meat and fish freshness. / BTB tabanlı kâğıt sensör, et ve balık tazeliğini hızlı, düşük maliyetli ve güvenilir şekilde izlemeyi sağlar.*

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A Colorimetric Sensor Approach for Accurate Meat and Fish Freshness Monitoring

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Abstract

Monitoring the freshness of meat and fish is crucial for enhancing food safety for consumers and protecting public health. In this study, a paper sensor using bromothymol blue indicator sensitive to colour change was developed. The sensor performance was compared in solutions prepared with different dye amounts and different holding times. Studies were conducted on meat and fish, and colour changes were analysedanalyzed using both RGB and ΔE values. In the first sensors produced on fish that had not yet come into contact with food, RGB values resembled grey-greenish tones. In spoiled samples, the colour changes to reddish tones. RGB analysis of meat transitions from beige to reddish tones in intact samples and from beige in spoiled samples. RGB is a method that provides objective results in monitoring spoilage. The findings emphasize that colour changes are not merely visual differences but can also be used as a highly effective parameter in determining product condition. Furthermore, solutions containing higher BTB showed more pronounced and rapid colour changes, particularly in response to alkaline compounds formed during spoilage. The paper sensor developed for determining the freshness of meat and fish products offers a practical, low-cost, rapid, and intuitive alternative. The study contributes to the optimization of sensor systems using pH-sensitive synthetic dyes and presents RGB, L*, a*, and b*-based data.

Et ve Balık Tazeliğini Doğru İzleme İçin Kolorimetrik Sensör Yaklaşımı

Makale Bilgisi

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Öz

Et ve balığın tazeliğini izlemek, tüketiciler için gıda güvenliğini artırmak ve halk sağlığını korumak açısından çok önemlidir. Bu çalışmada, renk değişimine duyarlı bromotimol mavisi indikatörü kullanan bir kağıt sensör geliştirilmiştir. Sensörün performansı, farklı boya miktarları ve farklı bekletme süreleri ile hazırlanan çözeltilerde karşılaştırılmıştır. Et ve balık üzerinde çalışmalar yapılmış ve renk değişiklikleri hem RGB hem de ΔE değerleri kullanılarak analiz edilmiştir. Henüz gıda ile temas etmemiş balıklar üzerinde üretilen ilk sensörlerde, RGB değerleri gri-yeşilimsi tonlara benzemektedir. Bozulmuş numunelerde renk kırmızımsı tonlara dönüşmektedir. Etin RGB analizi, bozulmamış numunelerde bejden kırmızımsı tonlara, bozulmuş numunelerde ise bejden kırmızımsı tonlara geçiş göstermektedir. RGB, bozulmayı izlemede objektif sonuçlar sağlayan bir yöntemdir. Bulgular, renk değişikliklerinin yalnızca görsel farklılıklar olmadığını, aynı zamanda ürün durumunu belirlemede oldukça etkili bir parametre olarak da kullanılabileceğini vurgulamaktadır. Ayrıca, daha yüksek BTB içeren çözeltiler, özellikle bozulma sırasında oluşan alkali bileşiklere tepki olarak daha belirgin ve hızlı renk değişiklikleri göstermiştir. Et ve balık ürünlerinin tazeliğini belirlemek için geliştirilen kağıt sensör, pratik, düşük maliyetli, hızlı ve sezgisel bir alternatif sunmaktadır. Çalışma, pH'a duyarlı sentetik boyalar kullanan sensör sistemlerinin optimizasyonuna katkıda bulunmakta ve RGB, L*, a* ve b* tabanlı veriler sunmaktadır.

1. INTRODUCTION (GİRİŞ)

Food safety and freshness monitoring are vital for preventing foodborne illnesses and protecting consumer health, while contributing to reducing food waste and economic sustainability. Meat is a rich source of animal-based food, rich in highly bioavailable proteins, energy-dense fats, vitamins, and various micronutrients. This rich nutrient content has led to a significant increase in global demand for meat in recent years. Spoilage in food products is often associated with increased pH levels, which can be easily monitored visually, particularly through colour-changing chemical indicators[1]. Monitoring the freshness and quality of fish is critical for consumers, retailers, and the fishing industry. Therefore, rapid, harmless, and economical methods must be developed to instantly

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and directly monitor the freshness of fish products. Thanks to its high nutritional content and unique flavour, fish is a healthy food that many people prefer in their daily diet. However, post-catch storage and processing processes can significantly impact fish's freshness and overall quality[2]. Despite its nutritional properties, fish products are highly susceptible to microbial spoilage due to their high water activity, neutral pH, and autolytic enzymes, making them among the most perishable foods. In recent years, increasing public awareness of the importance of a balanced and healthy diet has increased interest in fish consumption[3].

Sensitive foods such as meat and fish spoil rapidly due to microbial growth and enzymatic activity. This necessitates constant freshness monitoring. Spoilage in packaged foods can generally be detected by physical indicators such as colour, pH, and odour. Such changes occur due to the breakdown of proteins, fats, and sugars by microorganisms and endogenous enzymes. They vary depending on the food type and the storage conditions it is exposed to. Colour, in particular, is one of the most distinctive sensory attributes of foods, directly affecting the product's appearance and perceived quality. Therefore, colour is a determining factor in consumer preference and acceptance of a product [4].

The type of solvent used directly affects colour perception and visual visibility. Chemical properties of the solvent, such as its polarity and hydrogen bonding capacity, alter the light-absorbing behaviour of the substance, creating differences in colour tone and intensity. This interaction makes distinct colour changes visible to the naked eye[5]. Experimental studies conducted by Reichardt on solvatochromic dyes revealed that the type of solvent significantly affects colour. Significant colour changes were observed, particularly when solvents with different polarities, such as water, acetone, and dimethyl sulfoxide, were used. Therefore, treatments with different solvents were selected in this study to evaluate how solvents affect shape colour changes [5].

Bromothymol blue (BTB) is among the pH indicators widely used in numerous studies to monitor food freshness. The distinct colour transitions of BTB provide significant advantages for practical applications. A study involving bromothymol blue and examining the effects of solvent type on spectral properties and colour intensity found that solvent polarity causes shifts in λmax values and changes in absorption intensity [6]. BTB is a triphenylmethane derivative dye frequently used as a pH indicator. Its pH-sensitive colour change makes it widely used in chemical laboratories and environmental and biological analyses. The colour change ranges from yellow in acidic environments ($pH < 6.0$), through near-neutral green ($pH \approx 7.0$), and to blue in basic environments ($pH > 7.6$). It is soluble in water and alcohol. It is used in plant photosynthesis and respiration indicator applications, biochemical and microbiological indicators, innovative packaging and food spoilage indicators, and high-sensitivity bioanalytical applications [7–11].

Studies in the literature that directly provide L*, a*, and b* data using a BTB mixture are limited or nonexistent. Most existing binary system studies use ΔE or RGB analysis, omitting L*, a*, and b* values. In a study monitoring the spoilage of beef samples with and without garlic extract, findings showed that samples without garlic extract spoiled by day 6. In contrast, adding 15% and 20% extracts extended the spoilage time to day 12. The colour transition of the smart indicator label progressed from dark yellow (fresh) to reddish yellow (time of consumption) and then to pale red (spoiled) depending on the freshness level. Additionally, Romero and colleagues assessed the quality of cow's milk samples with two clever packaging prototypes incorporating a bromothymol blue-based pH indicator [1].

A paper-based pH sensor was designed by dip-coating bromocresol purple (BCP) and BTB onto filter paper. This sensor allows visual monitoring of fish spoilage by changing the colour of BCP from yellow to pink and then purple, and the colour of BTB from orange to green-yellow and finally to green-blue. This provides a dual-indicator label indicating whether the product is fresh, medium-fresh, or spoiled. The colourimetric pH sensor obtained by dip-coating onto filter paper allows easy and accurate detection of fish spoilage with the naked eye [2].

A study developing three-layer smart packaging indicators for food freshness monitoring designed the indicators to be sensitive to gases released during spoilage. The study evaluated the performance of different pH indicators. BTB exhibited relatively higher sensitivity and a visible colour change in the presence of CO_2, and BTB was reported to more clearly reflect pH changes associated with food spoilage. This finding is consistent with the visuals in our current study and supports the potential of BTB as a food freshness indicator [3].

Biopolymers, naturally occurring polymers derived from living organisms, are monomer units linked by covalent bonds that spontaneously degrade in the environment. Their biodegradable nature, non-toxicity, and high compatibility make them widely used in active and intelligent packaging applications [4]. Methods for determining food freshness include traditional biological analyses, physical and chemical detection techniques, sensory evaluations, and rapid, non-destructive testing technologies. However, traditional methods are often time-consuming, destructive, and costly, leading to various limitations in application. Rapid, non-destructive detection techniques used in food analysis can be categorised as optical, mechanical, and acoustic systems. Alternative methods based on detection mechanisms include X-ray, electromagnetic methods, sensor-based systems, and microbial or enzymatic indicators. Furthermore, innovative approaches such as electronic nose and tongue technologies, biosensors, and pigment-based sensors offer practical solutions for quality assessment of food products[4].

In a study of freshness indicator labels used to track fresh foods, pH indicators were used to analyse the colour change profiles of acetate buffers and similar liquids resulting from pH changes during storage. In this study, colour changes were measured quantitatively in the L*, a*, and b* colour space, and it was revealed that bromothymol blue transitions to blue, green, and yellow hues depending on the pKa values of the indicators. Methylcellulose-based colour-change labels were developed to monitor the freshness of coconut water. In addition to the colour changes observed on the labels, parameters such as pH, total dissolved solids, acidity, microbial load, and gas exchange rates (CO_2/O_2) in coconut water stored at room temperature were also monitored. Colour changes were reported visually, rather than numerically, using the L*, a*, and b* system [12]. One study designed a three-layer packaging system to monitor the freshness of chicken breast meat. The study reported that colour changes were numerically analysed using the parameters ΔE (colour difference) and ΔRGB (red-green-blue colour components). However, the study did not provide measurements for the L*, a*, and b* colour coordinates; colour changes were evaluated using RGB and ΔE values, and detailed numerical data were not included [13]. Colour changes in film systems prepared with anthocyanins and similar natural colour compounds were measured using the Hunter Lab colour space (L*, a*, b*) for natural pigment-based freshness labels used in various food groups, such as spices, fruits and vegetables, and meat products. Colour differences in these films produced with pigments derived from natural sources, such as red cabbage, blackberries, and butterfly pea flowers, were quantitatively analysed using the ΔE formula [4].

Reviews of natural pigments in freshness monitoring systems indicate that colour changes in natural dyes such as anthocyanins and betalains can be reported in detail using L*, a*, and b* coordinates. Some studies have also evaluated these natural dyes in comparison with synthetic indicators. However, synthetic pigments (e.g., bromothymol blue and methyl red) are still preferred in commercial applications[14]. In another study, a paper-based, dual-indicator label was developed for monitoring the freshness of milkfish products. This system, fixed on filter paper, used BTB and bromocresol purple. Although phenol red was not used in the study, the paper-based fixation technique was preferred. Color changes on the labels were monitored numerically using RGB color values, but the L*, a*, and b* color coordinates were not directly reported [2]. In another study, a mixed solution containing BTB and other ingredients was applied to a paper substrate for freshness monitoring in beef packaging. The developed smart label visually indicated changes in freshness, with dark yellow hues for fresh meats, reddish hues for those requiring immediate consumption, and light red hues for spoiled samples. It was emphasised that the colour changes followed a linear course over time, but measurements for L*, a*, and b* were not reported. This study emphasised the method's direct applicability to packaging paper [1]. In a study on chicken products, pH-sensitive labels monitored meat spoilage and CO_2 levels within the package. The findings revealed that BTB-based labels exhibited a higher ΔE colour difference—the results support using such paper- or film-based substrates in freshness monitoring systems [15].

Existing studies generally focus on RGB/ΔE-based numerical monitoring or visual assessment. Paper-based systems incorporating BTB have been successfully implemented for meat, fish, and poultry by visually monitoring colour changes using RGB and ΔE differences. However, numerical reports based directly on L*, a*, and b* values are unavailable. Studies in the literature use binary indicator systems, where colour changes are reported numerically using L*, a*, and b* colour coordinates. Some of the most significant studies, particularly in food freshness monitoring, are summarised below.

A BTB-based colour indicator system was used to monitor the freshness of only one type of bonito. Although colour changes were observed in the study, numerical data for the L*, a*, and b* colour coordinates were not included [3]. Whatman filter paper-based colour indicator systems were developed to monitor the freshness of meat and fish products. These studies tested the indicators in aqueous environments that come into direct contact with food products. Colour change analyses were performed using ΔE and RGB, and numerical data for L*, a*, and b* colour coordinates were not directly included [16].

In one study, monitored the spoilage process of meat products using RGB sensor arrays. The spoilage processes of various meat types, including chicken, beef, pork, and cod, were monitored using RGB sensor arrays. Colour changes reflect the stages of spoilage in meats, providing a method for monitoring the spoilage process. In this study, the colour changes of the sensor array were monitored using photographs taken over time. The RGB indices obtained from these photographs were used to monitor the spoilage process. Furthermore, the obtained data were modelled using principal component analysis (PCA) to compare the spoilage processes of different meat and fish types[17]. One study summarises the development and application of colourimetric sensor systems to determine food safety and quality. RGB sensors are an important tool for assessing the safety and quality of food products. Machine vision-based colourimetric sensor systems quantitatively measure colour changes in food products using RGB values. In other words, freshness or spoilage is numerically monitored as changes in each pixel's red, green, and blue channel values[18] . One article discussed the main color modeling methods used in the digitalization of colorimetric sensors. The article highlights the significant advancement of colourimetric sensors in food safety applications. It notes that digitalisation enables more precise and rapid sensor data analysis. However, it also notes the need for further studies comparing the effects of different colour modelling methods on sensor performance[19]. One study monitored the freshness of pork using low-cost RGB sensors. These sensors were used to track meat spoilage processes[20]. In another study, the spoilage process of chicken meat was monitored using pH-sensitive dyes such as bromocresol purple and BTB. These sensors were used to determine the spoilage levels of the meat[21].

In this study, an advanced A-motif-based colourimetric pH biosensor was developed. The biosensor detects pH by integrating specific nucleic acid sequences and pH-sensitive dyes. The sensor exhibits a distinct colour change in the pH range of 5.5–7.0, making it effective for monitoring food freshness [22]. The colour changes observed in the team's study are consistent with the findings in our current study. This agreement scientifically supports the usability and reliability of our developed sensor for food spoilage detection. In the study, which developed a paper-based pH indicator, the spoilage process in pork was investigated. It was noted that significant colour changes were observed when the ΔE value of the indicator exceeded 33 due to pH change [23]. These findings are consistent with the findings of our study and further confirm its effectiveness and reliability in monitoring freshness. The indicator system using curcumin monitors pH change and colour stability. These two properties are critical for determining food freshness [8]. The findings are consistent with the pH sensitivity approach in our study and support the effectiveness of our paper-based sensor system in monitoring food freshness.

Most existing methods for detecting food spoilage are complex and expensive, requiring laboratory facilities and sophisticated equipment, and are composed of layers not made of biodegradable natural polymers (in studies focusing on field availability). The need for systems that are easy to use in the field, biodegradable, cost-effective, and provide visually traceable results is increasing daily. Innovative solutions such as paper-based sensors offer significant potential for reliable food freshness monitoring in the food industry and at home. The previous study focused on spoilage in ayran[24] .Given the challenges of freshness monitoring in meat and fish foods, the potential of such sensors is significant. This study focuses on a paper-based sensor that boasts a fast, low-cost, visual detection time of just 15 minutes, enabling significant improvements in food safety. The study aims to develop a rapid and practical food freshness detection method. The study provides valuable insights into the literature, the fundamental principles, and the development of this innovative approach, and its effectiveness has been tested on various food types. The visual colour change allows spoilage to be easily detected without needing any specialised equipment.

2. MATERIALS AND METHODS (MATERYAL VE METOD)

2.1. Materials (MATERYAL)

Paper, bromine thymol blue (Edukim-100 mL), pure water, meat (hind leg, 85 g), fish (cay fish, 166 g) were used.

2.2. Sensor Preparation (Sensör Hazırlığı)

Cellulose-based paper is readily available and frequently preferred in studies on biopolymers like cellulose and pH-sensitive freshness indicators [2,4]. As indicated in Table 1, solutions containing different proportions of components were prepared by mixing for 15 minutes. Two different solvents, water and ethyl alcohol, were used to prepare the solutions (Figure 1).

Papers were cut into 2×8 cm dimensions, impregnated by dipping them into the prepared indicator solutions for two minutes each, and dried at room temperature (28 ± 2 ◦C). This process enables the papers to respond to food pH changes (Figure 2).

Table 1. Information on prepared solutions (Hazırlanmış çözeltiler hakkında bilgiler)

Solution number	Solution Content
1	100 mL of pure water + 5 mL of bromthymol blue
2	100 mL of pure water + 10 mL of bromthymol blue
3	50 mL of pure water + 5 mL of bromthymol blue
4	50 mL of pure water + 5 mL of bromthymol blue (waited for 1 hour)

Figure 1. Preparation of solutions (Çözeltilerin hazırlanması)

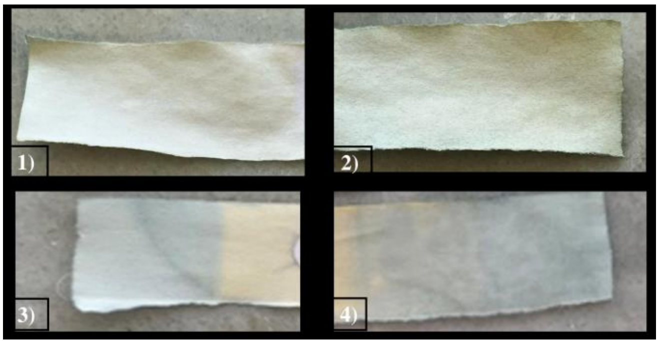

Figure 2. Prepared sensors (Hazırlanmış sensörler)

2.3. Colour Change Measurement in Samples (Örneklerde Renk Değişimi Ölçümü)

2.3.1. ΔE Measurement (ΔE Ölçümü)

The prepared sensors were placed in direct contact with food samples. Colour changes were observed and recorded in fresh meat, spoiled meat left to decompose at room temperature for 2 days, fresh fish, and spoiled fish left to decompose at room temperature for 2 days. The L*, a*, and b* values of colour indicators in foods were determined using a digital colourimeter program, and the total colour difference (ΔE) was calculated using these values. Three replicate experiments were performed for each measurement, and the mean and standard deviation values were calculated. In the L*, a*, and b* values, L* represents brightness (0 = black, 100 = white), a* represents the color transition from green to red (–a = green, +a = red), and b* represents the color transition from blue to yellow (–b = blue, +b = yellow). ΔE values were calculated by substituting these values into the equation in Equation 1. ΔE is a standard measure used to quantify the difference between two colours.

$$\Delta E = [(L_0 - L_1^*)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2]^{1/2} \quad \textbf{(1)}$$ [3,13]

where L0*, a0*, b0* are the initial color parameters and L1*, a1*, b1* are the color parameters in each analysis.

When evaluated with respect to temperature, L* generally decreases. High temperatures degrade or darken pigments, creating a duller/darker surface. In spoiled meat, L* decreases due to darkening. a* decreases in the meat and pigment systems. High temperatures degrade red pigments, resulting in a brownish/greenish hue. Reddish tones disappear as meat deteriorates. Temperature-related pH changes in BTB indicators can also affect a*. b* (Yellow/Blue Axis) generally increases (appears more yellow). Some pigments turn yellow with heat, increasing b* [25–29].

2.3.2. RGB measurement (RGB ölçümü)

Colour changes are directly related to food freshness and are an important indicator in evaluating sensor performance. This study examined the ΔE values of sensor solutions prepared using meat and fish food samples under different dye types and solution conditions. In experiments with different food samples, such as meat and fish, the ΔE values of the sensor solutions successfully reflected the level of food spoilage. In fish samples in particular, solutions containing higher amounts of BTB showed a stronger color response to the increasing pH changes associated with spoilage. This demonstrates that these solutions are practical sensor components for monitoring food freshness.

The study demonstrated that sensor solutions can be designed for highly pH-sensitive dyes, considering appropriate concentration and time. Measurements with the sensors used revealed that changes in solution pH are directly related to ΔE values over time. These results support the use of low-cost, fast-response paper sensors for food freshness monitoring. The next phase plans to improve system reliability through long-term stability testing, trials with different food matrices, and field applications.

The importance of the RGB model in food safety can be summarised as quantitative analysis of colour changes, shelf life estimation, and visual ease of reading. Sensor colour changes are digitally analysed to monitor food products' freshness and spoilage processes. RGB values provide a numerical representation of these changes. RGB data can be integrated into mathematical models to estimate the shelf life of food products. This is important for monitoring fresh food products and detecting spoiled products. The visual nature of the RGB model allows users to interpret sensor results quickly and easily. This is a significant advantage, especially in field testing[19].

3. RESULTS (BULGULAR)

In this study, a paper-based sensor was developed using cellulose-based paper and BTB dyes. The initial color difference (ΔE) and RGB values of paper sensors prepared using different combinations were compared. The results show significant differences between samples (Figure 3).

The colour change values for the samples were 18.9, 15.7, 23.4, and 29.5, respectively. A general upward trend was observed, with the highest colour change value achieved for the fourth sensor structure. These values indicate that the colour stability and colour change of the sensors vary depending on the sensor properties. The increase in colour change depends on the differences in solution content and holding time, as shown in Table 1. Considering this, the fact that the fourth sample exhibits the highest colour change suggests that this sample may have lower stability performance. This suggests that the preferred sensor structure (solution content) can also play a role in process optimization. Furthermore, the fourth sensor, with its high color change value, offers the ability to detect food spoilage more quickly and effectively, making it suitable for ongoing studies. With BTB-based sensors, the colour change is more pronounced and visually noticeable [13]

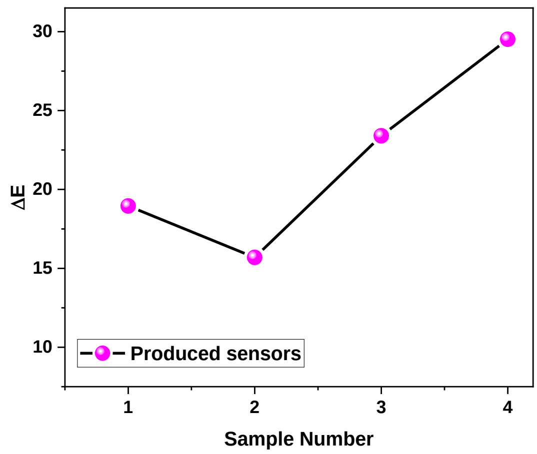

Figure 3. Initial ΔE values for paper sensors (Kağıt sensörler için başlangıç ΔE değerleri)

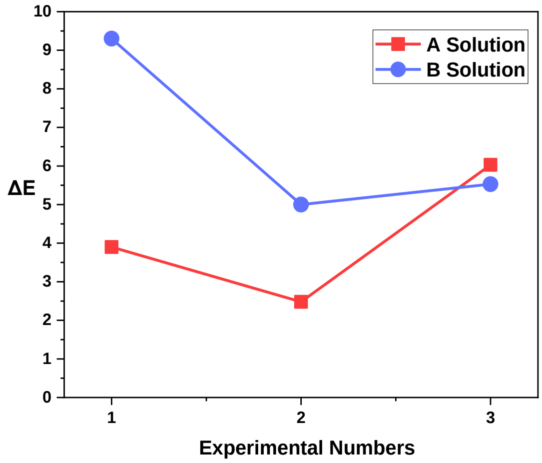

Figure 4. ΔE exchange for solutions A and B (A ve B solüsyonları için ΔE değişimi)

After evaluating the effect of sensor solution contents on color change, freshness determination experiments were conducted on meat and fish. In this step, two different solutions, solution 2 (100 ml distilled water + 10 ml bromthymol blue) and solution 4 (50 ml distilled water + 5 ml bromthymol blue), were examined. Papers were prepared with solutions A (100 ml distilled water + 10 ml bromthymol blue) and B (50 ml distilled water + 5 ml bromthymol blue) for meat, and solutions A (50 ml distilled water + 5 ml bromthymol blue) and B (100 ml distilled water + 10 ml bromthymol blue) for fish, and dried. Triplicate measurements were taken from the dried sensors, and ΔE and RGB values were monitored.

The color change graph for solutions a and b, with three repetitions, shows values of 3.9, 2.5, and 6 for solution a, and 9.3, 5, and 5.5 for solution b, respectively. This demonstrates that paper sensors exhibit rapid color change depending on pH (Figure 4).

Studies have been carried out on the traceability of pH changes caused by meat spoilage using bromotimol blue solutions and the measurability of this change using ΔE colour difference values. In this context, after the paper sensors were prepared, they were directly touched to the meat, and a noticeable colour change was observed (Figure 5. a-b). The effects of solutions containing different amounts of BTB on color sensor performance were analyzed (Figure 6).

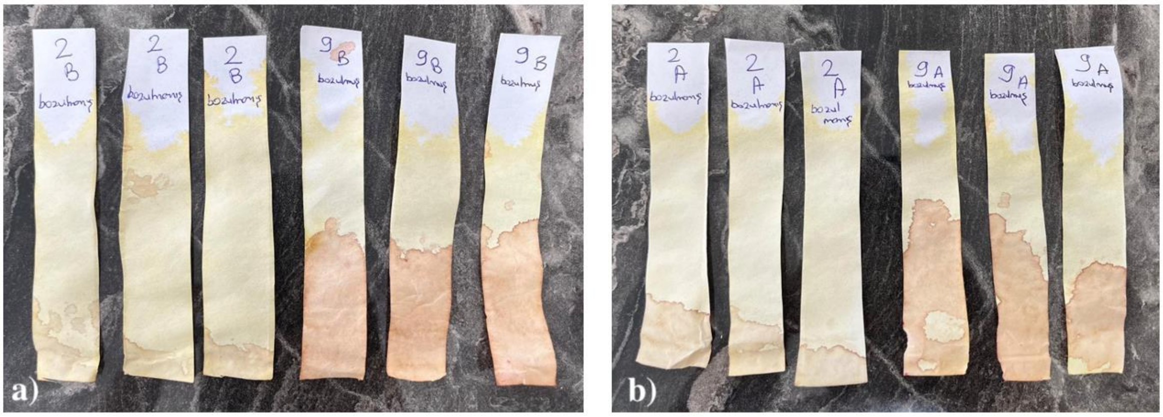

Figure 5. a-b) Visible colour changes in spoiled and unspoiled meat (a-b) Bozulmuş ve bozulmamış ette görünür renk değişiklikleri)

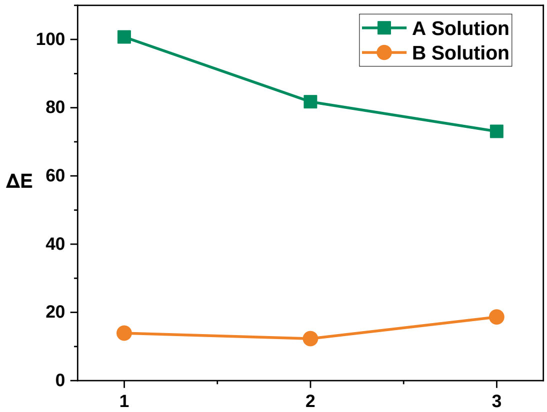

Figure 6. Comparison of ΔE of solutions A and B in meat samples (Et örneklerindeki A ve B çözeltilerinin ΔE'sinin karşılaştırılması)

Looking at Figure 6, the ΔE values for Solution A are 100.8, 81.7, and 73, respectively, while those for Solution B are 13.4, 12.6, and 18.4. Solution A exhibited a rapid ΔE value in the first experiment, but a gradual change over time in the third experiment. In contrast, Solution B exhibited lower ΔE values in the first experiment, but this value tended to increase over time. The high-amount solution exhibited a greater color change in the early stages of degradation, but the degradation process could be monitored more consistently with a high-amount indicator solution. A study based on ANOVA analysis of color sensors containing BTB reported a statistically significant effect of BTB concentration on sensor response ($F = 4.39$, $p = 0.01$) [26]. Other studies have shown that higher BTB concentrations make color transitions more pronounced and detectable [8,30,31].

After the paper sensors were produced for freshness experiments, they were placed in contact with fish, and visible colour changes were observed (Figure 7).

As fish spoil, they form alkaline compounds, triggering an increase in pH, which in turn causes colour changes. Figure 8 shows that the ΔE values for Solution A are 8.7, 10.3, and 9, while those for Solution B are 6.7, 11, and 3.4, respectively. 3Solution A exhibited more stable results, increases in sensitivity [2,30]. The method and results employed are consistent with and supportable in the literature, which establishes a direct relationship between BTB density and sensor sensitivity.

RGB results and interpretations were made separately for fish and meat. Evaluations and graphs were created first for fish and then for meat. The RGB values for each condition are similar in the first sensors and represent greyish-greenish tones. In spoiled fish samples, an increase in the R channel and a decrease in the B channel are observed, causing the color to shift to reddish or yellowish-cream tones. Increases in ΔR and ΔB values actually created a red dominance, while increases in ΔG neutralised the colour. When examining the RGB channel measurements and the resulting colours, these measurements yield quite objective results in detecting fish spoilage (Table 2).

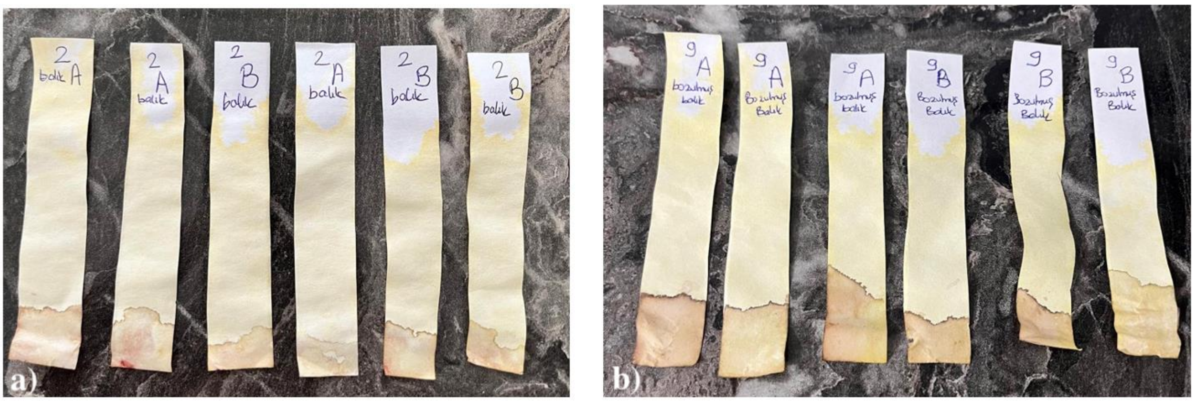

Figure 7. Visible colour changes in unspoiled and spoiled fish (Bozulmamış ve bozulmuş balıklarda görünür renk değişiklikleri)

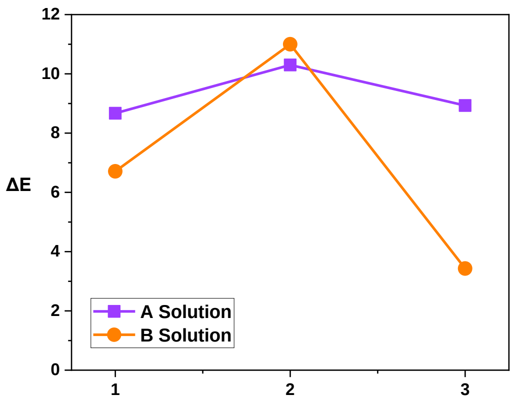

Figure 8. Comparison of ΔE of solutions A and B in fish samples (Balık örneklerinde A ve B çözeltilerinin ΔE'sinin karşılaştırılması)

Table 2. RGB values and interpretation for fish samples (Balık örnekleri için RGB değerleri ve yorumlama)

Sensor	Condition	R	G	B	Channel Differences (ΔR, ΔG, ΔB)
2B	Original	192	203	188	-
2B	Unspoiled	220	195	162	ΔR=+28, ΔG=-8, ΔB=-26
2A	Original	189	197	187	-
2A	Unspoiled	224	207	184	ΔR=+35, ΔG=+10, ΔB=-3
9A	Original	170	180	179	-
9A	Spoiled	185	160	160	ΔR=+15, ΔG=-20, ΔB=-19
9B	Original	155	165	164	-
9B	Spoiled	208	178	139	ΔR=+53, ΔG=+13, ΔB=-25

For comparing and better evaluation of the measurement results and the resulting colours, the results are presented grapically ın Figure 9.

When examining the meat, ΔR was observed to increase significantly in the spoiled samples (9A, 9B), while ΔB decreased. This resulted in a shift toward redder tones in the spoiled samples. The average RGB values show higher values in the degraded samples, indicating more vibrant colours. This data indicates that the colour change during degradation reveals visible differences in RGB (Table 3).

As with fish, a graph was created using the measurement results for meat (Figure 10). Colour blocks were placed along the horizontal axis of the graph to emphasise the visual changes.

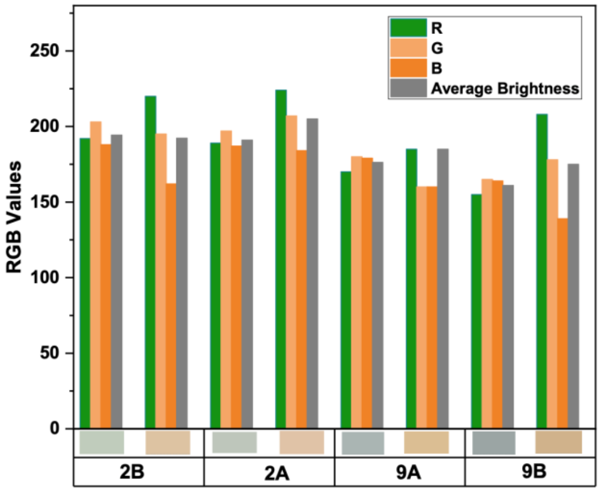

Figure 9. Comparative RGB analysis of fish samples (Balık örneklerinin karşılaştırmalı RGB analizi)

Table 3. RGB values and interpretation for meat samples (Et örnekleri için RGB değerleri ve yorumu)

Sensor	Condition	R	G	B	Channel Difference (ΔR, ΔG, ΔB)
9B	Original	155	165	164	—
9B	Spoiled	219	175	146	ΔR=+64, ΔG=+10, ΔB=-18
9A	Original	170	180	179	—
9A	Spoiled	231	198	164	ΔR=+61, ΔG=+18, ΔB=-15
2B	Original	192	203	188	—
2B	Unspoiled	226	209	177	ΔR=+34, ΔG=+6, ΔB=-11
2A	Original	189	197	187	—
2A	Unspoiled	224	207	184	ΔR=+35, ΔG=+10, ΔB=-3

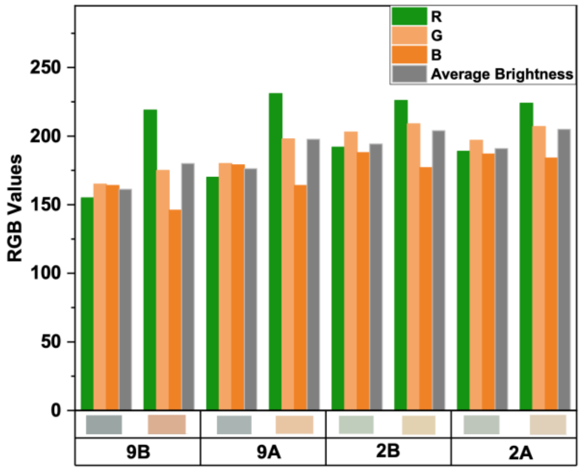

Figure 10. Comparative RGB analysis of meat samples (Et örneklerinin karşılaştırmalı RGB analizi)

The results obtained indicate that the developed paper-based sensor can quickly and easily detect colour changes in meat and fish, thereby ensuring consumer safety. The effectiveness of the paper-based colour sensors developed in this study was compared with studies in the literature that serve similar purposes (Table 4).

Table 4. Comparison of the literature with the presented study (*Advantage/disadvantage is created according to the presented study.) (Tablo 4. Literatürün sunulan çalışmayla karşılaştırılması (*Avantaj/dezavantaj sunulan çalışmaya göre oluşturulmuştur.)

Study Subject	Application Environment	Display Type	Sensor Type	Advantages	Disadvantages	Ref.
Freshness monitoring of coconut water with smart packaging	Coconut water	Phenol red, bromothymol blue, methyl red	Gel-based film	Actual packaging application, detailed analysis	Coconut water only, lack of direct-to-consumer reviews	[12]
Extending the shelf life and monitoring the quality of cold-stored beef with active garlic-extract packaging and pH indicator	Cold chain environment (4 ± 1°C), controlled laboratory experiment	Paper-based pH indicator, color change	Visual colour indicator	Delaying microbial spoilage with garlic extract	the effect of delaying microbial spoilage was not evaluated separately, lack of sustainability in plastic-based packaging, measurement was on the surface of the meat, not inside it	[1]
Recent developments of pH-sensitive freshness indicators with natural colorants and their applications in food packaging	General assessment – theoretically a variety of foods (meat, fish, milk, vegetables)	pH indicators made using natural pigments.	Natural pigment-based colour change sensors	Examination of different food groups	High moisture per-meability and risk of fading	[4]
Color label with dual pH indicator for real-time monitoring of fish (milkfish) freshness	Fish samples stored at room temperature and in the refrigerator (4°C)	paper-based dual indicator labels	Dual pH sensor based on colour change	Clearer colour change with dual indicators, Direct in-pack application	Unnatural dyes (synthetic)	[2]
Sensitivity determination of indicator paper as smart packaging elements in	Meat	Color Change (RGB)	Paper-Based-pH indicator	The combination of BTB with different solvents shows high sensitivity	L^*, a^*, b^* color data not reported; limited to cold storage conditions	[16]

monitoring meat freshness in cold temperature				to degradation volatiles		
Simulation of three-layer smart color-changing labels with different binders for monitoring the freshness of Atlantic bonito	Atlantic bonito	Four types of three-layer labels	Both pH indicator and TVB-N indicator	Both CO_2 and TVB-N based indicators were developed, performance comparison of different binders, various simulations	TVB-N labels did not show significant color change in real fish samples (due to the low TMA level in practice)	[3]
Developing CO_2-sensitive fresh labels for real-time monitoring of chicken breast meat spoilage	Real food packaging conditions	Three-layer color label	pH indicator	Tested in a real packaging environment, suitable for industrial applications	Only one product was examined: PA-PET + pH paper. The focus was solely on CO_2-based degradation.	[13]
Freshness monitoring in meat and fish with paper-based sensors	Meat and fish	BTB	Paper-based sensors	meat and fish applications, low-cost sensors, easy fabrication; ΔE and RGB values for different amounts of BTB; Systematic investigation of solution time sensitivity	Long-term stability testing is lacking	This work

In conclusion, the paper-based sensor used in this study is a low-cost, sensitive, rapid, and practical method for detecting spoilage in meat and fish. All these features make paper-based sensors particularly important for developing countries.

4. CONCLUSIONS (SONUÇLAR)

This study, which aimed to develop paper-based sensors for detecting meat and fish spoilage, utilised bromothymol blue in the preparation of the sensors, demonstrating a sensitive, inexpensive, and rapid system. BTB concentration plays a significant role in determining sensor sensitivity and colour change. The ΔE results obtained for meat and fish indicate distinct colour changes, which are also supported by the RGB results. These findings highlight that colour changes are not merely visual differences but can be used as a highly effective parameter for determining product status. It offers a practical, inexpensive, and rapid alternative to existing methods. Future research should be expanded to include sensors with a variety of food samples, as well as improving their stability and sensitivity. Evaluating these sensors will be beneficial in increasing confidence in paper sensors, better protecting consumer health, and expanding their application.

DECLARATION OF ETHICAL STANDARDS (ETİK STANDARTLARIN BEYANI)

The author of this article declares that the materials and methods they use in their work do not require ethical committee approval and/or legal-specific permission.

Bu makalenin yazarı çalışmalarında kullandıkları materyal ve yöntemlerin etik kurul izni ve/veya yasal-özel bir izin gerektirmediğini beyan ederler.

AUTHORS' CONTRIBUTIONS (YAZARLARIN KATKILARI)

Merve Okumuş SARI: Conceptualization, Investigation, Methodology, Visualization, Writing – draft & editing.

Kavramsallaştırma, Araştırma, Metodoloji, Görselleştirme, Yazma – taslak ve düzenleme.

Kübra KESER: Supervision, Conceptualization, Methodology, Writing – draft & editing.

Denetim, Kavramsallaştırma, Metodoloji, Yazma – taslak ve düzenleme.

CONFLICT OF INTEREST (ÇIKAR ÇATIŞMASI)

There is no conflict of interest in this study.

Bu çalışmada herhangi bir çıkar çatışması yoktur.

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